MAIZE GENETICS COOPERATION

NEWS LETTER

63

March 31, 1989

Department of Agronomy
and
U.S. Department of Agriculture
University of Missouri
Columbia, Missouri

Some sources of general information on maize genetics and cytogenetics:

Carlson, W. R., 1989. The cytogenetics of corn. Pp. 259-344 in G. F. Sprague and J. W. Dudley, eds., Corn and Corn Improvement, 3rd ed., Amer. Soc. Agron., Madison, Wisconsin.

Coe, E. H., Jr., Hoisington, D. A., Neuffer, M. G., 1987. Linkage map of corn (maize) (Zea mays L.). Pp. 685-707 in S. J. O'Brien, ed., Genetic Maps 1987, Vol. 4, Cold Spring Harbor Laboratory.

Coe, E. H., Jr., Neuffer, M. G., Hoisington, D. A., 1989. The genetics of corn. Pp. 81-258 in G. F. Sprague and J. Dudley, eds., Corn and Corn Improvement, 3rd ed., Amer. Soc. Agron., Madison, Wisconsin.

Emerson, R. A., Beadle, G. W., Fraser, A. C., 1935. A summary of linkage studies in maize. Cornell Univ. Agric. Exp. Sta. Memior 180.

Hallauer, A., Miranda, J. B., 1988. Quantitative Genetics in Maize Breeding, 2nd ed., Iowa State Univ. Press, Ames., Iowa.

Sheridan, W. F., ed., 1982. Maize for Biological Research. Plant Molec. Biol. Assoc., Box 5126, Charlottesville, Virginia.

Walbot, V., Messing, J., 1989. Molecular genetics of corn. Pp. 389-430 in G. F. Sprague and J. W. Dudley, eds., Corn and Corn Improvement, 3rd ed., Amer. Soc. Agron., Madison, Wisconsin.

Conservation of Valuable Genetic Resources

The National Plant Germplasm System (NPGS) takes seriously its mission to acquire and preserve valuable genetic resources. Scientists who have characterized valuable genetic stocks are encouraged to place a fresh sample in the National Seed Storage Laboratory (NSSL) at Fort Collins, Colorado. The originator or donor will need to supply characterization data. The sample will be held separately at NSSL as a backup to the scientist's working collection. The donor can request the seed returned for needed regeneration, but the sample will neither be germinated nor distributed except as needed for that purpose since it is a backup sample. The quantity of seed desired should be 200-500 seeds, if possible, but at least 25. Some stocks are extremely difficult or costly to manage and the small samples are acceptable under these circumstances. Comments relative to any unusual regeneration performance or handling requirement are essential to the sample's documentation. Please give this request your most serious attention. We thank you for your attention to this essential detail of our precious genetic resources.

STEVE A EBERHART
Director, National Seed Storage Laboratory
USDA-ARS
Colorado State University
Fort Collins, CO 80523

HENRY L. SHANDS
National Program Leader, Germplasm
USDA-ARS-NPS
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I. FOREWORD

"The research newsletter is particularly suitable for information not usually suitable for scientific journals: continuing updating of gene symbols and nomenclature, location of mutants, strains and stocks, ...Short technical notes, short research findings, observations, new ideas and compilations of data..."

-H. V. Wyatt, 1986

Sharing here our updating, technical data, short notes, short findings, observations, compilations, new ideas, gene symbols, location of mutants, strains and stocks, we contribute to the advancement of biology and to the power of shared technical knowledge. The working research information here is shared with the specific understanding that it is unpublished information, not to be cited in publications without the specific consent of the authors.

I thank you, dear reader, for your patience and forbearance (no, not forebearance, which must have something to do with pedigree) as we have struggled and stumbled out of the cocoon to unfold the butterfly before you. The utopian promise of "desktop" production is fulfilled only via a sustained learning curve; at long last you may evaluate the payoff. Because so many cooperators helpfully supplied their material in electronic form, we in fact have been able to concentrate more on form and content than previously. Both Zealand and the symbol index reflect changes due to that freeing-up.

Gifts to the Endowment Fund for support of the Newsletter total over $57,000. Please see the impressive 4-page listing, in the front of this issue, of donors whose generosity has made this total. The response has been wonderfully gratifying, and we are all grateful for the support of our colleagues and of organizations with which we have common interests. Part of the financial support this year also comes from the National Science Foundation; the mapping project centered at Missouri (see page 141) was funded by NSF at a level sufficient for highest-priority map integration work, including some funds for mapping-related compilations in the Newsletter.

A warm acknowledgement for advice and ideas is given to my colleague Dave Hoisington. Dave also assembled and developed the fine linkage maps in this issue, using his knack for encompassing the status of each research area. His participation with the News Letter, and many computer trickeries, make all the editing work feasible and efficient.

Shirley Kowalewski learned how to amuse the word-processor into doing what we wished it to do, single-handedly edited and refined the copy, kept up addresses and subscriptions and correspondence, and screened the literature for this year's compilation. Mary Brazil helped with literature entries and reprint requests. Suzanne O'Dell and Mary Ann Steyaert booked addresses and posted subscriptions with care. Kathy Chappell and Chris Browne efficiently carried out numerous vital tasks. Chang-deok Han, Masumi Katsuta and G. Madhavi Reddy helped with library work. At University Printing Services, Yvonne Ball and Dale Kennedy and their staff again efficiently and carefully made sure that the job was done promptly and well.

Included in this issue are impressive organelle maps, generously volunteered by Steven Rodermel and Lawrence Bogorad for the chloroplast and by Christiane Fauron for T mitochondria, and their contributions are gratefully acknowledged.

For submission of notes for the next issue (Number 64, 1990), please see details inside the back cover.

If you wish to subscribe to this News Letter please use the form in the back of this issue. Gifts to the Endowment Fund, toward our goal of $100,000, will be very much appreciated.

Ed Coe

ALBANY, CALIFORNIA
Plant Gene Expression Center, USDA-ARS

Location of *Adh1* and *Kn1* on the linkage map
--Julie Mathern and Sarah Hake

We undertook a RFLP mapping study of the long arm of chromosome 1 in order to place *Kn1* and *Adh1* on the map and to orient them with respect to the centromere. One of the purposes of this study was to characterize the endpoints of a deletion of *Kn1*.

The material used for segregation analysis was from self-pollinations of *Kn1-N1 Adh1-S Lw/kn Adh1-Fgamma25 lw* heterozygotes. Seedlings were green and knotted, or white and normal, unless there was recombination. Southern blots of DNA from these individuals were probed with a *Kn1* probe (Hake, Vollbrecht, Freeling in press), an *Adh1* probe and the RFLP markers shown in the table. We were able to place *Kn1* and *Adh1* on the RFLP map between UMC107 and BNL7.25. We found 2 recombinants, one green and normal and the other white and knotted, at the frequency of 1/200. The proximal marker, UMC 107, segregated with *Kn1* and the distal markers segregated with *Adh1* (and *lw*), demonstrating that *Kn1* is proximal to *Adh1*. In both cases the *Adh1* allele cosegregated with its linked *lw* marker, thus we have not yet determined the placement of *Adh1* relative to *lw*.

RFLP CLONE	# INDIVIDUALS TESTED	ESTIMATED MAP UNITS FROM *Kn1*
UMC 37	17	35
BNL 8.10	22	23 proximal
UMC 107	65	6.9
BNL 7.25	38	8 distal
BNL 8.29	17	26

We recovered a deletion of *Kn1* following X-ray mutagenesis that is not transmissible through the male. The deletion does not include *Adh1* or *lw* (Hake, Vollbrecht, Freeling, in press). Since we have placed *Adh1* and *lw* distal to *Kn1*, we know this deletion extends less than 1 map unit in the distal direction. Recombination to a proximal marker, *Bz2*, does not appear to be affected by the deletion. 68 recombinants were found out of 284, giving a distance of 24mu, similar to the distance on the genetic map of 22mu. Therefore, the deletion is relatively small. We tried to uncover the deletion by crossing it to the TB-1La translocation using *Adh1* markers to distinguish the progeny. We could not recover the genotype for the hypoploid/deletion double heterozygote in healthy kernels. The ear however did contain defective kernels. This suggests that the deletion is also homozygous lethal to the embryo. We are curious whether it is the absence of *Kn1* or a closely linked gene that makes it lethal.

The *Kn1-O* mutation is a 19kb tandem duplication
--Bruce Veit, Erik Vollbrecht and Sarah Hake

Kn1-O and *Kn1-2F11* are two dominant mutations, both closely linked to *Adh1*, that interfere with the differentiation of vascular cells in the leaf blade. Recently, DNA associated with the knotted allele *Kn1-2F11* was cloned by its association with the previously cloned transposable element, *Ds2*. Expression of the knotted phenotype in *Kn1-2F11* depends on the presence of *Ds2*; excision of *Ds2* results in reversion to a non-knotted phenotype (Hake, Vollbrecht and Freeling, in press).

We have used the *Kn1-2F11* clone as a hybridization probe to examine the organization of homologous DNA sequences associated with *Kn1-O*. In comparisons of knotted vs. normal siblings, no insertion sequences comparable to the *Ds2* of *Kn1-2F11* were found; however, by Southern analysis, this region appears to be duplicated in *Kn1-O*. Cloning of junction fragments unique to *Kn1-O* indicates the tandem duplication of a 19kb region. *Kn1-Ox*, an extremely knotted derivative of *Kn1-O*, shows a triplication of this same region, while *Kn1-Od*, a normal derivative of *Kn1-O*, is missing the region.

Studies are in progress to determine if novel transcripts associated with the unique duplication junction might be responsible for the knotted phenotype. The importance of this region is suggested by at least two separate instances in which the insertion of *Mu* related transposable elements within 1kb of the duplication junction is associated with the loss of the knotted phenotype. The junction is also implicated by its proximity (<2kb) to the site at which the insertion of *Ds2* in *Kn1-2F11* produces a knotted phenotype.

Interaction between mutations that influence inflorescence development
--Bruce Veit and Sarah Hake

We have begun to analyze the extent to which different mutations affecting inflorescence development interact with each other. We hope that knowledge of such interactions will suggest functional relationships between the gene products defined by these mutations.
Initial experiments indicate that the tassel morphology conditioned by *ts2* is modified by *Mpl* or *ts4*. *ts2* typically produces a normally branched tassel that bears only pistillate florets while *Mpl* conditions both dwarf and anther-ear phenotypes. In the *Mpl ts2* double mutant, both the dwarf and anther-ear phenotypes persist. Interestingly, the pistillate florets conditioned by *ts2* become perfect when *Mpl* is also present. Thus, in its response to *Mpl*, the terminal pistillate inflorescence produced by *ts2* behaves much like its normal axillary counterpart, the ear. *ts4* conditions the development of a highly branched tassel with the sporadic development of pistillate florets. In *ts2 ts4* double mutants, a highly branched, completely pistillate tassel develops.

With both the *ts2 Mpl* and *ts2 ts4* double mutants, the mutant phenotypes can be explained as simple superimpositions of the transformations associated with each of the single mutants (i.e., the elaboration of a given mutant phenotype is not precluded by the presence of a second mutation). It would seem that these mutations affect developmental processes that are relatively distinct from each other.

The insertion of a *Mu* element results in a new knotted mutation
--Richard M. Walko and Sarah Hake

A knotted mutation has arisen in a Robertson's Mutator line. This mutant exhibited mild to severe knotting as well as ligule displacement when homozygous. When outcrossed to a non-Mutator line, however, the heterozygotes exhibited very mild knotting on the first and second leaves, and then did not exhibit any knotting or ligule displacement

until the last one to three leaves below the tassel. These upper leaves exhibited additional ligules displaced outward on the leaf blade and generally severe knotting.

Southern analysis indicates the insertion of an element into the *Kn1* locus. The insertion is approximately 1.4kb in length with *Hin*FI sites close to its ends in the inverted repeat regions as indicated by probing Southern blots with a probe for *Mu* ends, pDTE1 (V. Chandler, C. Rivin, and V. Walbot, Genetics 114:1007, 1986). While this insertion is similar to *Mu1* in size and *Hin*FI restriction pattern, this insertion contains a *Pvu*II restriction site and does not exhibit homology to an internal fragment of *Mu1* (pA/B5; Chandler et al., 1986) as indicated by Southern analysis. The insertion appears to be located in the *Kn1* locus within 250 bases of the site where a *Ds2* insertion occurs to cause the knotted phenotype in another mutation, *Kn1-2F11* (Hake et al., EMBO in press). The insertion and its flanking sequences have been cloned and further analysis is in progress.

Continuous transposition of the maize *Ac* element in four generations of transgenic tobacco

--Reinhard Hehl and Barbara Baker

We have studied the activity of the maize transposable element Activator (*Ac*) in transgenic tobacco plants. Six transposed *Ac* elements have been cloned and are found to be integrated into and close to unique and low copy DNA. Five of these elements have been cloned from an R1 plant obtained through selfing of an original R0 transformant. These elements are designated *Nt-1::Ac-18*, *Nt-5::Ac*, *Nt-6::Ac*, *Nt-7::Ac* and *Nt-8::Ac*. Four of the five elements isolated from the R1 plant (*Nt-5::Ac*, *Nt-6::Ac*, *Nt-7::Ac* and *Nt-8::Ac*) can not be detected in genomic Southern blot hybridizations. Since the R1 plant does not contain *Ac* sequences in T-DNA this suggests that transposed elements continue to transpose somatically. One element (*Nt-1::Ac-18*) is stable and can be detected in genomic Southern blots in the R1 as well as the R0 plant. *Ac-18* sustained a 4bp terminal deletion and has never been found to transpose again.

The induction of an 8bp duplication of target sequences of *Ac* (*Nt-6::Ac*) upon integration reveals that the mechanism of integration of *Ac* in tobacco is similar to that in maize. The result that the R1 plant harbored transposing *Ac* elements has been verified by analyzing R2 offspring plants obtained through selfing of the R1 plant. Genomic Southern blot hybridizations showed that 10 out of 12 R2 plants harbored new and different *Ac* homologous restriction fragments distinct from those present in the R1 progenitor. This indicates that *Ac* has transposed frequently into new and unique target sites. To confirm that the new restriction fragments represent newly transposed *Ac* elements we cloned one of these elements (*Nt-2::Ac*) from an R2 plant harboring only *Nt-1::Ac-18* (see above) and *Nt-2::Ac*. The presence of an 8bp duplication adjacent to *Ac* at *Nt-2::Ac* reveals that *Ac* indeed transposed into this particular target fragment. Analysis of R3 offspring plants isolated by selfing of the R2 plant harboring only *Nt-2::Ac* and the stable *Nt-1::Ac-18* demonstrates that the *Ac* element at *Nt-2::Ac* is capable to transpose again, since new *Ac* homologous restriction fragments can be detected in genomic Southern blot hybridizations. Molecular analysis of tobacco DNR adjacent to *Ac* in *Nt-2::Ac* shows the presence of not only unique but also repetitive DNA sequences. Furthermore a transcript homologous to the unique DNA fragment of *Nt-2::Ac* can be detected in Northern blot hybridizations.

The observed continuous activity of *Ac* in transgenic tobacco also correlates with the detection of the *Ac* specific 3.5kb mRNA as well as with the lack of methylation of *Ac* internal sequences. The latter was analyzed by *Hpa*II and *Pvu*II digestion of DNR of the RO plant as well as 4 R1 and 12 R2 plants obtained through selfing of the RO plant. All 17 plants harbor *Ac* elements at different target sites and in none of these plants were *Ac* sequences found to be methylated.

These data suggest that *Ac* can be employed for transposon tagging in transgenic tobacco. We are currently using *Ac* in an attempt to tag the dominant TMV resistance gene *N*.

Delivery of *A1* and *Bz1* genes to intact aleurone tissue

--Ted Klein, Brad Roth and Michael Fromm

We have employed microprojectiles to deliver the *A1* and *Bz1* genes to cells within intact aleurone tissue. Aleurones from plants carrying *C1* and *R* and either *a1* or *bz1* were excised 15 days after pollination by peeling back the pericarp and removing the aleurone and underlying endosperm tissue. The tissue was placed on agar-solidified medium and bombarded with microprojectiles (Klein et al., Bio/Technology 6:559, 1988) coated with the genomic clones of the *A1* (C. O'Reilly et al., EMBO J. 4:877, 1985) or *Bz1* (N. Fedoroff et al., Proc. Natl. Acad. Sci. USA 81:3825, 1984) genes. After incubating the tissue for 1 day in the light at 26 C, a portion of the cells within the aleurone developed purple pigmentation (Fig. 1). Individual isolated

Figure 1. Expression of the *Bz1* genomic clone following its delivery into maize aleurone tissue from *bz1,C,R* plants. Anthocyanin pigment appears as dark spots on the black-and-white photograph.

purple cells could be visualized but more generally 3 to 5 adjacent cells developed pigmentation with a central most cell being more highly pigmented than surrounding cells. These pigmented spots were restricted to the aleurone layer. Up to 100 of these spots developed on a single *a1,C1,R* or *bz1,C1,R* aleurone following bombardment with either the *A1* or *Bz1* genomic clone, respectively. Such spots did not develop when aleurone tissue was bombarded with microprojectiles coated with pUC18. This indicates that the introduced *A1* or *Bz1* genomic clones complemented the *a1* and *bz1* mutations and that the isolated clones are functional when reintroduced into aleurone cells.

To analyze the expression of these genes in different genetic backgrounds, chimeric genes were constructed by fusing the firefly luciferase coding region to the 5' and 3' regions from the *A1* or *Bz1* genomic clones to form pA1L and pBz1L, respectively. These constructs were introduced into aleurones of the following genotypes: *C1,R*; *c1,R*; *C1,r*; *c1,r*; *C-I,R*. Levels of luciferase activity in permissive backgrounds (*C1,R*) were about 20 to 100 fold greater than those detected in tissue carrying either or both of the recessive alleles (*c1,r*) of these genes (Table 1). Low levels of luciferase were also observed following delivery of pA1L or pBz1L to *C-I,R* aleurones. As a positive control, aleurones from the various genotypes were bombarded with pAI_1LN (J. Callis et al., Genes Develop. 1:1183, 1987). The luciferase gene in this plasmid is under the control of the maize *Adh1* promoter. Therefore, its expression should not be influenced by the different genetic backgrounds that regulate anthocyanin production. Levels of luciferase activity were consistently high in aleurone tissue from all of the genotypes tested following delivery of pAI_1LN-DNA.

Table 1. Luciferase expression in aleurone tissue from various genotypes following bombardment with the chimeric *A1* or *Bz1* genes.

GENOTYPE	PLASMID	LUCIFERASE ACTIVITY ± S. E. (light units x 10^3)*
a1, C1, R	pA1L	20.4 ± 3.8
	pBz1L	26.1 ± 8.1
	pAI1LN	124.4 ± 21.9
bz1, C1, R	pA1L	22.6 ± 0.3
	pBz1L	33.3 ± 11.2
	pAI1LN	319.0 ± 20.4
c1, r	pA1L	0.5 ± 0.2
	pBz1L	0.2 ± 0.01
	pAI1LN	141.1 ± 56.4
c1, R	pA1L	1.2 ± 0.2
	pBz1L	0.6 ± 0.1
	pAI1LN	193.5 ± 23.4
C1, r	pA1L	0.3 ± 0.1
	pBz1L	0.5 ± 0.3
	pAI1LN	299.2 ± 85.6
C-I, R	pA1L	1.2 ± 0.1
	pBz1L	0.3 ± 0.0
	pAI1LN	446.2 ± 168.4

*Luciferase activity was determined according to Callis et al. (Genes Develop. 1:1183, 1987).

These results show that expression of DNA delivered to intact tissues by microprojectiles properly reflects the regulation of the native genes. The transfer of genes directly to intact tissues provides a rapid means for studying the genetic regulation of gene expression at the molecular level.

ALBANY, NEW YORK
State University of New York

Codon usage table for maize based on sequences of 25 nuclear genes

--David M. Bashe and Joseph P. Mascarenhas

The genes of various eukaryotic organisms exhibit marked differences in their patterns of choice for synonymous codons. A knowledge of the codon preference for a given organism is useful particularly with regard to determining preferred open reading frames of genes whose identities are unknown, and for back-translating from a known peptide sequence to produce a probe for the gene by which that protein is encoded. The pattern of codon choice is clearest when a large number of known genes are used in the preparation of the codon-usage table. Maruyama et al. (Nucl. Acid. Res. 14S, r151-r197, 1986) have tabulated codon usage tables for all organisms for which more than 5 genes were available using GenBank Genetic Sequence Data Bank Release 38.0, Nov 1985. At that time, only 8 complete coding sequences were available for maize, seven of which were storage proteins. Since cereal storage proteins are very deficient in certain amino acids, some amino acids were underrepresented in their table. A number of additional maize genes have now become available. The present work was undertaken to provide a meaningful table of codon usage. In the process of producing this table, it became apparent that at least one of the zeins (22kD) was not typical of corn in its codon usage profile.

In this study, 25 maize nuclear genes (Table 1) from the GenBank database were selected for analysis. In cases

Table 1. GenBank Sequences used in the preparation of the codon usage table.

GenBank locus	Function	GenBank locus	Function
MZEA1G	NADPH-dep reductase	MZEACT1	Actin 1
MZEADH1F	Alc. dehydrogenase1	MZEADH2NR	Alc. dehydrogenase2
MZEALD	Aldolase	MZEANT	ATP/ADP translocator
MZEEG2R	Endosperm glutelin	MZEH3C2	Histone 3
MZEH3C4	Histone 3	MZEH4C7	Histone 4
MZEH4C14	Histone 4	MZEHSP701,2	70kD heat shock protein
MZELHCP	Light-harvesting chloroplast protein	MZEMPL3	Major lipid body protein L3
MZEPEPCR	Phosphoenolpyruvate carboxylase	MZERBCS	RuBP carboxylase small subunit
MZESUSYSG	Sucrose synthase (shrunken)	MZETPI1	Triosephosphate isomerase1
MZEZE15A3	15kD Zein	MZEZE19B1	19kD Zein
MZEZE22A	22kD Zein	MZEZE22B	22kD Zein
MZEZEA20M	Zein	MZEZEA30M	Zein
MZEZEPCM1	Zein	MZEZEZ4G	Zein

Table 2. Table of codon usage derived from GenBank sequences, with the number of occurrences of each codon, and the occurrences per 100 codons for the same amino acid.

Amino Acid	Codon used	Occur-rences	% usage	Amino Acid	Codon used	Occur-rences	% usage
Arg	CGA	5	3	Val	GCA	16	5
	CGC	72	36		GTC	106	34
	CGG	20	10		GTG	125	40
	CGT	29	15		GTT	68	22
	AGA	17	9				
	AGG	57	29	Lys	AAA	23	9
					AAG	235	91
Leu	CTA	42	9				
	CTC	123	26	Asn	AAC	136	78
	CTG	139	29		AAT	39	22
	CTT	92	19				
	TTA	17	4	Gln	CAA	125	42
	TTG	68	14		CAG	171	58
Ser	TCA	31	11	His	CAC	57	61
	TCC	67	24		CAT	36	39
	TCG	32	12				
	TCT	40	15	Glu	GAA	43	17
	AGC	83	30		GAG	171	58
	AGT	21	8				
				Asp	GAC	119	64
Thr	ACA	25	11		GAT	67	36
	ACC	118	50				
	ACG	42	18	Tyr	TAC	133	86
	ACT	51	22		TAT	22	14
Pro	CCA	76	27	Cys	TGC	68	81
	CCC	61	22		TGT	16	19
	CCG	70	25				
	CCT	75	27	Phe	TTC	143	77
					TTT	42	23
Ala	GCA	60	13				
	GCC	145	32	Ile	ATA	13	5
	GCG	95	21		ATC	161	65
	GCT	151	33		ATT	74	30
Gly	GGA	41	12	Met	ATG	131	100
	GGC	152	45				
	GGG	43	13	Trp	TGG	31	100
	GGT	100	30				
Ter	TAA	3	21				
	TAG	7	50				
	TGA	4	29				

where more than one allele of a gene was available, and the different forms were very similar, only one was used. The results in Table 2 show the number of occurrences of each codon and the number per 100 codons for that amino acid. A strong preference is apparent for codons with a C or a G in the third position.

Ultimately, the usefulness of a table of codon usage is measured by its ability to distinguish a correct reading frame from an incorrect one. Therefore, this table was used to analyze several maize sequences whose reading frames were known. These results are shown in Table 3. The scoring was calculated as follows: for each codon in the sequence a score was assigned as the percent occurrence of that codon divided by the highest possible percent occurrence of any codon for that amino acid, as determined from the table. The scores for all the codons in the sequence to be scored were summed and divided by the total number of codons to give a percent similarity to the table. The genes in Table 3 were scored in each of the three possible reading frames, over the same region. The first frame is that which has been identified as the coding frame. With the exception of the genes MZEZE22A and MZEZE22B, coding for two 22kD zeins, all the genes tested were correctly distinguished by their codon usage. MZEZE22A and B, however, scored considerably higher in the second reading frame, indicating that their codon usage profile is significantly different from the consensus profile of all the maize genes.

Table 3. Scoring of some maize coding regions on the basis of the codon usage table.

Gene tested	Score for reading frame: 1	2	3
MZEACT1G	59.3	55.4	50.2
MZEH4C14	81.5	54.8	47.5
MZEALD	68.8	62.3	57.6
MZEH3C2	80.0	62.5	57.9
MZEHSP701,2	72.7	56.2	48.1
MZESUSYSG	64.9	56.4	49.0
MZEANT	61.4	55.6	52.5
MZEADH1F	63.3	53.1	46.6
MZEADH2NR	73.2	59.8	46.6
MZETPI1	85.3	38.4	55.7
MZEZE15	82.3	70.0	55.1
MZEZE22A	59.6	70.3	50.0
MZEZE22B	56.9	68.7	49.3

Permission of the authors is not required for citing the codon usage table.

AMES, IOWA
Iowa State University

The termini of *Ac* have prokaryotic promoters, homologous sequences with *E. coli* DnaA protein binding sites, and ARS sequences.

--James H. Zhou and Alan G. Atherly

The termini of Ac *have prokaryotic promoters.* The mechanism of transposition of plant transposable elements is not known, but the termini of the elements likely play an important role. We have found that an approximately 480bp DNA fragment of the 3' terminus of the maize transposable element Activator (*Ac*), when fused to the promoter-less gene encoding chloramphenicol acetyltransferase (CAT), turns on the CAT gene in *E. coli* strain DH5. Similarly, an approximately 178bp DNA fragment of the 5' end of *Ac* shows identical polarity and promoter activity as the 3' end. Both fragments are orientation-dependent in their promoter activity.

We inserted an approximately 1kb *Xho*I 3' end fragment of *Ac* element from pSB105 (Fig. 1) into vector pTZ18R at a *Sal*I site within the multiple cloning sequence between the T7 promoter and the alpha-complementation fragment of the ß-galactosidase gene (*LacZ'*) in two different orientations, giving rise to pZA20R and pZA20L. Similarly, the approximately 850bp 3' *Bam*HI fragment of pSB105, containing the 178bp 5' end of *Ac* element was inserted into the modified pTZ18R (pTZ18R') *Bam*HI site in two orientations, giving rise to pZA30R and pZA30L (Figure 1b).

The approximately 500bp *Hinc*II\ *Hin*dIII fragment of pZA20R, containing the 3' end of *Ac*, and the 850bp *Sma*I\ *Hin*dIII fragment of pZA30L, containing the 5' end of the *Ac*

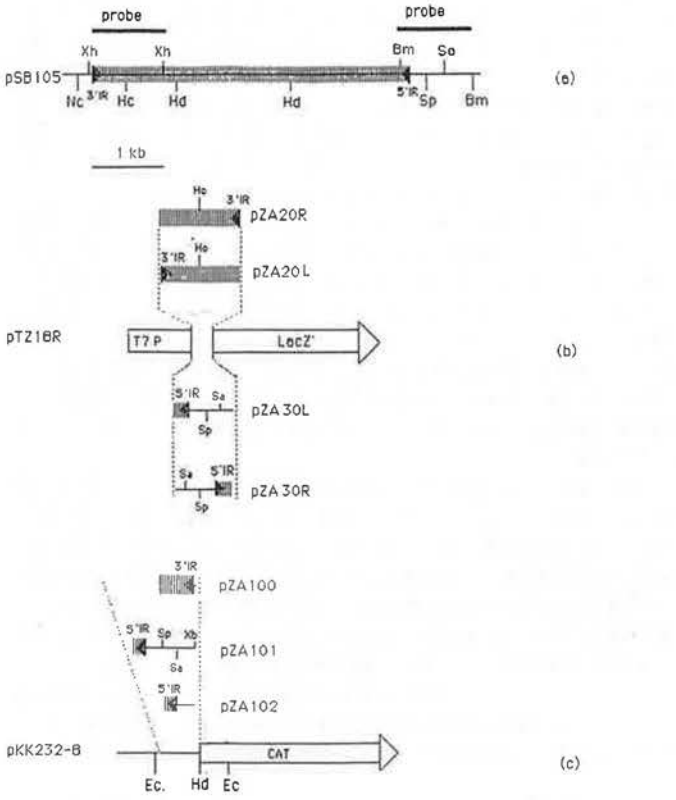


Fig. 1. Plasmid construction. a) The *Ac* fragment of *P-vv* present in pSB105. The position of *Ac* is shown as the vertical-hatched bar (same in b and c). The solid triangles indicate the direction of the inverted repeat sequences (IR) of *Ac* (same in b and c). The thin horizontal line extending out of *Ac* borders represents *P-vv* flanking sequences (same in b and c). The solid bars above the *Ac* element are the sequences used as probes of *Ac* termini in Figure 2. b) Schematic representation of pZA20R, pZA20L, pZA30L and pZA30R plasmids. Insertion of approximate 1kb *Xho*I fragment contained 3' end of *Ac* element with only 22bp *P-vv* flanking sequence in either orientation and insertion of approximate 0.85kb *Bam*HI fragment contained 5' end of *Ac* element in either orientation into the *LacZ'* gene in plasmid pTZ18R are shown. The white triangle represents the direction of *LacZ'* gene expression. c) Schematic representation of pZA100, pZA101 and pZA102 plasmids. Insertion of approximate 480bp fragment of PZA20R (see b) containing the 3' end of the *Ac* element with the 22bp *P-vv* flanking sequence, and insertion of approximate 850bp fragment of pZA30L (see b.) containing the 178bp 5' end of *Ac* element with the 662bp *P-vv* flanking sequence into the front of CAT gene. The pZA102 plasmid is derived from pZA101 in which the approximately 650bp fragment of *P-vv* flanking sequence is deleted at the *Sph*I site and *Xba*I site between the 5' end of *Ac* element and the 5' end of the CAT gene in pZA101.

Restriction endonuclease abbreviations: Nc, *Nco*I; Xh, *Xho*I; Hc, *Hinc*II; Hd, *Hin*dIII; Bm, *Bam*HI; Sa, *Sal*I; Sp, *Sph*I; Xb, *Xbh*I; Ec, *Eco*RI.

element, were each inserted into pKK232-8 (at the *Sma*I and *Hin*dIII sites, respectively) giving rise to pZA100 and pZA101, respectively. An approximately 650bp deletion was made in pZA101 (between the *Sph*I site and *Xba*I site) to delete a portion of the flanking *P-vv*_sequence from the 5' *Ac* end. This gave rise to pZA102 (Fig. 1c).

Expression of the CAT gene was determined by growing transformed *E. coli* DH5 cells on LB plates containing 30mg\l chloramphenicol.

Expression of LacZ' *gene is dependent upon the orientation of the inserted* Ac *termini.* In pZA20R the direction of 3' inverted repeat sequence (3'IR) of *Ac* is opposite to that of the *lacZ'* gene; and in pZA20L the direction of 3'IR of *Ac* is the same as that of *lacZ'* gene. pZA20R shows *lacZ'* gene activity (Fig. 1). In contrast, pZA20L does not show any *lacZ'* activity. In addition, when the *Bam*HI fragment of pSB105, containing the 5' end of *Ac*, was inserted into the *Bam*HI site of the multiple cloning sequence of the vector (pTZ18R), in the direction of IR opposite to *lacZ'* gene (see Figure 1b: pZA30L) gene expression was observed. In the opposite orientation, no gene expression was observed. These experiments suggest that the termini of the *Ac* element have polar effects on expression of *lacZ'* gene that is analogous to that of prokaryotic transposons.

CAT gene expression driven by the termini of Ac. The *Ac* termini-CAT gene fusion is expressed when present in *E. coli* strain DH5. These strains can grow on agar media containing chloramphenicol at a concentration of 30mg/l. The negative control (pKK232-8 in *E. coli* DH5) cannot grow, even at 10mg\l chloramphenicol. These experiments indicate that the termini of the *Ac* transposable element have strong orientation-dependent prokaryotic promoter activities.

At least four putative prokaryotic promoter consensus sequences appear in a 500bp sequence within the 3' terminus (see Figure 2), and one in the 5' end of the *Ac* element, both within the "extended inverted repeat" region and close to the 11bp inverted repeat (IR) (the 5' sequence is not shown). We cannot be sure which putative promoter sequence in the 3' end turns on the CAT gene activity. However, since the putative promoter sequence in the 5' end is close to the perfect inverted repeat sequence (IR), we can reasonably speculate that the putative promoter sequence

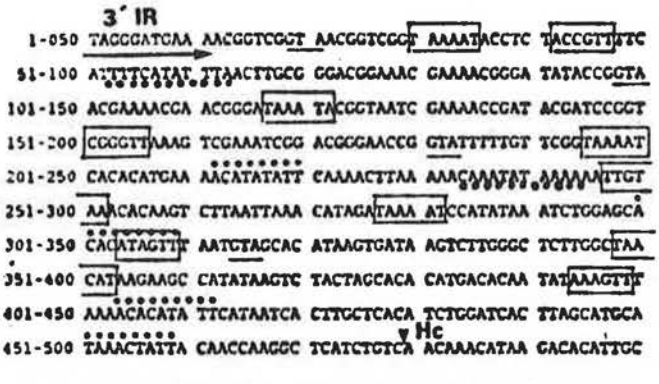


Fig. 2. The sequence of 500bp of the 3' end of *Ac* is presented. The numbers correspond to the position on the *Ac* sequence; the 3' correlates to the 3' of the 3.5kb transcript of *Ac*. The putative -10 and -35 consensus sequences are boxed. The ARS sequences are under-dotted. The possible start points (ATG) for transcription are underlined. The over-dotted sequences are homologous sequences with dnaA protein binding sites.

in the 3' end closest to the IR may turn on the CAT gene activity in pZA100. Thus, the two putative promoters in each end are likely located within the first mismatched "loop" of the secondary structure at the termini of the *Ac* element.

The probability by chance alone of a putative promoter consensus sequence residing in the 3' end of *Ac* element (pZA100) is approximately $1x10^{-4}$. Similarly, the probability of a promoter sequence in the 5' end is approximately $4x10^{-5}$. However this calculation assumes an equal distribution of the four bases in the sequences. These calculations suggest that it is unlikely for the promoter consensus sequences to appear by chance alone.

Sequence similarities between the 3' end region of Ac *and ori*C. A remarkable feature of the nucleotide sequence of the 3' end of the *Ac* transposable element is the abundance of TTATACACA sequences (four within a 250bp stretch, Fig. 2). This feature is reminiscent of the *ori*C region in which four consensus TTAT[C\A]CA[C\A]A sequences are present within a 245bp sequence and constitute the minimal origin of replication. These four inverted consensus repeats have been found to be DnaA protein binding sites. The DnaA protein is essential for DNA replication. Experiments with the 3' end of *Ac* as an origin of replication (*Xho*I fragment of pSB105 linked to a Kanamycin resistance gene, Fig. 1a) demonstrated that it was unable to function as an origin of replication when present in *E. coli* DH5 (data not shown). This could be due to several reasons: orientation of the binding sequences (direct repeats in *Ac* end and inverted repeats in *ori*C), the match with the DnaA protein complex (#2 is matched, but the remainder are not perfectly matched with the consensus sequence in *ori*C), lack of enough GATC methylation sites, or lack of "13 mer" sequences which are necessary for *ori*C function.

The 3' terminus of Ac *possesses an ARS sequence.* Examination of the sequence of the 3' terminus of the *Ac* element revealed the presence of two 11-mers consisting of the consensus sequence of the yeast ARS sequence (Figure 2). The 460bp 3' terminus of *Ac* was placed in a pUC18 vector lacking a yeast origin of replication (pUC-ura3,) creating pZA400. This vector possessed a ura3 gene for selection and was transformed into a ura3- yeast strain, selecting for growth in the absence of uracil. Controls included a similar vector with an ARS and one lacking ARS sequences. Transformants possessing pZA400 grew normally on uracil minus media, and segregated out when placed in media lacking uracil. These data strongly suggest that the 3' terminus of *Ac* possesses a functional ARS sequence.

An important question that arises from this study is how were the prokaryotic promoter-like sequences, DnaA protein binding site-like sequences, and ARS sequences in the termini of *Ac* element generated, and what functions do they exert? As suggested from the data of others and our own data, *Ac* termini when placed adjacent to promoter-less genes do not express activity in transformed plant cells. Our observations suggest that plant transposable element systems and prokaryotic transposons may share some similar "DNA signals" relating to the origin of DNA replication. This implies that the origin of the *Ac* transposable element family may have an evolutionary relationship with prokaryotic DNA replication origins and/or transposable elements. An attractive explanation for the presence of prokaryotic promoter sequences, ARS sequences, and DnaA

protein binding site-like repeat sequences within the termini of *Ac* is that they are conserved as secondary sequences required for transposition during evolution. Evidence to support this hypothesis is that TTATACACA sequence is found in TRL-*Tn5* and *dna*A mutations affect the transposition efficiency of *Tn5*. Alternatively, additional factors may be required for transposition of DNA elements in plants. This could be a protein which recognizes the homologous sites of the prokaryotic *Dna*A binding sequence. It is possible that DnaA protein-DnaA-box interaction plays an important role in transposition, or regulation of transposition, as suggested from findings with bacteria.

We are presently conducting deletion analysis of the 3' terminus to ascertain exactly which of these sequences are required for transposition.

The putative Mutator-induced deletion of the *A1* and *Sh2* loci on the long arm of chromosome 3, *a1-sh2-Mu*, is inviable in both the homozygous and hypoploid conditions

--Philip S. Stinard and Donald S. Robertson

In the 1988 MNL (62:14), we reported a deletion of the *A1* and *Sh2* loci on the long arm of chromosome 3, *a1-sh2-Mu*, that arose in our Mutator stocks. In our 1987-88 winter nursery, we planted colorless kernels from the cross *a1-sh2-Mu/A1 Sh2* X *a1 a1 Sh2 Sh2*. We self-pollinated the resulting plants (*a1-sh2-Mu/a1 Sh2*) in an attempt to obtain a homozygous deletion stock, and outcrossed them to standard *a1 sh2* testers to verify the presence of the deletion. Eight selfs were obtained, and of these eight selfed plants, six were successfully outcrossed (Table 1). The reduced

Table 1. Counts of plump and shrunken kernels on the selfs and outcrosses to *a1 sh2* testers of plants heterozygous for the deletion *a1-sh2-Mu* (*a1-sh2-Mu/a1 Sh2*).

Hetero-zygous	Selfed ears			Male outcross to a1 sh2			
parent	Sh	sh	%sh	Sh	sh	%sh	1:1 chi-sqr
4719-2	438	0	0.0	---	--	----	------
4720-1	235	0	0.0	78	23	22.8	29.95***
-2	173	0	0.0	---	--	----	------
-4	198	0	0.0	142	48	25.3	46.51***
-6	164	0	0.0	234	38	14.0	141.24***
-7	178	0	0.0	257	69	21.2	108.42***
-8	242	0	0.0	257	34	11.7	170.89***
-9	208	0	0.0	262	87	24.9	87.75***

Symbols: *** chi-square for 1:1 ratio significant at p = 0.001 level

frequency of shrunken kernels (less than 50%, $p < 0.001$) on the outcross ears verified the presence of the deletion in the selfed parents. One of the eight selfed ears segregated for a small, empty pericarp defective kernel mutant, but it is doubtful that this represents the deletion homozygote since seven of the selfed ears had no defective kernels. Had the deletion homozygote been viable, we would have expected a frequency of 0.464 x 0.185 = 0.086 (female transmission x male transmission) shrunken kernels. Since no shrunken kernels were observed, *a1-sh2-Mu* is most likely homozygous inviable, perhaps even in the zygote.

To further characterize this deletion, and to determine the phenotype of hypoploid deletion embryo and endosperm in the presence of hyperploid endosperm and embryo respectively, we crossed deletion heterozygotes (*a1-sh2-Mu/A1 Sh2*) by TB-3La (*a1* 3, 3-B, *A1* B-3, *A1* B-3). Plants heterozygous for the x-ray induced deletions *a1-x1* and *a1-x3* (L. J. Stadler and H. Roman, Genetics 33:273-303, 1948; both deletions are homozygous lethal) were also crossed by TB-3La for purposes of comparison. All plants were also outcrossed as males to standard *a1 sh2* testers in order to determine which plants carried deletions. (Plants grown from the *a1-sh2-Mu* deletion stock were either *a1-sh2-Mu/A1 Sh2* or *A1 Sh2/A1 Sh2*, and plants grown from the *a1-x1* and *a1-x3* deletion stocks were either *a1-x/A1 Sh2* or *a1 sh2/A1 Sh2*.) Plants heterozygous for a deletion are expected to have reduced male transmission of the deletion, resulting in fewer than 50% shrunken kernels on the male outcross ear.

The results of the testcrosses to *a1 sh2* are presented in Table 2. All outcross ears showing a significant reduction

Table 2. Counts of purple plump and yellow shrunken kernels from the male outcrosses to *a1 sh2* testers of plants heterozygous for either the standard *a1 sh2* alleles (*A1 Sh2/a1 sh2*) or various *a1 sh2* deletions (*A1 Sh2/a1-sh2-del*).

Hetero-zygous	Putative	Male outcross to a1 sh2			
parent	deletion	Pl Sh	y sh	%y sh	1:1 chi-sqr
9187-2	a1-x3	148	157	51.5	0.26
-6	"	294	0	0.0	294.00***
-8	"	123	143	53.8	1.50
9188-1	"	201	0	0.0	201.00***
-2	"	142	118	45.4	2.22
-3	"	127	136	51.7	0.31
-4	"	155	174	52.9	1.10
-5	"	148	180	54.9	3.12
-6	"	157	151	49.0	0.12
-8	"	212	243	53.4	2.11
9190-1	a1-x1	86	86	50.0	0.00
-2	"	184	194	51.3	0.26
-3	"	140	144	50.1	0.06
-4	"	239	57	19.3	111.91***
-5	"	138	81	37.0	14.84***
-6	"	136	117	46.2	1.43
-7	"	294	169	36.5	33.75***
-8	"	118	126	51.6	0.26
9191-1	"	523	76	12.7	333.57***
-3	"	240	94	28.1	63.82***
-9	"	193	190	49.6	0.02
-10	"	194	217	52.8	1.29
9193-6	a1-sh2-Mu	186	80	30.1	42.24***
-8	"	434	55	11.2	293.74***
9194-4	"	294	112	27.6	81.59***
-6	"	222	88	28.4	57.92***
-8	"	129	52	28.7	32.76***
-10	"	298	98	24.7	101.01***

Symbols: *** chi-square for 1:1 ratio significant at p = 0.001

from 50% in the number of yellow shrunken kernels ($p < 0.05$) were presumed to be outcrosses of deletion heterozygotes. Plants heterozygous for the *a1-x3* deletion showed no male transmission of the deletion, which is consistent with the data of Stadler and Roman. The percentage of yellow shrunken kernels on the outcross ears of *a1-x1* and *a1-sh2-Mu* deletion heterozygotes varied from 12.7% to 37.0%, and from 11.2% to 30.1% respectively, which is also consistent with previous data. Kernel counts for the outcross of *A1 Sh2/A1 Sh2* sibling plants of the *a1-sh2-Mu* heterozygotes are omitted from this table, but gave 100% plump purple kernels, as expected.

Kernel counts from the crosses by TB-3La of the heterozygous deletion plants (*a1-del/A1 Sh2*) and their normal siblings (*a1 sh2/A1 Sh2*) are presented in Table 3. In addition to the expected purple plump kernels, the normal plants segregated for yellow shrunken kernels (*a1 a1 sh2 sh2* 3-B hypoploid endosperm, with *a1 sh2*, 3-B, *A1* B-3, *A1* B-3 hyperploid embryo) and yellow plump kernels (*a1 a1 a1 sh2 sh2 Sh2* endosperm from pollination of *a1 a1 sh2 sh2* endosperm polar nuclei by the normal *a1* 3 homologue carried by the TB stock). A small number of germless kernels (under 2% in most instances) were also observed, probably induced by drought and heat stress on developing kernels, since germless kernels have not been reported by other workers making this cross.

Crosses of all three heterozygous deletions by TB-3La did not uncover a yellow shrunken phenotype as would be

Table 3. Counts of purple plump, yellow plump, yellow shrunken, purple germless, yellow germless, and sectored kernels on female outcrosses by TB-3La (*a1* 3, 3-B, *A1* B-3, *A1* B-3) of the heterozygous parent plants listed in Table 2.

Hetero-zygous parent	Deletion carried	Female outcross by TB-3La							
		Pl Sh	y Sh	y sh	Ny sh	Pl gm	y gm	N gm	sec
9187-2	N	235	31	54	16.6	3	0	0.9	3
-6	a1-x3	141	1	0	0.0	7	4	7.2	0
-8	N	174	22	37	15.7	2	1	1.3	0
9188-1	a1-x3	197	9	0	0.0	9	0	4.2	0
-2	N	98	12	21	14.8	9	1	7.0	1
-3	N	252	72	44	12.0	0	0	0.0	0
-4	N	262	29	60	16.8	2	0	0.6	3
-5	N	222	66	26	8.2	0	0	0.0	2
-6	N	112	16	34	20.7	1	0	0.6	1
-8	N	183	30	15	6.8	8	0	1.7	1
9190-1	N	262	41	56	15.5	0	0	0.0	3
-2	N	180	26	38	15.4	2	0	0.8	1
-3	N	74	13	20	18.7	0	0	0.0	0
-4	a1-x1	251	23	0	0.0	42	14	16.8	3
-5	a1-x1	248	25	0	0.0	39	2	12.9	7
-6	N	178	31	30	12.3	0	1	0.4	4
-7	a1-x1	95	9	0	0.0	22	2	18.3	3
-8	N	240	31	31	9.9	6	0	1.9	5
9191-1	a1-x1	195	23	0	0.0	42	9	18.7	4
-3	a1-x1	150	19	0	0.0	58	6	26.1	1
-9	N	254	32	27	8.5	2	0	0.6	4
-10	N	116	49	19	9.7	6	1	3.6	5
9193-6	a1-sh2-Mu	96	23	0	0.0	6	10	11.8	1
-8	a1-sh2-Mu	42	6	0	0.0	0	3	5.8	1
9194-4	a1-sh2-Mu	193	21	0	0.0	31	7	15.1	0
-6	a1-sh2-Mu	302	41	0	0.0	50	9	14.6	2
-8	a1-sh2-Mu	217	48	0	0.0	15	26	13.3	3
-10	a1-sh2-Mu	214	41	0	0.0	27	17	14.7	1

Symbols: N, parent carries standard a1 sh2 alleles

expected if the deletions were viable in hypoploid endosperm. Instead, the following exceptional phenotypes (and presumed genotypes) were observed: (1) yellow plump (*a1-del a1-del a1 Sh2* endosperm from the pollination of *a1-del a1-del* endosperm polar nuclei by the normal *a1* 3 homologue carried by the TB stock), (2) purple germless (*a1-del a1-del*, 3-B, *A1* B-3, *A1* B-3 hyperploid endosperm, with an inviable *a1-del* 3-B hypoploid embryo, and (3) yellow germless (*a1-del a1-del*, 3-B, *a1* B-3, *a1* B-3 hyperploid endosperm resulting from a crossover between *a1* and the B-3 breakpoint in the TB-3La parent, with an inviable *a1-del* 3-B hypoploid embryo). The frequency of germless kernels was significantly greater in the crosses of the deletion heterozygotes by TB-3La than in the crosses of the normal sibling plants by TB-3La for all three deletions, so we presume that the excess of germless kernels in the crosses involving the deletion heterozygotes represents lethality of these deletions in hypoploid embryos.

If these deletions are lethal in the hypoploid condition in both embryo and endosperm, we might expect to find a class of kernels complementary to the plump germless kernels, i.e., kernels with defective hypoploid endosperm but normal appearing hyperploid germs. It seems from the absence of this class that such kernels abort early in development. We did observe defective kernels on ears crossed by TB-3La, but these did not have germs, and appeared on both the deletion heterozygote ears and their normal siblings; these defective kernels may be drought induced, or perhaps the missing class of hypoploid deletion endosperm kernels is of similar phenotype to kernels aborted by drought (mostly empty pericarp). If the latter is true, then the hyperploid embryo does not develop in the presence of hypoploid deletion endosperm.

The endosperm sectored kernels that occur in rare frequency on the ears crossed by TB-3La (Table 3) provide some insight into the phenotype of hypoploid deletion endosperm. These kernels appear to be the result of chromosome breakage or loss during the development of *a1-del a1-del A1 Sh2* endosperms (B chromosome constitution unknown), resulting in sectorial hypoploidy for the deletion. When such sectoring occurs on the crosses of heterozygous normal siblings (*a1 sh2/A1 Sh2*) by TB-3La, plump kernels with sectors of yellow shrunken tissue result. When the sectoring occurs on the crosses of deletion heterozygotes by TB-3La, the sectors fall into two classes: (1) small sectors, generally less than one fourth of the kernel area, which are composed entirely of yellow shrunken tissue, and (2) larger sectors, which have a small border of yellow shrunken tissue at sector's edge, and collapsed defective endosperm toward the interior of the sector. (These two types of sectors have been observed in crosses of *a1-x1* and *a1-sh2-Mu* heterozygotes by TB-3La. They have not been observed in crosses of *a1-x3* heterozygotes by TB-3La, but the population size for these crosses is small.) Kernels have been observed that are almost entirely devoid of endosperm except for a small sector of plump tissue bordered by yellow shrunken tissue; these kernels had small, but normal appearing embryos. By extension, completely hypoploid endosperms most likely do not develop, and may not support the development of diploid or hyperploid embryos, which seem to require the presence of at least a small amount of normal endosperm tissue in order to develop properly. The presence of a small border of yellow shrunken tissue at the edge of hypoploid deletion endosperm sectors indicates that normal tissue adjacent to the sector is able to provide a diffusible substance that allows bordering hypoploid deletion tissue to produce an *a1 sh2* phenotype rather than a completely defective phenotype. This substance may be an essential metabolite produced by a biochemical pathway involving a product coded by a gene (or genes) which is (are) included in the deleted chromosome segment. If this is the case, then this metabolite is either not diffusible from hyperploid endosperm to hypoploid deletion germ, or is unable to overcome the defect which prevents the development of hypoploid deletion germs. This model predicts that a defective kernel mutant or mutants (or a zygotic lethal) will be found on the long arm of chromosome 3 that map to the segments deleted in *a1-x1*, *a1-x3*, and *a1-sh2-Mu*.

Allele tests of new putative Mutator-induced recessive kernel mutants with *ae*-like phenotype

--Philip S. Stinard

In the 1988 MNL (62:14), we reported on the allele testing of 12 independent sugary/translucent kernel mutants that arose during the course of our studies of the Mutator system. This past summer, we tested 12 additional putative *Mu*-induced sugary/translucent kernel mutants for allelism with *ae*. Of the 12, only 2 proved to be allelic to *ae* (Table 1);

Table 1. Results of allele tests of recessive *ae*-like kernel mutants with *ae*.

1988 Mutant Designation	Original Source	Allelic to ae?	New Designation
ae*-Mu13	Stand/Mu	No	su-sh*-3389
ae*-Mu14	Mu3 O. C.	No	su-sh*-5288
ae*-Mu15	Mu4 O. C.	No	su-sh*-5087
ae*-Mu16	Mu1/A632	No	su-sh*-2370
ae*-Mu17	c sh bz wx/Mu2	No	su-sh*-2424
ae*-Mu18	MO17 S ms/Mu O. C.	N. T.	N. C.
ae*-Mu19	Mu6 O. C.	No	su-bt*-3040
ae*-Mu20	y1-Mu/Y1	N. T.	N. C.
ae*-Mu21	y1 wx gl8/Mu	No	su-sh*-5178
ae*-Mu22	y1 wx Mu/Y1	Yes	ae-Mu8
ae*-Mu23	wx-Mum1	Yes	ae-Mu9
ae*-Mu24	y1 wx gl1/Mu2	N. T.	N. C.

Symbols: su, translucent or glassy; sh, shrunken like sh1; bt, brittle, like bt1; N. T., no test; N. C., no change in mutant designation.

3 did not receive a sufficient test, and will be retested. All mutants from both years of testing that were not allelic to *ae* are being propagated, and will subsequently be allele tested with the mutants *su1*, *su2*, *du*, and *sh1*, and crossed by a series of B-A translocations.

Two-point linkage data for *brn1* to *cr1* on 3S

--Philip S. Stinard

We report the results of a two-point linkage test for *brn1* to *cr1* in Table 1. The test was set up as a modified back cross. Plants were scored for crinkly at the time of shedding, and *brn1* was scored on the ears of selfed plants. The percent recombination (6.7 ± 1.1) is consistent with the linkage data for *brn1* with respect to *g2* (15.8 ± 2.0), *d1* (19.7 ± 1.6), *ra2* (26.7 ± 2.0), *cl1* (35.8 ± 2.3), and *Lg3* (39.6 ± 2.4) reported in MNL 61:6 and MNL 62:17, and would place *brn1* 7 cM distal to *cr1* on 3S. Using the 1988 linkage map as a guide, we propose the following placement of *brn1* on chromosome 3:

Table 1. Linkage data for *brn1* to *cr1*.

Testcross: cr1 cr1 Brn1 Brn1 × (Cr1 brn1 / cr1 Brn1)

Reg	Genotype		No.	Totals
0	cr	+	229	
	+	brn1	216	445
1	cr	brn1	16	
	+	+	16	32

% Recombination brn1 -- cr1 = 6.7 ± 1.1

g2-------*E8*---*brn1*---*cr1*------*Cg1*------*d1*---*ra2*--------*cl1*--*Lg3*-
0 14 19 26 35 44 49 60 65

A putative Mutator-induced *du1* mutant

--Philip S. Stinard

A kernel mutant from our Mutator selfing block with a translucent/shrunken phenotype reminiscent of *du1* (*du*-2197*, from the source B73 *Mu* Loss/*Mu2*) was allele tested with *du*, and proved to be allelic. We now designate this mutant *du-Mu1*.

Inbred line A188 is homozygous for *y1* and *Wc*

--Brent Buckner and Philip S. Stinard

The white cap locus (*Wc*) is a dominant gene which conditions white endosperm in a homozygous recessive *y1* background or pale yellow endosperm with a white crown in the presence of *Y1*. The inbred line A188 also carries a dominant gene for white endosperm which exhibits phenotypically similar epistatic interactions with the *y1* locus. We have conducted experiments to determine the allelic constitution of A188 for the *y1* locus and also to determine if the dominant white gene in A188 is allelic to *Wc*.

To determine the allelic constitution of inbred line A188 for the *y1* locus, we crossed A188 plants to plants that were homozygous for both the *Y1* allele and the recessive wild-type *wc* allele (*Y1/Y1, wc/wc*). The progeny kernels from this cross were all pale yellow endosperm with a white crown. The plants derived from these seeds were crossed to plants which were homozygous recessive for the *y1* and *wc* alleles (*y1/y1, wc/wc*). If A188 was homozygous for *y1*, the progeny kernels of this cross would be expected to segregate in a 1:2:1 ratio for pale yellow with white crown, white, and yellow endosperm. If, on the other hand, A188 was homozygous for *Y1*, the progeny kernels of this cross would be expected to segregate in a 1:1 ratio for yellow and pale yellow, white-crowned endosperm. The observed phenotypes and total number of kernels of each phenotype are found in Table 1. The chi-square values indicate that the data do not significantly deviate from a 1:2:1 ratio in six of seven crosses. In the seventh cross, the data deviated significantly at the 5% level. The total counts for all seven ears do not significantly deviate from 1:2:1. We conclude from these data that inbred line A188 is homozygous for the recessive *y1* allele.

Table 1. Kernel counts for the testcross [A188 x *Y1 Y1 wc wc*] x *y1 y1 wc wc*.

	Endosperm Phenotype			
Ear number	pale yellow-white crown	white	yellow	Chi-square value
4622-5/5696-6	49	103	54	0.242
4622-2/5724-3	74	106	57	5.076
4622-4/5696-6	61	127	53	1.232
4622-9/5696-6	59	121	60	0.016
4622-3/5622-1	31	56	37	1.742
4622-7/5696-6	17	53	20	2.794
4622-1/5724-3	48	80	64	8.000*
Totals	339	646	345	1.140

*Chi-square significant at $p < 0.05$.

To determine if the dominant white gene in A188 is allelic to *Wc*, A188 plants were crossed to plants homozygous for *Wc* and *y1* (*Wc/Wc, y1/y1*; inbred K55, supplied by Dr. Earl Patterson). The progeny kernels from this cross had white endosperm, which is in support of our finding that A188 is homozygous for *y1*. (If A188 was homozygous for *Y1*, this cross would have produced kernels with pale yellow, white-crowned endosperm.) The plants derived from these seeds were reciprocally crossed to plants homozygous for the *Y1* and *wc* alleles. If the dominant white gene of A188 is allelic to *Wc* all of the kernels produced in this cross are expected to have pale yellow, white-crowned endosperm. If, on the other hand, the dominant white gene of A188 is not allelic and is unlinked to *Wc*, this cross is expected to produce kernels segregating in a 3:1 ratio for pale yellow, white-crowned to yellow endosperm. We found that all kernels produced from this cross were pale yellow with a white crown, therefore, the dominant white gene of inbred A188 is allelic to *Wc*.

The source of the Mutator system and the first *Mu*-induced mutants

--Donald S. Robertson

The Source. Figure 1 summarizes what is known about the pedigree of the stock from which the Mutator system was derived. The earliest progenitor of record was a *P-VV* line propagated at the University of Wisconsin, Madison in Dr. Alexander Brink's pericarp project. A *P-RR* revertant found in this line was transferred from its original background into that of the inbred W23. After 6 generations of backcrossing, the *P-RR* stock tested negative for the presence of *Mp* (*Ac*). A self-pollinated ear of this conversion line, which segregated for a defective kernel mutant, was given to Dr. Jerry Kermicle. One of the self-pollinated plants produced by kernels from this ear segregated for a pale yellow endosperm mutant. Dr. Kermicle sent this ear to me in 1961, because of my interest in studying mutants affecting carotenoid synthesis. This mutant was mapped to the short arm of chromosome ten and was given the symbol

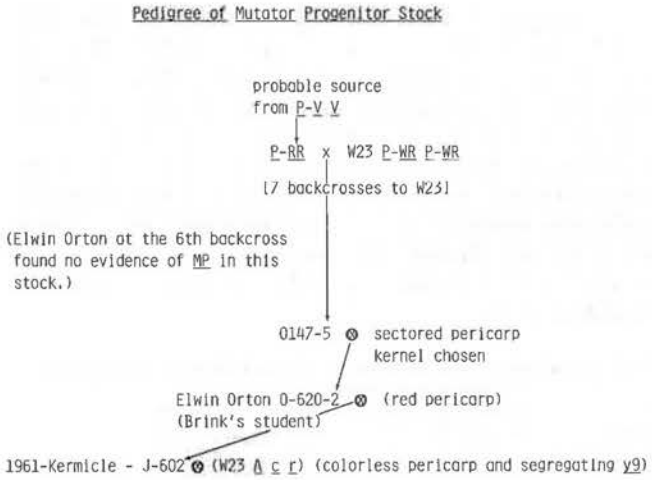


Figure 1.

y9. The phenotype of *y9* seedlings is quite variable. It ranges from normal green, to green seedlings with pale leaf tips, to pale green, to yellow- green seedlings. A single ear segregating for *y9* kernels can have *y9* seedlings all of which exhibit just one of these phenotypes, or various combinations of the phenotypes can be observed. None of these phenotypes is consistently transmitted and the conditions responsible for the induction of any of them are unknown.

The First Mutant. The first mutant of record in this stock was undoubtedly the defective kernel mutant that attracted Dr. Kermicle to this line. The second mutant was probably the *y9* mutant, which was responsible for the stock coming into my hands. The first mutants induced at Iowa State University were a pair of *w3* mutants found in 1963.

The first year the *y9* stock was planted at I.S.U. was 1961, and both yellow and pale yellow kernels were sown. All plants were weak, but selfs of plants from both classes were obtained. In 1962, kernels from a homozygous *y9* ear were sown and the resulting plants were crossed to a series of waxy marked chromosome nine translocations and to standard and inbred lines. Plant 62-1001-3 was crossed by the inbred N25 (male), while the sibling plant 1001-2 was crossed onto a waxy T3-9c stock (female). Ten kernels from the cross of plant 62-1001-3 were sown, and eight of the resulting plants were pollinated by other heterozygous *y9* plants. All ears from these crosses segregated for pale yellow *y9* kernels that produced seedlings showing the variable *y9* phenotype. One plant from this family was not pollinated. The tenth plant was self pollinated and segregated for pale yellow dormant kernels and pale yellow viviparous kernels. Among the seedlings produced by the pale yellow kernels were those that were pale green, albino, and albino with borders of yellow-green tissue on the leaves. Some or all of the pale green seedlings might represent *y9* seedlings but the other classes were not typical of *y9* seedlings.

Ten kernels from the cross of plant 62-1001-2 were planted. Seven plants resulted and these were all self-pollinated. Six of the ears segregated for pale yellow kernels that produced *y9* seedlings. One ear, however, in addition to segregating for dormant pale yellow kernels also had pale yellow viviparous kernels. The pale yellow dormant kernels segregated for green, pale green and albino seedlings. (Vivipary is not unexpected in *y9* material because this is one of the phenotypes occasionally associated with *y9*).

The green and pale green seedlings found on these two selfed ears might represent *y9* seedlings, but the other classes were not typical of *y9* seedlings. The albinos were very similar to the white endosperm-albino mutant seedlings (e.g., *lw1*, *vp5*, etc.). Were these albino seedlings just an extreme expression of the *y9* seedling phenotype or was a second mutant involved? Because the ratio of yellow to pale yellow kernels on these ears was 9:7, the latter possibility suggested itself and indeed proved to be the situation. These two mutants were not *y9*, but they proved to be allelic to each other and also to *w3*. The allele found in the progeny plants from the cross to waxy T3-9c (*w3-Mus1*) was phenotypically very similar to *w3* (i.e. pale yellow and/or white kernels, which are frequently viviparous and produce albino seedlings only). However, the other allele (*w3-Mum1*), unlike *w3*, had seedlings that were quite variable in phenotype. After these new mutants were crossed out of the *y9* background, the mutant phenotype resulting from these alleles could be clearly delineated. The following kinds of phenotypes were observed associated with the pale yellow kernels of the *w3-Mum1* allele: 1) vivipary, 2) white seedlings only, 3) pastel (pale green) and albino, 4) pastel seedlings only, 4) pastel, albinos and albinos with varying amounts of yellow-green tissue (albescent-like pattern). Some of these mutant seedlings had the late-occurring mutable pattern typical of Mutator-induced mutants. In crosses to plants with the *w3-Mus1* allele and *w3* tester stocks, the pastel, mutable and albescent-like phenotypes were expressed. The *w3-Mum1* mutant has the characteristics expected of Mutator-induced mutants (i.e., variable mutant phenotype and somatic mutability).

The molecular basis for mutability has been determined for at least 14 different Mutator-induced mutants with the typical late mutability pattern of Mutator mutants. All of these have been found to have an insertion belonging to the *Mu* family of elements. To date, no mutant with a late sectoring pattern from a Mutator stock has been found that has been shown not to have an insertion belonging to the Mutator family of elements. Also, a stable Mutator mutant, which has been characterized molecularly, was found to have *Mu1*-like elements present. The phenotypic variability of *y9* and *w3-Mum1*, as well as the somatic instability of *w3-Mum1*, are typical of the Mutator-induced mutants that have been characterized molecularly. We anticipate that these two mutants, and perhaps *w3-Mus1* as well, will be found to contain *Mu* family inserts at the mutant loci. We are setting up these mutants for molecular analysis.

A model for heterosis

--Donald S. Robertson

It has been suggested that many quantitative trait loci (QTLs) may be wild type alleles of loci for which qualitative mutants involving quantitative traits have been found--e.g., dwarfs (plant height), defective kernels (seed size), etc. (MNL 58:10, 1984; J. Theor. Biol. 117:1, 1985). This hypothesis suggested that quantitative patterns of inheritance occurred because there were slight differences in the efficiency of gene products as a result of the molecular polymorphism found in wild type alleles. Such slightly different alleles, all of which produce basically the same, but

nonetheless slightly different phenotypes, are called isoalleles. The effect of such isoalleles has been shown by Hageman et al. (Adv. Agron. 19:45, 1967) to be primarily additive. An additive model will explain the classical quantitative inheritance pattern (see Figure 1). Such a simple model, however, does not explain heterosis.

Figure 1. The classical model for the inheritance of quantitative traits assuming additivity.
Assumptions:
1. All quantitative genes for a trait are completely additive.
2. 10 loci
3. Two lines are crossed
   a. Line X - each allele contributes 10 quantitative units above the basic wild type of 1000. Line X would equal 1200 units.
   b. Line Y - each allele contributes 5 quantitative units above the basic wild type of 1000. Line Y would equal 1100 units.

Results expected in the F1 and F2 populations:
1. The F1 population would show very narrow variation around a value of 1150.
2. The F2 population would show the typical bell-shaped curve for the distribution of phenotypes in the F2 populations. The extreme values of this curve would approach those of the parents.

Figure 2 presents the basic assumptions for a proposed model (the Biochemical Pathway Model) to explain how wild type isoalleles might explain both the classical model of quantitative inheritance and heterosis. Figure 3 is a schematic presentation of the model. The biochemical pathways involved in the expression of any quantitative trait are probably much more complicated than this illustration. It is known that there are other mechanisms regulating biochemical pathways besides the efficiency of the gene products of the individual genes in the pathway (e.g., feedback inhibition, end product inhibition, suppressors, inducers, etc.). As implied above, for most quantitative traits there may be more than one pathway involved in the expression of a trait. For example, it is known that the gibberellin biosynthetic pathway is involved in the expression of plant height. Yet not all plant height qualitative mutants (e.g., dwarfs, brachytic, etc) are gibberellin responsive.

Figure 2. The biochemical pathway model of quantitative inheritance and heterosis.
Assumptions:
1. A given quantitative trait is the end result of a product or products produced by one or more biochemical pathways.
2. Each step in these pathways is under the control of unlinked genes and the products they produce (enzymes).
3. The gene products for each step can vary in efficiency depending upon variation in the wild type isoalleles.
4. All variant gene products function above the minimum level necessary for the expression of wild type.
5. For a given step, some wild type isoalleles produce products that function at higher levels above the minimum wild type level than others.
6. The final amount of the end product for any pathway is determined by the most inefficient reaction in the pathway.
7. The effect of gene products from isoalleles is additive but the total effect of the products from two isoalleles can not exceed 100% of the potential wild type phenotype.

If this model is correct, inbreds most likely have many biochemical steps controlled by low efficiency gene products. Two inbreds that show good combining ability as hybrids have in toto all steps functioning at a higher level than either inbred considered alone. This would mean that for each low efficiency gene product functioning for one step in inbred X, the other inbred, Y, will have a high efficiency product functioning for the same step. For those inbreds that do not have good combining ability, they both probably

Figure 3. The biochemical pathway model of quantitative inheritance and heterosis: an example.

Definitions:

1. A, B, C, etc. : genes in a pathway

2. ——→ : steps regulated by gene products of genes A, B, C, etc.

3. (+1), (+2), (+5), (+20), (+80), etc. : The amount (%) a given gene product functions over the minimum needed to result in the wild type phenotype at the low end of the normal curve for wildtypes.

4. I, II, III, etc. : precursors in pathway

Pathway:

A B C D E F
→ I → II → III → IV → V → End product

Inbred X - height 4% above minimum
[A=(+2) B(+80) C(+75) D(+10) E(+35) F(+25)] X2

Inbred Y - height 10% above minimum
[A=(+90) B(+5) C(+15) D(+80) E(+65) F(+70)] X2

F_1 - height 85% above minimum
A(2) B(80) C(75) D(10) E(35) F(25)
A(90) B(5) C(15) D(80) E(65) F(70)

F_2 - heights could be: 4%, 10%, 20%, 30%, 50%, 70%, 100%, etc.

F_2 individuals will exhibit the wild type phenotype permitted by its least efficient step. Because of independent assortment, there will be numerous different levels of wild type expression depending upon which pair of isoalleles happen to determine the limiting step.

have relatively low efficiency gene products functioning for one or more of the same steps.

The effect of gene products from isoalleles does not have to be completely additive. The model will still work even if dominance is found for some loci.

Recent results reported in Science (Stewart et al., Science 241:1216, 1988) support The Biochemical Pathway Model. These workers demonstrated that the level of gibberellin production was low in the four inbreds tested and, as expected, the inbreds were shorter than hybrids. In addition, the inbreds showed significant growth response to exogenously applied gibberellin. Most of the hybrids between these inbreds, on the other hand, were taller than either parent (heterosis) and showed very little if any response to exogenously applied gibberellin. B. O. Phinney has shown that some dwarf mutants block steps in the gibberellin biosynthetic pathway (Plant Growth Substances, Springer-Verlag, p. 55, 1985). Thus the wild type alleles at these dwarf loci produce enzymes regulating steps in this pathway. It is obvious that in the inbreds studied by Stewart et al., the gibberellin pathway was not functioning to its full potential. Otherwise, the inbreds would not respond to exogenously applied gibberellin. This low gibberellin production could be the result of wild type isoalleles in the inbreds at one or more steps that produce low efficiency gene products that limit the total amount of gibberellin produced. Most hybrids had complementing isoalleles that would allow full or near full production of gibberellin (as suggested by the model). In such a situation, exogenously applied gibberellin has little effect. One hybrid did not show a heterotic response as measured by gibberellin production in seedlings. This hybrid had a correspondingly greater response to exogenously applied gibberellin than the other hybrids tested. Again, this is a result predicted by the model for inbreds with poor combining ability for plant height. Such inbreds have isoalleles responsible for enzymes with low efficiencies for the same step(s) in the gibberellin biosynthetic pathway.

This model is not proposed as the sole explanation for heterosis. There is undoubtedly more than one mechanism responsible for this complex phenomenon. Enhanced

biosynthesis due to enzymatic polymorphism, as proposed by Schwartz and Laughner (Science 166:626, 1969), also could be responsible for a portion of the heterotic response, along with other, as yet, undiscovered mechanisms.

Evidence for Mutator activity in the spore-zygote interval

--Donald S. Robertson and Philip Stinard

In the 1985 (MNL 59:14-15), 1987 (MNL 61:10-11) and 1988 (MNL 62: 21-22) Newsletters, evidence was presented suggesting that Mutator could induce mutants in either the male gametophyte or the zygote. The principal support for such a conclusion came from the observation of a number of white endosperm discordant kernels generated in the cross of *y1 y1 wx wx gl1 gl1* x *Y1 Y1 Wx Wx Gl1 Gl1 Mu2*. The frequency of such discordant kernels was 16.69×10^{-5}.

In the 1988 report, we presented the results of tests for discordant kernels among the yellow kernels from this same cross. Plants from this discordant class would be expected to be *y1 y1*. In order to score a large plant population, yellow kernels were sown in an isolation plot and the resulting plants allowed to open pollinate. Plants from discordant kernels would segregate yellow to white kernels in a 1:1 ratio, while those from nondiscordant kernels would segregate in a 3:1 ratio. Seven putative 1:1 ears were found in a population of 37,975 plants screened (frequency = 18.43×10^{-5}). However, of these seven ears, all but one were semi-sterile. It was suggested that the induction of an ovule lethal mutation closely linked to *Y1* would result in such semi-sterile ears with 1:1 ratios. Also, a discordant kernel that was heterozygous for a translocation would produce a plant with such an ear.

In our studies of over 200 independent Mutator-induced *y1* mutants, 71.94% have been shown to have the pastel (pale green - zebra) phenotype when grown at 35 C. Thus if the 1:1 ears on plants from putative discordant yellow kernels are the result of a Mutator mutational event, there is a good likelihood that the white kernels should give pastel mutable plants. If the 1:1 ears indeed carry a Mutator-induced *y1* mutant, one half of the yellow kernels should carry this mutant. Yellow kernels from the normal 1:1 and two of the semi-sterile 1:1 ears were sown and the resulting plants self-pollinated. The yellow and white kernels were separated and seedlings tested at 35 C for the expression of the pastel phenotype. Three of the nine selfed ears of plants from yellow kernels of the 1:1 ear with normal seed set segregated for mutable pastel seedlings. Similar tests of nine plants each from yellow kernels of the two semi-sterile 1:1 ears failed to segregate for pastel seedlings. In addition, white kernels from these same 1:1 ears were sown and the resulting plants outcrossed to *Y1 Y1* standard plants. For each, 4 to 5 different outcross progeny were grown and the plants self-pollinated and seedling tested as above. Only the selfs of outcrosses from the 1:1 ear with normal seed set segregated for white-endosperm, pastel-seedling mutants.

The occurrence of a 1:1 ear with normal seed set demonstrates that the reciprocal discordant class (yellow endosperm - *y1 y1* embryo) does occur as would be predicted if Mutator induced *y1* mutations in the male gametophyte or embryo. However, the question is still open as to the origin of the semi-sterile 1:1 ears. The fact that pastel mutants have not been found in this class of mutants that they are not Mutator-induced *y1* mutants, because about 28 percent of Mutator-induced *y1* mutants do not have the pastel phenotype. The two semi-sterile ears tested could be carrying such a non-pastel mutant along with a reciprocal translocation, or some other genetic phenomenon (e.g., a large unlinked inversion, unlinked ovule lethal, etc.) could be responsible for the semi-sterility. Alternatively, these ears may not involve a *y1* mutation, but instead result from the induction of an ovule lethal tightly linked to the *Y1* allele. Evidence supporting the idea that some of the semi-sterile 1:1 ear ratios are due to an ovule lethal closely linked to *Y1* is the occurrence of several semi-sterile ears in both sets of reciprocal tests with a very low number of white kernels per ear (2-7). These may represent the same ovule lethal mutant linked to the *y1* allele.

If there is only one true yellow endosperm discordant kernel out of 37,975 tested, the frequency of this class of discordant kernels (2.63×10^{-5}) is significantly different (at the 5% level of significance but not at the 1% level ($\chi^2 = 6.4193$)) from the frequency of white-seeded discordant kernels (16.69×10^{-5}). Because the difference is significant at the 5% level and just escapes significance at the 1% level, this difference might be real. If so, it may have a biological basis such as preferential fertilization, if gametophytic mutations indeed are occurring. However, because rare events are involved and because there is a seven fold difference in population size, sampling error could have a profound effect. If one more 1:1 normal ears had been found, the difference between these two populations would not have been significant at the 5% or 1% levels.

Because the two cells the female parent contributes to the endosperm come from two different cell lineages in the developing embryo sac, discordant white endosperm kernels are expected only very rarely or not at all in the reciprocal cross (i.e., *Y1 Y1 Wx Wx Gl1 Gl1 Mu2* x *y1 y1 wx wx gl1 gl1*). However, because only one cell, the egg cell, is contributed by the female to the embryo, discordant yellow kernels can be expected in this cross. In the female gametophyte, there are three cell divisions involved in the production of the egg. In each division there is the potential for a mutational event, and thus yellow discordant kernels are expected 2.7 times as frequently in this cross as in the reciprocal cross in which the *Mu2* parent is a male. In the past summer, yellow kernels from the crosses with *Mu2* plants as females were planted, the resulting plants allowed to open-pollinate and the ears were scored for the presence of a 1:1 ratio (Table 1). The same three classes of ears (1:1 ratio of yellow to white, normal seed set; 1:1 ratio, semi-sterile seed set; and 2:1 ratio, semi-sterile seed set) seen in the reciprocal cross reported upon in the 1988 MNL were observed in this cross. Each class occurs in a higher frequency when the *Mu2* parent is used as a female. Class 1 (1:1 ratio, normal seed set) is six times as frequent. Class 2 (1:1 ratio, semi-sterile seed set) is 6.4 times as frequent. These two classes are those that are most likely to include mutations at the *y1* locus (see MNL 62: 21-22 for an explanation of the putative origin of these classes). Class 3 ears (2:1 ratio, semi-sterile seed set) occur with a frequency 1.5 times greater in the female crosses compared to the male crosses. Until all 1:1 ears from both crosses are tested further for the presence of pastel mutants, a clear estimate of the fre-

Table 1. Results of test for putative yellow discordant kernels from the cross of *Y1 Y1 Wx Wx Gl1 Gl1 Mu2* X *y1 y1 wx wx gl1 gl1* and its reciprocal cross (data from MNL 62:21-22, 1988)

Class 1	Class 2	Class 3
Normal or near normal seed set	Semi-sterile or near semi-sterile seed set	Semi-sterile or near semi-sterile seed set
Y1 Y1 Wx Wx Gl1 Gl1 Mu2 X *y1 y1 wx wx gl1 gl1*		
1:1* ratio	1:1* ratio	2:1* ratio
3 (8.9×10^{-6})	34 (101.3×10^{-5})	9 (26.8×10^{-5})
% of total white kernels for each class		
47.77	49.00	36.24

Total ears scored - 33,561

y1 y1 wx wx gl1 gl1 X *Y1 Y1 Wx Wx Gl1 Gl1 Mu2*		
1 (2.6×10^{-5})	6 (15.8×10^{-5})	7 (18.4×10^{-5})

Total ears scored - 37,975

* The ears in these categories do not differ from the indicated ratio at the 1% level, and the vast majority do not differ at the 5% level (only three ears differed from the expected ratio at the 5% level).

quency of *y1* mutations in these crosses will be impossible. However, whether Mutator is inducing mutants at the *y1* locus or linked loci (e.g, ovule or gametophytic lethal loci) on chromosome six, these mutations are occurring in a higher frequency when the *Mu2* parent is a female than when it is a male. The presence of two classes of discordant kernels when *Mu2* plants are crossed as males, and the presence of only the yellow endosperm, *y1 y1* class of discordant kernels when *Mu2* is used as a female supports the hypothesis that Mutator can induce mutants in the development of the gametophyte or in the zygote and triple fusion nucleus. However, the results from the crosses when *Mu2* plants are used as females suggest that few if any of the *Mu*-induced mutants studied are being induced in the zygote. If the mutations found in these studies were occurring at this time (i.e., the zygote), the frequencies of mutations of classes 1,2 and 3 would be expected to be equal in the reciprocal crosses. This is not the case. They are more frequent when *Mu2* plants are used as females. Again, this is expected because there are three times as many divisions in the development of the female gametophyte, each with the potential for producing discordant kernels, as there are in the development of the male gametophyte. In the latter, only in the division of the generative nucleus would a mutation occur resulting in a situation in which discordant kernels would be produced.

If, as the results of these studies suggest, Mutator does induce mutants in the developing gametophyte, progeny of Mutator plants used as female parents would be expected to exhibit more mutants overall than those in which Mutator plants are used as male parents. This has not been observed to be the case even when the male data are corrected for the presence of white discordant kernels. In fact, just the opposite is found to be true (D. S. Robertson, MNL 61:10-11, 1987). This may only be an apparent contradiction, however, because the new mutants found in these outcross progenies include those from other possible stages in development during which mutations can occur (i.e., premeiotic and meiotic).

In the crosses of the *Mu2* parents as a male, the corrected mutation frequency (i.e., without discordant kernels included) is 3.22×10^{-4} (MNL 61: 10-11, 1987). When the *Mu2* plants are the female parents, the frequency of white mutant kernels is 1.99×10^{-4}. There is a 1.6 fold frequency difference in favor of the male. The female data only counted mutations giving rise to a mutant ear sector as one event. In the male crosses, however, similar sectors occurring in the tassel could not be recognized and thus all kernels resulting from all mutant gametes produced by a sector were counted as separate events. Thus the male mutation frequency would be expected to be higher. The exact increase in frequency expected as a result of not being able to distinguish kernels derived from tassel sectors can not be determined. It is known, however, that ear sectors are usually small. Fifty-seven percent are two-kernel sectors, while 86% consist of 5 or fewer kernels. Tests of male transmitted mutants suggest that sectors in the tassel are equally small (D. S. Robertson, Genetics 94:969-978, 1980). Because the difference between female and male derived mutants favors the male (i.e., male 1.6 times that of female), it could well be that the higher male frequency results from not being able to distinguish mutants coming from tassel sectors.

The data from the tests for gametophytic mutants, however, create another problem. Data from Table 1 suggest that events that seem to be the result of mutations happen 3 to 6 times as frequently in the female as male. However, at this time we do not fully understand the nature of the genetic change responsible for some phenotypes observed. If classes 2 and 3 are the result of some event independent of the *y1* locus (e.g., a translocation, or an abortive ovule mutant, etc.), there is no a priori reason to consider these as gametophytic mutants. All or part of them could be meiotic or premeiotic in origin. The Class 1 (Table 1) mutants seem to be the most likely candidates for gametophytic mutants, and these occur in the female with a frequency 3.4 times that of the male. Thus the overall female mutation rate would be expected to be higher. It may be that the sample of 1:1 normal ears (Class 1) is too small to support any definite conclusion in this regard. It should be noted, however, that female gametophytic mutants may not account for the bulk of transmitted mutants. If this is so, gametophytic mutants may not markedly affect the balance between the mutants transmitted by the two sexes in reciprocal crosses. If it is assumed that the 1:1 ears with normal seed set in Table 1 (Class 1) are the result of gametophytic mutants (male data suggest this is a reasonable assumption), then it is possible to estimate the frequency of gametophytic events relative to pre-gametophytic events. In MNL 61:10-11, 1987, it was determined that the pre-gametophytic events occurred in a frequency 1.99×10^{-4} (*Mu2* as female). The tests reported in Table 1 involved a subset of the same population of kernels scored to obtain the frequency of 1.99×10^{-4}. Thus, if this frequency is applied to the population 33,561 (Table 1), it is possible to estimate how many pre-gametophytic mutants would be expected in that population. This number is 6.7. Thus only 30.9 percent (3/(3 + 6.7)) of the total mutants that are found in the eggs of *Mu2* plants are gametophytic. Thus over two thirds of all mutants are pre-gametophytic. However, our database is too small, and much more data are needed before definite conclusions can be made on the

full effect of gametophytic mutants on the mutation frequency of Mutator plants.

Results of tests of an alternative explanation for 1:1 ratios involving Mutator-induced mutable *a1* mutants
--Donald S. Robertson

We first reported the occurrence of 1:1 ratios (i.e., mutable : stable mutant phenotypes) in some outcross progeny of *a1-Mum2* and *a1-Mum3* in MNL 61:11-13. Last year (MNL 62:20-21) we suggested that such ratios could be expected if these stocks and the *a1 sh2* stocks used in outcrosses had a second aleurone gene (e.g., *r*) that was segregating. Last winter ('87-'88), we tested plants from yellow stable kernels from 1:1 ears from each of ten families included in our original report (MNL 61:10-13) for their genotype with respect to the aleurone genes *a2*, *c*, *c2*, and *r* (Table 1).

Some, but by no means all, 1:1 ears were segregating for *r* in addition to *a1*. In several crosses, the segregation for *r* could have been responsible for the 1:1 ratio. Because *r* is involved, heterozygous kernels of the genotype *a1-Mum a1 a1 R r r* would be given lower mutability scores due to *R* mottling, which would result in less pigmented tissue in which reversion sectors could be observed. Thus, the mutability counts in all crosses segregating for *r* should be suspect and have been dropped from further study. Analyses, however, have continued for those lines in which the aleurone genes are under control, and in newly occurring 1:1 stocks.

It is interesting that none of the other aleurone genes were found to be segregating. Why is *r* the only one causing trouble? The answer involves a longstanding problem we have faced with our summer nursery. We have our plots adjacent to a wildlife preserve, which serves as a safe haven for raccoons that constantly visit our field during pollinating season. To keep them out of our experimental material, we have provided a very generous border of Illini XTRA Sweet corn around our field. Tests have shown that this line is *a1 a1 r r*. It is obvious that at some time in the past there has been some contamination of some of our *a1 sh2* tester stocks by Illini XTRA Sweet. We have crossed our *a1 sh2* stocks to purple aleurone and reextracted new tester lines with all genes under control. These tester stocks will be continually monitored to avoid contamination in the future.

***a-m877527* - *Cy* controlled**
--Peter A. Peterson

In the second outcross yielding the genotype *a-m Sh2/a sh2* and crossed by *a sh2* the progenies included the following range of frequencies (%) of round colorless (non-mutable) (*Sh2*) kernels: 2, 4, 7, 13, 26, 50. Other progenies show a zero to 8% distribution. This could mean a range in the number of functional elements that activate the receptor allele of *a-m877527*.

To test this possibility, a **reconstitution test** of the colorless segregants crossed with plants derived from the shrunken segregants (*a-o sh2* ± element) showed spotted kernels confirming the activation of the allele by a second element and thereby verifying two-element control of mutability of this allele among these progenies.

System relationship: Assorted tests confirm that this mutability is not under *En* or *Mrh* control. The number of *Cy* in these progenies parallels the frequencies of mutable kernels among the *a* testcross progenies. This is illustrated in the following cross.

bz-rcy no *Cy* x *A/a-m*	Colored F1 (88 1418)
88 1418-1 x *bz-rcy*	2% *bz*--no spots
88 1418-lt x *a sh2*	1.8% colorless - non spotted

The *Cy* relatedness of *c-m877527* can be changed from putative to near confirmed.

Systems relationships - *Mrh* and *Mut*
--Peter A. Peterson

In view of the relatedness (molecularly) of the terminal inverted repeats of ever increasing numbers of maize elements (CACTA sequences, etc.), it is significant to have firm data on tests of activation (mutability) of genes with known receptors. The *Mut* (Rhoades and Dempsey, MNL, 1982) element was tested against the *a-mrh* element at the *a* locus. The cross was the following:

a-mrh bz-mut Mut X Colored F1 *A/a-mrh Bz/bz-mut Mut/+*

This F1 was crossed by *a-mrh bz*.

None of the colorless kernels were spotted (6/6 crosses) but *bz* spotted kernels were segregating on these same ears. This confirms that *a-mrh* was not responding to the presence of *Mut*.

bz-877803* - a correction, not *En* but likely *Cy
--Peter A. Peterson

The *bz* spotted (*bz-m/C sh bz wx, A/a sh2*) reported in MNL 62:3 were used in crosses with *bz-rcy*; in the progeny, 1/2 the kernels were spotted indicating that one *Cy* is present. When crossed by *bz/bz*, 1/4 are spotted. When tested with an *En* tester, no spotted kernels were uncovered. This would indicate that *bz-m877803* is not *En* controlled but likely *Cy* controlled.

Basis for *Cy* control: When co-segregation is examined for *bz-m* mutability and *a2-rcy* mutability, 3 out of 3 were co-segregating.

Basis for independent control: When *bz* spotted from the cross *bz-m/bz* x *bz/bz* are again testcrossed by *bz/bz*, the segregating progeny as shown in the table are indicative of independent control of mutability.

	bz		
2nd testcross	bz spotted	non-spotted	% spotted
88 2653-1 x 2701	67	218	23
2653-2 x 2701	122	324	27
2653-6 x 2701	77	242	24
2654-1 x 2703	93	275	25

***c2-m857213* - System relation**
--Peter A. Peterson

This *c2* mutable allele is phenotypically uniquely different than other *c2-m* alleles in our collection in that the mutability spots are on a palish background. In tests of system relationship, the results are confounded by the presence of many *Cy* elements that are triggering the reporter allele, *bz-rcy*. However there is a non-correlation of *c2-m* spotting and *bz* spotting. In crosses containing a very late *c2-m*

Table 1. Tests for the constitution of the aleurone genes of stable kernels from 1:1 stocks.

87 - 88 #	86' 1:1 Test (see MNL 61:10-13)	Cross	% Class 3 + Class 4	Aleurone Tests[a] r	c	a2	c2
4604	9349-2 / 8436-2	Class 4[e] X al sh2	43.88	8R	2C	6A2	3C2
4606	9442-1 / 8442-1	Class 4 X al sh2	51.02	8R	0	4A2	4C2
4605	9442-5 / 8442-2	Class 4 X al sh2	44.07	10R	0	5A2	5C2
4608	9442-8 / 8442-4	Class 4 X al sh2	45.09	7R	1C	1A2 Motts.[b] / 4A2	4C2
4607	9442-6 / 8442-5	Class 4 X al sh2	33.33	9R	1C	4A2	5C2
5604[c]	2274-3 / 3274-7	Stable X Seg.[d]	37.22	1R 1R/r 3rr	4C	3A2 Motts.[b] / 1A2	2C2 Motts.[b]
5605	2274-5 / 3274-11	Stable X Seg.	47.50	3R 3R/r	3C	2A2 Motts.[b] / 3A2 Motts.[b]	2C2 Motts.[b] / 2C2
4613	3275-1 / 2275-1	Seg. X Stable	54.67	5R/r	3C	2A2 Motts.[b] / 1A2	2C2/c2 ? / 3 C2
5606[c]	2275-2 / 3275-7	Stable X Seg.	53.39	2R 1R/r 5rr	1C	3A2 Motts.[b] / 1A2	3C2 Motts.[b] ((1c2/c2))=?
4614	3275-6 / 2275-5	Seg. X Stable	49.10	1R	1C	5A2 Motts.[b]	1C2 Motts.[b] / 1C2
4615	3275-11[d] / 2275-6	Seg. X Stable	38.12	4R/r	3C	2A2 Motts.[b] / 42a	3C2 Motts.[b]
5607	2280-1 / 3280-6	Stable X Seg.	51.32	5R	4C	4A2	4C2
5609	3280-8 / 2280-3	Seg. X Stable	50.60	8R	1C	5A2	4C2
5612	3280-2 / 2280-4	Seg. X Stable	59.17	5R	0	4A2	3C2
5613	3280-7 / 2280-5	Seg. X Stable	49.60	7R	1C	5A2	4C2
5608	2280-5 / 3280-7	Stable X Seg.	33.33	3R	2C	4A2	4C2
5614	3280-9 / 2280-7	Seg. X Stable	48.07	4R	2C	4A2	3C2
5615	3250-13 / 2280-8	Seg. X Stable	45.38	5R	2C	4A2	4C2
4616	3280-1 / 2280-9	Seg. X Stable	62.30	6R	1C	6A2	3C2
4617	3280-10 / 2280-10	Seg. X Stable	88.82	4R	3C	1A2/a2? / 9A2	5C2
5616	3281-12 / 2281-3	Seg. X Stable	46.57	3R 2R/r	2C	4A2 Motts.[b] / 1A2	2C2 Motts.[b] / 2C2
4609[c]	2281-7 / 3281-13	Stable X Seg.	43.51	8rr 2R/r	0	3A2 Motts.[b] / 2A2	4C2 Motts.[b] / 1C2
4612	2282-7 / 3282-4	Stable X Seg.	42.01	5R	3C	5A2	3C2
5617	3282-9 / 2282-9	Seg. X Stable	86.17	4R	1C	2A2	2C2

Illini XTRA Sweet = a a, r r

a. A single dominant symbol = homozygosity for that allele. The numbers before the symbols indicate the number of different stable plants tested.
b. Motts. = ears that were either segregating for r mottled kernels or were homozygous r mottled.
c. Ears where the segregation of r could affect the ratio of mutable:stable kernels
d. seg. = An al sh2 kernel from a 1:1 ear resulting from the outcross of al Mum Sh2/al sh2 X al sh2/al sh2.
e. Class 4 mutability = The most intense mutability pattern.

spotting (7aa,-Reddy and Peterson MGG, 1984), *c2-m/c2, Bz/(bz/Bz)* x *c2*, backcrossed on *bz-rcy*, the resulting progeny ears could be classified in the following way.

	c2-m		no	
	bz	bz no	bz	bz no
Set 1	7	3	2	0
Set 2	6	5		

The presence of *c2-m* spotting accompanied by *bz* no spotting would indicate a non-relation of *c2-m* and *Cy;* however, this *c2-m* allele mutability is quite ephemeral (note, though 7aa type was planted, there were two plants with no spotting for the *c2* allele).

With numerous tests with the *En*-reporter allele, no *En* could be detected.

P-VV alleles

--Peter A. Peterson

P-VVAc1162 (Zemun). This unstable pericarp allele was found among some Yugoslavian accessions. The phenotype of this allele is a *P* to *p* expression with large colorless sectors. Current evidence would eliminate *En* and it is not likely to be *Ac* (though not absolutely confirmed).

P-VVAc1163 (Zemun). This also arose among Yugoslavian accessions. Here, the sectors are not as large as *P-VVAc1162*. This allele does not show any relation to *Ac*, *En*, or *Uq*. That is, in plants where this allele is sectoring, the *C-I Ds*, the *c-m(r)* and the *c-ruq* allele do not show sectors or spots.

P-VV878401. This allele arose as an ear sector on a plant that was *P/p A/a-mpapu*. The sectoring is very low and unlike the two Zemun alleles. All progenies carry a number of *En* but confirmatory crosses have not been completed.

a2-m668144 - *a2-rcy* responds to *Cy*

--Peter A. Peterson

This *a2-m* allele arose in a 1965 isolation plot. It was originally cited in the 1975 Urbana Maize Symposium (Walden, ed. 1978). The early segregation pattern showed colorless segregants and the nature of the mutation pattern suggested segregation rather than mutation-induced loss of mutability events. Early observations eliminated *En* as the active element though many *En* were present in the genome.

With the availability of the *bz-rcy* reporter allele, tests were made to test the involvement of *Cy* in *a2-m668144* mutability. From a segregating progeny, from the cross *a2 bt* x *a2-m/A2, Bz/bz-rcy, Cy*, the following phenotypes were selected and intercrossed:

colored round	*A2 Bt/a2 bt + Cy* '88 2359-2360
colorless round	*a2(?), Bt/a2 bt* '88 2357-2358

The cross:	round			bt		
	Cl	sp	cl	Cl	sp	cl
2360-10 x 2358-2	207	96	119	9	1	110
2359 x 2357-1	215	47	108	7	0	115

This is a **reconstitution test** and confirms that the colorless segregants are *a2-rcy* (responsive allele) and they respond to the presence of *Cy*. The role of *Cy* in the mutability of these crosses had previously been established as well as the lack of involvement of *En*. This is the second *a2-m* allele to be established as a *Cy*-related allele from that TEL population in the 1965 series.

C-I-m836519 - Insert induced loss of a male transmitted color inhibition capacity

--Peter A. Peterson

This *C-I* mutable allele arose from a *C-I* allele in a population containing *En*. This allele gives a low (few, late spots) spotting pattern. The insert caused a loss of *C-I* potency when transmitted as a male (1 dose) but does show suppression when transmitted as a female (2 doses) when challenged with a *C* allele.

C-I-m836976 - an autonomously mutable *En* allele

--Peter A. Peterson

In a previous report (MNL:59) it was established that the mutability of this allele is *En*-dependent. The phenotype of this allele (without mutability) is a basic colored background that mutates to colorless sectors (*C* to *C-I*). This would indicate that the insert suppresses the *C-I* transcript (no suppression of anthocyanin color in *C-I-m/C*) and subsequent excisions lead to release of that suppression followed by restoration of the *C-I* effect (inhibition of color). That *En* causes the sectoring is supported by the correlative effect of *En* on *wx-m8*, a reporter allele for *En*. In the cross *C* x *C-I-m/C sh bz wx* colored and colored-with-sectors kernels were tested. In all cases, the colored selections were the *C sh bz* chromosome and the sectored kernels were the *C-I Sh Bz* chromosome. This is not absolute that it is *En* at the locus since sectoring should always be accompanied by *wx* sectoring with the *wx-m8* allele. The exception (lack of *wx* sectoring) may be due to change in *wx-m8* to a non-responsive allele, which is a common event.

Df C-I Sh-846571

--Peter A. Peterson

This deficiency arose as a full colored shrunken kernel from the cross *C-I Sh Bz Wx* x *C sh bz wx*. In confirmatory crosses full-colored-shrunken and bronze-shrunken ear progeny appeared. In the reciprocal crosses, where the plant from the colored shrunken seed was used as a male on a *C sh bz wx* plant that was used as the female, only bronze-shrunken seed appeared among the progeny. This change to a double mutant from *C-I Sh* to *C sh* and its nontransmissibility as a male indicates a deficiency of this segment of the chromosome.

The transmission is also affected from the female side of the cross. In the cross *Df Bz Wx/C sh bz wx* x *C sh bz wx*, the progeny included:

Bz sh	*bz sh*	*T*	*Bz*
72	149	221	32%

This shows an impaired transmission of this chromosome even as a female. Though the tests are not extensive, all the *sh Bz* plants show this behavior. An extensive testing of *sh Bz* plants would uncover a possible crossover. Thus far, none has been found; all the *sh Bz* plants show this behavior.

a-m(Au)871618W1, a-m(Au)871618W2 and *a-m(Au)flow*

--Peter A. Peterson

The *a-m(Au)* allele (a very dark heavily mutating background with large colorless areas) is one of the numerous alleles at the site of the original *En* insert at the *A1* locus. Its position is similar to the *a-m(papu)* allele of the *A1* gene, 22bp in from the 5' end of the second exon (Schwarz-Sommer et al., EMBO J. 6:287-294, 1987). From the cross *a-m(Au) Sh* x *a sh2* two exceptional kernels (low-spotting) arose.

In confirmatory testcrosses, the following progeny types were observed.

	Round		
Ear	Pale	Spotted	Total
#1	119	117	236
#2	107	121	228

It is clear that this allele is a basic pale colored that responds to an independently segregating *En*. This is one of numerous such alleles and brings up several points.

One, the functional component of *En* was lost leaving a remnant responsive allele. Therefore, a transposon undergoes numerous events other than complete excision. Thus it responds to *En* by retaining the terminal inverted repeats.

Secondly, the allele includes an insert that does not impair gene functioning. Its position in the exon is such that it must be included in the full transcript and is unlike the *a-m15719* allele which is positioned at the end of the 3' end of the second exon (Tacke et al., Maydica 31:83, 1986). Apparently, additional amino acids in the first third of the second exon do not interfere with the near wild-type gene expression of the *a-m(Au)871618W1* allele, which provides additional support for diversity in protein products generated from transposon inserts at a gene.

a-m(Au)871618W2. This allele is similar (pale, without *En*) to W1, though it occurred as an independent event. A similar segregation pattern supports the control of this allele by an independently segregating *En*.

a-m(au)-flow. A series of progenies have been uncovered from the *a-m(Au)* allele that exhibit a flow behavior (spotting, very late confined to the gown and/or shoulder of the kernel but absent in the crown). This phenotypic expression is quite transient and cannot be assured of its heritability in succeeding generations. This phenotype with a single seed descent crossing program ranges from near completely colorless to every kernel on the ear showing this phenotype. Obviously, this full allele is still present because the typical *a-m(Au)* allele is uncovered at random times. Thus, the flow behavior is transient and is probably a consequence of an inhibitory methylation pattern.

wx-m867674 - an *En* insert at the *Wx* locus

--Peter A. Peterson

This *wx* allele expresses *wx* to *Wx* events. It arose from the cross *C-I Sh Bz Wx* x *C sh bz wx* as a colorless *wx*-mutable kernel. The question of the basis of this control of mutability was tested by crossing the heterozygote, *C-I Sh Bz wx-m/C wx, A/a-o* by *sh wx/wx, a-m1/a-m(r)*. The male tester represents a reporter allele for *En* of kernel endosperm *a*-gene mutability. The rationale is to correlate *a*-gene reporter allele mutability with *wx*-mutability in order to test *En* inheritance. A small sample of progenies of this cross is given below.

	Colorless		Spotted	
	wx-m	*wx*	*wx-m*	*wx*
Ear				
#1	122	150	14	0
#2	117	127	9	0
#3	155	201	26	0
#4	90	79	10	0

Two items of interest are apparent. The distribution of *wx-m* to *wx-o* is close to 1:1 in this testcross indicating that the *wx-m* allele is 1/2 of the *wx* alleles and 1/2 are showing mutability. If the element was independent, this 1:1 ratio would be distorted.

More convincing is the sampling of *wx*-mutability among the *a*-reporter alleles. All the spotted that were tested were *wx*-mutable. This supports the contention that the only *En* is cosegregating with *wx*-mutability.

C-I836978 - a weak *C-I* allele

--Peter A. Peterson

This allele arose in a cross of *C-I/C-I-K55* x *C sh bz wx*. Initially, it appeared *C*-mottled. In reciprocal crosses a difference is seen: 87g 293-1/326-1 *C-Iweak/C-Iweak* x *c2-m2/c2-m2 C/C* gave all full-colored, while 87g 293-2 selfed *C-Iweak/C sh bz* gave a range: colorless, weak suppression. Crossed on *C/C*, all are full color - i.e , there is no suppression of color. The *c2-m2* line has a *C* allele that cannot be suppressed by this weakened *C-I*. Possibly, a *C-super* is included here. When used as a male, plant-2 is ineffective in suppression.

c-m(r)860904 - a mutant controlled by *En-I* system, the origin of a receptor allele from *c-m655437*

--Ch. Jayaram, A. R. Reddy* and Peter A. Peterson

*University of Hyderabad

The mutability at *c-m655437* is controlled by an autonomous *En* transposable element (MNL 1988). Crosses of homozygous spotted round kernels *c-m Sh Wx/c-m Sh Wx* with *c sh wx* tester give approximately 87.83% spotted round kernels among progeny ears. However, in one particular instance (86 0904 x-3), the progeny ear exhibited the segregation of spotted round and colorless round kernels in approximately a 1:1 ratio. To determine whether these colorless kernels represent a case of the origin of a receptor element of *c* from the autonomous *En* element, these colorless were then crossed with *c sh wx En* line which has more than

Table 1. The cross *c-m(r)?.Sh Wx/c sh wx* x *c sh wx En En*

	Spotted round	Colored round	Colorless round	Shrunken	Total
4601-21 x 2756-3	120	1	5	135	261
-22 x 2753-4	164	0	22	172	358
-23 x 2755-8	92	0	4	106	202
-24 x 2753-3	145	0	12	174	331
-25 x	61	0	10	85	156
-26 x	25	0	2	29	56
4602-21	37	0	1	41	79
-23	138	0	16	160	314
-24	151	0	10	165	326
-25	94	0	8	99	201
-26 x	63	0	7	60	110

two *En* elements segregating independently in the genome, to find out whether these colorless kernels represent a case of the origin of a receptor element of *c* from the autonomous *En* element. Table 1 shows the segregation of spotted round kernels among the progeny ears confirming that these colorless kernels have a receptor element which respond to a trans-active *En* element.

Use of commercial ink marker pen in genetic analysis

--Yong-Bao Pan and Michael G. Muszynski

Waxy marker pencils are used routinely in maize genetic analysis to assist accurate counting. The marker is difficult to remove and creates certain problems in photography if the maize ear or kernel is to be photographed. Recently, we found an alternative but also efficient way to mark the maize kernels simply by using the ink marker pens of various colors available on common market. The ink marker can be easily removed by any type of tissue paper shortly after the counting. Therefore, unlike the waxy marker pencil, the ink marker pen leaves no problem for photography.

***Uq* activation: recovered germinal events**

--Yong-Bao Pan and Peter A. Peterson

An additional set of germinally activated *Uq* elements has been isolated since our first report (MNL 62:5). These newly-arisen *Uq*'s arose either as single or as two separate spotted kernels on a colorless progeny ear upon crossing *a-ruq/a-ruq*, sectored (or few-spot or colorless sibs) (for terminology see Genetics 119:457) with an *a-ruq* tester. They all elicit a heritable unique and distinguishable spotting pattern when interacting with the *a-ruq* reporter allele (Table).

Table. Properties of germinally activated *Uq* elements.

Uq designation	Spotting Pattern[1]	a-ruq/a-ruq in original mutant	Transactivation of c-ruq
Uq-870801U	Heavy, C1-like	Yes	Yes
Uq-870621Y	6-7b-c/10aa, with d-e pale sectors	"	"
Uq-870829U	varies (some C1-like spotting, some 2-4b/6-7aa)	"	"
Uq-870829Y	C1-like spotting	"	"
Uq-870829V	varies (few C1-like spotting, majority 2-4b/4-6aa)	"	No
Uq-870834Y	C1-like spotting	"	?
Uq-870621X	3-4c/4b	No	Yes
Uq-870622X2	5-6b/6aa	Yes	"
Uq-870622Y[2]	varies (few C1-like majority 1-3b/2-5aa)	"	No
Uq-870830Z	3-7b/5-9aa	"	Yes
Uq-870831X	6-7b-c/10aa with d-e pale sectors	"	No
Uq-870832X	3-4b/9aa	"	Yes
Uq-870834C	4-5b	"	?

[1]genotype = a-ruq Sh2/a sh2 Uq/+

[2]the progenitor sectored kernel has been treated with 5-aza-2-deoxycytidine

The *Uq* nature of this exceptional activity has been tested using two approaches. First, in order to prove *a-ruq* was the only reporter allele in the original mutant, twelve colorless sibs, derived from the confirmation ear (the spotted mutant x *a sh2* (no *Uq*)), were crossed with a standard *Uq* element. The results of these crosses confirm that *a-ruq* was the only allele present. Secondly, an *a sh2/a sh2 Uq/Uq* (or +) line was constructed and this was followed by crossing this line to the standard *a-ruq*, *a-m1*, *a-m(r)h*, and *a-mdt* reporter alleles. A spotting phenotype was produced only from the interaction between the *a-ruq* reporter allele and the element in the *a sh2* line thereby confirming the *Uq* nature of the original mutant. In addition, the ability of these new germinally activated *Uq* elements to transactivate a *c-ruq* reporter allele was also tested (Table) and this gave variable results.

Allelism tests between a standard *Uq* and four germinally activated *Uq*s (*Uq-870801U*, *Uq-870621Y*, *Uq-870829U* and *Uq-870829Y*) are underway.

The *Uq* at *mn-866248U* is not a standard *Uq* element

--Yong-Bao Pan and Peter A. Peterson

Our genetic tests have indicated that the activated *Uq* element at the *mn-866248U* mutant locus (MNL 61:6; 62:5-6) is not the standard type of *Uq*. This conclusion is derived from the following observations: 1) The *mn Uq*, whether in the original mutant or in an *a sh2/a sh2* construct, is capable of transactivating the standard *a-ruq* reporter allele resulting in a miniature spotted phenotype which distinctly differs from the spotting type produced by the standard *Uq/a-ruq* interaction (data not shown); 2) it does not transactivate a *Uq*-responsive *c-ruq* reporter allele (data not shown). This is in contrast to most standard tests with *Uq* elements, where *a-ruq* and *c-ruq* always show corresponding responses.

***Dee*-m857345* and *Dee*-m857513*: two mutable *de*fective endosperm *m*utants**

--Yong Bao Pan and Peter A. Peterson

Recently, a number of mutable *de*fective *e*ndosperm *m*utants (*Dee-m*) arose in several isolation plots containing active transposable elements. These mutants impair the normal endosperm development leading to sectors of chalky endosperm tissue and, as a result, to colorless sectors of aleurone layer overlying the chalky endosperm tissue (Figure 1). On the other hand, the mutant kernels have normal embryos and grow to normal plants. Genetic tests indicate that the phenotype (chalky-endosperm-and-aleurone-variegation) is a dominant trait and is independent of the genes involved in anthocyanin pathway (Figure 2).

Two of these mutants, *Dee*-m857345* and *Dee*-m857513* that were recovered as single variegated kernels from a *Cy*- and an *En*-containing maize population respectively, have been studied. This report summarizes the relations of these two mutants to several known families of

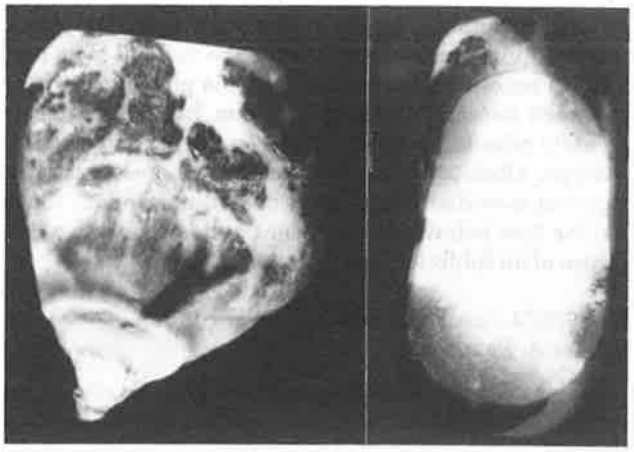


Figure 1. A chalky-endosperm-and-aleurone-variegation phenotype is shown by side (left) and top (right) views of a sectioned kernel of *Dee-m*/+ (line C) genotype.

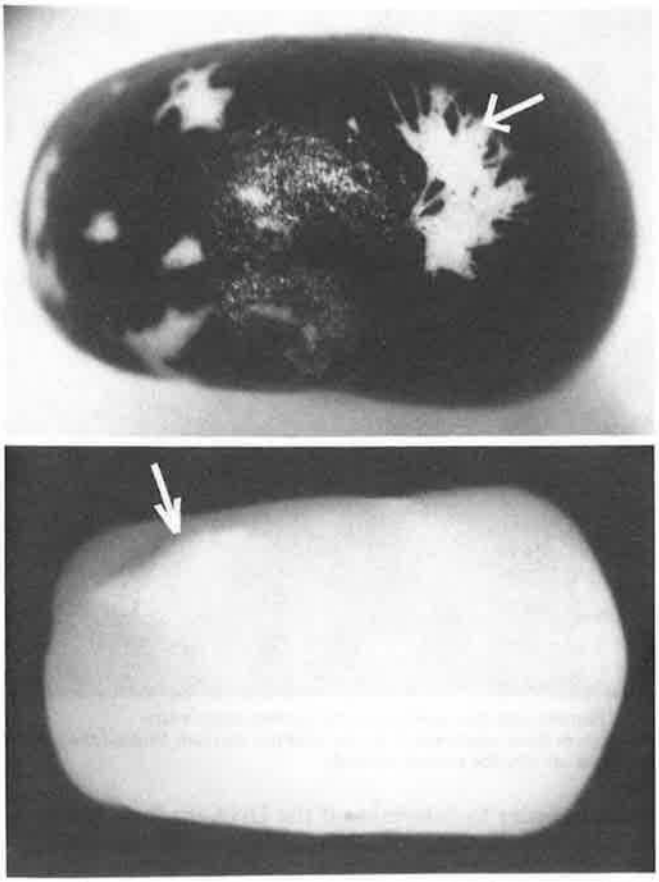


Figure 2. Photographs of two *Dee-m* kernels showing the *Dee-m* trait is independent of aleurone coloration. Top: *Dee-m/+, C/c-ruq*. Bottom: *Dee-m/+, c-ruq/c-ruq*.

maize transposable elements by using appropriate reporter allele testers (Table).

The mutant *Dee*-m857345* has active *En* and *Ac* elements in addition to *Cy*, and *Dee-m857513* has active *Ac* in addition to *En* (Table). Nevertheless, the mutable *dee* trait is

Table. Transposable element (TE) content of *Dee-m857345* and *Dee-m857513*

Mutant	Source	Reporter allele	Element target	#of Dee-m gametes[1]	TE Content present	TE Content absent
Dee-m857513	En	a-ruq	Uq	27(5)	0(0)	27(5)
		c-ruq	"	29(6)	0(0)	29(6)
		C1-Ds	Ac	(4)	(4)	(0)
		bz-rcy	Cy	33(6)	0(0)	33(6)
		r-cu	Fcu	9(3)	0(0)	9(3)
		c2-m2	En	36(6)	2(1)	34(5)
		a2-m(r)	"	54(9)	3(2)	51(7)
Dee-m857345	Cy	a-ruq	Uq	51(9)	0(0)	51(9)
		c-ruq	"	62(11)	0(0)	62(11)
		C1-Ds	Ac	(9)	(9)	(0)
		bz-rcy	Cy	43(8)	43(8)	0(0)
		c2-m2	En	58(13)	11(4)	47(9)
		a2-m(r)	"	30(5)	5(2)	25(3)

[1]number in parentheses represents the number of parental Dee-m gametes involved in the test.

shown not to be correlated to the activity of any of these residing active transposable elements (data not shown).

C-I-m846079

--Yong-Bao Pan and Peter A. Peterson

Since our first report on *C-I-m846079*, a highly mutable allele (MNL 61:5), several genetic experiments have been completed. To understand how this mutable allele acts, we crossed this allele with both *C* (of different sources) and *c* (of *c sh wx* tester) alleles. Although a mutable phenotype was always observed for a *C/C-I-m846079* genotype, the *c/C-I-m846079* only yield a colorless phenotype (data not shown). This observation leads to two conclusions. First, *C-I-m846079* does not encode a functional *C* gene product; secondly, the normal inhibitive function of the *C-I-m* allele is interrupted by an insertion at the *C-I* locus unless the insert excises. Therefore, the highly mutable phenotype observed for a *C/C-I-m846079* genotype represents many colorless sectors on a colored background, indicating that an excision event at *C-I-m* yields a normal *C-I* allele leading to a colorless sector.

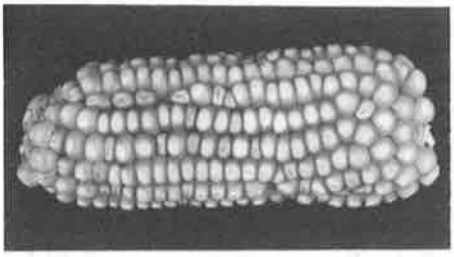
The transposable insert of this mutant has also been studied. By crossing to appropriate reporter allele testers, we observed no other families of active transposable elements except *Cy* elements in this mutant. These tests included *Ac, En, Uq, Dt, Mrh, Fcu, Bg* (data not shown). In addition, the mutant contains at least 5 independently segregating active *Cy* elements in its genome (data not shown). Exceptional colorless derivatives of *C/C-I-m846079* arise and these are not transactivated by *Cy*.

Uq-10aa-SS: a second 5-aza-2-deoxycytidine induced *Uq* element

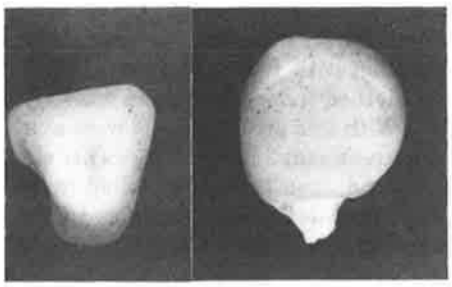
--Yong-Bao Pan and Peter A. Peterson

In the progeny test of the dominant mutant *Mn-866248U* (MNL 61:6), another example of *Uq* element activity (independent of the *Uq* element at the *mn* locus) was found. Upon selfing, half of the progeny ears, arising from either the miniature spotted or the colorless normal sib kernels of the ear 866248U/5121-7, segregated for smaller seeds with very small, near single-cell spots (10aa, Reddy and Peterson, MGG 194:124, 1984) at about .25 frequency (data not shown). An example of this type ear is shown in the Figure with top and side views of a 10aa spotted smaller seed.

Since both parents of the cross (866248U x 5121-7) had the *a-ruq* reporter allele (*a-ruq/a-ruq*) genotype and were normal, this 10aa spotted smaller-seed phenotype must be due to an interaction of the *a-ruq* reporter allele with two



A



B

Figure. Phenotype of *a-ruq/a-ruq Uq-10aa-SS/Uq-10aa-SS*.

homozygous activated *Uq* elements. On the other hand, since the selfed ear of plant 865121-7 produced only 333 colorless normal kernels, the activation event must have occurred in the maternal plant 866248U that had been treated with 5-aza-2-deoxycytidine. This *Uq* element is tentatively designated as *Uq-10aa-SS* (Smaller Seed).

Our effort in studying the genetics of the element was hampered by the fact that when the 10aa spotted smaller seeds were planted none of the 72 established a plant either in the greenhouse or in the field. This brings up a very interesting question of whether this element affects the fitness of the genotype *a-ruq/a-ruq Uq-10aa-SS/Uq-10aa-SS*.

Pollen transformation: improving seed setting

--Yong-Bao Pan, Hua Zhou, Peter A. Peterson and Alan G. Atherly

In our initial attempt with maize pollen transformation, there were five treatments (see footnote in Table) that were conducted under two different conditions. Using the original method at 29 C, maize pollen paste of one genotype from the 5 treatments was directly applied to freshly nicked silks of another genotype in a "pollen-free" greenhouse room for 3 consecutive days. Under a revised procedure in a 20 C "pollen-free" room, pollen samples of the same genotype were put through the 5 treatments and were applied to the freshly nicked silks pretapped at their base (i.e., ear tip) in order to hold the applied pollen paste. On day 2 after the treatments, the same procedure was repeated an additional time. On day 4, these plants were moved into the 29 C greenhouse room and grown to maturity.

Table. Effect of temperature (T C), pollination method and treatment on seed setting in pollen transformation.

T°C	Pollination method[a]	Treatment[b]	Number of ear tried	Total number seeds	Average number of seeds per ear
29	**Original**	1	2	0	0
		2	6	7	1.2
		3	2	0	0
		4	2	0	0
		5	4	0	0
20	**Revised**	1	2	2	1
		2	12	318	26.5
		3	2	0	0
		4	8	27	3.4
		5	6	25	4.1

[a]For a description of **"original"** and **"revised"**, see text.

[b]Treatment: 1 = pollen + buffer

2 = pollen + buffer + DNA[c]

3 = pollen + buffer + electroporation

4 = pollen + buffer + DNA[c] + electroporation

5 = pollen + buffer + DNA[c] + 5-minute-germination + electroporation

[c]DNA = plasmid DNA pGA482::OD9 (containing NPT II and luciferase genes) or pLE392 (containing NPT II and nopaline synthesase genes) + carrier DNA

Upon harvest, we observed a very strong effect of temperature, pollination method, and treatment on the average seed set per ear (Table). First, a lower temperature at around 20 C in combination with two-day cycles of nick-tape-pollen application method indeed improved seed setting (Table and Figure). With this procedure, we were able to increase seed setting in treatment 2 from 1.2 seeds per ear to 26.5 seeds per ear. Second, even though we failed to obtain any seed from electroporated pollen samples in the original experiment, we were able to get 3.4 to 4.1 seeds per ear from two electroporated pollen samples under the revised procedure. Currently we are analyzing these maize kernels in order to determine if the DNA markers are present that will be a confirmation of successful transformation.

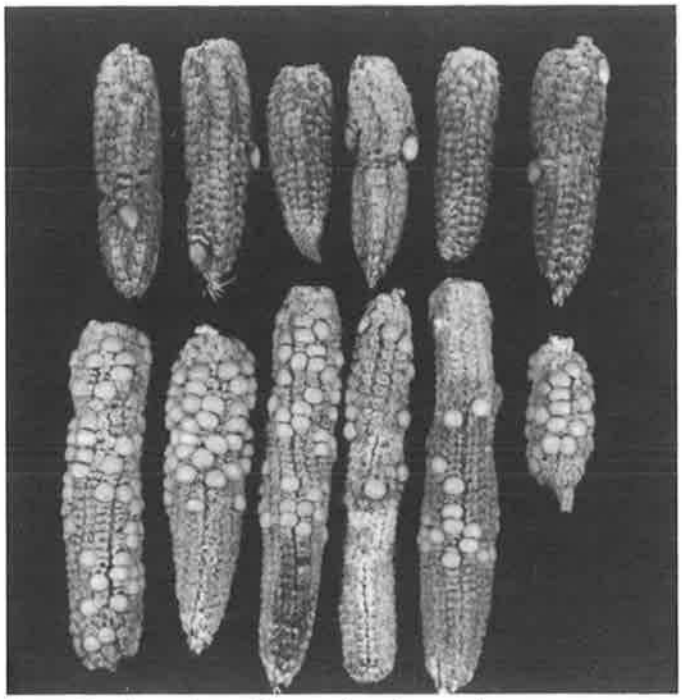


Figure. Sample ears from pollen transformation experiment. Top row: ears from treatment 2 by the original method; Bottom row: ears from treatment 2 by the revised method.

The "*a-m(r)* effect" on *wx-844* mutability

--Kim Hagemann and Peter A. Peterson

The *a1-m(r)102* allele reduces the excision of *En-1*. The *wx-844* allele containing *En-1* is known to exhibit a **coarse** *wx* mutability pattern in genotypes not carrying the *a1-m(r)102* allele. When genotypes carry *wx-844* and *a1-m(r)102* a distinctly distinguishable *wx* mutability pattern *a-m(r)* effect and a heavy kernel spotting pattern occur (Cuypers et al., EMBO J. 7:2953, 1988). Kernels having lower spotting patterns than normal in the presence of *a1-m(r)102* were isolated from the following cross: *a1-m(r)102 wx-844* x *a1-m15719A Wx*. These kernels were then crossed onto homozygous *a1-o wx*. These exceptions yielded phenotypes that are designated in the table below. These mutants of *a1-m(r)102* will be useful in identifying the sequences of *a1-m(r)102* which alter and contribute to the suppression of *En-1* excision, i.e. a coarse to fine pattern of *wx*-mutability.

No. of exceptions	wx mutability: coarse	wx mutability: medium	wx mutability: fine	al-m(r) coloration: colorless	al-m(r) coloration: decreased spotting	**a-m(r) effect**
9	X				X	lost
3	X	X			X	modified
2	X		X	X		transferred
10	X		X		X	unchanged

(Other exceptions produced ears with transposed En-1 elements, ears with normal a1-m(r)102 activity (non-heritable changes), and ears having changes which cannot simply be attributed to a change in a1-m(r)102.)

The transposable element *Uq* in BSSS(R) and BS13(S) corn breeding populations

--Lisa Lorenzen and Peter A. Peterson

Iowa Stiff Stalk Synthetic populations have found a seemingly unending source of genetic variability in long term recurrent selection (Hallauer et al., 1983). One possible source of this variation is the insertion and excision of transposable elements, which can lead to changes in the base pair sequence of a gene (Schwarz-Sommer et al., 1985). Activity of the transposable element *Uq* has been found in Iowa Stiff Stalk Synthetic (BSSS) (Cormack et al., 1988). Eleven cycles of BS13(S) (seven cycles of half-sib selection, four cycles of S2 selection) and eleven cycles of reciprocal recurrent selection (BSSS(R)) were analyzed to determine at what frequency the element *Uq* was present, and how that frequency varied between cycles. Statistical analysis showed a positive linear relationship in the BS13(S) series and a negative linear relationship in the BSSS(R) series.

Crossingover frequencies between several translocations and four *En*-containing alleles

--Ruying Chang and Peter A. Peterson

In an effort to relocate highly transposing En-containing alleles to certain chromosome regions, crosses were made between several translocation stocks (shown in Table 1) and four *En*-containing alleles. These mutable alleles include the *a1-m(papu)*, *c2-m*, *a2-m* and *wx-m* alleles. The F1 progenies from those crosses were testcrossed with the corresponding recessive alleles. Spotted kernels from semi-sterile ears of the testcross progenies were planted in order to obtain crossovers. This was tested by examining pollen fertility. Semi-sterility in pollen of those *En*-containing plants is an indication of a crossover between the specific allele and the breakpoint on the specific arm being labeled.

Specific mutable alleles are being linked to one of the seven translocation stocks. A description of the translocations, the mutable alleles, the crossover percentage, as well as the recorded map distance is given in Table 1.

Table 1. Translocation and crossingover data

Translocation	Break points	Mutable allele	Map distance*	Sterile plants/total	%	χ^2
T1-3 5597	1S.77 3L.48	a1-m(papu)	±18	17/48	35.4	16.82
T1-4 8602	1S.41 4L.81	c2-m	±3.5	24/96	25.0	132.07
T2-3 d	2L.67 3L.48	a1-m(papu)	±18	1/48	2.1	14.04
T2-5 032-9	2L.40 5S.31	a2-m	0-15	5/24	20.8	2.24
T4-5 e	4S.41 5S.32	a2-m	0-15	17/96	17.7	0.49
T4-6 033-16	4L.50 6S.90	c2-m	±20	43/96	44.8	30.75
T6-9 5454	6ctr. 9S.75	wx-m	±39	15/48	31.2	1.56

*Map distances between specific mutable alleles and specific arms being labeled, which are the estimates according to Maize Genetics Newsletter V.52:129-145, 1978

The ratio of pollen-sterile plants vs. total plants gives the crossover percentage. As shown in Table 1, the cross between T6-9(5454) and *wx-m* allele is consistent with the map distance recorded ($\chi^2 = 1.56$). Crosses T2-5(032-9) x *a2-m* and T4-5e x *a2-m* were also in agreement with the map distances if we consider the map distance for both as 15 ($\chi^2 =$ 2.24 and 0.49, respectively). The other four were highly discrepant from the published map distances. Three of the four had higher values than that expected [T1-3(5597) x *a1-m(papu)*, $\chi^2 = 16.82$; T1-4(8602) x *c2-m*, $\chi^2 = 132.07$; T4-6(033-16) x *c2-m*, $\chi^2 = 30.75$)]. The other one had a lower value than that expected [T2-3d x *a1-m(papu)*, $\chi^2 = 14.04$]

Chemical methods for direct gene transfer to maize protoplasts: I. Efficient transient expression after treatment with the polycation Polybrene

--N. M. Antonelli and J. Stadler

Although methods for introduction of foreign genes directly into intact cells of tissues may eventually become the most desirable method for producing transgenic maize plants, methods for direct transfer into protoplasts remain necessary, especially for preliminary studies of gene structure and regulation (for example, Callis et al. Gene Dev. 1:1183, 1987). We have therefore continued our studies of methods for simple and reliable gene transfer to maize protoplasts.

We report a novel and efficient chemical method for direct gene transfer to maize protoplasts. Freshly isolated protoplasts are treated for 6 or 12h with transfecting DNA and the polycation Polybrene (hexadimethrine bromide: Kawai and Nishizawa, Mol. Cell Biol. 4:1172, 1984). At the end of the incubation period, the transfection mixture is simply diluted by addition of growth medium and the cells are then incubated further for 30h before being assayed for transient gene expression. This gentle method involves little handling of the cells and no physical disruption of the membrane, a feature of both electroporation (Fromm et al., Proc. Natl. Acad. Sci. USA 82:5824, 1985) and microprojectile bombardment (Klein et al., Proc. Natl. Acad. Sci. USA 85:4305, 1988) procedures for transformation of maize cells. And, probably as a consequence of the gentleness of this treatment, we do not detect loss of viability beyond that seen in untreated protoplast control populations by 24h after the transfection treatment (Table 1).

Table 1. Viability after transfection treatments.

	Time after treatment (h)			
	0	6	12	24
Control	92	82[a]	85	71
Polybrene[b]	92	98	70	70
Electroporation[c]	92	59	54	52

[a]These values indicate the percentage of viable cells relative to control at time 0. Each data point is derived from counts of 300 cells in 3 replicate samples. Viability is ascertained by staining with fluorescein diacetate.
[b]$2x10^6$ protoplasts were combined with 30ug polybrene and 20ug DNA.
[c]$2x10^6$ protoplasts were transformed with 20ug DNA at 150V and 800u F for 12.0m sec.

Although it is difficult to make accurate comparisons of the efficiency of direct gene transfer by electroporation (Fromm et al., Nature 319:719, 1986), polyethylene glycol (PEG: Antonelli et al., MNL 62:7, 1988), and Polybrene methods, representative experiments are shown in Fig. 1A, 1B, and 1C. In each case, protoplasts were isolated as described and transformed with 20 to 50ug plasmid DNA, pCaMVI1CN (MNL 62:7, 1988). Assays for transient chloramphenicol acetyl transferase activity were performed 30 to 40h after CAT gene transfer. In all instances (Fig. 1A lane 2; 1B lane 2; 1C lane 2) treated control protoplasts without DNA showed no acetylation of ^{14}C chloramphenicol. However, electroporation of 25ug pCaMVI1CN DNA (Fig 1A lane 4), PEG treatment with 50ug (Fig. 1B lane 3), and

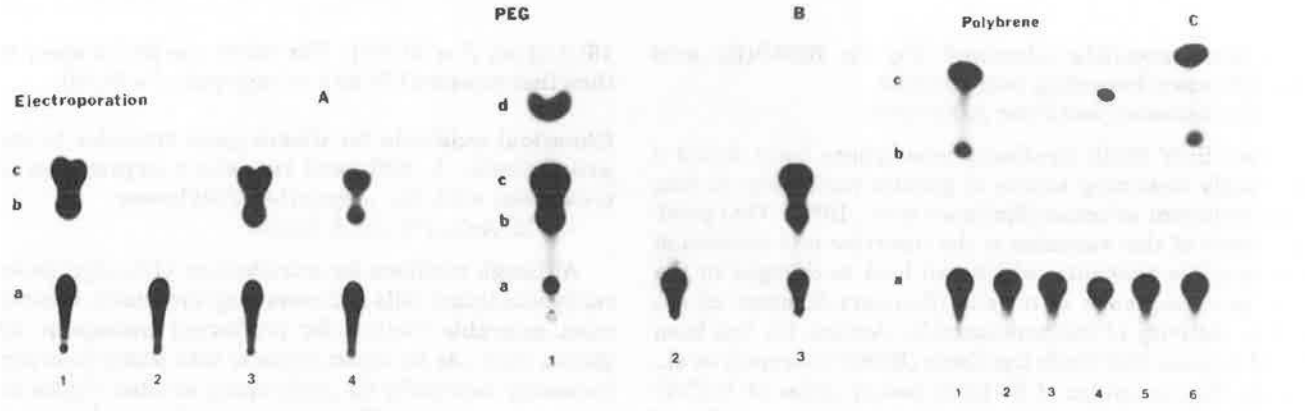


Fig. 1. Chloramphenicol-acetyl transferase activity in BMS-M protoplasts transformed with plasmid DNA pCaMVI1CN by electroporation (A), by polyethylene glycol (PEG) (B), or by Polybrene (C). CAT activities were determined by incubating heat-treated lysates with 4mM acetylCoA and 0.1uCi of 14 [C] chloramphenicol. The reaction products were separated on silica gel TLC plates and detected by autoradiography. Reaction products: (a) chloramphenicol; (b) 1-acetylchloramphenicol; (c) 3-acetylchloramphenicol; (d) 1,3-diacetylchloramphenicol. Lanes A: 1, bacterial standard; 2, electroporated control cells without DNA; 3, electroporation with 50ug of pCaMVI1CN; 4, as lane 3 but with 25ug of the same DNA. Lanes B: 1, bacterial standard; 2, PEG-treated control protoplasts without DNA; 3, PEG-treated protoplasts with 50ug of pCaMVI1CN. Lanes C: 1, bacterial standard; 2, Polybrene-treated protoplasts without DNA; 3, control cells with 20ug of pCaMVI1CN without Polybrene; 4, $3x10^6$ protoplasts treated with Polybrene and 20ug pCaMVI1CN, diluted after 6h; 5, $2x10^6$ Polybrene-treated protoplasts with 20ug of the same DNA, but diluted after 18h; 6, $2x10^6$ Polybrene-treated protoplasts with 20ug pCaMVI1CN, diluted after 6h.

Polybrene treatment with 20ug of the same DNA (Fig. 1C lane 6) each effect direct gene transfer. In each of these instances, respectively, 6.4%, 37%, and 22% conversion of ^{14}C chloramphenicol to acetylated products was obtained. With all three direct gene transfer treatments we have obtained in different experiments 60% to 95% chloramphenicol acetylation.

Transfection methods using polycations to aid DNA transfer to plant cells are new, although similar techniques have been used for several years in transfection of animal cells (Chaney et al., Somatic Cell and Mol. Genet. 12:237, 1986). Polycations probably enhance adsorption of the transfecting DNA to plasma membrane by interacting with the negative charges of both the DNA molecules and the membrane surface. The adsorption of the extracellular DNA may be followed by endocytosis. When Polybrene was first successfully used in animal transfection it was necessary to follow the polycation treatment with dimethyl sulfoxide (DMSO) treatment to achieve gene transfer. In our initial experiments with the Polybrene/DMSO procedure, we found that the recommended DMSO concentrations (30% v/v) caused protoplast lysis and that Polybrene treatment alone, without DMSO permeabilization, was sufficient to obtain efficient transfection as measured by the transient expression of CAT genes.

The detailed procedure is as follows:

1. Prepare protoplasts, and resuspend 2 x 10^6 cells in 0.5ml Murashige Skoog-based growth medium with 8% mannitol (MS2D8M) (Somers, Plant Sci. 53:249, 1988).
2. For each experiment prepare a fresh Polybrene (Aldrich) stock solution (10mg/ml in phosphate buffered saline, pH 7.0). This is an extremely hygroscopic chemical and the manufacturer's safety instructions must be rigorously applied. The stock solution is then diluted to yield a final concentration of 30ug Polybrene in 0.1 ml MS2D8M.
3. The desired concentration of transfecting DNA is suspended in 0.4ml MS2D8M.
4. Mix the 0.1ml (30ug) Polybrene solution with the resuspended protoplasts and transfer to a 60 mm Petri dish.
5. Immediately add (dropwise) the 0.4ml DNA suspension. The protoplast/Polybrene/DNA mixture (total volume 1.0ml) is rotated gently (25rpm) on a gyrotary shaker for 15 min and then incubated (stationary) at 28 C for 6h.
6. After the 6h incubation, dilute the above mixture with 4.0ml MS2D8M, seal the Petri dish, and follow procedures for assaying transient gene expression or for selection of stable transfectants.

The uptake of DNA following Polybrene treatment requires that the recipient cells (and recipient plasma membranes) be as healthy as possible. When adapting these procedures to your cell lines, be sure that the cells are growing optimally (our BMS-M line has a doubling time of 26 to 37h) and that the time of cell-wall digestion is as short as possible.

Phagocytosis of latex beads by protoplasts

--J. Stadler and Huang H. C. Lin

The engulfing of foreign substances (phagocytosis or pinocytosis) by mammalian cells is a normal process involving plasma membrane (Berlin et al., Cell 15:327, 1978). We were curious to see if maize protoplasts would take up large particles since natural or induced phagocytosis of foreign DNA aggregates might eventually prove to be the least cytotoxic and most efficient method for direct gene transfer to these cells. To test the phagocytic ability of Black Mexican Sweet (line BMS-M) protoplasts, the cells were presented with Fluoresbrite-labeled, 100μ polystyrene latex beads (Polysciences) after removal of the cell wall (MNL 62:7, 1988). The latex beads were prepared by a 1:250 dilution with growth medium to yield approximately 10^9 beads in 1.0ml. One ml of the bead suspension was gently mixed with 10^6 protoplasts in 1.0ml growth medium with 8% mannitol (Somers et al., Plant Sci. 53:249, 1988) and incubated at 28 C without shaking. At various times after addition of beads a protoplast sample was removed and exam-

ined with a Leitz Dialux 20 microscope equipped with a Ploem illuminator and a filter combination designed for wide-band blue fluorescence. At least 100 protoplasts were examined to establish each data point.

The results of two preliminary experiments are shown in Table 1. In the first, labeled beads were added to the pro-

Table 1. Uptake of 100 u polystyrene latex beads by maize (BMS-M) protoplasts.

		Cells with latex beads (%)	Average number of latex beads per cell
A. EXPERIMENT 1			
Beads added at: 0h		31	8.0
Beads added at: 16h		9	3.4
Beads added at: 24h		0	0
(All cells examined 24h after addition of beads.)			
B. EXPERIMENT 2			
(All beads added at 0h)			
Cells examined at:	2h	23	2.0
	6h	36	2.9
	12h	31	4.5
	24h	32	4.8

toplasts immediately after cell wall removal, or 16h, or 24h later. Protoplasts were then examined microscopically 24h after the addition of the beads. When the beads were added at 0 time, 31% of the protoplasts took up an average of 8.0 beads, but when the beads were added to cells 16h after protoplasting only 9% took up the particles. No beads were taken up by the treated cells if they were presented 24h after cell wall removal. These data suggest that the latex particles are efficiently taken up until major portions of the cell wall are reformed by 16h.

We do not yet have microscopic proof (by EM or counterstaining) that the brightly fluorescing 100μ beads are in the cells rather than accidentally attached to the surface of the protoplast. However, several observations have convinced us that most cell-associated beads have been phagocytized and do lie within the cells. First, very often the engulfed particles have been moved through the tonoplast and concentrated in the vacuole. Also, in time course experiments like the one just described (Table 1A) accidental association of the beads with the cell surface would be expected to give a high "background" at all times. However, this background is not seen when beads are added to 24h-old protoplasts and allowed to incubate for an additional 24h.

The second experiment (Table 1B) followed a time course for particle uptake when the beads were added immediately after cell wall removal. Twenty three percent of cells had taken in an average of 2 particles per cell by 2h. By 6h 30% of the cells had engulfed an average of 3 beads. The percentage of cells with beads remained the same between 6 and 24h although the average number of beads per cell increased slightly. These data again suggest that maize cell protoplasts are quite capable of phagocytosis of large 100μ polystyrene latex beads before cell-wall formation interferes. If so, the cells should probably also be capable of the endocytosis of large DNA aggregates.

Chemical methods for direct gene transfer to protoplasts. II: Recovery of stable transformants after direct gene transfer using polyethylene glycol (PEG)

--N. M. Antonelli and J. Stadler

A method for transfection of Black Mexican Sweet protoplasts (line BMS-M) with polyethylene glycol was adapted from Krens (Krens et al., Nature 296:72, 1982) and reported in this Newsletter (MNL 62:7,1988). In that article we described the detection of transient expression of transfected chloramphenicol acetyl transferase (CAT) genes. We now report the recovery of stable kanamycin-resistant microcalli obtained after polyethylene glycol (PEG)-induced transfection of BMS-M protoplasts with the neomycin phosphotransferase II gene (pCaMVNeo; Fromm et al., 1986). This frequency of stable transformation of maize protoplasts is higher than that reported by others (Fromm et al., Nature 319:791, 1986) and may reflect the somewhat higher average viability of protoplasts 24h after PEG treatment (70%) compared to electroporated protoplasts (50%).

To obtain stable transformants we used two methods of protoplast culture after PEG-mediated gene transfer. In the first procedure the protoplasts were resuspended immediately after treatment in growth medium containing 8% mannitol and 25% conditioned medium (CM)(Somers et al., Plant Sci. 53:249, 1988) and incubated in the dark at 28 C for 14 d. Then, to allow more cell division under nonselective conditions, 1ml aliquots of cells in liquid were placed on solid growth medium (containing 0.3% Gelrite and 25% CM) for an additional 7d. After this time 150 individual microcalli were picked at random and transferred to selective solid medium containing either 100 or 200ug/ml kanamycin and 25% CM, and were incubated further for 7 to 14 d. At the end of the second period on selective medium, some microcalli were faster growing than others (Fig. 1), and these

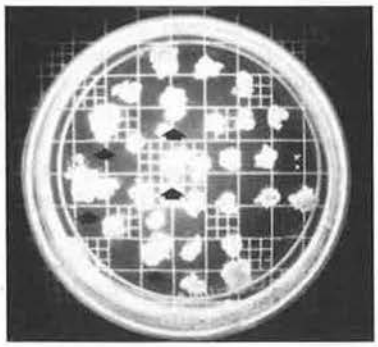


Fig. 1. Selection for stable transformant colonies. The arrow indicates some fast-growing calli on 200ug/ml kanamycin.

were harvested for molecular analysis when they were approximately 10mm in diameter with 300-400mg tissue. In the experiment presented in Table 1, 31 of the 150 microcalli picked were fast growers. These microcalli, however, may not have been of unique clonal origin since treated protoplasts tend to aggregate in liquid culture after PEG treatment, and during growth in liquid for 14d the larger aggregates often subdivide to form smaller (possibly genetically identical) clusters. There was either no growth in selective conditions of microcalli from control cultures that had received PEG treatment but no DNA or a limited amount of

Table 1. Selection of kanamycin-resistant cell lines after treatment of BMS protoplasts with PEG and pCaMVNeo (50ug.)

	a	b	c	d	e
Control	$5x10^6$	$2x10^6$	50	0	0
Liquid	$5x10^6$	$2x10^6$	150	5	3.3
Agarose slabs	$5x10^6$	$2x10^6$	150	5	3.3

a. Number of treated cells.
b. Cell number after PEG treatment.
c. Total number of colonies picked at random.
d. Transformed colonies from population identified by fast growth in selective conditions.
e. Transformation frequency (% of randomly recovered colonies).

cell division followed by browning of the callus in a few weeks. Genomic DNA from the faster growing microcalli was isolated (Mettler, Plant Mol. Biol. Rep. 5:346, 1987) and digested with several restriction enzymes including *Hin*dIII and *Bam*H1. A probe containing the *Bam*H1 fragment of the nptII gene was radioactively labeled and hybridized. The 1.0kb diagnostic fragment demonstrating nptII genomic insertion was present in 5 of the 31 faster- growing colonies (Table 1; Southern blot not shown).

In the second procedure, protoplasts were plated immediately after PEG treatment in a 2ml slab of 0.6% low melting agarose (Paszty and Lurquin, BioTechniques 5:716, 1987) in growth medium with 6% mannitol and 25% CM which was then overlaid with less than 0.5ml liquid growth medium also containing 6% mannitol and 25% CM. After 2 weeks, the slabs were sectioned and placed as above on solid nonselective growth medium for additional proliferation of cells for 7d before selection. Rapid growth of discrete microclusters was observed during this second round of growth on nonselective agar. These microclusters are called "colonies" since the original aggregates of PEG-treated protoplasts were maintained as unique entities embedded in agarose. This plating method does not allow further division into sib aggregates during growth on nonselective medium. Further, it is also possible that each original cluster of PEG-treated cells may have contained no more than one transfected cell. A random sample of the colonies arising was transferred to the described kanamycin-containing selective medium, and after 7d the faster-growing transfected microcalli were harvested for Southern blot analysis. There was again little or no growth of colonies originally derived from PEG- treated cells without DNA. Five of the 7 fast-growing colonies picked showed the 1.0kb band expected after hybridization of genomic DNA containing integrated nptII genes with the *Bam*H1 nptII probe (Fig. 2). Other bands of larger size in Fig. 2 are the result of probe contamination with radioactively labeled residues of the vector (pUCPiAN7) that contains the nptII gene in pCaMVNeo.

The common feature shared by these two methods for recovery of transformants is the extended time for proliferation of treated cells in nonselective medium. When in both instances a random population of healthy, actively growing microcalli or colonies was later transferred to kanamycin-containing medium, the selective medium allowed identification after 7d of a subpopulation of fast growers. In the experiments presented (Table 1), 31 of the 150 microcalli isolated from populations of treated cells initially grown in liquid medium were fast growing, as were 7 of the 150

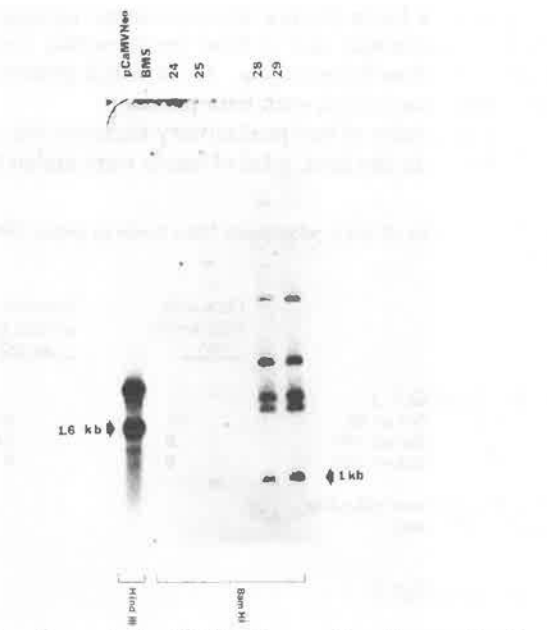


Fig. 2. Southern blot analysis of BMS-M kanamycin-resistant calli. The pCaMVNeo was digested by *Hin*dIII, generating a 1.6kb fragment containing the nptII gene (on the left side). The probe used was the 1.0 kb *Bam*HI fragment containing the nptII gene. No hybridization occurs in BMS-M control DNA. Samples 24, 25, 28, 29 show the diagnostic 1.0kb fragment as well as larger bands which are due to probe contamination with residues of pUCPiAN7. Rehybridization of the same filter with a labeled 3.4kb *Bam*HI fragment of pCaMVNeo gave the same pattern minus the 1.0kb fragment (data not shown).

colonies picked from the population plated in agarose slabs. Rapid growth on kanamycin identified transformed cells exhibiting stable drug resistance since 16% to 71% of these fast growers were proved transgenic by molecular analysis. The overall frequency of transgenic isolates was at least 3.3% of randomly selected microcalli and may be higher since some slower growing microcalli may also have been transformed. This frequency of transformation of corn cells is greater than reported by others (Fromm et al. 1986). We have established cell lines from the microcalli that were positive for nptII gene insertion, and these continue to grow briskly on medium containing 200ug/ml kanamycin.

Possible reactivation of *Mu*-loss line through tissue culture and regeneration

--M. James, P. Stinard, D.S. Robertson, and J. Stadler

Regenerant plants from A188/Mutator and H99/Mutator embryogenic callus lines were tested for Mutator activity (James and Stadler, MNL 62:9,1988). All Mutator parents used in the crosses to establish these lines were presumably active second generation inbred *Mu* parents (*Mu2*); however, subsequent genetic tests for Mutator activity (Robertson, Mutat. Res. 51:21-28, 1978) revealed one *Mu* parent (86-657-2) to be transpositionally inactive (a *Mu*-loss plant). Calli from an embryogenic line (86-657-2B) which was derived from the cross of this *Mu*-loss plant with an H99 inbred were regenerated to whole plants. Most primary regenerants (R0) exhibited mutant phenotypes (typically, tassel-seed tassels and stunted growth), but two regenerants developed normal tassels and ears and were self-pollinated. The R1 progeny of one of these R0 plants were normal in appearance, while the R1 progeny of the other segregated for a knotted mutant of variable

expressivity. This was probably a culture-induced mutation, but whether it was *Mu*-induced is unknown. The R1 plants were self-pollinated, outcrossed to a standard line, or both. At least 50 F1 progeny from the outcrosses were then planted and self-pollinated. As in Robertson's standard test for Mutator activity (Robertson, Mutat. Res. 51:21-28, 1978), progeny ears were examined for the appearance of new seedling mutants in the sandbench. A new mutant frequency of 8% was observed when one of the R1 plants (87-399-2) was crossed as a female. Four of the 50 ears tested segregated for new seedling mutants of five types (pale green, yellow green, yellow green with zebra banding, pale yellow necrotic, and white). Mutability of these phenotypes (revertant sectors), however, was not observed. No new seedling mutants were observed when this same R1 plant (87-399-2) was crossed as a male. Seeds from the segregating ears were grown in the field the next summer and self-pollinated, and their progeny ears were also observed to segregate for seedling mutants when tested in the sandbench. New mutant frequencies of 2% were seen in two additional R1 plants also crossed as females, but the reciprocal outcrosses were not available. All other R1 plants tested showed no new seedling mutations.

The observation that R1 plant 87-399-2 generated new seedling mutants only when crossed as a female suggests that the events which gave rise to the mutations occurred in the female gametes of this plant, but not in the male. This could be due to reactivation of *Mu* elements through the culturing and/or regeneration process, with *Mu* transpositional activity taking place only in the female gametes. The frequency of new mutants generated in this outcross is similar to that seen in other active Mutator lines, and the variety of seedling mutants is also indicative of an active Mutator system. The possibility that the generation of new mutants was due to culture-induced rearrangements (somaclonal variation) seems unlikely for two reasons. First, the data suggest that the mutations occurred as post-culture events in the female gametes of the R1 plant. Second, the variety of mutations observed, as well as the appearance of each mutant phenotype in only one ear apiece, is suggestive of mutations caused by independent events, such as transposable element insertions.

Preliminary molecular analysis of the callus tissue and tissues of plants regenerated from this *Mu*-loss line (86-657-2) was performed by digestion with *Hin*fI, an enzyme which cuts within *Mu* element inverted repeats, and hybridization with a *Mu1*-homologous probe. This showed that DNA from the initial 86-657-2B callus tissue had a mixed population of *Mu* elements with respect to *Hin*fI modification. Approximately 1/3 to 1/2 of the *Mu1*-hybridizing elements were unmodified at the *Hin*fI sites, while the remainder were modified. *Hin*fI site modification has been correlated with inactivity of *Mu* elements following inbreeding (Chandler and Walbot, PNAS 83:1767-1771, 1986). The finding that at least some of the *Mu* elements in the callus DNA were unmodified leaves open the possibility that, despite the genetic evidence, the original *Mu* parent may not have been a true *Mu*-loss plant. Interestingly, *Hin*fI digestion of the DNA of various tissues of two mutant R0 somaclones derived from this callus line indicates that the degree of *Hin*fI modification of the population of *Mu* elements varied between tissues, with unmodified *Mu* elements ments seen only in the immature cob. Molecular analysis of R1 plants and their outcross progeny with *Hin*fI as well as with enzymes which are external to *Mu1* may help determine whether active *Mu* elements were involved in the generation of new mutants.

Genetic tests for Mutator activity in a primary regenerant from an A188/Mutator callus line (86-646-8C) (derived from a cross with a transpositionally active *Mu* parent, 86-207-8) revealed that Mutator activity in the R0 plant was maintained. Although a limited number of seeds from the outcross of this R0 plant prevented a full-scale test for Mutator activity, two of five ears tested segregated for new seedling mutants in the sandbench, with one of the mutants (striate) showing a mutable phenotype.

These initial results demonstrate that Mutator activity can be maintained through the culture and regeneration process. They also suggest that an in vitro system might possibly be instrumental in the reactivation of a Mu-loss plant, although further molecular and genetic studies are necessary.

ATHENS, GEORGIA
University of Georgia

Background effects on the expression of Mutator-induced mutant alleles

--Daniel Ortiz, Robert Gregerson and Judith Strommer

The mutant allele of alcohol dehydrogenase designated *Adh1-S3034* was produced by insertion of a *Mu1* element into the first intervening sequence of the progenitor *Adh1-S* allele. It was shown to produce 30-40% normal levels of ADH1-S peptide, while a derivative, *Adh1-S3034b*, was reported to exhibit three-fold lower levels of ADH1-S (Freeling et al., Devel. Genet., 1982). Despite the differences in expression, we found the two alleles indistinguishable at the level of Southern mapping. A comparison at the DNA sequence level seemed likely to provide a means of learning how minor changes in intervening sequences could result in dramatically different levels of gene expression.

We therefore cloned the *S3034b* allele for comparison to the previously cloned and sequenced *S3034*. Our surprising finding was that DNA sequences of the two putative alleles are indistinguishable. Re-examination of allozyme ratios revealed that levels of ADH1 produced by both "alleles" are highly variable and also indistinguishable. We conclude that *S3034* and *S3034b* are the same allele.

The survey of allozyme ratios did reveal a correlation between genetic background and level of *Adh1-Mu* expression (relative to expression of the progenitor allele). *Mu* disruption of the first intervening sequence of *Adh1* has a much greater effect in a background contributed by a Boone County White line than in a background contributed by Funk Fast, for example. The same pattern was found to apply to an independent mutant allele, *Adh1-S4477*, produced by insertion of *Mu1* a few hundred nucleotides downstream from the site of insertion in *S3034*.

The background effect is attributable to differences in levels of steady-state RNA. It is not related to differences in DNA methylation. Preliminary evidence is that few loci are responsible for the observed variation.

Aberrant processing of Mu1-induced mutant alleles

--Daniel Ortiz, Robert Gregerson and Judith Strommer

Mutant alleles of *Adh1* and *Sh1* produced by insertion of *Mu1* transposable elements produce decreased levels of steady-state RNA, largely due to decreased levels of transcription. But in addition, at least some of the transcripts produced by these alleles are aberrantly processed, resulting in a family of polyadenlyated RNA species with altered splicing and polyadenylation sites. Some of the aberrant transcripts are unstable. The general pattern appears to fit that observed for other transposable element systems of maize (see esp. Simon and Starlinger, Mol. Gen. Genet., 1987).

The extent of aberrant processing appears to depend to some degree on genetic background, raising the possibility that polymorphic loci for components of the processing systems might be responsible for background-related differences in susceptibility to the mutational effects of *Mu1* insertion (see preceding letter).

We are currently defining the splice junctions and polyadenylation sites used to produce aberrant transcripts of *Adh1-Mu* alleles and attempting to verify the association between genetic background and selection of processing sites.

AUSTIN, TEXAS
University of Texas

A technique for spreading maize microsporocyte pachytene chromosomes for silver staining and EM viewing of synaptonemal complex core and lateral elements

--M. P. Maguire

The following describes a successful procedure for a number of maize stocks which combines parts of techniques set forth separately by Stack, Holm, and Jones and collaborators (mostly for other organisms) and contains a few original alterations. This technique produces relatively intact, complete-complement configurations which seem generally free of distortion.

New microscope slides are coated in advance with plastic by dipping them in a chloroform plastic solution (4g broken Falcon petri dishes: 400ml chloroform) and standing them on end in a test tube rack to dry. For this procedure plastic coated slides must then be treated with a glow-discharge unit to render the plastic surface hydrophilic, at a time no more than about three days before use. (This step may be omitted in procedures which call for use of a swelling medium containing a detergent.)

Fresh anthers at pachytene stage are macerated in a deep depression slide in 5µl of a freshly prepared ice cold medium: 2.5% sucrose, 1% polyvinylpyrrolidone and 2.5mM acid EDTA, adjusted to pH 4.6 - 4.7 with KOH; after maceration 60µl of an ice cold solution (6% paraformaldehyde, 1.5% sucrose, adjusted to pH 8.6) is quickly added. The depression slide is then covered and placed over an ice water bath for at least 30 minutes. The paraformaldehyde solution may be prepared in advance and kept in a refrigerator for 5 or 6 days. If the pH declines below 8.4, it should be discarded.)

After 30 minutes to 1 hour, the depression slide is removed from over the ice bath, and quickly warmed to room temperature on a laboratory bench. The contents are then micropipetted to plastic coated slides, sucking out the liquid from around the anther remnants. The plastic coated slides are then vibrated for 10 seconds by touching an electric vibrating engraver to the surface of the ground glass end, and these slides are then allowed to air dry, leaving them overnight at room temperature (protected from roach and ant demolition). Then the dried down preparations on the slides are rimmed with nail polish (to prevent loss of plastic during fixation and staining), and the drying process is completed by placing the slides for 3-4 hours on a slide warmer at 37 C. These slides can be stored indefinitely before fixation and staining (as described here, almost entirely the procedure of S. Stack and L. Anderson, J. Hered. 78:178-182,1987).

Immediately before staining, slides are treated with an ice cold, freshly prepared solution (4% paraformaldehyde, 1.5% sucrose, adjusted to pH 8.6) for 10 minutes (changed for fresh solution after the first 2 minutes). Then slides are briefly washed in 0.4% photoflo and air dried. Slides are then individually placed on props over 1mm of distilled water in petri dishes, and one drop of a freshly prepared 50% water solution of silver nitrate is added to each from a Pasteur pipette. A siliconized coverslip is applied to each slide, and petri dishes are covered and placed overnight in an oven at 60 C. Coverslips are then readily floated off, and slides are air dried and ready for scanning. This procedure may produce a rather dark stain so that reducing the staining time somewhat may be desirable.

Slides are scanned with phase contrast microscopy (with at least a 20X to 25X objective). Copper grids (Pelco IGC 50) are made slightly sticky by dipping them in dichloroethane (in which a short piece of scotch tape has been briefly swished), and drying them on parafilm. Such grids are carefully positioned on good synaptonemal complex configurations. (It is a good idea to tilt the slides slightly at this stage to determine whether the grids are securely positioned so as not to slip during additional manipulations.) The plastic film is then scored in a circle around and about 2mm from the grids. Next the grids on their plastic rafts are floated on a drop or two of distilled water. (The water is placed at the edge of the scoring and with luck will creep under the plastic). Then the slide is carefully immersed at a slant under the surface of distilled water in a bowl in such a way that the rafts carrying their grids are floated on the surface of the water, and the slide is then withdrawn. Plastic rafts with grids are then picked up on weighing paper (push down on the rafts from above, pushing them momentarily below the surface, and deftly invert and withdraw the weighing paper so that the plastic is on top of the grids on top of the weighing paper.) The weighing paper is propped on edge for drying. Later, after careful removal of the dried grids from the weighing paper, it helps to examine them on a microscope slide with phase microscopy and map the positions of the configurations to be observed. Store the grids in grid boxes, and you are ready for the EM.

Some cautionary notes: Coating slides with plastic film works best in relatively low humidity (below 60%). In high humidity I consistently get a cloudy plastic film.

Floating plastic rafts (with grids) off of slides works best at relatively high humidity (above 60%). In low humidity, apparently, static electricity frequently inspires grids just floated to make incredible flip dives below the water surface back to the slide from which they have just been removed, before it can be withdrawn. This is enough to wreck an otherwise placid disposition. I am told by others that sometimes grids resist being stored in grid boxes by jumping back out again as fast as you can put them in; I have not seen this action yet, and do not really need it.

Additional directions: Plastic petri dish pieces are quickly dissolved in chloroform under sonication.

Maceration medium pH adjustment is performed with KOH and HCl, avoiding addition of sodium which is considered toxic to plant cells.

A 25mM acid EDTA stock solution is prepared in advance (and stored indefinitely). Dissolution is accomplished by adding 4 or 5 pellets of KOH; pH is then adjusted to 4.1 with HCl and KOH.

Paraformaldehyde is dissolved by addition of 1N NaOH (2ml to every 100ml of solution) and heating while stirring to no hotter than 50 C. The solution is then cooled to about 4 C, and the pH is adjusted to 8.6 with formic acid, NaOH and sodium borate.

One additional piece of advice: Do not undertake this kind of silver staining unless you are fortified with grim determination, but if you are, you should eventually be duly rewarded.

BALTIMORE, MARYLAND
Carnegie Institution of Washington

Studies on the transposase function of *Spm* in tobacco

--P. Masson, G. Rutherford and N. Fedoroff

The *Spm* family of maize transposable elements consists of both autonomous and defective elements. The former are capable of promoting their own transposition, as well as the transposition of the defective elements (*dSpm* elements). The latter are totally stable in the absence of a trans-acting autonomous *Spm* element in the same genome and are deletion derivatives of the autonomous element (reviewed by Fedoroff, in Howe, M. and Berg, D., eds., "Mobile DNA", in press).

The wild-type autonomous *Spm-s* element is 8.3kb long; its structure is shown in Figure 1 (Pereira et al., EMBO J. 5:835-841, 1986, Masson et al., Genetics 177:117-137, 1987). Between the 13bp terminal inverted repeats and the 250-300bp subterminal regions, the *Spm-s* contains two long open reading frames (ORF1 and ORF2). Element transcription yields one major 2.5kb transcript, whose structure is also represented in Figure 1, and two minor 6kb transcripts (Pereira et al., 1986, Masson et al., 1987, Banks et al., Genes & Develop., 2:1364-1380, 1988). The 6kb transcripts overlap the two ORFs and the right end of the element.

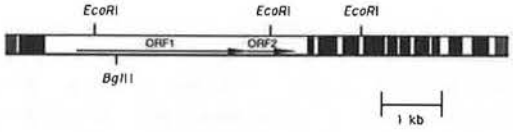


Figure 1. Structure of the *Spm-s* element. The two 13bp inverted repeats and 250-300bp subterminal regions are represented by the stippled boxes on each end of the element. The sequences encoding the exons of the major 2.5kb transcript are represented by black boxes. The two long open reading frames are represented by arrows. The *Eco*RI and *Bgl*II sites are also indicated (Pereira et al., EMBO J. 5:835-841; Masson et al., Genetics 177:117-137).

Despite a good knowledge of the *Spm* structure, very little is known about the actual *Spm* sequences encoding the transposase. To gain more insight into this function, we have tested the transposition of *Spm* in tobacco. The *Spm-s* element was introduced into tobacco by *Agrobacterium*-mediated transformation, and its transposition was analysed by the detection of flanking plasmid sequences lacking *Spm* sequences (empty donor fragments) in the DNA extracted from transgenic plants (Masson et al., MNL 62: 26-27, 1988). We have cloned and sequenced fifteen empty donor sites from one transgenic plant (Table 1). The empty donor site fragments can be classified into three different categories, deriving from either precise excision or imprecise excision of the *Spm* element. All sequences are among the types of empty donor fragment sequences observed after *Spm* transposition in maize (Schwarz-Sommer et al., EMBO J. 4: 591-597). This observation, coupled with the detection of transposed copies of *Spm* in the DNA extracted from the same transgenic tobacco plants, demonstrates that *Spm* transposes in tobacco as it does in maize (Masson and Fedoroff, PNAS, in press).

Table 1. The nucleotide sequences of empty donor sites.

Original sequence:
...TTTGAATATT-Spm-AATGGA...
Empty donor fragment sequences:
-Group 1: precise excision of Spm
...TTTGAATATT AATGGA... (2 sequences)*
-Group 2: imprecise excision of Spm
...TTTGAATATT ATGGA... (6 sequences)*
-Group 3: imprecise excision of Spm
...TTTGAATAT TTAATGGA... (7 sequences)*

*For each group of empty donor fragments, the number of cloned fragments having the sequence shown is indicated in parentheses.

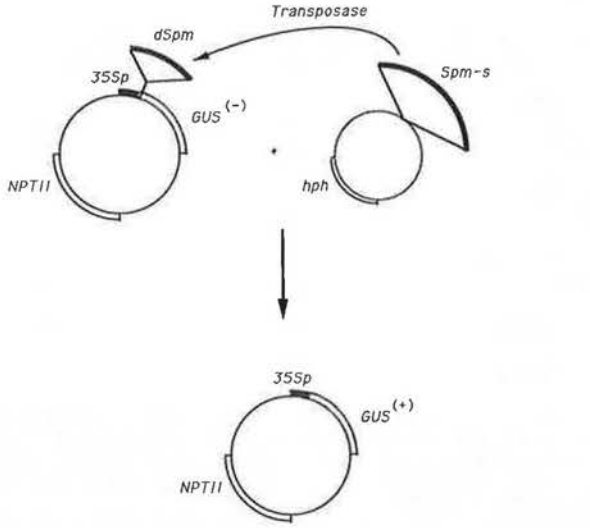


Figure 2. Schematic representation of the assay for *Spm* transposition in tobacco. The *Spm-s* element, inserted in a plasmid conferring hygromycin resistance (*hph* gene) to the plant cell, provides the transposase function necessary to induce the excision of *dSpm* inserted within the GUS gene on a plasmid conferring kanamycin resistance (NPTII gene). 35Sp refers to the 35S promoter from CaMV (Shah et al., Science 233:478-481). (+) and (-) refer to the activity of the GUS gene. The vectors were generously provided by Dr. S. Rogers (Monsanto Co.).

To study the *Spm* sequences encoding the transposase function, we have developed a functional assay which allows us to test the ability of in vitro-mutagenized *Spm* elements to promote the transposition of a defective element in tobacco (Masson and Fedoroff, PNAS, in press). The assay is based on the restoration of GUS gene expression after *dSpm* excision (Figure 2). The *E. coli* GUS gene, coding for ß-glucuronidase, is functional in plants when fused to the appropriate control sequences (Jefferson et al., EMBO J. 6: 3901-3907). The ß-glucuronidase enzyme converts the chromogenic X-GLU substrate into an insoluble blue compound which accumulates in the transformed plant cells expressing the GUS gene. A *dSpm* element inserted between the promoter and the open reading frame of the GUS gene disrupts GUS gene expression. However, when the same plasmid is introduced in tobacco together with a second plasmid carrying a wild-type *Spm-s* element, the latter induces *dSpm* excision. This results in the appearance of cells expressing the GUS gene (Figure 1). During development, such cells divide and generate sectors of X-GLU-stainable, GUS-positive cells, whose number and size reflect, respectively, the frequency and timing of transposition.

Using this assay, we have shown that *Spm-s* induces the excision of a defective *dSpm* element introduced on a separate Ti plasmid. *Spm* transposition occurs late and infrequently in tobacco. However, earlier transposition often occurs when the trans-acting *Spm-s* element is expressed from the CaMV 35S promoter (Masson and Fedoroff, PNAS, in press). In vitro -mutagenized *Spm* elements are being tested for their ability to promote the excision of a standard *dSpm* element inserted in the GUS gene. Preliminary data indicate that ORF1 and ORF2 are necessary for the transposase function of *Spm*: frameshift mutations in the *Eco*RI and *Bgl*II sites in ORF1, or in the *Eco*RI site in ORF2 (see Figure 1 and Table 2) alter the transposase function of the element. On the other hand, a frameshift mutation in the *Eco*RI site located in the intron sequence separating the fourth and the fifth exons of the major 2.5kb transcript (Figure 1) does not affect this function (Table 2). However, our previous analysis of several *Spm* derivatives in the *a-m2* alleles of maize indicates that the right end of the element, including sequences encoding the last ten exons of the major 2.5kb transcript, is also necessary for the transposase function of the element (Masson et al., 1987).

Taken together, the data presented here indicate that ORF1, ORF2 and sequences in the right half of the *Spm* element are necessary for the transposase function of the element. These various sequences are present, at least in part, in the minor 6kb transcripts encoded by the element. We have recently cloned partial cDNAs corresponding to internal portions of the 6kb transcripts produced in maize. Preliminary results indicate that several RNA species are produced by alternative splicing between sequences in ORF1, ORF2 and the sequences encoding the translated exons of the major 2.5kb transcript (not shown). We are currently cloning the remaining portions of the cDNAs for the 6kb transcripts.

Table 2. Transposase function of in vitro-mutagenized *Spm* elements in tobacco.

Site of frameshift mutation (1)	Number of calli analysed	Number of calli showing GUS-positive sectors (2)
ORF1:		
*Eco*RI	14	1#
*Bgl*II	14	0*
ORF2:		
*Eco*RI	9	0
4th intron:		
*Eco*RI	20	9#
Wild-type:	12	10#
Defective (3):	25	0

(1) The frameshift mutagenesis was done by cutting the element with the appropriate restriction enzyme (see Figure 1 for their position on the *Spm* sequence), filling in the ends using the Klenow fragment of the *E. coli* polymerase and religation (Maniatis et al., In "Molecular Cloning: A Laboratory Manual", CSHL).
(2) The in vitro-mutagenized *Spm* elements were tested for their ability to promote excision of *dSpm* in tobacco. *dSpm* excision was monitored by the detection of X-GLU-stainable, GUS-positive sectors, as described in the text. # The positive calli showed multiple GUS-positive sectors. * Two calli showed one single GUS-positive sector.
(3)In the negative control experiment, the reporter plasmid containing the *dSpm* element inserted within the GUS gene was co-transformed in tobacco together with the second vector which contained no *Spm* element.

BEIJING, CHINA
Beijing Agricultural University

Identification of a sweet corn mutant from the People's Republic of China

--Dai Jingrui, Chen Liufang and Han Yashan

It was found that one-fourth of the kernels on the ear of dent corn inbred Chao23 had wrinkling and a transparent phenotype and looked like sweet corn kernels (Fig. 1). The phenotype of the other three-fourths of the kernels on that ear was the same as normal Chao23. Those two types of kernels were planted in 1986. The results showed that the plants from variant seeds produced only variant kernels. On the other hand, the plants from normal seed were of two types. About 1/3 of them produced only normal kernels; the remaining 2/3 produced both normal and sweet kernels in the proportions of roughly 3/4 and 1/4 (Fig.2). These results indicated that the sweet corn mutant was controlled by one pair of recessive genes.

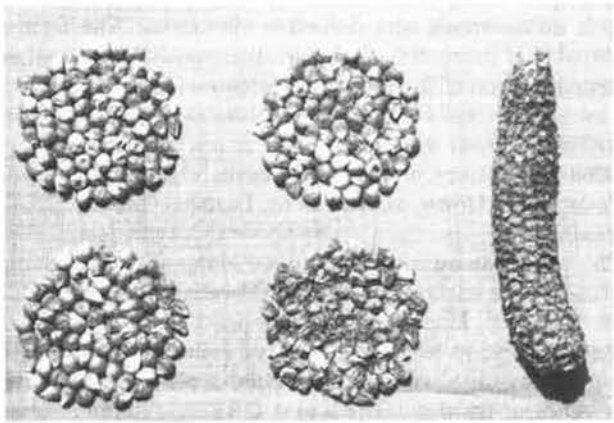


Figure 1. Two types of kernels from mutant plant.

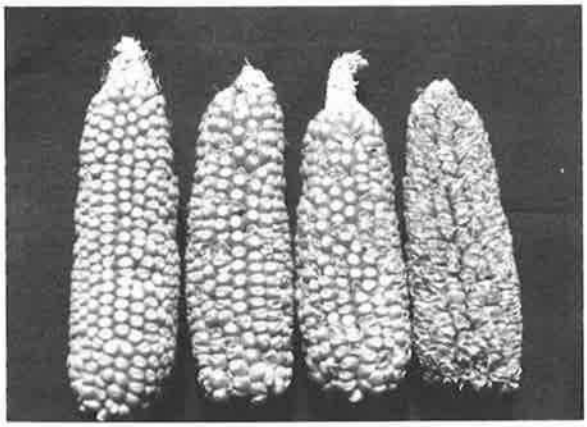


Figure 2. Ears from sweet and normal kernels.

The cross test between pure mutant (Chao23S) and other inbreds with *su1 su1* genotype and the sugar content test were made in 1987. The results of the cross test showed that only one phenotype of sweet kernels was observed from the F1 generation. So this sweet mutant is *su1*. The total sugar content of Chao23S was higher than those of other inbreds according to the average of six tests on different dates after pollination. The test results 27 days after pollination also demonstrated that Chao23S could maintain the highest level of the total sugar and sucrose contents for a longer period. This phenomenon is very similar to the characters of inbred Ill779 (*su1 su1 se se*) and significantly different from those of inbred T48S (*su1 su1 Se Se*). The dry matter weights in the different period also appeared less different between inbred Chao23S and Ill779 and significantly different between inbred Chao23S and T485 (*su1 su1*). It is deduced that Chao23S may have genotype *su1 su1 se se* according to the above results. But studies are still in progress to determine whether the characters of Chao23S are due to genotype *su1 su1 se se* or due to another genotype. Two possibilities may be as follows:

1. Inbred Chao23 originally contained recessive gene *se*, and mutation only from gene *Su1* to gene *su1* occurred in that plant observed.

2. The genotype of Chao23 in itself contains a gene which differs from *se* and also enhances the appearance of *su1*. Anyway the inbred Chao23S is good material for studies on genetics and biochemistry because Chao23 and Chao23S are isogenic lines. If Chao23 originally contained gene *se*, it will be interesting because gene *se* is seldom seen in normal corn. This will inspire corn breeders working with sweet corn breeding.

BERGAMO, ITALY
Istituto Sperimentale per la Cerealicoltura

The b-32 protein from endosperm: characterization of genomic sequences

--H. Hartings, N. Lazzaroni, A. Spada, R. Thompson[1], F. Salamani[1], M. Motto, J. Palau[2], N. Difonzo
[1]Max-Planck Institut, Cologne
[2]University of Barcelona

The b-32 protein of endosperm has been described as a putative regulatory factor for the synthesis of zeins, the major group of storage proteins. Recently, the cDNA coding for the b-32 protein has been cloned and the complete amino acid sequence of the protein derived (Di Fonzo et al., Mol. Gen. Genet. 212:481, 1988). The cloning and sequencing of three *b-32* genes from two different inbred lines (W64A and A69Y) as well as the Southern analysis, demonstrate that the *b-32* genes form a polymorphic gene family that, in the case of W64A, is constituted by at least three genes.

The two isolated W64A genes and the previously reported cDNA clone are extremely similar and most of the observed nucleotide variations account for an amino acid replacement or for an insertion/deletion of 1-2 residues within the amino acid sequence. The genes *b-32.129* (W64A) and *b-32.152* (A69Y) differ from the *b-32.120* gene (W64A) and *b-32.66* cDNA clone (W64A) in three 1-nucleotide insertions, covering the central part of the coding region. The reading frame changes three times within 219 nucleotides and, as a result, the translation of the central domain of the b-32 protein changes markedly from one to the other set of clones. This contrasts with the preservation in all cases of the N-terminal and C-terminal domains.

The central region of the b-32 protein is a highly hydrophilic sequence, very rich in acidic residues and, probably, poorly structured. These characteristics are also valid for the protein sequence that is derived from the *b-32.120* gene. However, the amino acid sequences of the central domain deduced from the nucleotide sequence of the *b-32.129* and *b-32.152* genes display a very different character. In fact, the central sequence is less hydrophilic, contains a rather low proportion of acidic residues and an increased amount of basic amino acids.

All *b-32* genes described appear to be functional genes, as they possess the typical characteristics for it. The presence in the developing endosperm cell of two types of b-32 proteins, having two alternative central domains, is of great interest for these molecules in relation to possible regulatory mechanisms in which they could be involved. In fact, it has been reported that acidic surfaces of regulatory proteins can interact with the chromatin of the promotor region causing a local relaxation and facilitating the expression of the gene. If this is the case for the b-32 protein, a molecule possibly involved in the regulation of zein genes, the simultaneous presence within the cell of two molecular species with an alternative central domain affords a possible mechanism of switching on and off via local chromatin relation, depending on which of the two molecules is present nearby the promotor region of a given gene.

The two *b-32* genes isolated from the inbred line W64A are very similar with regard to the flanking sequences and they possess the same motifs that apparently are relevant for gene expression. Therefore, it seems likely that they are coordinately expressed, probably being regulated by the same transacting factors. In addition to the typical elements that participate in the overall activation of the transcriptional machinery (CATAGA and TATA boxes), a long stretch of DNA rich in A+T is present at about 410 nucleotides upstream of the first ATG codon. Two additional motifs are present, corresponding to a proximal inverted repeat (PIR) and to a far inverted repeat (FIR) flanking the A+T rich element. It is probable that some if not all of these motifs are cis-acting elements participating in the regulation of the *b-32* genes.

A comparison between gene *b-32.129* (W64A) and gene *b-32.152* (A69Y) is of special interest. From the coding sequence they could be considered as polymorphic genes from two different inbred lines showing a high level of homology. However, the 5' flanking region upstream the CATAGA motif shows great divergence, since the *b-32.152* gene possesses several duplications and long inverted repeats, when compared with the *b-32.129* gene. To date, we have no further sequence 5' upstream region of the *b-32.152* gene. However, our data indicate that an important polymorphic variation exists between the two genes that may correlate with a different regulatory mechanism for each case. From the above considerations, it is proposed that the *b-32* genes represent a family of regulated regulatory genes that play a role as intermediate elements of an unknown regulatory chain. The fact that the same mutants (*o2* and *o6*) control the expression of both *b-32* and zein genes, makes it of interest to investigate a possible mechanism of gene regulation of zeins in which the *b-32* genes and their protein products might be involved.

Molecular properties of the *wx-m32* allele: a *Bg*-induced unstable mutation

--M. Maddaloni, G. Ponziani, N. Di Fonzo, F. Salamini*, R. Thompson* and M. Motto
*Max-Planck Institut, Cologne

A newly isolated unstable mutation *wx-m32* was proven by genetic analysis to be caused by the insertion of the *Bg* autonomous transposable element into the waxy (*Wx*) gene. To identify the molecular structure of the *wx-m32* allele, genomic Southern blots were performed using plant DNA extracted from a heterozygous *wx-m32 / wx* plant and from the parental lines (A69Y *A C R wx* and A69Y *o2 R Bg-m Wx*). The DNAs were digested with several restriction enzymes and investigated at the molecular level by blot hybridization to *Wx* and *Bg* probes. The molecular probe of the *Wx* gene was a 2.0kb *Sal*I fragment corresponding to the central region of the *Wx* gene, while as *Bg* probe we used a 4.0kb *Xho*I-*Eco*RI fragment corresponding to the *Bg* receptor of the *o2-m(r)* allele (Thompson et al., unpublished).

In the hybridization experiment shown in Fig. 1A, *Pvu*I digested genomic DNAs were hybridized with the 2.0kb *Sal*I *Wx* probe. It was clearly evident that the progenitor *Wx* allele and the stable recessive *wx* allele contain, respectively, a 3.1 and 5.8kb *Pvu*I fragment. The *wx-m32* allele, however, contains, in addition to the 5.8kb *Pvu*I fragment of the stable *wx* allele, a new fragment of ~ 8.8kb in size, indicating an insertion of approximately 5.7kb into the 3.1kb *Pvu*I fragment of the *Wx* wild-type allele. Moreover, in the same lane of the *wx-m32* allele, a less intense band was visible, similar in size to the 3.1kb *Pvu*I fragment of the *Wx* wild-type allele. This result can be explained considering that, if somatic reversion events take place, induced by the autonomous *Bg* element present at the locus, they should restore the wild-type fragment size of the parental *Wx* allele after excision of *Bg*. Because plant DNA of the *wx-m32* allele was prepared from young leaves, we concluded that *Bg* activity is not restricted to the endosperm tissue.

When the same *Pvu*I digests were hybridized with the *Bg* probe (Fig. 1B) it was clearly evident that a unique restriction fragment could be correlated with the mutable phenotype of the *wx-m32* allele. This mutable allele contained a novel 8.8kb *Pvu*I band absent in the parental lines. The band migrated in the gel at the same position as that of the *Pvu*I fragment of 8.8kb lit up by the *Wx* probe. To prove whether the insertion is entirely contained in this *Pvu*I fragment, digested genomic DNAs of the previous genotypes were also hybridized with different probes of the 5' and 3' region of the *Wx* locus. The resulting data revealed no apparent alteration of the expected 5' and 3' *Pvu*I fragments. Therefore, the insertion is located in the *Pvu*I fragment and its size is 5.7kb. The screening of a genomic library from the *wx-m32* allele is now underway.

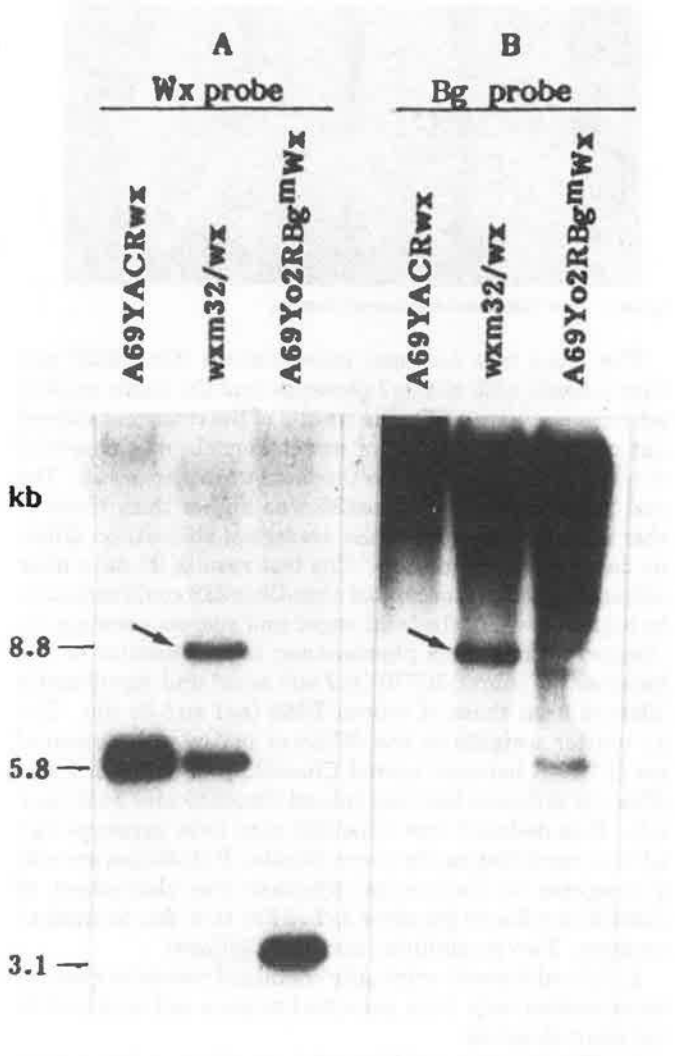


Figure 1. Southern blot analysis of the *wx-m32* allele. Genomic DNAs, prepared from the leaves of plants with the genotypes indicated above each lane, were digested with *Pvu*I. Each DNA sample (~12ug) was divided in two parts, electrophoresed through an 0.8% agarose gel in parallel experiments, Southern blotted and probed with the 2.0kb *Sal*I, *Wx* probe (A) and *Bg* probe (B). Sizes are shown in kb.

Characterization of in vitro salt-tolerant embryogenic cultures

--E. Lupotto, M. C. Lusardi, D. Bartels* and M. Mongodi
*Max-Planck Institut, Cologne

The in vitro selection scheme followed for the isolation and regeneration of salt tolerant somaclones (STSC) has been previously reported (Lupotto et al., MNL 62:30, 1988). At the end of the selection, 18 embryogenic calli, perfectly regenerable and tolerant to 85mM NaCl in the culture medium, were obtained. A total of 142 regenerates were produced, 93 (65.5%) on MS (Murashige and Skoog, 1962) hormone-free medium without NaCl, and 49 (34.5%) on salt-containing medium. Plantlets developed in both cases and were transplanted to soil; 78 of them (54.9%) were grown to maturity in the greenhouse. Of 27 flowering plants, complete with tassels and ears, only four set seeds, three by selfing and one by crossing with pollen from anther regenerates. About fifty percent of the regenerates were plants which only developed ears and 29 could be outcrossed with pollen from control plants: four of them set seeds. Five regenerates only developed tassels which were sterile and seven plants showed evident phenotypic abnormalities and failed to develop. In Table 1 the breeding procedures followed for obtaining progenies of the regenerated plants which set seeds are reported. R2 populations are consistent for in planta analyses and comparisons with nonselected progenies, in order to evaluate if any trait conferring salt tolerance has been transmitted to the progenies of the regenerates.

Table 1. Salt tolerant regenerates-situation.

R0 = direct regenerate
R1 = ♀ or cross of R0
R2 = ♀ of R1

STSC	R0	Operation	N° of seeds set	N° of plants grown (R1)	N° of plants setting seeds	N° of seeds R2
28	1	♀	6	4	3	202-171-2
28	9	x STSC 24/R5	9	7	7	365-463-192-273-337-302-296
28	12	x STSC 23/R2	4	3	3	395-166-371
29	2	♀	11	10	4	86-169-233-237
29	9	outcrossed (A69Y ♂)	4	not considered in summer '88		
14	3	" "	18			
15	5	" "	8			
16	11	" "	5			
20	7	" "	9			
21	1	" "	12			
21	4	" "	19			

Some experiments have been developed in vitro for testing the nature of the trait of salt tolerance acquired by some STSC. A curve of sensitivity to NaCl drawn on non-selected embryogenic somaclone no. 10 (SC10) and the correspondent STSC10 tolerant to 85mM NaCl, showed that the STSC could easily tolerate higher doses of salt, the relative growth being reduced to 50% of the control at 230mM for STSC10 against 90mM NaCl for SC10. Furthermore, the effective embryogenesis of the callus culture resulting in plant regeneration in STSC10 disappeared around 220mM, while it was at 85mM in the case of SC10. As reported before, some STSC tolerant to 85mM NaCl also resulted in derivatives spontaneously tolerant to higher dosages of NaCl, and three clones, STSC10-II, STSC20-II and STSC21-II, are currently maintained on 128mM NaCl; their embryogeny and capability of plant regeneration remained intact. A test of stability of the character in vitro was performed by recording the growth curve as GI (growth index: increment in fresh weight during subculture with respect to the initial fresh weight) of STSC20-II and STSC21-II in the absence of salt, in the presence of 128mM NaCl, and in the presence of salt after a period of three months on 0mM NaCl. This was done for evaluating the possible maintenance of the trait of salt tolerance also after a rather long period of growth in absence of NaCl. Indeed, STSC20-II and STSC21-II grew better on NaCl-devoid medium, suggesting their independence from NaCl in the culture medium; in addition the curve relative to their growth on 128mM NaCl and on salt after a period on non-salt medium was practically identical, confirming the stability in vitro of the acquired trait. In each case calli were embryogenic and regenerable. However, in STSC10 and STSC10-II cultures grown respectively on 85 and 128mM NaCl, dependence on NaCl supplement in the medium was recorded when drawing the curves of sensitivity to a range of NaCl dosages. This might suggest, in the case of STSC10, an in vitro halophytic behaviour.

The appearance of specific proteins typically expressed by salt tolerant cell lines (e.g. Singh et al., Plant Physiol. 79:126, 1985) has been investigated on some STSC grown on 128mM NaCl by one- and two-dimensional gel electrophoresis analysis. While the appearance and disappearance of specific protein bands were difficult to interpret with one-dimensional SDS polyacrylamide gel electrophoresis, though revealing several evident differences between each SC and the corresponding STSC, a better analysis could be performed by 2D gel electrophoresis according to O'Farrell (J. Biol. Chem. 250:4007, 1975). Indeed 5 STSC stably growing on 128mM NaCl expressed four different peptides of deduced molecular weight respectively of 24, 26, 31 and 36.5kDa. These peptides were clearly detected and common to the STSC 20, 21, 16, 41 and 3 and absent in the non-selected counterparts. Peptides of 24-26kDa are clearly expressed in salt tolerant suspension cultures of *Nicotiana tabacum* and in the plant roots (King et al., Plant Mol.Biol. 7:441, 1986). Current investigations are focused on the analysis of the expression of these peptides and their role in salt tolerance; furthermore, studies on the detection of their eventual presence in the regenerated plants are also underway.

Isolation of L-glufosinate-tolerant embryogenic lines in various genotypes

--E. Lupotto, M. C. Lusardi, E. Nielsen* and G. Forlani*
*Dipartimento di Genetica e Microbiologia, Universita di Pavia

The active principle of the herbicide BASTA (Hoechst AG) is L-glufosinate or L-phosphinotricine (L-PPT), an analogue of L-glutamic acid and two L-alanine residues. Upon removal of these residues by peptidases, PPT results in a potent inhibitor of GS (glutamine synthetase). This enzyme plays a central role in the assimilation of ammonia and in the regulation of nitrogen metabolism in plants (Miflin and Lea, Ann. Rev. Plant Physiol. 28:299, 1977). L-glufosinate belongs to the so-called "total herbicides" which represent, to date, the most important classes of compounds of different chemical nature, for chemical control of weeds. These compounds can be applied in low dosages, are stable, safe for animals, and completely degraded by the microor-

ganisms in soil, and act on specific target sites in the biosynthetic pathways of the plant cell (Comai and Stalker, Oxford Surv. of Plant Mol. Cell. Biol. 3:166, 1986). Resistance to L-PPT has been reported in alfalfa cells, after a stepwise selection on growing levels of L-PPT, resulting in gene amplification (Donn et al., J. Mol. Appl. Genet. 2:621, 1984), and by introgression in tobacco, potato and tomato plants, via *Agrobacterium*-mediated transformation of the bar gene, encoding for phosphinotricine acetyltransferase (PAT), a detoxifying enzyme (De Block et al., EMBO J. 6:2513, 1987). In vitro selection for L-glufosinate in cultures may lead to the isolation of resistant cell lines differing from the control counterparts in different features. Resistance to L-glufosinate can be due either to a mechanism of gene amplification or to the presence of altered forms of the target enzyme glutamine synthetase as well as to the presence of naturally occurring detoxifying enzymes.

Maize cultures were established from immature embryos of different genotypes. They were chosen because the derived callus was typically of type II (Armstrong and Green, Planta 164:207, 1985) highly embryogenic and friable. This was done in order to enhance the homogeneity of the material grown in selective conditions, the cellular population to be subjected to selection, and the subsequent regeneration of the selected cell lines. A derivative of B79 culture was also considered (Lupotto et al., MNL 62:31, 1988) because of its high regenerability and friability compared to the direct callus culture obtained in B79. Also a cell line of type I callus was considered, LC10, derived from a F2 population of the cross W64AxA188, which was particularly suited to this work because it was established as long-term highly regenerable culture. The genotypes considered are listed in Table 1. The curve of sensitivity to L-glufosinate in the culture medium was drawn for each genotype.

Table 1 - Level of selection of different maize genotypes on L-glufosinate.

Genotypes	Dose of L-glufosinate (mM)	Results s = stable r = in selection	Regeneration p = plants
TYPE II			
B79	0.05	S	+
	0.1	S	+
	0.2	S	-
Va85 x B79	0.05	S	+
	0.1	S	+
	0.2	S	-
154/LC12	0.05	S	+
(BC_1A188 x B73)	0.1	S	+
	0.2	S	-
B73 x A188	0.05	S	+
	0.1	S	+
	0.2	R	-
TYPE I			
LC10 (W64A x A188)	0.05	S	+
	0.2	S	+
	0.3	S	+ (p)

The effect of L-glufosinate varies depending on the genotype considered. The LD_{50} in the various cases lies between 0.05 and 0.2mM with the exception of LC10, which displayed a higher LD_{50} (around 0.15mM). Resistant clones were obtained in all cases at different levels of selection (0.05, 0.1 and 0.2mM for type II calli and 0.05, 0.2 and 0.3mM for type I LC10 culture) (Table 1). A total fresh weight of 4 grams embryogenic callus was considered for each genotype and spread over 30ml agar N6P medium (Lupotto et al., MNL 62:31, 1988) in 90mm petri dishes. Growing isolates were subcultured 8-9 times after the first passage before being classified as stable resistant clones, in the presence of L-glufosinate. At that time their GI on glufosinate was identical to the non-selected counterparts grown on control medium.

When a L-glufosinate tolerant isolate was considered stable, it was also embryogenic, at least at the histological level checked by observing the presence of well defined embryogenic structures. Regeneration was stimulated in all the resistant cell clones in each genotype by transferring callus portions on MS (Murashige and Skoog, 1962) hormone-free medium and subculturing them each week onto fresh medium in the light. Regeneration was easily obtained in B79 and Va85xB79, while a longer time in MS hormone-free medium was required for 154/LC12 and B73xA188. Regeneration was also obtained in LC10 type I callus culture at higher levels of L-glufosinate (0.2 and 0.3 mM). In this case, plants were easily established in soil. The only evident abnormality registered in them was the presence of tassel-seed plants, a very common abnormal feature of in vitro regenerated plants not connected with the selection.

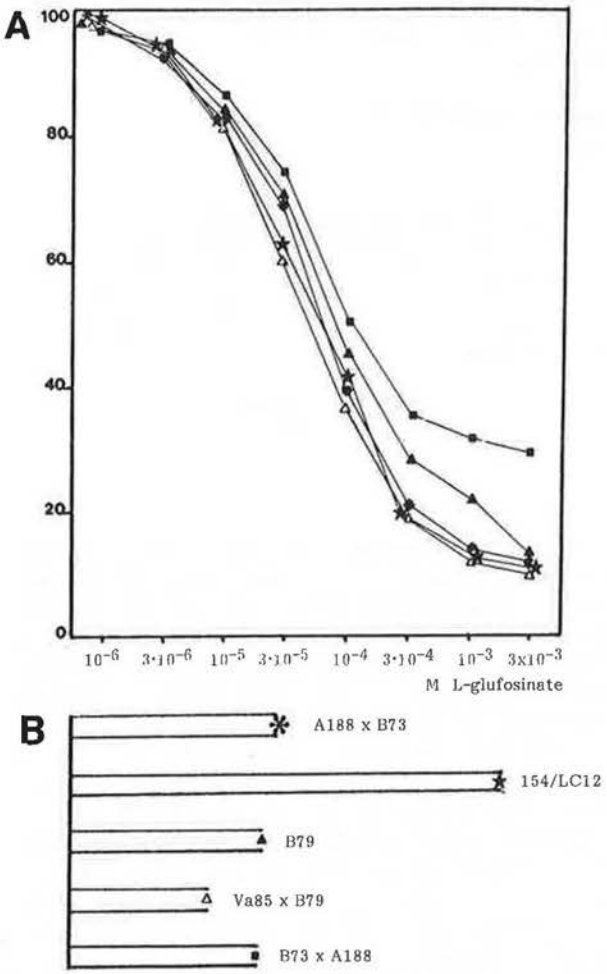


Figure 1. A: in vitro sensitivity to L-glufosinate of the enzyme glutamine synthetase in the various selected cell clones. B: levels of the specific activity of GS in 5 isolates compared among each other.

Analysis of the level of specific activity and curve of sensitivity to L-glufosinate in vitro of glutamine synthetase in selected calli was performed on frozen and fresh callus tissues. Calli were extracted in Tris-HCl 100mM, β-mercaptoethanol 6mM pH 7.4 (1 mg^{-1} FWT), mixed with PVPP and dialyzed overnight at 4 C. Two hundred ul of each extract were tested in the presence or absence of L-glufosinate in a reaction mixture consisting of Tris-HCl 100mM pH 7.4, β-mercaptoethanol 6mM, ATP 10mM, L-Glutamic acid 100mM, NH_20H-HCl 100 mM, $MgCl_2$ 20mM. The reaction was run 3 hrs at 35 C, then stopped by addition of 750µl colorimetric dye: $Fe(CNO3)_3$ 10% + TCA 5% + HCl 6.7%. Each sample was read at 535nm (coeff. molar est. 761 M^{-1} cm^{-1}). Total protein content was measured with the Lowry method. Specific activity of glutamine synthetase varies according to each genotype, with the highest value detected in 154/LC12 selected on 0.2mM L-glufosinate. The glutamine synthetase sensitivity to L-glufosinate reflects in all the cases the LD50 of the tissues, being around 10^{-4}M (Fig. 1). However, because the tolerant calli do not show a real enhancement in the level of their glutamine synthetase activity in comparison to controls, further analyses are in progress to ascertain the nature of the tolerance obtained.

Isolation of Bg-induced unstable mutations at the *Wx* locus

--F. Salamini*, L. Asperti, C. Balconi, N. Di Fonzo, R. Thompson* and M. Motto
*Max-Planck Institut, Cologne

The *Bg-r* transposable element system was reported originally by Salamini (Mol. Gen. Genet. 179:497, 1980) to be responsible for controlling the instability of the mutable allele *o2-m(r)*. In this system *Bg* (Bergamo) is the self-mobile or autonomous element and *r* (receptor) the nonautonomous element. Though both elements can transpose, only *Bg* can induce transposition of both itself and *r*, that is, only *Bg* has a transacting transposition function. It has also been shown that the *o2-m(r)Bg* system displays an apparent specificity for the *02* locus (Montanelli et al., Mol. Gen. Genet. 197:209, 1984), and mutations at loci under the control of an autonomous *Bg* element have not been yet identified. To study the *Bg* element at the molecular level we set up a transposon tagging experiment to move *Bg*, located somewhere in the genome, into the waxy (*Wx*) gene for which molecular probes are available.

In order to induce instability at the *Wx* locus in the A69Y *o2 R Bg-m* strain, two experiments were performed in the summer nursery of 1986 and 1987 by crossing homozygous *o2 R Bg-m Wx* plants to a stable *wx* tester line. An F1 population of approximately 350,000 seeds was screened for mutable *wx* (*wx-m*) kernels, i.e. variegated sectors of *Wx* over *wx* background in mature ears. Plants originating from these kernels were selfed to confirm presence of variegated phenotypes in the F2 generations. In two cases the variegated phenotype observed in the F1 kernels was heritable giving rise to the unstable alleles termed *wx-m32* and *wx-m33*. They arose with a frequency of 5.7 x 10^{-6} (two out of 350,000 kernels).

The *wx-m32* mutant appeared interesting because the selfed progenies of plants grown from variegated seeds of the F2 generation gave ears with a great majority of variegated kernels. Therefore, genetic crosses with the *wx-m32* allele were initiated to test for the presence of *Bg* insertion and the autonomous nature of the element present in the *wx-m32* strain was confirmed by the following experiments.

Variegated kernels from the 87-7087-9 selfed ear (genotypes *wx-m32/wx* and *wx-m32/wx-m32*) were planted in the 1988 winter nursery and the plants resulting from these kernels were selfed and backcrossed to the A69Y *wx/wx* female tester. Out of the 19 plants considered, upon selfing and backcrossing, 10 ears segregated variegated and waxy kernels, while the remaining 9 ears were found to be homozygously variegated. In the segregating ears, ratios near to 3 variegated: 1 waxy were obtained from such selfed plants, while ratios of 1 variegated: 1 waxy were obtained from the backcross to *wx* females (Table 1). The conclusion was that *wx-m32* is an unstable allele induced by an autonomous transposable element.

Table 1. Results of the backcross of *wx-m32/wx* plants used as pollen donor to wx/wx females

	Phenotype of seeds in the backcross to a *wx* female parent			
Plant	vitreous	variegated	waxy 1:1	$\chi^{2(1)}$
S50/47-21	2	47	46	0.09
-22	4	45	46	0.09
-23	3	84	75	0.88
-26	1	13	10	0.66
-28	1	31	41	1.11
-29	4	93	1 00	0.04
-30	1	18	25	0.81
-33	2	87	84	0.14
-36	4	49	51	0.03
38	1	35	32	0.23

(1)Vitreous seeds are counted in the variegated class

Tests were also done by crossing the *wx-m32* stock onto *o2-m(r)+Bg* tester plants. Results indicated that *wx-m32* stock carries an active *Bg* element by virtue of its ability to induce *02* sectors in *o2-m(r)*.

BERKELEY, CALIFORNIA
University of California

Cell-free protein synthesis with maize polyribosomes and wheat germ translation factors

--Julia Bailey-Serres and Michael Freeling

A heterologous in vitro translation system which uses maize polyribosomes and wheat germ translation factors has been made. The system faithfully elongates proteins from mRNAs which have already initiated translation on polysomes, but does not efficiently promote initiation of translation. Attempts to make a heterologous system capable of initiating translation have shown that wheat germ elongation factors, but not initiation factors, are compatible with maize ribosomes.

The system capable of in vitro, run-off translation of mRNAs present on polysomes was constructed as follows: polysomes were extracted from maize seedling roots in a buffer containing 400mM KOAc, 20mM HEPES (pH 7.6), 35mM MgOAc and 5mM BME; concentrated by centrifugation at 100 x g through a buffered 2M sucrose cushion (Lincoln et al., PNAS 84:2783-2792, 1986); and resuspended in translation buffer (100mM KOAc, 20mM

HEPES (pH 7.6), 3.5mM MgOAc, 5mM BME). Wheat germ extract from Promega Biotec was made 130mM KOAc and centrifuged at 100xg to obtain the S-100 supernatant fraction. This fraction should contain factors sufficient for initiation and elongation of translation and lack wheat germ ribosomes. Run-off translation of polysomal RNA was carried out in 25ul reactions containing: 50-100ug polysomal protein in translation buffer, 2-4ul wheat germ S-100 fraction, 2ul 1mM amino acids minus methionine, 1.23ul [^{35}S-methionine (1200 Ci/mmole) and sufficient KOAc to maintain a concentration of 100mM. The reaction was carried out at 25 C for 1hr and translation products were analyzed by SDS-PAGE.

To test for initiation of protein synthesis the reaction mix included poly A$^+$ mRNA (1ug) encoding CAT (in vitro synthesized and analog-capped pSP6CAT-A$^+$ per Callis et al., 1988), and the presence of CAT activity was tested. No initiation of translation was observed when polysomes extracted in 400mM KOAc, which should lack initiation factors, were combined with the 130mM KOAc treated wheat germ S-100 fraction, which should contain initiation factors. Also, no initiation of translation was observed when polysomes extracted in 100mM KOAc, which should maintain initiation factors, were combined with 100mM KOAc treated wheat germ S-100 fraction, which should contain elongation factors but not initiation factors (these elute at 120mM KOAc). Since the maize polysomes and wheat germ translation factors appeared to be incompatible for the initiation of translation, the effect of maize translation factors on the total wheat germ translation system was tested. A maize S-100 fraction was eluted from polysomes isolated in 20mM KOAc (at 20mM KOAc both initiation and elongation factors should remain associated with polysomes). Addition of this fraction to the total wheat germ extract dramatically inhibited the initiation of translation by the extract. In conclusion, certain factor(s) required for initiation of translation are unique to maize and others are unique to wheat germ. Nevertheless, run-off of maize polysomes by the wheat germ S-100 fraction is efficient and has proven useful.

A testable site-specific recombination model for *Mu3* excision

--Barbara Kloeckener-Gruissem and Michael Freeling

Germinal revertants of the *Mu3*-induced *Adh1* mutant *3F1124* (C-H. Chen et al., Genetics 116:469-477, 1987) were isolated. Homozygous mutant plants were grown in isolation from *Adh1-3F* progenitor plants; these plants were selfed or sib-crossed. Approximately 30,000 resulting kernels were germinated in aerated water for 7 days. Only revertants with partial or fully reverted ADH activity can germinate under these conditions.

Six revertants were recovered that express about 100% ADH1 activity in the scutellum. DNA sequences (100bp) surrounding the *Mu3* insertion site of 2 of the 6 revertants were amplified in the polymerase chain reaction (PCR), cloned and sequenced. The DNA sequence of both revertants is indistinguishable from the *Adh1-3F* progenitor sequence. Yet another revertant, *Adh1-3F1124r53* (see B. DeFrancisci et al. this issue), was also found to have no *Ac/Ds*-typical footprint left upon excision of *Mu3*.

One possible mechanism to explain clean *Mu3* excision is a recombination event similar to phage lambda excision, and is illustrated in Figure 1. DNA folds to achieve alignment between the 9bp direct repeats (that resulted from the

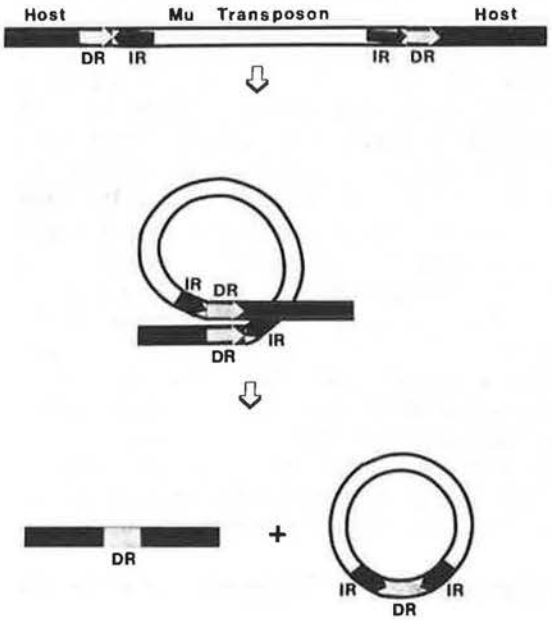


Figure 1.

duplication of host DNA upon insertion of the element). Stabilizing proteins are most likely required to keep the 200bp inverted repeats at the ends of the element from aligning to each other as well as to hold the "loop-structure" in place. A recombination enzyme (not necessarily an enzyme encoded by a Mutator element) may catalyze the cutting and rejoining of the ends yielding the chromosomal copy which is identical to the *Adh1-3F* allele and a hypothetical circular molecule of the *Mu3* element that carries the 9bps from the host DNA duplication between the terminal inverted repeats of *Mu3*. Thus, the exact sequence of the progenitor allele--shown in the lower left corner of Figure 1 as carrying its single, normal direct repeat (DR)--is restored after an excision. A population of *Mu* transposon circles of varying sizes has been identified previously by Sundaresan and Freeling (Proc. Natl. Acad. Sci. 84:4924-4928). We are now looking for the DNA sequence between the inverted repeats using the PCR. We predict that each *Mu* circle will carry a different 9bp insert.

Analysis of a partial *Adh1-3F1124* germinal revertant

--Brandon DeFrancisci, Barbara Kloeckener-Gruissem and Michael Freeling

In order to learn about the activity, excision and transposition of the *Mu3* transposable element and to gain information about the promoter region of *Adh1*, revertants of the mutant *Adh1-3F1124* were isolated. These revertants were isolated from *Adh1-3F1124/Adh1-2F11* heterozygous seeds. *Adh1-3F1124* is a conditional anaerobic lethal mutant caused by the insertion of a 1.85kbp *Mu3* transposon resulting in the duplication of the TATA box (C-H. Chen et al., Genetics 116:469-477). It has 6% of wild type ADH1 activity in primary roots and scutellum but normal activity in pollen. *Adh1-2F11* has no ADH1 activity and is caused by

the insertion of *Ds* (Doring et al., MGG 193:199-204). Over one hundred thousand seeds were screened under partially anaerobic conditions. Six *Adh1-3F1124* revertants were recovered.

One of these revertants, *Adh1-3F1124-r53*, has 50% of wild type ADH1 activity in the scutellum and normal activity in the pollen. Using *Adh1* and *Mu3* specific probes, Southern analysis indicates the expected restriction sites for the *Adh1-3F* progenitor allele and that *Mu3* has excised. Approximately 100bps surrounding the excision site were sequenced after amplification of this DNA fragment using the polymerase chain reaction (PCR) according to Perkin Elmer Cetus. The sequence was found to be identical to the progenitor allele, *Adh1-3F*. To see whether the phenotype of *r53* is linked to *Adh1-3F1124* mutant allele, linkage analysis was done by crossing *Adh1-3F1124-r53/Adh1-1S* heterozygotes to *Adh1-3F1124* homozygotes. ADH1 activity in the scutellum segregated 1:1 for *Adh1-1S/Adh1-3F1124* and *Adh1-3F1124-53/Adh1-3F1124* indicating linkage between the revertant and mutant allele. In order to determine the cause of *r53*'s phenotype, the allele will be further examined by thermostability studies of the protein and by obtaining and analyzing a large genomic clone.

Organ and tissue specific expression of 6-phosphogluconate PGD2 · PGD2 dehydrogenase (6-PGD)

--J. Thom, J. Bailey-Serres and M. Freeling

The three cytosolic 6-PGD isozymes, PGD1 · PGD1, PGD1 · PGD2, and PGD2 · PGD2, are encoded by *Pgd1* and *Pgd2* (Phenotype and genetic analysis provided by Stuber and Goodman, Maydica 29: 453-471, 1984; C. Stuber per. comm.). We resolved 6-PGD dimer banding patterns in a number of tissues and organs by native polyacrylamide gel electrophoresis (Sachs and Freeling, Mol. Gen. Genet. 161:111-115, 1978), followed by PGD activity staining(0.2mg/ml NADP+, 0.2mg/ml NBT, 0.02mg/ml PMS, 1mg/ml Na_3 6-phosphogluconate, 0.05 M Tris (pH 7.5), at RT for ~ 60').

Our analysis suggests that the relative steady state levels of 6-PGD isozyme dimers exhibit organ and tissue specificity. When comparing the two heterodimers, we observed the PGD2 · PGD2 to be more abundant relative to PGD1 · PGD1 in pollen, root, coleoptile, immature scutellum, and quiescent embryo axis. However, in the milky endosperm we observed the two homodimers, PGD1 · PGD1 and PGD2 · PGD2, to be present in equal amounts while the heterodimer, PGD1 · PGD2, was approximately fourfold more abundant.

In situ staining of 6-PGD activity in B73 and two *Pgd2-5* null families from our Robertson's Mutator stock confirmed specific expression of *Pgd2-5* in scutella. In B73, 6-PGD stained evenly throughout the scutella whereas scutella from our *Pgd2-5* null homozygotes failed to stain, except for cells associated with the preprovascular bundles. This result suggests that *Pgd1-3.8* expression is localized to the preprovascular bundles of the scutella. In the future, we hope to determine the precise distribution of the 6-PGD isozyme dimers within the plant tissue and organs.

At the moment, we are in the process of determining whether our two *Pdg2-5* null lines originated via *Mu*-induced mutations or pre-existed in our Robertson's Mutator lines.

Detailed analysis of the aerobic and anaerobic tissue distribution of ADH1 activity in the primary root

--Julie Vogel, John Fowler and Michael Freeling

It has long been known that alcohol dehydrogenase-1 (ADH1) is expressed in the aerobic primary root (Schwartz, MGG 127:215, 1971), and that the level of enzyme activity in the root as a whole is induced at least 10-fold upon anaerobiosis (Freeling, Genetics 67:411, 1973). More recently, Christie Williams examined in more detail the distribution of ADH among a number of tissues of the seedling primary root as part of her Ph.D. thesis work in this laboratory ("Organ-specific Expression of ADH in *Drosophila melanogaster* and *Zea mays*", UC-Berkeley, 1987). Using in situ staining of longitudinal root sections to assay ADH enzyme activity, Williams found that, under aerobic conditions, ADH1 is expressed only in the root cap and the stele (probably only the inner parenchymous tissue). However, upon 18hr of anaerobic treatment, not only was the staining more intense in these same tissues, but de novo ADH1 activity was apparent in the cortex, meristem, epidermis, and some longitudinal elements of the vascular system. We have extended this initial analysis, using both longitudinal and transverse sections, and we have made a number of further observations about the tissue distribution of ADH1 in the primary root. All of our results are controlled in two ways. First, we compare staining patterns with and without ethanol to make sure that our NADH source is indeed from the ethanol-driven ADH reaction. Second, since we have null alleles for both *Adh1* (which encodes ADH1 subunits) and *Adh2* (which encodes a very low level of ADH activity that is below the level of detection of this assay), we know that ADH1 subunit activity alone is being monitored.

First, we observed that the same anaerobic pattern of ADH1 expression could be seen after only 8hr of anaerobic treatment, performed by immersing 5-day-old seedlings in water saturated with bubbling argon gas. However, at this 8hr time point, the two different maize *Adh1* genotypes examined, 1S (of the 1s2p inbred line) and 1FB73 (of the B73 inbred line), exhibited consistent differences in the apparent levels of ADH1 induction in the various root tissues. Although the absolute levels of ADH activity could not be quantified by this visual assay, the level of staining in B73 appeared to be at least 2-fold greater in all induced tissues than in 1s2p. It is possible that cis-acting sequences at these *Adh1* alleles are responsible for these quantitative differences, or, alternatively, that one or more unlinked "modifier" genes differing in these lines may confer such phenotypic variation. These two possibilities are to be tested.

In addition, our tissue distribution analysis has revealed that ADH1 is anaerobically induced, apparently de novo, in the endodermis, the innermost cell layer of the cortex that directly surrounds the pericycle. However, activity in this cell layer is most pronounced only in the elongation region of the root; the endodermis does not stain appreciably in the meristematic region nor in the root maturation zone. We also observed ADH1 induction in the epidermis, but only within a limited region of the root, which appears to correspond roughly to the elongation zone. In our 5-day-old anaerobic seedlings, with roots 3-5cm long, epidermal ADH1 activity was detected only within a distance of ~0.5-0.7cm from the root tip. There were no root hairs in the

elongation zone, and hairs in the older portion of the root, like the rest of the surrounding epidermal cells in this region, did not stain for ADH.

The root cap and stele express ADH1 activity under normal growth conditions; all other tissues or tissue regions induce ADH1 in response to anaerobiosis. We do not know the functional significance (evolutionary advantages) of these observations.

Preliminary analysis of the pollen cell lineage

--R. Kelly Dawe and Michael Freeling

Rhoades observed that when purple anthers from *a1-m* plants were crossed to an *a1* tester, about half transmitted the revertant phenotype through the gametes (Genetics 23:377-397). We have confirmed his result and have further shown that pollen derived from larger sectors--up to one half of the tassel -- transmit the revertant phenotype with the same likelihood as single anther sectors (50%, see Table 1). The results suggest that the cell lineages leading to the coloration of the anther wall and to the formation of the pollen mother cells are separable as early as the tassel two-cell stage.

Table 1. Transmission of *Bz2* gametes from purple tassel sectors in *bz2-m*, *Ac-2F11* plants[1].

sector size	# sectors crossed	# heritable	# not heritable
one half anther (purple half only)	7	3	4
whole anther	4	2	2
one half floret	12	6	6
whole floret	21	13	8
groups of florets (<half branch)	23	13	10
one half tassel branch	9	2	7
whole tassel branch	5	3	2
one eighth tassel	13	3	10
one fourth tassel	5	2	3
one half tassel	3	2	1
(subtotal)	102	49	53
more than one half tassel	3	3	0

[1]Seeds homozygous for *bz2-m* and heterozygous for *Ac-2F11* and *Pl* were grown and screened for sectors. Pollen from locules or anthers of colored sectors was emptied onto the silks of homozygous *bz2*-stable, homozygous *Ac* testers. For sectors larger than one floret, three anthers from single florets were used. Only crosses yielding 30 or more seeds were tabulated. Sectors were considered heritable if 40% or more of the kernels from a cross were purple. Sectors were considered not heritable if less than 5% of the kernels were purple. Intermediate ratios (greater than 5% but less than 40% purple) were sometimes found, but are not considered here.

We have used the genetically unstable *Ds* insertion mutant *bz2-m* (M.G. Neuffer, MNL 27:67, 1953) to induce purple revertant sectors in the tassel. A number of *Ac* elements mapping to different places on chromosome 9S (in conjunction with *Pl*) were categorized according to when during tassel development excision events at *bz2-m* occurred. Most *Ac*s permit frequent but late excision events only. However, the *Ac* present in the line that generated *Adh1-2F11* (see Doring et al., MGG 193:199-204), *Ac-2F11*, is 20 map units from *Wx* and acts exceptionally early during plant development. In plants homozygous for *bz2-m* (and carrying *Pl*), purple tassel sectors of one half tassel branch or larger occur in about 15% of the plants when *Ac-2F11* is present.

Purple anthers on *bz2-m* plants generally transmit either 1) a low basal level of *Bz2* pollen -- the reversion frequency of *bz2-m* pollen is about 5%, with roughly half of the reversion events occurring in bronze anthers -- or 2) 50% *Bz2* pollen, the expected frequency from a germinal revertant heterozygote. The two possibilities occur with approximately equal frequency among sectors ranging in size from one half anther to one half tassel (Table 1; see "subtotal"). However, all of three sectors including more than half of the tassel were heritable. This result suggests that a single-celled tassel primordium forms developmentally distinct cell layers immediately following its first cell division. Additionally, results using *B Pl* stocks indicate that neither anther wall color nor the pollen mother cells are derived from the epidermal cell layer (L1).

When *bz2-m* plants were outcrossed to *bz2* testers, the most common derivative kernels were *bz2-stable*, not purple. However, colorless sectors in *bz2-m*/*bz2-stable* (with *Pl*) tassels are extremely rare. Pollen shed from seven such colorless sectors of various sizes has been testcrossed and all seven transmitted the *bz2-stable* phenotype. Our working hypothesis is that anther wall color is derived from two different cell layers, one of which also generates the pollen mother cells. Under this hypothesis, colorless sectors are rare because *bz2-stable* events must occur simultaneously in two adjacent cell layers.

An explanation of the sporadic behavior of morphological mutants

--Michael Freeling and Nicholas Harberd

This laboratory has been working with several dominant morphological mutants that specify the common phenotype of foci of extra cell division within the leaf. *Kn1* (in blade), *Kn2* (over auricle), *Rs** (in sheath and ligular region) and *Hsf** (sheath transforms blade at margins of blade) are examples. Each of the 16 mutants included within the four loci designations above shows a *sporadic* phenotype: the patterns of extra cell division are not always the same and genetic background modifies expression. We have logically sound explanations for most aspects of these mutants' phenotypes (see Freeling et al., 1988. In: Plant Gene Research; Temporal and Spatial Regulation of Plant Genes, D.P.S. Verma and R.B. Goldberg, eds., Springer-Verlag, Wien, pp. 41-62), but their sporadic behaviors were unexplained. Since the sporadic aspects of phenotype characterize genes rather than individual mutants--all the mutants are sporadic--transposons are not likely to be involved.

We now think it likely that there is a default cell division rule for the developing leaf (and, presumably, each other organ of the plant): the slower-dividing cells communicate with those adjacent cells that could divide faster and say "slow down so as not to tear the leaf apart." The signal might well be physical stress and strain, transmitted wall-to-wall and transduced into molecularly meaningful second messages. The existence of this default cell division rule is not proved, but there are excellent data. Harberd and Freeling (1989, Genetics, in press) have described the basic genetics of *D8* and *Mpl*, two closely linked, GA-insensitive, dominant dwarf mutants that derepress anther primordia in the ear. Plants carrying one copy of *D8* are late-maturing and have leaves that have about 1/3 the nonmutant number of cells in length and in width; leaf primordial cells divide more slowly than do cells in leaves of normal sibs. Using genetic mosaic analyses, genetic markers and x-irradiation, we were able to obtain plants that were almost entirely dwarf (*D8*/+), but had sectors of normal tissue. Loss of *D8* in

the epidermis didn't matter in any case, but loss from the internal tissues of the plant often mattered. The question: did the normal tissue outgrow the nearby dwarf tissue? The answer: yes, *D8* behaved autonomously in sectors including several phytomers of ear or tassel,or when comprising large portions of glume (unpublished). However, the autonomy argument grew circular when smaller sectors within leaves were examined. Most behaved as if they were dwarf; that is, *D8* behaved nonautonomously. However some (2/9) overgrew the dwarf leaf at the margin only. *D8*-loss sectors within the glume (unpublished) were often large. In these cases, the normal sector did attempt to grow faster (was larger), but there were always smooth morphological contours between the faster-growing and slower-growing regions rather than a sharp boundary reflecting genotype.

If there were a default cell division rule operating in the leaf, as we suggest, the sporadic mutant phenotypes are explained. For example, the mutant *Kn1-0* conditions a phenotype that often includes many sporadic protuberances over lateral veins; these are called knots. Our explanations of knotted phenotype have centered on age-identity problems involving the cells surrounding lateral veins: these cells "think they are younger than their positions would indicate" (Freeling et al., 1988, ibid.). Thus, these retarded cells differentiate to younger fates (sheath in blade) or keep dividing when they ought to stop (knots). How come just some of the cells keep dividing?; i.e. why the sporadic knot formation? Suggested answer: all the too-young cells surrounding lateral veins *do* have the autonomous capacity to continue division, but the default leaf cell division rule usually keeps them in check. Sometimes the default rule is overridden, and a knot occurs which, itself, would exacerbate or relieve cell-cell strain and lead to quite complex and sporadic cell-cell strain relationships. We imagine similar explanations for the sporadic phenotypes specified by the other dominant leaf cell division/cell identity mutants.

Perhaps "developmental compartments" in plants might be defined as the domains within which this hypothetical default cell division rule operates. Groups of cells within different compartments would not communicate to each other their rates of growth and coordination would not happen, as with cells in different phytomers.

Use of the scanning electron microscope to ascribe leaf regional identities even when normal anatomy is disrupted

--Phil Becraft and Michael Freeling

For developmental investigations it is essential to recognize the constituent cells and tissues of the particular system. Scanning electron microscopy (SEM) is proving useful for leaf developmental studies. Using SEM, we have found that the epidermis of most leaf parts has characteristic and distinctive surface features, and it has been possible to distinguish leaf tissues which otherwise appear very similar. For example, the gross morphology of sheath and midrib is nearly identical; each are thick, rigid, and have a smooth, shiny adaxial surface. In cross section, both have thick mesophyll layers with sparse chloroplasts, and vascular bundles, and are associated with prominent fibers localized toward the abaxial surface. Genotypes causing anthocyanin pigmentation in the sheath often affect the midrib as well. Thus it has been difficult to discern sheath and midrib by means other than position on the leaf. However, SEM images of the adaxial epidermis show clear distinctions between sheath and midrib. Sheath epidermal cells (Figure 1) have wispy wrinkles running longitudinally, are rectangular in shape, and have narrow cell borders. The midrib epidermal cells (Figure 2) have smooth wall surfaces, are rectangular to somewhat diamond shaped, and have a distinctive weld-like appearance at the cell borders. No other cells with these surface characteristics were found in normal maize leaves.

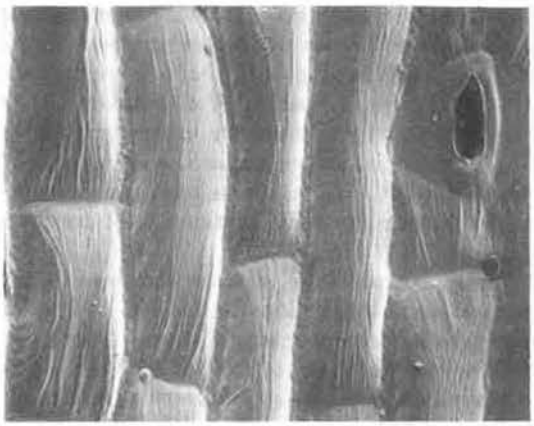


Figure 1. Scanning electron micrograph of adaxial sheath epidermis. 507X.

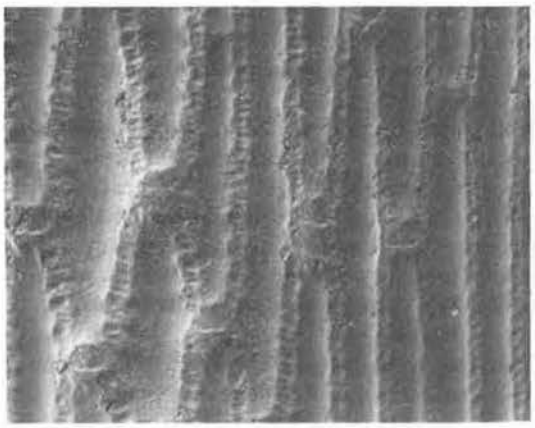


Figure 2. Scanning electron micrograph of adaxial midrib epidermis. 520X.

Several mutations exist in maize which appear to transform tissue of one organ component into that of another organ component (e.g. *Hsf1* transforms blade to sheath--older to younger). Knotted plants (the phenotype of *Kn1*) have patches of sheath-like leaf, often bounded by ligular fringes, along lateral veins of the blade. SEM provided conclusive evidence that this sheath, although located in the blade, is actually sheath rather than midvein or any other leaf component. Thus, we have established leaf cellular identities independent of position within the leaf.

A *Mu*-suppressible *hcf* mutant, and non-reciprocal transmission of *Mu* activity

--Rob Martienssen, Alice Barkan, William C. Taylor and Michael Freeling

The high chlorophyll fluorescence maize mutant *hcf*-106* is a recessive, pale green, seedling lethal that arose in a Robertson's Mutator line and shows somatic instability

(namely small, late dark green sectors) typical of a *Mu* insertion. Molecular analysis and cloning has shown that a 3.7kb *Sst*I fragment containing a *Mu1*-like element probably corresponds to part of the *hcf*-106* locus (Martienssen, Barkan, Freeling, and Taylor, submitted).

The mutant *hcf*-106* phenotype is only observed in seedlings with *Mu1* elements that are active and unmodified at the *Hin*fI sites in their terminal inverted repeats. Seedlings homozygous for the mutant allele that have modified elements are normal in appearance and survive to maturity. This suppression of the mutant phenotype results in its disappearance from pedigrees that are losing *Mu* activity in successive generations of selfing and outcrossing. However, suppressed plants can be backcrossed to *Mu*-active plants that are heterozygous for *hcf*-106* and, typically, these crosses re-activate inactive *Mu1* elements. Consequently, backcross progeny that are homozygous for *hcf*-106* adopt a mutant phenotype and are seedling lethal. We have termed *hcf*-106* "*Mu*-suppressible", by analogy with McClintock's *Spm*-suppressible mutants in maize (Masson et al., Genetics 177:117-137, 1987).

Occasionally, mutant seedlings are observed that have large sectors of dark green, low-fluorescent leaf tissue on pale green high-fluorescent mutant leaves. These sectors are much larger than those typical of *Mu* excision, and Southern analysis shows no sign of the revertant allele diagnostic of revertant sectors. However, these wild-type sectors contain hypermodified *Mu1* elements relative to adjacent mutant leaf tissue (Martienssen et al., submitted). This shows that leaf sectors containing modified *Mu1* elements can arise from single somatic cells during development.

Sectored plants display an interesting relationship between the size of inactive sectors and leaf position. While lower leaves are mainly mutant, the proportion of "wild-type" tissue increases in successive leaves until the upper leaves are entirely normal in appearance. Apparently, as plant development proceeds, dividing cells within the meristem "turn-off" progressively to generate larger sectors of "wild-type" tissue until, in the upper leaves, most cells contain inactive *Mu* elements. The more meristematic divisions a cell undergoes, or the longer a given cell lineage spends in the meristematic condition, the more likely it is to adopt an inactive phase.

This developmental pattern may provide an explanation for the reciprocal effect observed in crosses between *Mu*-active and *Mu* -inactive plants (Walbot, Genetics 114:1293-1312, 1986; Bennetzen, Mol.Gen. Genet. 208:45-51, 1987; our unpublished results). That is, some active plants (but not all) are more likely to lose *Mu* activity through the male rather than the female flower. Because of the relationship between developmental position and size of inactive sector, the male flower at the apex of the plant may contain larger inactive sectors than the female flower, resulting in a higher proportion of gametes carrying inactive *Mu*. According to this model, crosses involving unsectored plants would show no reciprocal effect. Reciprocal effects observed in the cycling of some *Spm* elements (Fedoroff and Banks, Genetics 120:559-577, 1988), and in the inheritance of paramutable phenotypes of *B* (booster) (Coe, Genetics 53:1035-1063, 1966) could reflect a similar mechanism.

Novel derivatives of *Adh1-3F1124*

--Rodney G. Winkler and Michael Freeling

Seedlings that are low or null for ADH1, in contrast to normal seedlings, do not germinate under partially anaerobic conditions. The Freeling lab has utilized this behavior to select revertants of transposon-induced mutants of *Adh1* (C.-H. Chen, M. Freeling, Maydica 31:93-108, 1986). *Adh1-3F1124* is a novel mutant that has a *Mu3* insertion that duplicates the TATA box and has been used in a number of selections for revertants (B. Kloeckener-Gruissem, M. Freeling, MNL 1989). Under the conditions used *Adh1-3F1124*, which has 6% of the ADH1 activity of the *3F* progenitor, does not germinate, although revertants with approximately 30% or greater activity do germinate. Although this selection has yielded a number of revertants, most of the germinating seedlings show no detectable ADH1 by starch gel analysis. Preliminary experiments eliminated the possibility that these mutants are simple ADH2 overproducers. In the simplest terms the phenotypes of the 25 mutants being analyzed are: anaerobic germination positive (unlike the progenitor), less than 6% ADH1 activity by starch gel analysis in both the dry scutellum and submerged root, normal levels of ADH1 in pollen (like the progenitor). In situ analysis of the dry scutellum is also consistent with most of these derivatives being lower in ADH1 than the progenitor. Several of the derivatives are still mutable, yielding full ADH1 revertants. Currently these derivatives are being analyzed at the molecular level to understand their altered pattern of expression. One of these, *Adh1-3F1124d104,* has been shown to delete all but approximately 600bp of the *Mu3* insertion.

An anaerobic protein (ANP) that may not be a glycolytic enzyme

--Wolfgang Kammerer and Michael Freeling

Anaerobiosis induces the expression of a specific set of genes yielding a highly changed pattern of protein biosynthesis. The expression of these newly synthesized anaerobic proteins (ANP's) is regulated at the transcriptional as well as the posttranscriptional level (Sachs, Freeling and Okimoto, Cell 20:761, 1980). Work done in this and other labs has shown that the ANPs are glycolytic enzymes (Bailey-Serres et al., Plant, Cell and Environment 11:351, 1988). We report here progress in the study of a 31kD protein, which is one of the prominent ANPs and is rapidly induced as mRNA and protein after the onset of anaerobiosis (ANP31; Hake et al., JBC 260:5050, 1985). We have isolated an apparently full length cDNA clone and sequenced it in order to get a clue about the function of the protein product. The full length clone was isolated by D.C. Bennett from a cDNA library prepared by P. Kelley (unpublished) using the Hake cDNA probe (this laboratory). According to the sequence, the protein is 29.8kD in size, has a 119bp untranslated 5' leader region as well as 191bp untranslated sequence at the 3' end, containing two possible polyadenylation signals; the second one is located about 20bp upstream of the polyA sequence.

Sequence comparison using the EMBL/Genbank database revealed no good similarities with any of the available sequences, including glycolytic enzymes. There was only weak similarity with some viral proteins and protamines, which is most likely caused by the unusually high

content of the positively charged amino acid arginine (19%). Another quite unusual feature is the very high G+C content of the gene, which is about 75% overall and about 85% in the middle third. We are currently trying to express this protein in order to raise antibodies, etc. While most ANPs are certainly glycolytic enzymes, perhaps this one has a binding function.

_Mu3_ may cause chromosomal rearrangements at a distance from the insertion site

--Yvonne Thorstenson and Michael Freeling

Adh1-3F1124 is a mutation at the *Adh1-3F* locus caused by a *Mu3* insertion at the TATA box within the promoter region upstream of the gene. This mutation confers a tissue-specific alteration in ADH1 expression; the mutant plant exhibits 6% of normal activity in the scutellum, but 100% activity in pollen (Chen et al., Genetics 116:469-477, 1987). Several derivatives of the mutant, selected via allyl alcohol resistant pollen grains, are null in both pollen and scutellum (Chen, Ph.D. dissertation, UC Berkeley, 1986).

In an effort to determine the structural changes at the locus that resulted in a null phenotype, Southern analyses of some of the null derivatives recovered by Chen were performed. At least two derivatives had restriction map differences that were not immediately adjacent to the *Mu3* insertion even though *Mu3* was still present at its original location. One derivative had a 1.3kb insertion about 1kb downstream from *Mu3*. A second derivative had changes in a region beginning at about 3kb downstream from *Mu3* . In neither case were the changes associated with the new insertion of a transposon carrying *Mu* ends.

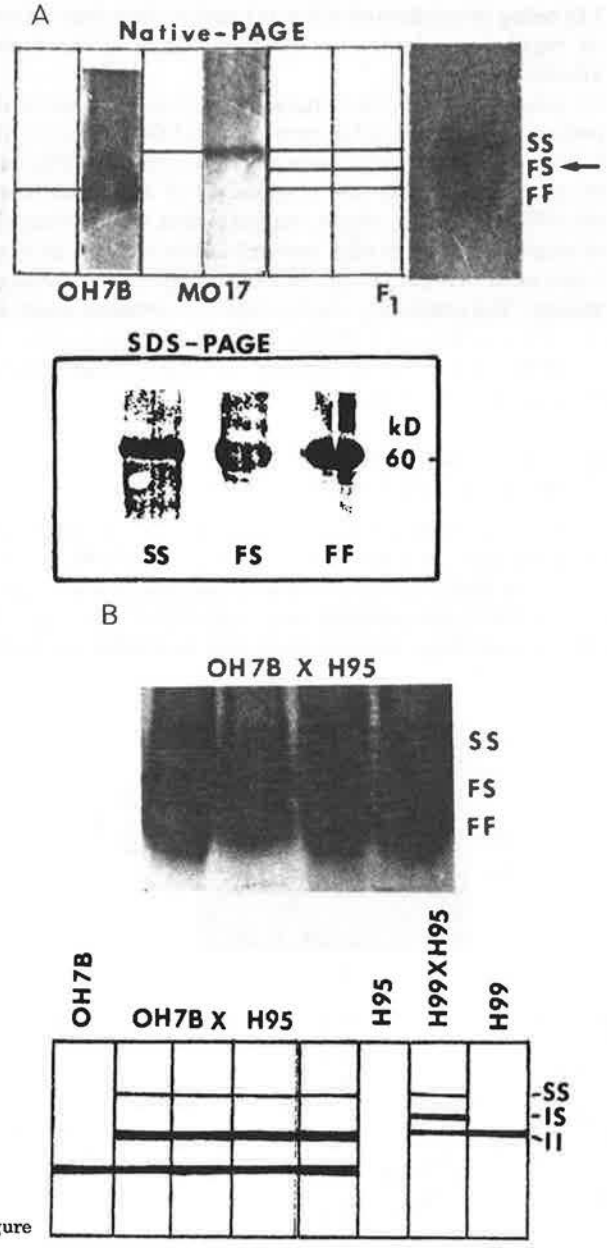
This phenomenon, if widespread, could cause trouble for persons attempting to tag genes with *Mu*. If *Mu* routinely causes such rearrangements in distant regions, it may knock out genes far from its insertion site. The *Mu* element responsible for the mutant gene would still cosegregate with the mutational lesion, but the *Mu*-containing clone could completely miss the mutant gene.

BLACKSBURG, VIRGINIA
Virginia Polytech. Institute and State Univ.

Cis and trans acting elements control the organ specificity of _Glu1_ expression

--Mahmoud M. Rifaat and Asim Esen

β-glucosidase (β-D-glucoside glucohydrolase, EC. 3.2.1.21) is encoded by the *Glu1* locus and its expression exhibits sporophytic specificity. The locus has an unusually high degree of polymorphism, heterozygosity per collection, total panmictic heterozygosity, and null alleles (J. F. Doebley et al., Amer. J. Bot., 1985). The accompanying Figure summarizes the behavior of maize β-glucosidase allozymes encoded by the *Glu1* locus. (A) The F1 seedlings (coleoptiles) resulting from crossing two maize inbreds, Oh7BFF and Mo17SS, having electrophoretically distinguishable enzyme variants (fast and slow, respectively), showed three β-glucosidase allozymes; i.e. two parentals and one with an intermediate mass/charge-dependent mobility. Each β-glucosidase allozyme (FF, FS, and SS) revealed a single subunit size of 60kD by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE). This supports the dimeric quaternary structure of the enzyme and indicates that *Glu1* encodes a polypeptide subunit of about 60kD in size (in preparation). (B) Three β-glucosidase activity bands were resolved from the coleoptilar extracts of all the F1 progeny resulting from crossing normal and null inbreds (Oh7BFF X H95OO and H99II X H95OO). The null inbred H95OO lacks the enzyme subunit (not shown). The slow-migrating activity band (SS) was (1) absent from all parents and common to all F1 progenies resulting from both crosses, and (2) lower in protein amount compared to other bands but indistinguishable from normal *Glu1*-encoded activities by the criteria of subunit size and peptide structure by limited proteolytic hydrolysis (not shown). This suggested that the null mutation in the inbred H95OO was (1) complemented in trans in the F1 progeny; i.e. due to the lack of a trans-acting regulatory element required for sporophytic expression, and (2) not a true allele to β-glucosidase structural gene. The allelism of this mutation to



Figure

Glu1 is being re-confirmed since it implies that this trans-acting regulatory element is tightly linked to the locus (pseudoallele).

It is interesting that the mutation in this null inbred is accompanied by an unexpected expression of *Glu1* activity in the pollen grains (contrary to any normal inbred). This observation, added to the low abundancy of the autodimer protein (SS) in the sporophyte, suggests that a cis-acting element might be "abnormally" present at the locus in this inbred and specifies gametophytic, but limited sporophytic, expression. The possibility that a single mutational event at or near *Glu1* is mediating both the loss and acquisition of respectively trans- and abnormal cis-acting functions, is currently under investigation.

Basis of β-glucosidase multiplicity

--Mahmoud M. Rifaat and Asim Esen

Multiple β-glucosidase electrophoretic variants were resolved from the sporophytic tissues (coleoptiles, roots, scutellum) of maize inbreds. The zymogram presented in part (A) of the Figure indicates the existence of a slower migrating β-glucosidase activity in maize coleoptile extracts

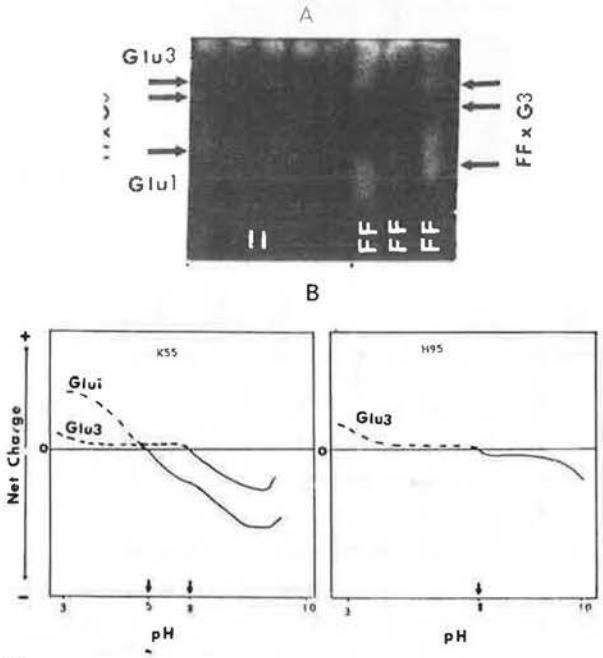


Figure.

(referred to as *Glu3*-encoded enzyme or activity hereafter) with the following characteristics: (1) electrophoretically invariant regardless of the allelic variant at *Glu1*, and (2) present in inbreds with *Glu1* null activity. These observations suggest that *Glu1*- and *Glu3*-encoded activities might not share a subunit in common. Furthermore, activity bands with mobilities intermediate between those of *Glu1*- and *Glu3*-encoded activities are detectable in zymograms of inbred genotypes and developed with the sensitive fluorogenic substrate 4-methyl umbelliferyl β-D-glucopyranoside. The electrophoretic mobilities of these intermediate bands are, however, affected proportionately by that of the allelic variant at *Glu1* (compare II and FF slots in the Figure). This suggests that these intermediate bands represent hybrid intergenic enzyme molecular forms and that *Glu1*- and *Glu3*-encoded enzyme must have different polypeptide subunits. Moreover, the presence of more than one hybrid molecular enzyme form in an inbred genotype rules out the possibility that *Glu3*-encoded enzyme could be a dimer molecule. The electrophoretic titration curves presented in part (B) of the Figure indicate that *Glu1*- and *Glu3*-encoded enzymes have isoelectric points (pI's) of 5 and 8, respectively, with a lower net charge on *Glu3*-encoded enzyme at most of the pH range. The two activities are further distinguishable by native molecular size (K_r estimated from Ferguson plots are 0.06 and 0.134, for *Glu1*- and *Glu3*-activities, respectively), pH optimum, thermal stability, kinetic constants and sensitivity to reducing and sulfhydryl reagents, subunit molecular weight and structure. The occurrence of multiple sporophytic β-glucosidase activities with distinguishable subunit size and structure in inbred genotypes suggests that multiple non-allelic structural genes are encoding maize β-glucosidase multiplicity. Moreover, *Glu3*-encoded enzyme appears to show little or no activity in *Glu1* heterozygous genotypes, with a concomitant absence of its presumptive subunit. Therefore, it appears that the two activities undergo compensatory regulation. The questions currently under investigation are (1) What is the molecular basis of the epistatic interaction between *Glu1* and *Glu3* genes? (2) Is *Glu3* expression determined by a genetic element at *Glu1*? and (3) Is *Glu3* a gene member within a complex *Glu1* structure?

Problems in maize-MDMV genetics

--C. W. Roane and S. A. Tolin

Maize plants infected with maize dwarf mosaic virus (MDMV) have a variety of symptoms some of which are difficult to interpret. The typical pattern of symptoms of completely susceptible plants is a mosaic extending from leaf blade margin to margin and sheath to blade tip. Usually, under conditions of natural infection by aphids transmitting the virus in areas where there is a severe infestation of johnsongrass, plants will have 100% of their leaf area showing mosaic. Lower leaves are sometimes free of symptoms because plants may escape early inoculation. Under conditions of artificial infection, it is difficult to achieve symptoms in more than 90% of the blade area. Usually the lowest leaves remain symptom free and only 70 to 80% of the blade area is symptomatic. When resistant inbred lines are crossed and progeny are artificially inoculated with MDMV and grown under conditions at Blacksburg, there is a range of responses observed among inbred lines having resistance genes. Some lines are apparently immune from MDMV as judged from the lack of symptoms appearing either under natural or artificial conditions, e.g. Oh7B, Pa405. Others are highly resistant in that a few plants become symptomatic with a minimum of leaf area (2 to 5%) exhibiting narrow stripes of mosaic, e.g. Va35. Other resistant inbred lines consistently display a range of symptoms and severity of mosaic in those plants that are infected. Attempts to stabilize these lines to give uniform symptom expression by strict inbreeding have been unsuccessful. For example, the majority of the plants of the resistant line T8 are symptomless; those plants showing symptoms are quite variable in symptom expression. We have been interested in the inheritance of reaction to MDMV, and where we tried to publish our

interpretation of the genetics of T8, we were chided by journal editors for choosing poor parents and thus we chose to delete all reference to T8. We feel, however, that it would be better to air the data and have it challenged than to pretend such a situation does not exist.

Genetics of MDMV reactions have been a frustrating topic. Different results and different interpretations from different workers make one wonder why genetics of MDMV reactions is such a difficult topic. Several factors may be involved:

1) *Inadequate genetic information about parental lines* - Most parents used in MDMV maize genetics may not have been selected under conditions which assure their homozygosity for characters to be studied. In other words, most are breeding materials, not genetic materials.

2) *Use of uniform inoculum* - MDMV exists as a number of strains, A, B, etc. There has been no demonstration that there may be a further subdivision of strains, A, B, etc., into "races" in sensu *Puccinia graminis* on wheat. There is evidence for a gene-for-gene relationship between bean and bean common mosaic virus (Drifjhout, Agri. Res. Rep. 872, Wageningen, 1978) and soybean and soybean mosaic virus (Roane, Tolin and Buss, Soybean Genet. Newsl. 13:136-139, 1986). Since there is evidence for more than one gene conditioning reaction to MDMV (Scott and Rosenkranz, Crop Sci. 22:756-751, 1982; Mikel, D'Arcy, Rhodes and Ford, Phytopathology 74:467-473, 1984), there may also be a gene-for-gene relationship, yet to be demonstrated, for maize and MDMV. If true, MDMV-A from Missouri, Mississippi, Ohio or Virginia used as inoculum might not yield like results in genetic experiments. Rust workers are well aware that an isolate of *Puccinia graminis* from one area may key out to a particular race (a waning concept) but an isolate from another area keying out to that same race may not be genetically identical. Therefore, the importance of assuring virus strain identity and integrity must be recognized and a system of preserving and utilizing virus cultures must be invoked in future studies of genetics of maize-MDMV relationships.

3) *Use of virus mixtures and strain mixtures* - In genetics of pathogen-host relations, the genes of two entities are interacting. Unknown factors which interfere with arriving at correct interpretations cause geneticists enough problems when they are studying the characters of a single species. When two genotypes are interacting the problems are enhanced in a more than additive fashion. Thus, as demonstrated many times, for many diseases, use of a single, genetically pure pathogen is essential for successful analysis of host-pathogen genetics.

The items above can be controlled but if maize-MDMV genetic experiments are to be conducted in the field, many factors are beyond control. From year-to-year and location-to-location, temperature, light intensity, edaphic factors and other influences affect the expression of MDMV symptoms and, consequently, the genetic conclusions. Secondary genes in both host and pathogen may unpredictably modify expression of major genes. Thus, a uniform environment, a uniform pathogen, and a uniform system of recording of data are needed if the genetics of this relationship is to be understood either at the classical or molecular genetic level.

Stripes and the fate map concept in the maize-MDMV relationship

--C. W. Roane and S. A. Tolin

In our paper on inheritance of reaction to maize dwarf mosaic virus (MDMV) in crosses of the resistant inbred line Oh7B with two susceptible lines (Roane, Tolin and Genter, Phytopathology 73:845-850, 1983), we described a scale for recording the symptoms induced by the virus. Our scale had 7 categories. Here we are concerned with reaction types 2, 3, and 4. In type 2 plants, narrow stripes of mosaic tissue occur on leaves below the ear; in type 3 plants, narrow stripes occur in leaves above the ear; and in type 4 plants, narrow stripes occur in leaves above and below the ear. We reported that nine F2 plants scored in the 2 to 4 range. Upon selfing, in F3 two plants were homozygous resistant, six were considered heterozygous and one produced a majority of susceptible plants. Prior to our report, plants with any expression of symptoms had been regarded as susceptible but we took the approach that if a plant restricted the development of symptoms, it must have some resistance and restrict replication or movement of the virus. We also suggested that striping might occur in resistant plants if somatic mutations for susceptibility occurred early in plant development to produce cells which permitted virus replication. If this were true, striping might conform to the fate map concept proposed by Walbot et al. (Proc. 34th Annual Corn and Sorghum Conf. 92-103, 1979). To gain evidence for somatic mutations, in 1985 we selfed a number of plants in some breeding lines which displayed symptoms in the 2-4 range. Type 2 and 4 plants were of special interest because the susceptible tissue might also extend into the ear. If such were the case, by dissecting ears such that pairs of kernels originating from the same spikelet and rows of spikelets were harvested as units, we could plant, inoculate and observe to see if a higher frequency of susceptible plants could be obtained from some pairs of kernel rows. Eight type 2 and type 4 plants were selfed. The ears were harvested and dissected and kernels planted as described above. Oh7B was the source of resistance but the plants were of different and complex pedigrees. We expected that, if our assumption that a somatic mutation for susceptibility occurred and extended into the ear, most rows of spikelets would produce a low frequency of susceptible plants and one or two rows of spikelets would produce a high frequency of susceptibles.

The results did not support our assumption. Susceptible plants were randomly distributed and we could find no evidence that a putative somatic mutation extended into the ear. Yet this assumption seems logical to us because the patterns of striping are not random. If blades may be described as left and right in their alternate phyllotaxy, stripes on the right side appear on the same side of the midrib as do those on the left side. In addition, the stripe of mosaic tissue usually extends halfway between adjacent secondary veins, indicating a presence of the virus in particular secondary veins and, consequently, in particular stalk vascular bundles. These should extend into the ear. Our limited observations do not support the assumption but perhaps experiments designed to test the assumption might be more conclusive.

Tillering and resistance to MDMV

--C. W. Roane and S. A. Tolin

In our work on genetics of breeding for resistance to maize dwarf mosaic virus (MDMV), we observed that there is a strong tendency toward tillering (suckering) in some segregating populations. Usually tillering occurs in relatively thin stands when there has been excellent distribution of moisture prior to anthesis. The year 1985 was such a year and it was noted that the reaction of a tiller to MDMV was sometimes different from that of the main stalk. In F1 of crosses of MDMV-resistant x susceptible lines where resistance was dominant, 51 tillered plants were observed; main stalks and tillers were healthy (= reaction type 1) in 46 plants. The other five plants, a through e, were scored main stalk and tiller, respectively, a-1,4; b-1,6; c-1,7; d-2,1; e-6,1 (1 = healthy, 2 = narrow stripes of mosaic tissue below ear, 3 = narrow stripes above ear, 4 = narrow stripes most leaves, 5 = broad stripes or mosaic below the ear, 6 = broad stripes or mosaic above the ear, 7 = broad stripes or mosaic above and below the ear; only types 6 and 7 are considered to be susceptible). Plants a-d were considered resistant by virtue of their main stalk behavior, but the tillers of plants b and c created an element of doubt. Plant e was in a row behaving atypically for its cross, was presumed to be a rogue because resistance of the resistant parent, Oh7B, is completely dominant and F1 plants remain healthy (= type 1). However, it illustrates what may happen. In F1 of crosses of resistant x resistant lines, 106 tillered plants were observed. All main stalks and tillers were healthy. Thus, only plants heterozygous for resistance behaved inconsistently.

The genetics of MDMV reaction has proven to be a difficult and controversial problem. When one is trying to determine the number of genes contributing to resistance, inconsistency of main stalk-tiller behavior, if of high frequency, can create yet another problem for the researcher. Fortunately, in our case the frequency of inconsistency was very low. In maize breeding, tillered plants are usually discarded; in genetics they must be regarded. Our observations raise the interesting question that if resistance of a plant is not expressed in its tillers, would it be expressed in plants produced by clonal propagation?

BLOOMINGTON, INDIANA
Indiana University

Transposons and paramutation

--Drew Schwartz

Paramutation, as defined by Brink, is a directed heritable change in one allele induced by the presence of a second allele in a heterozygote. According to this definition, the reactivations of inactive, methylated forms of transposable elements induced by the introduction of an active element into the genome, such as has been described for *Spm*, *Mu* and *Ac*, also represent cases of paramutation. Homologous transposable elements must be considered alleles even if they have been transposed to other sites in the genome. This communication is concerned with the direction of the paramutational change. Whereas, in the case of non-jumping genes, such as *R* and *B*, the change is from the active to the inactive condition, the direction is the reverse for the transposable elements, from inactive to the active state. I suggest that all the cases of paramutation involve the same basic mechanism; that *R* and *B* paramutations also involve transposable elements and the direction of the change depends upon whether one scores the state of the element or the state of the gene locus in which the element resides. This hypothesis is based on the principle that the presence of a transposable element in a gene locus can suppress the function of the gene when the element is in the active state, but that the same gene can be functional when the element is in the methylated, inactive condition. The inverse relationship between the functioning of the *Wx* gene and the methylation state of the *Ac* element in the *wx-m7* allele is a prime example (McClintock, Carnegie Inst. Wash. Year Book 64:527-536, 1965; Chomet et al. EMBO J. 6:295-302, 1987). The hypothesis will be developed for paramutation at the *B* locus but the same scheme could apply for *R*. I propose that there is a methylated, inactive form of a transposon-like element in the *B* locus. The transposon is also defective in that although it has the potential to make transposase it itself cannot transpose, possibly as a result of a mutation in one of the termini. In the paramutated *B'* allele, the element is in the non-methylated, active form, causing a shut-down in the functioning of the *B* gene. Activation of the methylated transposon in the *B* allele, induced by the transposase produced by the active element in *B*, would cause the *B* -> *B'* paramutational change observed in *B/B* heterozygotes. This requires 100% activation of the inactive transposon and a report of such a high level of reactivation for *Ac* is in press. In the case of *R*, the presence of a transposon in the paramutagenic *R-st* allele is indicated by its variegated phenotype.

Further studies on K10-I and K10-II

--M. M. Rhoades and Ellen Dempsey

Abnormal chromosome 10, type I, in addition to its primary effect as an inducer of neocentromeres and preferential segregation, also influences crossing over in sensitive regions of the genome. For example, Nel (Theor. Appl. Genet., 1973) reported an increase from 6.1% to 16.9% for recombination in the *A2-Bt* interval of chromosome 5 when K10-I was present. An attempt to localize the enhancement effect to a particular region of the foreign chromatin of K10-I was made by J. Miles (Indiana University Ph.D. Thesis, 1970). She found that various derivative chromosomes 10, missing portions of the large knob, still increased recombination in chromosome 9 (*sh-wx*) and in chromosome 3 (*gl6-lg2-a*). One derivative, K°VII, which retained the differential segment possessing three prominent chromomeres but lost all of the large knob, was no longer able to affect crossing over.

We have recently confirmed the Miles finding, making use of a well-studied chromosome (Df(H)) derived from K10-I. This chromosome possesses the differential segment, as well as the inverted distal region homologous to a segment of normal 10, but is missing the *Sr2* locus and the large knob. We chose the sensitive *A2-Bt* region including the centromere of chromosome 5 to study recombination. The data are given below:

Female parent	*A2 Bt*	*A2 bt*	*a2 Bt*	*a2 bt*	Total	*A2-Bt*%
R N10/*R* N10	847	42	58	814	1761	5.7
r Df(H)/*R* N10	1180	83	65	1190	2518	5.9

The control and experimental plants were identified by testing full siblings as the male parent in crosses with *r*-tester females. The closely linked *r* allele marks the Df(H) chromosome and those plants heterozygous for Df(H) segregated for *R* and *r* in testcrosses. The data indicate the crossover enhancement factors found in K10-I are not located in the differential segment since Df(H) shows no increase in *A2-Bt* recombination over the control.

The K10-II chromosome, transferred from teosinte to maize, was also tested for its ability to increase crossing over. K10-II differs structurally from K10-I, both in the differential segment and in the distal knobbed segment (see MNL 62:33, 1988). K10-II is preferentially segregated to the basal megaspore in chromosome 10 bivalents heterozygous for K10-II and N10. It also causes preferential segregation of the chromosome 9 with the large knob in bivalents of K^s9/K^L9 constitution. In these respects, K10-II closely resembles K10-I. The question remained whether K10-II also possesses the crossover enhancement factors. The data presented below address that question.

Female parent	*A2 Bt*	*A2 bt*	*a2 Bt*	*a2 bt*	Total	*A2-Bt%*
R N10/*R* N10	260	18	16	280	574	5.9
r K10-II/*R* N10	304	74	60	310	748	17.9

While the data are not as extensive as one might wish, the K10-II clearly causes a considerable increase in *A2-Bt* recombination in chromosome 5. The extent of the increase is very similar to that found by Nel for K10-I. Only in one functional aspect do the two chromosomes differ. The K10-II chromosome is pollen-transmitted more successfully than is K10-I when each is opposed by a normal chromosome 10 (MNL 62:33). No explanation is at hand to account for this difference.

BOMBAY, INDIA
Bhabha Atomic Research Centre

A case of somatic instability at *C-I*

--Shehalata B. Allagikar, S. E. Pawar and N. K. Notani

We reported a case of somatic instability for aleurone color in a local maize (MNL 61:26) (Fig. 1). Testcrosses with recessive anthocyanin markers (*a*, *a2*, *c1*, *c2* or *r*) and with a local *A C R* stock suggested that the instability is at *C-I*, on the short arm of chromosome 9. The plants raised from the dotted kernels after selfing showed segregation in an approximate ratio of 1 colored:2 dotted:1 colorless, indicating that the mottling is expressed in heterozygous state (*C-Im/C*).

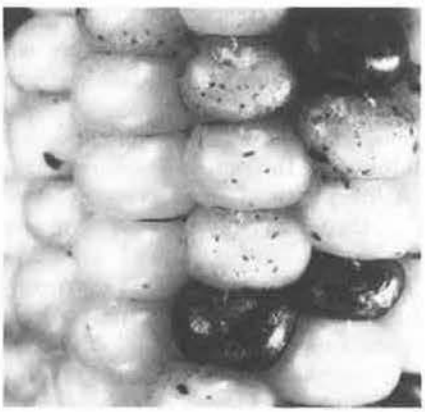


Figure 1.

Genetic tests were conducted to detect the presence of transposable elements in the unstable lines with *Ac* and *En* testers. Results obtained indicated the presence of *En*.

To detect the presence of *En* at the DNA level, genomic blots were prepared using DNA extracted from dotted kernels. This DNA was cut with the restriction enzyme *Eco*R1. The plasmid containing *En* element, provided to us by Prof. Dr. H. Saedler, was also cut with *Eco*R1. A 3kb fragment was isolated and used as a probe. Southern hybridization revealed an intense hybridizing signal at 3kb position indicating the presence of *En* element.

Breeding behaviour of shrunken kernels

--Shehalata B. Allagikar, S. E. Pawar and N. K. Notani

In the above unstable cob, occasionally a few shrunken kernels appeared. These upon self-pollination did not give fully shrunken kernels. In fact, most of them phenotypically appeared to be like wildtype. However, in testcrosses to *sh1* stock, all the progeny kernels were fully shrunken (Fig. 2). Thus Bombay *sh1* allele (*sh1-B*) is somewhat different from the standard *sh1* allele in expression.

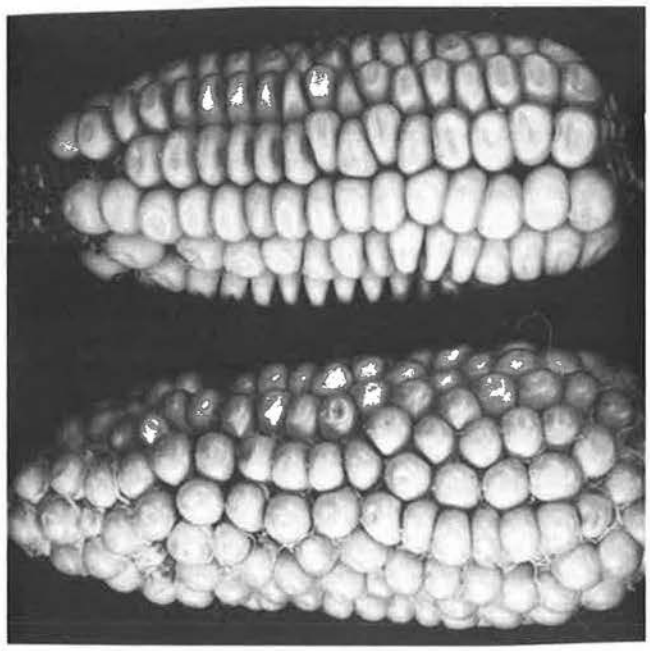


Figure 2.

Sucrose synthetase activity in developing kernels

--Shehalata B. Allagikar, S. E. Pawar, R. K. Mitra and N. K. Notani

Developing kernels of normal, standard *sh1* and *sh1-B* kernels at 10, 14, 16 and 22 days after pollination were harvested. Enzyme activity was assayed in the direction of sucrose synthesis. Peak sucrose synthetase activity was noted at 16 days after pollination. However, the enzyme activity

was markedly reduced in *sh1-B* and standard *sh1* phenotypes. The peak activity for *sh1-B* and *sh1* phenotypes was 57% and 40%, respectively, of the wildtype.

BOZEMAN, MONTANA
Montana State University
MADISON, WISCONSIN
University of Wisconsin

Inheritance patterns of *bz-m13* "*CS1*-like" phenotypes suggest asymmetric, strand-specific DNA methylation

--Victor Raboy and Oliver E. Nelson Jr.

The *bz-m13* allele of the bronze-1 (*bz*) locus contains a 2.2kb defective Suppressor-mutator (*dSpm*) insertion in the second exon of *bz*. In the presence of an active, standard *Spm* (*Spm-s*), the *dSpm* insertion in *bz-m13* is excised early during development, resulting in 1) large revertant sectors in the aleurone and 2) high rates of germinal excision. Six change-in-state (CS) derivatives of *bz-m13* have been isolated and studied in some detail (Schiefelbein et al., PNAS 82:4783, 1985). Their *dSpm* inserts, in response to *Spm-s*, are excised later in development, resulting in characteristically small revertant sectors (spots) in the aleurone and few if any germinal excisions. A molecular analysis revealed that five of the six CS's *dSpm*s were deletion derivatives of *bz-m13*'s *dSpm*. The sixth CS, *bz-m13CS1* (*CS1*), is identical in sequence to *bz-m13*. The delay in timing of *dSpm* excision characteristic of this CS is attributable to the fact that certain sequences within *CS1*'s *dSpm* are highly modified, whereas they are not in *bz-m13*. The DNA modification probably consists of methylation of cytosine in CG and CXG sequences. The sequence-specific DNA modification we observe and its relationship to the activities of *bz-m13* and *CS1* is very similar to that found between active and inactive *Spm* elements as described by Banks and Fedoroff (MNL 62:27).

To test the correlation between DNA modification and the *CS1* phenotype we sought to isolate a number of novel "*CS1*-like" derivatives. Plants of the genotype *bz-m13*/*bz*; *Spm-s*/*Spm-s* were crossed by and on tester stocks of the genotype *bz*/*bz*; *Spm-s*/*Spm-s*. New isolates appeared either as 1) one or a few individual kernels on a testcross ear, representing events which occurred relatively late during development, or as 2) the result of an event which occurred relatively early during development, so that all male and female gametes receiving the *bz-m13* allele contained *CS1*-like derivatives. In 1985, five of 82 plants tested gave this latter result. In one exceptional case, the testcross of 85510-5, approximately half of the variegated kernels displayed a *bz-m13*-like aleurone phenotype (few large sectors), and half displayed a *CS1*-like phenotype (numerous small sectors) (Table 1). How is it that when we testcross a plant of the genotype *bz-m13*/*bz*, we observe two variegated phenotypes in approximately equal numbers?

Kernels representing each of these two classes were grown out in 1986 and crossed onto *bz*/*bz* tester plants that were either homozygous for *Spm-s* (+*Spm-s*) or lacking in *Spm-s* activity (-*Spm-s*) (Table 1). It is relatively easy to distinguish the difference in phenotype between *bz-m13* and *CS1* when the endosperm contains one copy of a given allele, but difficult to distinguish when it contains two copies. Regardless of tester, plants grown from kernels that were *CS1*-like (86181-3, -5, -6) reproduced the initial result observed with 85510-5. With plants grown from kernels that were *bz-m13*-like (86181-9, -10, 11, -14), essentially all progeny receiving the *bz-m13* allele were *bz-m13*-like.

Table 1. Testcross analyses of the initial exceptional plant 85510-5 and its variegated progeny.

Plant[a]	Aleurone Phenotype[b]	bz Tester[c]	Total Progeny	Progeny Aleurone Phenotypes[b] bronze	Purple	Variegated Sectors	Spots
Initial Observation: Testcross of 85510-5							
85510-5	sectors	+Spm-s	218	124	9	44	41
Tests of 85510-5 Testcross Progeny							
86181-1	spots	-Spm-s	160	87	1	40	32
86181-3	spots	-Spm-s	162	90	2	35	35
		+Spm-s	90	46	4	24	16
86181-5	spots	+Spm-s	121	70	4	20	27
86181-6	spots	-Spm-s	247	141	11	45	50
		+Spm-s	95	52	3	22	18
86181-9	sectors	-Spm-s	110	63	12	35	0
		+Spm-s	101	71	1	29	0
86181-10	sectors	-Spm-s	215	125	16	74	0
		+Spm-s	168	105	11	52	0
86181-11	sectors	+Spm-s	141	88	18	35	0
86181-14	sectors	+Spm-s	95	56	2	33	4

[a] Plants were of the genotype bz-m13/bz:Spm-s/Spm-s and were used as pollen parents in testcrosses onto the indicated testers.
[b] Spots: numerous small sectors typical of bz-m13 CS1. Sectors: few large sectors typical of the initial state of bz-m13.
[c] +Spm-s: bz/bz tester plants homozygous for an active Spm-s. -Spm-s: bz/bz tester plants containing no Spm-s activity.

Our first hypothesis was that either an altered *Spm* element such as an *Spm-weak*, or a dominant modifier of *Spm-s*, was segregating independently of *bz*, and was responsible for this unusual pattern of inheritance. We know that *bz-m13*, in response to an *Spm-w* or *En-low*, is excised late during development, mimicking the *CS1* phenotype. We therefore tested a total of 34 *bz* kernels from the testcrosses of 85510-5, 86183-3 and 86181-6 for the presence of a modified *Spm* or a dominant modifier of *Spm-s*. The results indicate that these plants were homozygous for *Spm-s*. No altered *Spm* or modifier of *Spm* was found. Thus the phenomenon responsible for this exceptional pattern of inheritance appears to be specific to the *bz* locus.

Our current hypothesis is that this phenomenon is an outcome of strand-specific, asymmetric DNA methylation. Such a phenomenon has been proposed as responsible for the developmental asymmetry of daughter cells in fission yeast (*Schizosaccharomyces pombe*) (Klar, Nature 326:466, 1987). Perhaps the interaction of *Spm*-encoded products and DNA methylases results in this strand-specific modification. The separation of chromatids following the second division of meiosis might then produce two daughter cells which contain the same *bz-m13* sequence, but which represent different patterns of methylation, which in turn results in the different phenotypes.

A second possibility is that the modification event occurs frequently following the second microspore division, resulting in a high rate of noncorrespondence in DNA methylation pattern between germ and endosperm, coincidentally approximating 1/2 of *bz-m13* progeny. Subsequent tests of 86181-3 and 86181-6 progeny have not ruled out this possibility. *CS1*-like kernels from testcrosses of these two plants mostly produce all *CS1*-like progeny, and occasionally reproduce the results observe with 85510-5. *bz-m13*-like kernels from these two plants often produce only *bz-m13*-like progeny, but occasionally produce *CS1*-like progeny and combinations of the two. Whatever the mechanism, this phenomenon is not unique to 85510-5 and

its progeny. We have identified several additional independent cases.

BOZEMAN, MONTANA
Montana State University
ST. PAUL, MINNESOTA
University of Minnesota

Molecular evidence for the hybrid origin of *Tripsacum andersonii*

--Luther E. Talbert and John F. Doebley

The genus most closely related to *Zea* (x=10) is *Tripsacum* (x=18). At least thirteen species are recognized in *Tripsacum*. Although hybrids between *Zea* and *Tripsacum* species have been produced experimentally, good evidence for natural hybridization is wanting. The cytology of *Tripsacum andersonii* provides some evidence for hybridization. This species contains 2n=64 chromosomes, and is hypothesized to contain three genomes from *Tripsacum* and one genome from *Zea*.

Two lines of evidence have suggested that *Mu* transposable elements may be useful as molecular markers to monitor hybridization between *Zea* and *Tripsacum*. First, *Mu* elements are dispersed throughout the *Zea* genome, and approximately 40 elements may be visualized using a probe specific for the *Mu* termini (e.g. Chandler et al., Genetics 114:1007-1021, 1986; Talbert et al., J. Mol. Evol., in press). Second, our results suggest that *Mu* elements may have become a component of the *Zea* genome after the divergence of the genera *Zea* and *Tripsacum* (Talbert and Chandler, Mol. Biol. Evol. 5:519-529, 1988). Thus, the presence of *Mu* elements in the genome of a *Tripsacum* species would be strong evidence for hybridization with *Zea*.

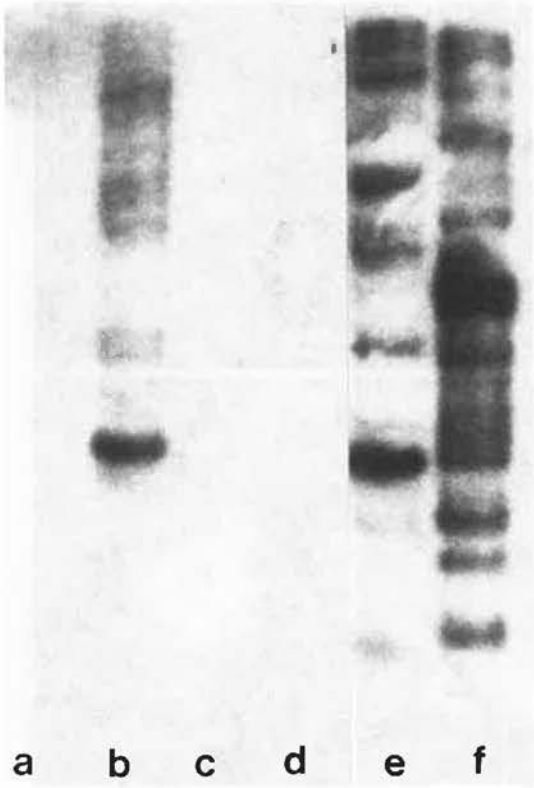


Figure 1. Southern blot with *Tripsacum* and *Zea* DNAs restricted with *Eco*RI/*Hin*dIII and hybridized to a probe for the *Mu* termini. Lane a: *Tripsacum dactyloides*, b: *Tripsacum andersonii*, c: *Tripsacum pilosum*, d: *Tripsacum peruvianum*, e: *Zea luxurians*, f: *Zea mays* ssp. *mays*.

A Southern blot with DNA from several *Zea* and *Tripsacum* species hybridized to a probe for the *Mu* termini is shown in Figure 1. Hybridizing sequences are seen in the *Zea*s and *Tripsacum andersonii*. No hybridizing sequences are seen in the other *Tripsacum*s. These data strongly support the hypothesis that *Tripsacum andersonii* is a hybrid with a *Zea*. Apparent similarity of banding patterns for the *Mu* termini in *T. andersonii* (lane b) and *Z. luxurians* (lane e) provides preliminary support for our morphological evidence that *Zea luxurians* was the *Zea* species involved. Studies are currently underway to confirm this by determining the numbers and types of *Mu* elements present in the respective genomes. Additionally, analysis of maternally inherited chloroplast DNA sequences may enable us to determine the maternal parent of this *Tripsacum-Zea* hybrid.

BUFFALO, NEW YORK
State University of New York
ROCHESTER, NEW YORK
University of Rochester

The use of x-ray contact microradiography in the study of silica deposition in the leaf blade

--Ping-chin Cheng and Hyo-gun Kim

The ability to absorb soluble silica from the soil, and subsequently translocate and deposit it in various parts of the plant (Lanning and Linko, J. Agric. Fd. Chem. 9:463, 1961) in the form of $SiO_2.nH_20$, has been observed in many species, but particularly in grasses, for decades (Jones and Handrek, Adv. Agron. 19:107,1967). It was reported that silicon in living rice plants is present in three basic forms: 1) insoluble silica (90%), 2) silicate ions (0.5-8%) and 3) colloidal silicic acid (0-3.3%). Furthermore, the mobility of silicon in rice plants is poor. In other words, reutilization of silicon, once deposited in plant tissue, is very unlikely to occur (Yoshida et al., Soil Sci. Plant Nutrition 8(3):15, 1962). The insoluble silica in plant tissue is generally a clear, colorless and isotropic deposit with an index of refraction of 1.42 to 1.44 (Jones and Handreck, 1967). X-ray diffraction studies revealed an amorphous pattern similar to opals. Because of these physical properties, silica deposits in plants are believed to be similar to opal minerals; hence, they are frequently referred to as being biogenic opals.

Jones and Handreck (1967) pointed out that the deposition of silica in plant tissue cannot be readily studied under the microscope without special treatment of the plant material. Traditionally, six methods are used for the microscopic determination of silica deposits in plants. They are: 1) formation of sodium silicofluoride crystals by treating the tissue with NaCl and HF; 2) wet ashing; 3) dry ashing (spodogram); 4) staining with basic fuchsin, safranin-phenol and malachite green; 5) hydrofluoric acid etching of plastic-embedded tissue (Yoshida et al., 1962); and 6) mounting the tissue in a high refractive index medium. Recently, Dayanandan et al. (Amer. J. Bot. 70:1079, 1983) reported the use of histochemical reactions based on the reactivity of the silanol (SiOH) group of silica and the use of polarized microscopy to detect a special type of silica body (phytoliths) in plants. The methods listed above involve ei-

ther harsh chemical treatment or high temperature ashing of the tissue. In addition, x-ray microanalysis (EDS) has been used extensively in the detection and mapping of mineral deposits including silica. The EDS method works well on detecting silica on the surface of bulk specimens, sectioned or fractured sample. However, the EDS technique is restricted to the very surface layer of the specimen. Therefore, detection of silica in deeper layers of tissue is not possible. Due to the deeper penetration, back scattered electron (BSE) imaging in a scanning electron microscope has also been used to image silica cells.

Due to the properties of x-rays and the availability of various high intensity x-ray sources in recent years, x-ray microscopy becomes a useful tool in the study of silica deposits in plants. We report here the use of x-ray contact microradiography using a pulsed x-ray source for the study of the silica deposition in the leaf blade of corn. This method not only allows the examination of silica deposition in dry specimens, but most importantly, it makes imaging of living specimens possible (X-ray Microscopy, eds. P. C. Cheng and G. J. Jan, Springer-Verlag, 1987). In contrast to conventional electron beam microanalysis (EDS), which is restricted to the very surface layer of the sample, x-ray imaging allows the study of silica deposits deep in the tissue.

The maize used in this study was greenhouse grown plants of Golden Beauty. Either fresh leaf blade or aldehyde-fixed (Cheng et al., Natl. Sci. Counc. Monthly, ROC 7:1001, 1979) tissue were used. Fixed leaf blades were dehydrated in acetone and critical point dried in CO_2. For controls, fixed leaf blades were treated in a 5% HF solution for 48 hours to remove the silica deposits, and then washed, dehydrated and critical point dried. The silica composition of the deposits was confirmed by using energy dispersion spectroscopy (EDS) in a scanning electron microscope.

A laser-produced plasma x-ray source was used in this study. A frequency tripled high intensity Nd:glass laser beam (Glass Development Laser at the Laboratory for Laser Energetics of the University of Rochester) was focused onto a Mo thin target (Figure 1). A one nanosecond pulse was delivered to the target, which generates a high temperature (10^7K)plasma. Laser output ranging from 20 to 100J of ultraviolet radiation was used in this experiment. The x-ray source size was on the order of 100μm and the specimen was placed approximately 25cm away from the source.

The experimental set-ups are shown in Figure 1. The x-ray contact imagings of dehydrated specimens were done in vacuum, and fresh tissues were imaged under atmosphere pressure by using a 2μm thick Mylar film as the vacuum window. An Al filter (two 250nm thick Al layers evaporated on both surfaces of a 2μm thick Mylar film) was used to block the UV and visible radiation emitted from the high temperature laser-produced plasma. The samples were sandwiched between two Mylar foils, and held in close contact with the photographic plate. The x-ray contact microradiographs were recorded on Agfa-Gavaert holographic plates (emulsion 8E75); the plate has a resolution of the order of 2000 lines/mm. The holographic plates were developed in Kodak D-19 for 10 min. The contact images were magnified by using either a macrophotography set-up or a compound microscope (Cheng et al., In: Modern Microscopy, eds. P. Duke and A. Michette, Plenum Press, 1989).

The dense silica deposits in the leaf blade can be easily detected by x-ray contact microradiography (Figure 2). As revealed by the x-ray imaging technique, silica is initially deposited in specialized dumbbell-shaped silica cells (Figure

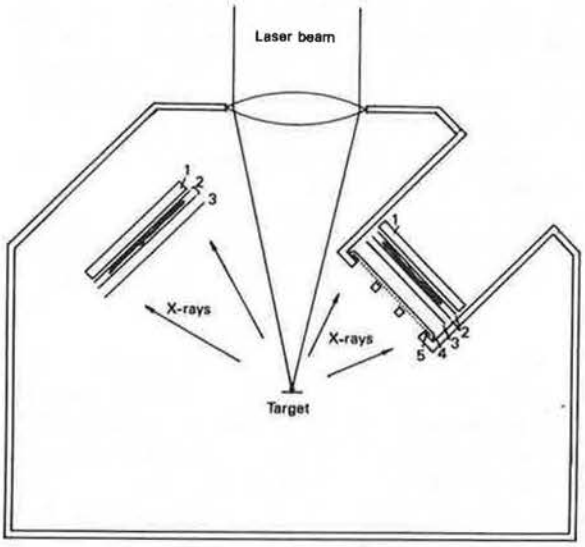
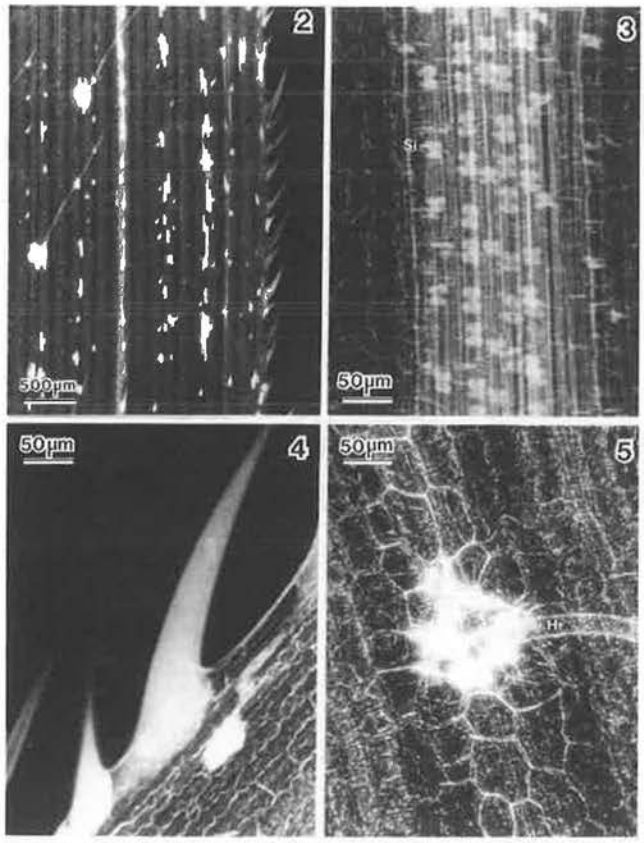


Figure 1. Diagramatic representation of the x-ray contact microradiography set-ups. Left: for the imaging of dehydrated specimen. Right: for the imaging of fresh sample. 1: film plate, 2: two sheets of Mylar film with specimen sandwiched between them, 3: Al filter, 4, 2um thick Mylar vacuum window, 5 1mmx1mm stainless screen. The target is in vacuum.



Figs. 2-5. Figure 2. X-ray contact image of a leaf blade of maize. Figure 3. Dumb-bell-shaped silica cells (Si). Figure 4. Siliceous hairs. Figure 5. Siliceous epidermal cells at the base of a hair (Hr).

3) which are in close association with the vasculature, excluding the main vein. In the mature leaf, it is common to find that silica deposits in the hairs (Figure 4), the epidermal cells surrounding the base of the epidermal hairs (Figure 5), and in selected patches of epidermal cells (Figure 2). Figure 6 shows the x-ray image of silica deposition in a fresh leaf blade.

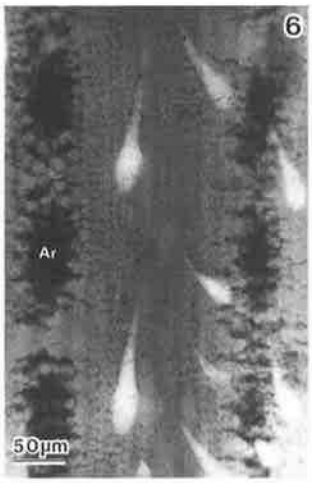


Figure 6. Siliceous hairs in a fresh leaf blade. Ar: air space in leaf blade.

The use of x-ray contact microradiography in conjunction with a high intensity x-ray source offers a new technique to study the silica deposition in fresh tissue. Technical simplicity is not the only advantage that x-ray contact microradiography can offer over other methods, the most important of which is that the technique allows the study of living specimens with a very large "field of view". At the present time, we can image a leaf area as large as 4x4cm, and the limit of the field of view is only restricted by the size of the photographic plate. Studies have shown that there is a correlation between a high concentration of silica deposits and insect resistance in many plant species. For instance, Ponnaiya (Madras Univ. Jour. XXI, Sect. B., No. 2: 203, 1951) reported that the deposition of irregular silica particles in the leaf sheath of a variety of sorghum correlated with resistance to the larva of *Antherigona indica*. High silica content in rice has also been correlated with resistance to the Asiatic rice borer, *Chilo suppressalis* (Djamin and Pathak, J. Econ. Ent., 60:347, 1967). Lanning et al. (Ann. Bot. 45:549, 1980) suggested that breeding for high silica content could be a potential factor for developing insect-resistant corn varieties. Therefore, the large "field of view" offered by x-ray contact microradiography can be used to screen large areas of leaf to obtain statistically meaningful data, such as patterns of silica cell distribution, needed in breeding programs. Furthermore, the technical simplicity of x-ray contact imaging also allows the possibility of screening large numbers of samples in a short time.

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CAMPINAS, SAO PAULO, BRAZIL
Instituto Agronomico

Resistance to Angoumois moth and corn weevil in husked ears

--Luiz Eugenio Coelho de Miranda, Luiz Torres de Miranda and Sylmar Denucci

In MNL 54:15-19 we reported on inheritance and linkages of multiple aleurone layering. Using ACRE 134, and AMAZONAS unnumbered collection, and Entrelacado from the same source, and a waxy source, versions with *Mal*, *wx* on our opaque-2 versions of IAC Maya and IAC 1 were obtained. The pedigrees simplified are *Mal wx* x IAC Maya *o2*(4) *-o2 Mal wx* and *Mal wx* x IAC 1 *o2*(4) *-o2 Mal wx*, *Mal* being "multiple aleurone layering". After the original cross and 3 backcrosses pure versions of *o2 Mal wx* were obtained. The original *Mal* collections are from a super humid very hot climate with no distinct dry season limiting for maize growing and optimum to insects. They are very badly adapted to our region down to pollen semi-sterility. In the beginning of June 5 ears of each entry of the acclimated material were husked and put in pairs touching in split plots, with their opaque-2 only versions on a shelf board, each pair about 30cm from the other in an ambient room condition. At the beginning of November the unholed and holed kernels were counted. The attack was by both *Sitophilus oryzae* or *S. zeamais* (Motschulsky), which are prevalent in our area, and *Sitotroga cerealella* (Oliver), not discriminating damage of one pest from the other. The data and analysis are presented in Table 1. For the IAC Maya

Table 1. Number of unholed (U), and holed (H) grains in the pairs studied with the calculated relevant parameters.

I-IAC Maya Mal o2 wx				IAC Maya 02			Difference	t
	U	H		U	H			
1	13	434	2.9±0.8	72	529	11.0±0.3	-9.0**±1.5	6.00**
2	160	219	42.2±2.5	200	311	39.1±2.2	+3.1±3.3	0.94
3	47	135	25.8±3.2	13	445	2.8±0.8	+23.0**±3.3	6.97**
4	3	336	0.9±0.5	15	463	3.1±0.3	-2.2*±0.9	2.44**
5	74	143	34.1±3.2	137	339	28.8±2.1	+3.2±3.8	0.84
I	297	1267	18.9±1.0	487	2087	17.3±0.8	+1.6±1.2	1.33
II-IAC 1 Mal o2 wx				IAC 1 o2				
	U	H	% U	U	H	% U	Difference	t
6	18	355	4.8±1.1	11	300	3.5±1.0	+1.3±1.5	0.87
7	17	165	9.3±2.2	65	382	14.5±2.7	-5.2±2.7	1.93
8	1	155	0.6±0.6	10	134	6.9±2.1	-6.3**±2.2	2.86**
9	138	63	68.7±3.3	120	266	31.1±2.4	+37.6**±4.0	9.40**
10	113	162	41.1±3.0	22	194	10.2±2.1	+30.9±3.6	8.58*
II	287	900	24.2±1.2	228	1278	15.2±0.9	+9.0**±1.5	6.00**
I	297	1267		437	2087			
I+II	584	2167	21.2±0.8	665	3365	16.5±0.6	+4.7±1.0	4.70**

the total effect of *Mal* over *mal* was +1.6±1.2, non-significant, but in the IAC 1 with +9.0±1.5, significant at P<0.01. In the 3 ears of each, which were better than the original opaque-2 versions, 11 grains each of both holed and unholed kernels were characterized in the number of aleurone layers by a third party. The results are presented in Table 2. Under binocular aleurone layer classification, a few kernels changed class, and that is why there are not equal numbers in unholed and holed classes.

Although we planted what we thought were pure *Mal* by analysis of 11 kernels per ear of selfed mothers, checking two or more layers, the readings gave initially 4 classes: 1) clearly only 1 layer, 2) with tendency to have more than 1

Table 2. Determination in the ear families 1, 3, 5, 6, 9, and 10 with *Mal* from Table 1 of the number of layers in the unholed (U), and holed (H) classes with the calculated relevant parameters. As seen, the families were not pure for multiple aleurone layering, perhaps because of environmental interaction effects.

(I) Maya	2 layers		1 layer		χ^2
o2 Mal wx	U	H	U	H	
1	10	1	2	9	+11.73**
3	8	3	3	8	+ 4.55*
5	10	2	1	9	+11.73**
Sum					28.01
Total	28	6	6	26	26.70**
% Unholed	82.4±6.5		18.7±6.9		
Interaction	(095>P>050)				1.31
(II) IAC 1					
o2 Mal wx					
6	2	3	6	11	+ 0.04
9	7	3	7	5	+ 0.32
10	4	7	3	8	+ 0.21
Sum					0.57
Total	13	13	16	24	0.64
% Unholed	50.0±0.8		40.0±7.7		
Interaction	(0.98>P>0.95)				0.07
I+II Total	41	19	22	50	18.72**
% Unholed	68.3±6.0		30.6±7.3		
Difference	37.7±8.1		t=4.65**		

layer, 3) clearly 2 layers and 4) with more than 2 layers. By a contingency test in the 2x4 factorial it was seen that best results were gotten by pooling 1) with 2) and 3) with 4) (Table 2). Curiously for IAC Maya, which gave a lesser effect, now we have greater significant effects and vice versa for the IAC 1. It seems clear that now selecting within a population with variability, for the stabilization and enhancement of multialeurone expression, a much greater effect will probably be achieved since now we have a mean unholed % of 68.3±6.2 for 2 or more layers as opposed to the first total of 1, 3, 5, 6, 9, 10 of 23.8±1.0 in whole segregating ears, a difference of 44.5±6.3. Parallel populations with the factors were field tested for grain yield in 4 localities with 4 reps per place and in the fourth backcross, now to the normal endosperm, yielded in IAC Maya 87% and in IAC 1 119% in relation to their counterparts.

Allometric genetics: in a factorial with results expressed in logarithms the linear effects are the coefficients in the allometry expression

--Luiz Torres de Miranda and Luiz Eugenio Coelho de Miranda

Since MNL 55:18-19 we have tried by allometrical genetics to develop algorithms more biologically minded. To begin to achieve this it is necessary to transform the original data in logarithms, but generally after this there is no change of procedure. This report deals with how the data could be analysed and interpreted. The solution of a factorial is achieved as:

	B	b
A	$(\log b_o+\alpha+\beta-\log a)^2$	+ $(\log b_o+\alpha-\beta-\log b)^2$ +
a	$(\log b_o-\alpha+\beta-\log c)^2$	+ $(\log b_o-\alpha-\beta-\log d)^2$

Where B and b are different alleles of the same locus, A and a different alleles of another locus affecting the same trait, a, b, c, d the experimental results, and b_o the center of the design in this case the mean in which to aply +1 or -1 of the linear effects. Differentiating in relation to α, and to β and equating to 0 we get

$$\frac{\partial F}{\partial \alpha} = 4\alpha-\log a-\log b+\log c+\log d=0$$

$$\frac{\partial F}{\partial \beta} = 4\beta-\log a+\log b-\log c+\log d=0$$

The solution is straighforward for each line for α and β

One sees that b_o vanishes from the solution which gives α or β

Expressing by allometric genetics the treatments are, See MNL 55:18-19) remembering that it could be expressed in relation to a center b_o, and differentiating in relation to α and β we get the same solutions as before.

	B	b
A	$2(1-\alpha-\beta)+2\alpha+2\beta=\log a$	$2(1-\alpha-\beta)+2\alpha=\log b$
a	$2(1-\alpha-\beta)+2\beta=\log c$	$2(1-\alpha-\beta)=\log d$

Taking the calculated values a and b in the factorial, we calculate $2(1-\alpha-\beta)$ as the allometric origin A of the design, and $F=A^{+\alpha x+\beta y}$ is the antilogarithmic solution of the table where $2\alpha+2\beta$ is for a, 2α for β, 2β for c and A is d, having no coefficient beyond 1 itself. This works at least with data expressed up to 100%.

Allometric genetics: a summing up

--Luiz Torres de Miranda and Luiz Eugenio Coelho de Miranda

In mathematical models measuring genetic parameters there are oversimplifications. One of them is *a*, in genetics half of the effect of the difference between the homozygotes from the same locus. The classic definition is a point in space time. To give *a* greater dimension it is necessary to express it in angle terms, which is possible by the relation of allometry.

The whole work consists of 6 theorems.

1) Graphically it was shown that for $X=AY^{\alpha}$, with $X'=X\div Y$, $X'=AY^{\alpha-1}=(100\div Y)^{1-\alpha}$ and that A is the antilogarithm of $2(1-\alpha)$, MNL 55: 18-19.

2) In quantitative genetics a, d.a, and -a, become $1-\alpha$, $\delta(1-\alpha)$ and $\alpha-1$, MNL 55: 18-19.

3) For the calculus of linkages the allometric coefficients are put in the theoretical frequency expectations as expoentes and equated to the observed ones, MNL 61:32.

	q	b
A	$\left(\frac{1-p}{2}\right)^{1+\alpha+\frac{\beta}{a}}=\frac{b}{n}$	$\left(\frac{p}{2}\right)^{1+\alpha-\frac{\beta}{b}}=\frac{b}{n}$
a	$\left(\frac{p}{2}\right)^{1-\alpha+\frac{\beta}{c}}=\frac{c}{n}$	$\left(\frac{1-p}{2}\right)^{1-\alpha-\frac{\beta}{d}}=\frac{d}{n}$

The logarithm maximum likelihood expression becomes

$$L=\frac{a}{n}(1+\alpha+\beta)\ln\frac{1-p}{2}+\frac{b}{n}(1+\alpha-\beta)\ln\frac{p}{2}+\frac{c}{n}(1-\alpha+\beta)\ln\frac{p}{2}+\frac{d}{n}(1-\alpha-\beta)\ln\frac{1-p}{2}$$

And the solution is

$$p=\frac{b(1+\alpha-\beta)+c(1-\alpha+\beta)}{a(1+\alpha+\beta)+b(1+\alpha-\beta)+c(1-\alpha+\beta)+d(1-\alpha-\beta)}$$

And the variance of $p=-1\div\frac{\delta^2 L}{\delta p^2}$ as usual

4) The variance of α and β can not be obtained directly since the equation does not follow the Cramer-Rao inequality. The solution is by Fisher indirect method of calculating the variance of one parameter in funtion of the variance of another. It turns out the direct solution as being $V_{(\tilde{\alpha})}=\left\{\Sigma_1^4\ 1/(\delta p/\delta\alpha)^2\right\}^{\frac{1}{4}}$, MNL 62: 37-38

5) It was shown that A is the constant of integration C, MNL 61:31

6) Transforming the values of p in cM (Haldane) and than calculating the distances by differences increases enormously the resolution power of the analysis. This is shown in various examples involving the elusive pd and tr factors, MNL 61:29-35 which determine the kernel row number in maize.

7) Last but not least, in this number it is demonstrated how the linear effects in a factorial in logarithms can be interpreted as the allometrical coefficients of A. A being the antilogarithm of 2(1-a-ß). This permits the summing up of different sets of data.

In all here are the tools to link RFLP and QTL.

Allometric genetics and linkages of teosinte characters in 4S

--Luiz Eugenio Coelho de Miranda and Luiz Torres de Miranda

W. C. Galinat (MNL 49:102) reported linkages of two new genes, *Ph* and *Ri*. The recombinational distances were *Ph*--27--*Ri*--39--*Su*--20--*Gl3*. Taking *Ph* as the 0 (zero) position *su1*, the reference gene, was put in position 66=(27+39). Transforming the p values into Haldane's centimorgans we would have *Ph*--31--*Ri*--50--*Su*--22--*Gl3*, with *Su* in the 81 position. We think this is not the most probable position. Taking Haldane values and obtaining the new values, appropriately paired, we use all six values of cM for the estimates; thus with the original data the distance *Su Gl3* is obtained as:

Ph Gl3	45	66.0	-	*Ph Su*	43	59.6	=	*Su Gl3*	6.4
Ri Gl3	40	52.2	-	*RiSu*	39	50.0	=	*Su Gl3*	2.2
									8.6/2=4.3±1.9

And with the same procedure *Ri Su*=29.8±1.7 and *Ph Ri*=11.7±2.0, which leads to *Su* in position 41.5. Calculating p by the product moment method we arrive at *Ph*--10.6--*Ri*--30.7--*Su*--4.2--*Gl3* and *Su1* would be in position 41.3 with *Ph* at the zero position. The new value cM=4.2-4.3 for *Su Gl3* strikingly brings out the proposed interference of a heterozygous inversion; it would be *Su Gl3* p=0.34 with cM=42.6 without interference. This fact could be used to quantify inversion and translocation effects by developing a theoretical model. The new rearrangement also would bring to closer position the teosinte factors *pd4* and *tr4*, which we proposed (MNL 61:32-34) to be in positions 3 and 7 respectively considering *Ts5* to be in 53 position.

CAMPINAS, SAO PAULO, BRAZIL
Universidade Estadual de Campinas

Relationship between zein and coixin

--Laura M. M. Ottoboni, Adilson Leite, Maria Luiza N. Targon, Marcio J. DaSilva and Paulo Arruda

The prolamins from seeds of maize and Job's tears (*Coix lachryma-jobi* L.) were extracted with 55% isopropanol containing 2% of 2-mercaptoethanol and subjected to SDS-PAGE on gradient gels. The prolamins of *Coix* were subdivided into five bands namely, C1 - 27kDa, C2 - 25kDa, C3 - 22kDa, C4 - 17.5kDa, and C5 - 15kDa (Fig. 1A, lane 1). These coixin bands are in the same molecular weight range as zeins which were subdivided into the well-known molecular weight classes of 27kDa (Z1, gamma zein), 22kDa (Z2, alpha zein), 19kDa (Z3, alpha zein) and 15kDa (Z4, Z5, Z6, Z7, beta zein) (Fig. 1A, lane 2).

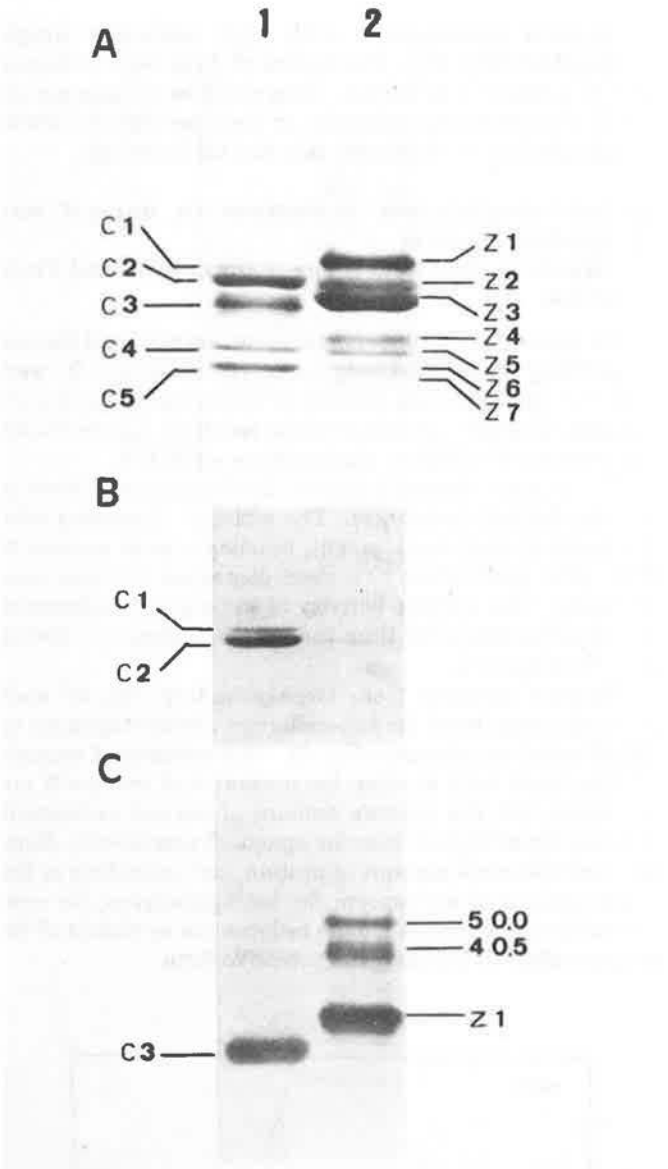


Figure 1. SDS-PAGE and Western blots of zein and coixin extracted from endosperms of maize var. Maya and *Coix lachryma-jobi* var. Adlay. (A) SDS-PAGE of coixin (lane 1) and zein (lane 2). (B) Western blot of coixin (lane 1) and zein (lane 2) of replicate gel from (A) using C2 antiserum. (C) Western blot of coixin (lane 1) and zein (lane 2) of replicate gel from (A) using C3 antiserum.

Polyclonal antibodies raised against coixin C2 and C3 were used to study the immunological properties of coixin and zein. Two replicate gels containing the same samples as in Fig. 1A were transferred to nitrocellulose sheets and blotted separately with C2 and C3 antiserum. C2 antiserum recognised both C1 and C2 coixins (Fig. 1B, lane 1), but did not cross-react with any zein band (Fig. 1B, lane 2). C3 antibodies reacted with the C3 coixin and cross-reacted with a 40.5kDa protein present in the *Coix* extract (Fig. 1C, lane 1). Surprisingly the C3 antibodies cross-reacted strongly with gamma zein and also with proteins of 40.5 and 50.0kDa present in the maize extract (Fig. 1C, lane 2).

The coixins and zeins were extracted with 55% isopropanol with and without 2-mercaptoethanol and analysed by SDS-PAGE. Both C3 and gamma zein presented similar behaviour as they were not extracted in the absence of the reducing agent. A further similarity in the properties of the gamma zein and C3 is that antibodies raised against

C3 protein cross-reacted with high molecular weight polypeptides (Fig. 1C). The nature of these high molecular weight proteins is unknown. They could be an aggregation of C3 or gamma zein subunits, or genuine high molecular weight proteins with gamma zein and C3 homology.

Lysine-ketoglutarate reductase in normal and opaque-2 endosperm

--Marcia R. Brochetto-Braga, Adilson Leite and Paulo Arruda

An inbred line, L1038, homozygous normal, and the corresponding mutant L1038, homozygous opaque-2, were used to investigate the activity of lysine-ketoglutarate reductase. The enzyme catalyses the reaction: Lysine + α-ketoglutarate + NADPH to saccharopine + NADP+.

The enzyme showed a typical developmental pattern of activity for both genotypes. The activity increased with the onset of seed development, reached a peak around 20 days after pollination and then decreased towards seed maturity. The enzyme activity of the mutant endosperm was 2 to 3 times lower than the normal endosperm during seed development.

Enzyme extracts from twenty endosperms of each genotype were used for ion-exchange chromatography on DEAE-cellulose columns (Fig. 1). The patterns of enzyme elution were very similar for normal and opaque-2 endosperms, but the enzyme amount of normal endosperm was two times higher than the opaque-2 endosperm. Since we used the same amount of protein, corresponding to the same amount of endosperm for both genotypes, we concluded that the opaque-2 gene reduces the synthesis of the enzyme when in homozygous recessive form.

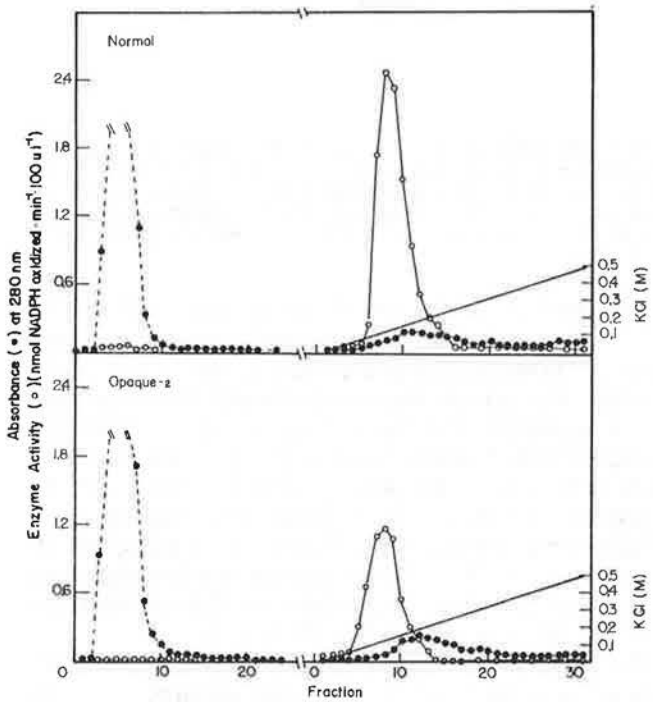


Figure 1. Elution patterns of enzyme activity and protein from DEAE-cellulose columns: (o—o) lysine-ketoglutarate reductase activity; (•—•) protein measured by absorbance at 280 nm; (—) potassium chloride concentration gradient.

Polyclonal antibodies raised against the purified enzyme from normal endosperm were used to study the immunological properties of normal and mutant enzymes. Western blot analysis of purified extracts from both genotypes revealed a single common band of 134kDa. Based on these results we concluded that the lysine-ketoglutarate reductase gene is under the transcriptional or translational control of the opaque-2 gene.

CHAPINGO, MEXICO
CIFAP

A realistic model for selection limit in maize

--Fidel Márquez-Sánchez

With mathematical formulae based upon gene frequencies, the theoretical selection limit in allogamous plants is asymptotically reached at generation infinity. There, frequencies of the favourable alleles of the involved loci are supposed to be one. However, because linkage fixation occurs for both favourable and unfavourable alleles, there is more on the former than in the latter. This is why breeders try to avoid inbreeding by recombining selected plants at the end of each selection cycle. As in most autogamous plants recombining is not possible, inbreeding can not be avoided, thus the result of selection are superior inbred lines chosen among large numbers of them.

On the other hand, as massive crossing between populations in maize is quite possible, inbreeding is used as a means of utilizing heterosis by crossing inbred lines. Such utilization may be through either different types of F1 hybrids or through multi-line F2-synthetics.

If an F1-synthetic is subjected to random mating inbreeding depression occurs depending mainly on the degree of inbreeding of parental lines and on their number (S. Wright, USDA Bull. 1121, 1929; Gilmore, Crop Sci. 9:102, 1969; Busbice, Crop Sci. 10:265, 1970). For diploid plants the smaller the inbreeding of the parental lines and their number the higher the inbreeding depression (Busbice, 1970).

If selection of desirable plants could be made before pollination (called individual selection by D. S. Falconer, Intro. Quant. Genet., 1961, as opposed to mass selection), a recurrent selection process may be visualized as a cyclic F2-synthetic methodology. In each cycle the F1-synthetic is the composite of selected plants (conceptual single-cross hybrids) while the result of its random mating recombination is the F2-synthetic, called by maize breeders the ith cycle of selection (C_i). According to Wright's formula the prediction of an F2-synthetic is

$$F2 = F1 - \frac{F1 - P}{n}$$

On the other hand, any population can be considered as n/2 conceptual single-cross hybrids (the individual plants) resulting from crossing n conceptual homozygous lines (the male and female gametes); therefore if the base population, in which selection is going to be practiced is large, i.e., n is large, random mating does not cause any inbreeding depression (Márquez-Sánchez, Crop Sci. 19:439, 1979). However, as soon as selection takes place, the saved portion (selection pressure) consists of a relatively small number of plants (n is small), and inbreeding depression occurs. As

selection advances, even if the selection pressure is kept constant, the number n of involved conceptual selected lines gets progressively smaller gradually increasing the inbreeding depression.

A mathematical function that describes the F1-synthetic behaviour through selection cycles is of increasing nature, for instance, an exponential function. Its higher limit is the conceptual single cross (n = 2) of highest theoretical yield, and reaches asymptotically its lower limit, the base population, at n=∞.

In order to find out a corresponding function for the F2-synthetic Márquez-Sánchez (1979) used Wright's formula, demonstrating that both F1 and F2 functions approach asymptotically the base population (V). However, while the F1 function is of increasing nature, the F2 curve has a minimum at n - 2 and maximum at n = k. That is to say, with k specific lines the F2-synthetic of maximum yield is obtained. In Fig. 1 the graphical description of this model is shown (n: 2, 3, ..., k,... ∞).

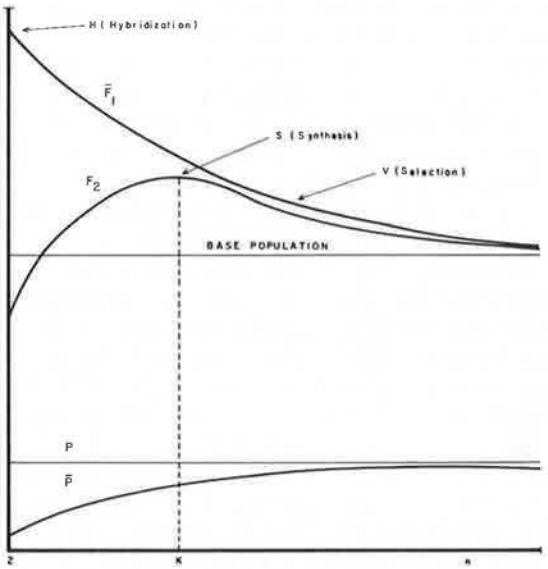


Figure 1. General model for maize breeding through selection (V), synthesis (S), and hybridization (H). P, average of homozygous lines.

The model proposed for the selection process is shown in Fig. 2; it is the same as Fig. 1 but now the involved curves are drawn from right to left (n:∞, ..., k, ..., 3, 2). If the selection limit is defined as the point where inbreeding causes the yield of the selected sample to decline, then the limit is reached when the number of conceptual selected lines is k and the number of selected plants is k/2.

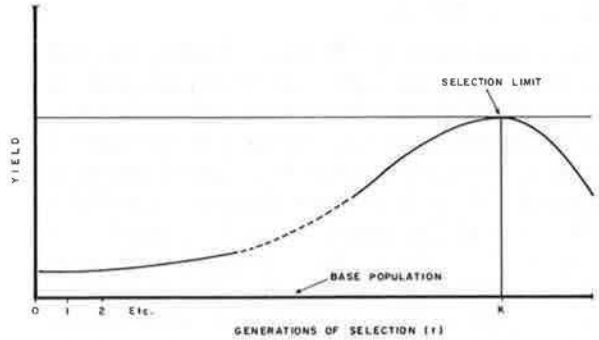


Figure 2. Proposed model for selection limit in maize.

Empirical studies on maize use numbers of selected plants around 200 (out of 4000 or 5000), which for the first cycle of selection would mean an F2-synthetic of 400 conceptual lines, a number which causes a negligible inbreeding depression. However, as phenotypic similarity of selected plants tends also to cause gene and genotypic resemblance, sooner or later the number (n) of conceptual selected lines will have to decrease gradually down to k, where the selection limit for practical purposes has to be reached.

If the number of conceptual selected lines can be associated to the number of cycles or generations of selection through a mathematical function, the generation at which the selection limit so described is reached may be predicted. A simulation study with such a purpose is being carried out at our institution.

CHESTNUT HILL, MASSACHUSETTS
Boston College

Chromosome doubling in anther culture-derived progeny plants

--Y. C. Ting and Stephen Schneider

In the last summer, 78 out of 182 plantlets derived from anther culture in vitro grew into adult plants. Those plants developed both female and male inflorescences, even though some of those inflorescences were very small.

Male inflorescences of all the above plants were collected and fixed in an alcohol and acetic acid fixative. Up to the present, meiotic observations were performed for 31 plants. It was found that among them, 15 plants were diploid (20); nine haploid (10); three triploid (30); and two aneuploid (one extra chromosome). The remaining two plants had only a very small amount of inflorescences and chromosome studies were not adequate.

Of the above progeny plants about 50 percent (15 plants) were identified to be diploid with 20 chromosomes in spite of the expected 10 chromosomes. It is significant. This high frequency of spontaneous chromosome doubling in the microspore plants is different from several previous studies. These doublings may be accomplished either by fusion of adjacent nuclei or by endomitosis. The latter is a process by which a doubled number of chromosomes was achieved by a successful nuclear division but without cytokinesis. Even though this is a preliminary observation, it immediately suggests that for future corn breeding mediated by anther culture in vitro, chromosome doubling by colchicine could be eliminated. If this can be realized, it would save not only lots of tedious labor but also a great deal of money.

More studies on anther culture

--Y. C. Ting and A. H. Mu

During the last year, 44 cultivars and hybrids were employed for anther culture. More than 60,000 anthers were inoculated on Zheng-14 medium (Ting et al., 1981). The anthers of five hybrids, Ch91, Ch92, Ch93, Ch94 and Ch96, responded positively to culturing by growing into embryoids or calli, or both. The percent of responses varied from 2.9 such as for Ch92 (2.3% + 0.6%) to 18.7 such as for Ch96 (18.6% + 0.1%). This is shown in Table 1. Eleven of the remaining 39 cultivars and hybrids also responded

Table 1. Different responses of anthers of five corn hybrids to Zheng-14 medium during the years 1987- 1988.

Hybrids	No.Anthers Inoculated	Response Embryoid %	Callus (%)
Ch91	1640	212 (12.9)	9(0.5)
Ch92	1515	35 (2.3)	10(0.6)
Ch93	305	31 (11.4)	7(2.2)
Ch94	930	102 (10.9)	15(1.6)
Ch96	2545	474 (18.6)	3(0.1)
TOTAL	6935	854 (12.3)	44(0.6)

favorably but only at a low frequency, on an average of less than 1 percent.

Upon transferring the above embryoids and calli onto regeneration medium, many grew into plantlets. Eventually approximately 80 plantlets grew into adult plants in the field with fully developed inflorescences. On the average, the height of those plants was only about half of that of the parental plants grown on the adjacent plots. After self-fertilization had been performed for most of the plants, only four of them bore well-filled kernels. The others were completely sterile. Consequently the genetic stability of the progeny plants is currently under examination.

CLEMSON, SOUTH CAROLINA
Clemson University

B37N mitochondria lack a mature transcript for URF-25

--J. Wang, J. Barth and A. Abbott

The study of mitochondrial gene expression in higher plants is hampered by the inability to easily manipulate the system; therefore, we have chosen to study maize lines which have naturally occurring variation in gene expression. Comparative molecular analyses of these expression variants with normal systems will define components essential to the process of mitochondrial gene transcription, processing and translation. Using Northern hybridization analysis, mitochondrial RNA's from diverse cytoplasmic backgrounds have been screened for variations in expression of a number of cloned genes or unassigned reading frames. Most sources of N cytoplasm have identical transcript patterns for the probes examined (N. H. Walker et al., Theor. Appl. Genet. 74:531, 1987). One line, however, B37N, shows an altered transcript pattern for the URF-25 reading frame originally described by R. E. Dewey et al. (Cell 44:439, 1986). In this line the mature URF-25 transcript is not present at the 2200 nucleotide size seen in B73N cytoplasm and all other lines examined. The major transcript for this URF in B37N is found at the probable precursor size of approximately 3800 nucleotides. Using flanking and reading frame specific probes in Northern hybridization analysis the putative precursor transcript has been characterized in B37N and B73N mitochondrial RNA's. These transcripts appear to be identical. One plausible explanation for the lack of a 2200 base transcript in B37N is the loss of a processing sequence on either the 5' or 3' end of the transcript. To examine this possibility clones containing URF-25 and flanking sequences from B37N and B73N mitochondrial DNA have been isolated and comparative restriction fragment analysis has been carried out. There does not appear to be any gross difference in the structural organization of this transcription unit in either cytoplasm. Using cloned probes, the 5' end of the mature transcript has been mapped and is being sequenced in both B37N and B73N to search for minor sequence changes. Similar experiments will be done on the 3' end. We are also testing the possibility that nuclear background may influence the expression of this URF. These experiments will delimit salient features involved in mature transcript size determination in higher plant mitochondria.

Presence of pBR322-homologous transcripts in maize

--D. Gupta, M. Tanzer and A. G. Abbott

In order to study transcriptional patterns in higher plants, cloned probes were used for hybridization on Northern blots. These studies revealed the presence of transcripts homologous to the bacterial plasmid pBR322. This homology was observed in both mitochondria and whole cell RNA extracts. The pBR322-homologous transcripts appear to be in a higher copy number in the mitochondrial RNA as compared to the whole cell RNA. These transcripts have been identified in several different strains. The plasmid pBR322 when used as a probe hybridizes to two major transcripts that are approximately 3400 bases and 1800 bases in size. RNA from different tissues of the plant was also used in Northern blot analyses to study pBR322 homology. These studies revealed differences in transcript pattern between coleoptile and stem tissue. The larger transcript is present in a lower copy number in the stem tissue as compared to the coleoptile tissue. This difference in transcripts may be important as a developmental regulation mechanism.

The next set of experiments was designed to identify genes that encode for the pBR322 homologous transcripts. A pBR322 probe was hybridized to a Southern blot with mitochondrial and genomic DNA. DNA fragments homologous to pBR322 were identified in both the samples of DNA. The major homology to pBR322 lies on two *Hin*dIII fragments, 6.0kb and 3.2kb in size, in a strain of B73N. This homology is observed only in DNA preparations that are not run on a CsCl gradient. This suggests that pBR322 homologous DNA has a GC ratio different from the GC ratio of the genomic DNA. Other strains have also been studied. A variation in the hybridization pattern among these different lines is observed.

We do not know if these DNA molecules encode the transcripts that hybridize to pBR322. Experiments are currently being done to investigate this.

CLERMONT-FERRAND, FRANCE
INRA - Domaine de Crouelle

Anther culture

--D. Barloy and M. Beckert

Phenotypic observation of DH lines. During the last summer we observed in the field more than two hundred selfed progenies of DH plants obtained through anther culture from different genetic stocks. Some of them resulted from spontaneous chromosome doubling, the others were colchicine treated. These first observations concerned the aspects of the lines at the agronomic and morphological levels. Whatever the origin of the material, it looks very homogeneous and stable. A wider experiment planned this summer will characterize more precisely these different DH lines and their different selfed generations for homo-

geneity and stability. By observing DH lines derived from well-responding homozygous lines in androgenesis it seems, in a first assumption, that gametoclonal variation might not play a major role in creating genetic variability.

Genetics of androgenesis. The androgenetic potential of such lines was also evaluated. The androgenetic response expressed as the percentage of responsive anthers, ranges from zero to more than 50% and this phenomenon is clearly not continuous: some lines show a null or close to null response, another group is close to 10 or 20% of response and the rest close to 40 to 50%. The data support the hypothesis that only a few genes might be responsible at least for the induction process. The quite good level of androgenetic potential of hybrids made between null or good responding material (Maydica, submitted) indicates a quite dominant or additive system. The fact that it is possible to get material with null response via androgenesis leads us to postulate a possible role of the anther wall in the physiological control at the mother plant level. In addition we did not find large differences between reciprocal crosses concerning the percentage of responding anthers or plantlet regeneration, suggesting that only nuclear genes might be involved.

COLD SPRING HARBOR, NEW YORK
Cold Spring Harbor Laboratory

Dosage of *Mu*

--V. Sundaresan

It has been observed that the mutability of *Mu*-induced mutant alleles can exhibit considerable variability from one generation to the next, and even between kernels on the same ear (for example, Robertson, MNL 60:9-10, 1986). As a result relationships of dosage to mutability are not easily measured in the case of *Mu*. Bennetzen (in Plant Genetics, UCLA Symposia 35:343-354, 1985) has demonstrated that mutability of a *Mu* insertion in *bz1* does not correlate with the copy number of *Mu1* elements in the genome. We have asked a different question: Is the mutability of a *Mu*-induced allele correlated with the dosage of that allele? This question was provoked by the following observation: In a cross of a *bz-Mum9/bz* female to a *bz-Mum9/bz-Mum9* male, the resulting ear was segregating approximately 1:1 medium mutable (300-400 spots/kernel) and highly mutable (>1000 spots/kernel) kernels. The sizes of the spots were the same in all kernels, i.e., small, covering 1-20 cells. In our experience the *bz-Mum9* allele (a *Mu* insertion at *bz1* isolated by D. Robertson) is relatively uniform in its mutability within a single ear and does not exhibit the extreme variability from kernel to kernel that is seen with some other *Mu* alleles (e.g., *a1-Mum2*). Therefore, the above segregation of two classes of mutable kernels could reflect the difference between 1 dose and 3 doses of *bz-Mum9* in the aleurone, depending on whether the kernel inherited *bz* or *bz-Mum9* from the female. If so, the medium mutable kernels should be genotypically *bz-Mum9*/bz and the highly mutable kernels should be *bz-Mum9/bz-Mum9*. A few kernels from each class were planted and crossed to *bz* testers to determine their genotype. As shown below, the results were consistent with the above interpretation.

		Genotype	
Kernel Phenotype	Number Tested	*bz-Mum9/bz-Mum9*	*bz/bz-Mum9*
High mutable	5	5	0
Medium mutable	4	1	3

Although the number of plants examined was small, and there was one exception (i.e. a medium-mutable kernel that was *bz-Mum9/bz-Mum9*), it does appear that *bz-Mum9* exhibits an additive dosage effect. This observation might be exploited in identifying the genotypes of progeny from a cross using their kernel phenotype. However, this rule breaks down when kernels from different crosses are compared (for example, we have observed many cases where the kernels from a *bz-Mum9* self exhibited lower mutability than the outcross progeny of the same plant).

R-locus imprinting is tissue-specific and not positional

--Joe Colasanti, Amar J. S. Klar and V. Sundaresan

The *R* locus of maize is involved in the regulation of anthocyanin pigment production in the seed and plant. A functional *R* allele allows the expression of purple pigmentation in the seed aleurone. In the R-mottling phenomenon, the expression of *R* in seeds of *R/r* heterozygotes depends on the direction of the cross. Certain alleles of *R* (*R-r*, for example) exhibit normal seed pigmentation when transmitted through the female gametophyte; however, when transmitted through the male gametophyte, *R* is expressed incompletely and the aleurone pigmentation is mottled. Kermicle (Genetics 66: 69-85,1970) has shown, using B-chromosome translocations, that the absence of mottling when *R* is transmitted through the female is not due to an extra dose of the *R* allele in the triploid endosperm, suggesting that during the development of the male gametophyte, the *R* allele is "imprinted" or altered in an epigenetic fashion such that its expression is suppressed in the next generation. The molecular basis of this temporary change in the activity of this allele is presently unknown.

In an attempt to determine whether *R*-allele imprinting in the pollen occurs as a result of the position of the tassel in the plant or whether the imprinting is gametophyte-specific, we have utilized the tassel seed mutant *Ts6*. *Ts6* is a dominant mutation that results in the formation of a female flower at the position where the male flower would normally form. The tassel seed ear is a large multi-spike, tassel-like structure with extensive silks; the ear produces no pollen but it can be cross-pollinated to yield mature kernels capable of expressing full color.

The question we asked was: "Do the kernels of a tassel seed ear carrying the *R-r* allele exhibit the *R*-mottling phenotype?", i.e., will *R-r* be imprinted in female gametophytes derived from what is normally the cell lineage leading to male gametophytes. The *Ts6* stock obtained from the maize co-op carried the recessive *r-g* allele. This was crossed to a *R-r* stock, and the progeny that inherited *Ts6* were crossed to pollen from *r-g* and *R-g* testers. Both the tassel seed ear and the normal ear of the *Ts6* plants were pollinated. From several such crosses it is apparent that the tassel seed ear behaves much like a normal ear in this respect. That is, when the *R-r* allele is transmitted through kernels of the

Cross	Ear	Phenotype
Ts6/+, R-r/r-g x *+/+, r-g/r-g*	tassel seed	full color: colorless, 1:1
Ts6/+, R-r/r-g x *+/+, r-g/r-g*	normal	full color: colorless, 1:1
Ts6/+, R-r/r-g x *+/+, R-g/R-g*	tassel seed	full color: mottled, 1:1
Ts6/+, R-r/r-g x *+/+, R-g/R-g*	normal	full color: mottled, 1:1

tassel seed ear they express full color, and mottling occurs when *R-r* pollen is crossed to a tassel seed kernel with an *r-g* genotype. From these observations we conclude that the position of the flower has no effect on imprinting; and that *R*-locus imprinting is male gametophyte-specific, probably occurring late in the development of the tassel.

Molecular analysis of the *P* locus

--Christa Lechelt and Thomas Peterson

The maize *P* locus controls pigmentation of certain floral tissues, including the pericarp and glumes of the cob. Interestingly, *P* can be expressed independently in pericarp and cob; thus, the *P-RR*, *P-RW*, *P-WR*, and *P-WW* alleles specify red pericarp/red cob, red pericarp/white cob, white pericarp/red cob, and white pericarp/white cob, respectively. The *P-VV* allele, which specifies variegated pericarp and cob, comprises the transposable element *Ac* inserted in a *P-RR* gene.

Using the *Ac* element as a transposon tag we have isolated 27kb of genomic DNA from the *P* locus. With an overlapping genomic *Bam*HI clone of 10kb (kindly provided by Jychian Chen and Stephen Dellaporta) the entire cloned region comprises 34kb. The cloned DNA contains two 5.8kb homologous regions, in direct orientation, separated by 6.6kb. The *Ac* element in the original *P-VV* allele is inserted in the 6.6kb of DNA between the 5.8kb direct repeats.

We knew from previous experiments that the *Ac* insertion in *P-VV* is correlated with a change in transcriptional pattern around the *Ac* insertion site (MNL 62:47). For a complete transcriptional analysis of the cloned *P*-locus DNA, restriction fragment probes spanning the 34kb region were hybridized to Northern blots of RNA from plants carrying the mutant *P-VV* and functional *P-RR* alleles. The results allowed a coarse determination of transcribed regions that are most probably specific for the *P* gene.

We found that probes from a region of 7.3kb around the *Ac* insertion site detect five transcripts of 7kb, 6.5kb, 2kb, 1.4kb and 1kb in RNA of *P-RR* and *P-RR* revertants derived from *P-VV*. The multiple RNA molecules may be formed by differential splicing. None of these transcripts is found in RNA from the *P-VV* allele. Instead, a transcript of 9.5kb in size is detected in *P-VV* RNA probes located 5' of *Ac* and by *Ac*-specific probes. The 9.5kb RNA is a chimeric transcript containing *P*- and *Ac*-specific sequences that most likely terminates within the *Ac* element, since it is not detected by probes 3' of *Ac*. Hybridization with single strand specific M13 probes demonstrated that the direction of transcription of the *P* gene is identical to the *Ac* gene in the cloned *P-VV* allele (MNL 62:47). Thus, the transcriptional start site(s) of the *P* gene is located 5' of the *Ac* element in the *P-VV* allele used for these studies. The transcript sizes reported previously (MNL 62:47) are overestimates, and the sizes given here are derived by side by side comparison with labelled RNA ladder and *Hin*dIII-cleaved lambda DNA.

Probes made from DNA fragments outside the 7.3kb region around the *Ac* insertion site do not detect differences in RNA from the *P-VV* and *P-RR* RNA. These transcripts do not seem to be specific for the *P* gene since they do not correlate with the phenotype, but we cannot exclude the possibility that they are somehow involved in *P* gene expression.

Although the 7.3kb region around the *Ac* insertion site is able to code for the largest transcript of 7kb, we do not yet know whether the promotor of the *P* gene is also located in this region, or whether RNA synthesis starts further 5'. At present it is also unknown which of the five transcripts are important for the expression of the *P* gene, or whether all of them may be.

Short-range transposition of *Ac* from the *P-OVOV* allele

--Thomas Peterson

Last year we reported the isolation and preliminary characterization of an allele termed *P-OVOV* (*o*range *v*ariegated pericarp and cob) derived as a change in state of *P-VV* (MNL 62:42). *P-VV* specifies colorless pericarp with red sectors, whereas *P-OVOV* specifies orange pericarp with many dark red sectors, and some colorless sectors. Southern analysis showed that the *Ac* transposable element is in the opposite orientation in *P-OVOV* relative to *P-VV*.

Cloning and sequencing show that inversion of *Ac* occurred by short range transposition and reinsertion in an inverted orientation. The *Ac* element has transposed 160 base pairs towards the 5' end of the *P* locus. Although the *Ac* element in *P-VV* is not bordered by host direct repeats, the *Ac* element in the *P-OVOV* allele is flanked by 8bp direct repeats of a sequence which is present once in the progenitor *P-VV* allele.

The orange variegated pericarp phenotype specified by *P-OVOV* may be considered as a mosaic of three phenotypes:

1. The orange background color may be due to a dilution of the red phlobaphene pigments, resulting from a reduced level of expression of *P*. Although we do not yet know why *P-OVOV* allows a moderate level of *P* expression, an orientation-dependent splicing mechanism similar to that proposed by Wessler, Baran and Varagona (Science 237:916) seems plausible. That is, in *P-VV* transcripts from *P* terminate within the *Ac* element, while in *P-OVOV* the orientation of *Ac* may allow splicing out of element sequences during RNA processing.

2. The numerous red sectors may be due to excision of *Ac* from *P-OVOV*, thereby restoring a *P-RR* allele. We know that this is the case for one germinal *P-RR* revertant from *P-OVOV*.

3. The occasional light sectors may result from a variety of mutations at *P*, including deletions (see below) and short-range transpositions. We have characterized two germinal *P-VV** mutants which have variegated pericarp and cob resembling the progenitor *P-VV* allele. Both cases were derived from kernels with variegated pericarp on otherwise orange variegated ears. In one case (*P-VV*-4177*) *Ac* has transposed from the site in *P-OVOV* and inserted at a site approximately 700bp towards the 3' end of the *P* locus, in the opposite orientation as in *P-OVOV* (i.e., in the same orientation as the *P-VV* "grandparent" allele). In the second case (*P-VV*-4189*) *Ac* has transposed from the site in *P-OVOV* and inserted at a site approximately 4kb towards the 5' end of the *P* locus. The orientation of *Ac* in *P-VV*-4189* is not yet known. In these experiments, we have not detected a strict polarity of transposition as might be predicted from Greenblatt's results showing a 4 map unit region proximal to *P* which contained no *Ac* insertions following transposi-

tion from *P-VV* (Greenblatt, Genetics 108:471, 1984). Rather, our results indicate that *Ac* can transpose in either direction from the site in *P-OVOV* to other sites within the *P* locus.

We thank Susan Allan for technical assistance.

***P-OVOV* mutates to *P-WW* by deletion**

--Prasanna Athma and Thomas Peterson

As mentioned above, the *P-OVOV* allele carries an *Ac* element in the inverted orientation with respect to *P-VV*. The *P-OVOV* allele shows both somatic instability (pericarp sectoring; see above) and germinal instability, as evidenced by the progeny of the cross: *P-OVOV/P-OVOV* x *P-WW/P-WW* (both directions).

Among 10,820 progeny ears, 697 (6.4%) had red pericarp and cob (*P-RR*), and 89 (0.8%) had white pericarp and cob (*P-WW*). These frequencies can be compared to those previously reported by Brink (Genetics 43:435): among 4575 offspring of the mating of *P-VV/P-VV* x *P-WW/P-WW*, 125 (2.7%) had red pericarp and cob and 8 (.17%) had white pericarp and cob. Thus, both *P-OVOV* and *P-VV* mutate to *P-WW* at low but detectable frequencies. It is not known whether the different frequencies arise from background effects, direction of the cross, or actual differences in mutation frequency of *P-VV* and *P-OVOV*.

We have investigated the molecular basis of seven *P-WW* alleles derived from *P-OVOV*. Each allele was obtained independently from kernels with mutant pericarp sectors from separate orange variegated ears. Southern analysis indicates that six of the seven *P-WW* mutants have a large deletion at the *P* locus; the seventh mutant has a more complex structure and will not be considered further here. In order to map the deletion end points, restriction fragments to the right and left of the *Ac* insertion site in *P-OVOV* were used as probes. Southern analysis with probes spanning a 3kb region to the right of *Ac* showed that this region was deleted in the mutants. Similarly the probes to the left of *Ac* representing a 9.5kb region were also deleted.

Molecular analysis shows that the *P* locus contains two direct repeats of 5.8kb, separated by 6.6kb (Lechelt et al., in preparation). The *Ac* element in the *P-OVOV* allele is situated in the 6.6kb of DNA between the two 5.8kb repeats. In the *P-WW* mutants the deletion end points lie within the two 5.8kb homologous direct repeats, on either side of *Ac*. We suspect that the deletions may have occurred by homologous recombination between the two direct repeats such that the 17kb of intervening DNA, including *Ac* and part or all of the *P* gene, is deleted.

The possible involvement of the *Ac* element in the occurrence of deletions is suggested by the apparent stability of the *P-RR* allele. Although *P-RR* contains the 5.8kb direct repeats, we do not know of any reports of *P-WW* mutants arising from *P-RR*. On the other hand, the *P-WW-1112* allele, obtained directly from *P-VV*, has a deletion of the same type as the six *P-WW* mutants derived from *P-OVOV*. We do not know whether the deletions are somehow induced by the presence of an active *Ac* element, or the increased length of DNA between the direct repeats.

We thank Rob Fincher and Ruth Meier of Pioneer Hi-Bred for overseeing the maize crosses and isolation fields.

COLLEGE PARK, MARYLAND
University of Maryland

Further evidence for the maternal inheritance of plastids

--Joseph L. Corriveau

Geneticists seeking to manipulate plastid genome-encoded traits need to know the mode of plastid inheritance for their respective plant species of interest. Genetic evidence is available describing the mode of plastid transmission for only about 60 genera of angiosperms (see reviews by Sears, Plasmid, 4:233, 1980; Smith, Plant Breed. Rev. 6:361, 1988). For the majority of these species, including maize, plastids appear to be maternally inherited.

Recently, further evidence for the maternal inheritance of plastids in maize was reported by Corriveau and Coleman (Amer. J. Bot. 75:1443, 1988). Using the DNA fluorochrome DAPI in conjunction with epifluorescence microscopy, Corriveau and Coleman examined the generative and/or sperm cells of pollen from 235 plant species, including maize, for the presence or absence of detectable plastid DAPI-DNA aggregates. Species were scored as potentially capable of biparental plastid transmission if plastid DNA was detected in the male reproductive cells. Conversely, species were scored as being maternal for plastid transmission if no plastid DNA was detected. There was a striking correlation between the known genetic and new cytological evidence for the mode of plastid transmission for 42 of the species examined. Plastid DNA was detected in the cytoplasm of generative and/or sperm cells of pollen from 13 species known genetically to be biparental for plastid transmission, but no plastid DNA was detected in the male reproductive cells of pollen from 29 species known genetically to display maternal inheritance. Plastid DNA was not detected in the sperm cells of over 500 mature pollen grains of maize inbred line B37 examined using this novel cytological methodology.

Although there is now genetic (Anderson, Bot. Gaz. 76:411, 1923), molecular genetic (Conde et al., J. Hered. 70:2, 1979), and cytological (Corriveau and Coleman, 1988) evidence for maternal inheritance of plastids in maize, there is precedent for the possibility that trace biparentalism may still be detected in the genus *Zea*. For example, there is now evidence for trace biparental transmission of plastids for petunia (Cornu and Dulieu, J. Hered. 67:40, 1988), tobacco (Medgyesy et al., Mol. Gen. Genet. 204:195, 1986), *Epilobium* (Schmitz and Kowallik, Curr. Genet. 11:1, 1985), and *Pisum* (Corriveau et al., Pisum Newsl. 20:5, 1988), genera previously thought to be maternal for plastid transmission. Perhaps previous analyses of plastid inheritance in maize did not take into consideration the possibility of paternal plastids being contributed to progeny at a very low frequency. An additional consideration is the possibility of genetic variability for plastid transmission patterns within the genus *Zea*. Such genetic variability is known to exist and has been genetically analyzed in *Oenothera* (Chiu et al., Curr. Genet. 13:181, 1988) and *Pelargonium* (Tilney-Bassett and Birky, Theor. Appl. Genet. 60:43, 1981). The novel DNA fluorochrome/epifluorescence microscopy protocol used by Corriveau and Coleman to screen pollen from 235 plant species could be used to screen pollen samples rapidly from maize accessions for potential biparental plastid in-

heritance. If biparentalism is to be detected within the genus *Zea*, one may expect to find it amongst uncultivated accessions because early maize breeders may have unknowingly selected for maternal inheritance of plastids.

COLLEGE STATION, TEXAS
Texas A&M University

Wrp: A new dominant dwarf mutation

--A.J. Bockholt and J.D. Smith

An unusual dwarf plant was found by A.J. Bockholt a few years ago in a composite population of Mexican maize races. The original plant was heterozygous, and, since no similar mutants were observed in this population before or since the plant was discovered, we believe it represented the original mutation.

Dominance for dwarfism is not complete. Homozygotes are about 14-20 inches tall, while heterozygotes are occasionally as tall as 3 feet. Both homozygotes and heterozygotes are capable of producing viable pollen and functional silks, although individual plants may be either male or female sterile. Silking tends to lag behind anthesis from a few days to a week or more. These tendencies are more pronounced in homozygotes, but selfed seed can be produced on some homozygous plants.

The most striking phenotypic characteristic of this mutation is the wrinkled appearance of the plant, which is more extreme in homozygotes than heterozygotes. The leaves appear to be longitudinally corrugated. This effect extends from the base of the leaf sheath to the tip of the leaf and even the culm in some plants.

Although we have not assayed these plants for gibberellins (GA), it seems unlikely that this mutation affects GA synthesis or functions. Dominance of the mutation rules out GA deficiency, and the wrinkled phenotype is very different from known GA-deficient or GA-insensitive mutations in maize (*D8*) and sorghum.

Thus, we propose to designate this mutant allele as *Wrp*, to distinguish it from GA related dwarfs. We have not yet mapped the *Wrp* locus, but small quantities of seed of this mutant can be supplied upon request.

Proteolytic enzyme activities in viviparous-1 and normal kernel tissues during development

--Kien Tjhen, J.D. Smith and D. G. Bai

Electron micrographs reveal that protein bodies in aleurone tissue, but not endosperm, are seriously degraded by 25 days after pollination (DAP) in viviparous kernels. As expected, these protein bodies appear to be intact through maturity in normal kernels.

Enzymatic activities of carboxypeptidase (CP) and endopeptidase (EP) were determined from purified extracts of aleurone, endosperm, and embryo tissue of developing maize kernels at 15, 20, 25 and 35 DAP. These ages correspond to stage 4, stage 6, mature embryos and black layer, respectively (MNL 61:40). The activities of CP and EP were investigated using routine TCA methods, at pH 5.0 with phenylalanine and at pH 5.4 with zein as respective substrates, followed by ninhydrin reaction for CP.

The CP and EP in *vp/vp* and normal kernels showed similar patterns of activities in all tissues. The activity of CP was about 0.06 mol/g/h and EP was about 0.1 mol/g/h during early seed development (15 DAP). The highest activity was seen in embryo tissue for CP (0.085 mol/g/h) and in aleurone tissue for EP (0.6 mol/g/h) at 25 DAP.

The activity of CP in aleurone tissue decreased significantly after 15 DAP, indicating that this enzyme was very active in aleurone tissue during early development. The activities of EP in aleurone and embryo tissues showed that this enzyme was very active at this point but turned off after 25 DAP. The endosperm tissue had low or almost negligible CP and EP activities at all stages of seed development.

Table 1. Carboxypeptidase (CP) activity in viviparous-1 and normal maize kernel tissues.

		CP activity (mol/g/h)		
	DAP	Embryo	Endosperm	Aleurone
Mutant	15	0.0588	0.0671	0.0632
	20	0.0646	0.0579	0.0605
	25	0.0960	0.0201	0.0215
	35	0.0379	0.0028	0.0037
Normal	15	0.0729	0.0926	0.0677
	20	0.0696	0.0562	0.0662
	25	0.0824	0.0229	0.0242
	35	0.0525	0.0040	0.0219

Table 2. Endopeptidase (EP) activity in viviparous-1 and normal maize kernel tissues.

		CP activity (mol/g/h)		
	DAP	Embryo	Endosperm	Aleurone
Mutant	15	0.0983	0.1829	0.1428
	20	0.2039	0.1038	0.1629
	25	0.6525	0.1234	0.9086
	35	0.6289	0.0820	0.4062
Normal	15	0.0690	0.1785	0.1149
	20	0.3142	0.2298	0.3298
	25	0.5136	0.1462	0.9129
	35	0.5040	0.0641	0.5645

Although some differences were observed, the similarities in the activity patterns of these proteolytic enzymes indicate that the degradation of aleurone protein bodies in *vp/vp* kernels is not due to *Vp* regulation of these enzymes.

Suppression of lipase activity by *Vp* and abscisic acid during kernel development

--D.G. Bai, C.W. Magill and J.D. Smith

Reserves, stored as starch, protein and lipid bodies, in endosperm, aleurone and scutellum tissues during seed development, are the main source of nourishment for axis growth during germination. Accumulation of reserves begins at about 13 DAP and continues to maturity. In mature maize kernels, aleurone tissue is characterized by a large number of protein bodies surrounded by lipid bodies. During the early stages of germination the primary source of both phospholipid precursors and the energy required for increased synthetic activity is derived from triacylglycerols in lipid bodies. Lipase, which hydrolyzes storage lipids, is located in the lipid bodies, and changes in lipase activity during germination have been reported.

Electron micrographs of aleurone tissues from wild type, *vp/vp* and *vp5/vp5* kernels were compared at 16, 25 and 35 days after pollination (DAP). The structural integrity of protein and lipid bodies was maintained

throughout development in wild type aleurone tissue. The degradation of lipid bodies in *vp/vp* aleurones was apparent at 16 DAP, and most of the lipid bodies were gone at 25 DAP. Visible degradation of protein bodies was also observed at 25 DAP. At 35 DAP very few lipid bodies were detectable, and most protein bodies were fused together and visibly degraded.

In *vp5* aleurone tissue, visible degradation of lipid and protein bodies lagged behind *vp*. Minimal effects were observed at 16 DAP, and 25 DAP *vp5* tissue was similar to that observed at 16 DAP in *vp*.

Crude homogenate of aleurone and scutellum tissue was assayed for lipase activity. Autolytic activity, degradation of its own lipid bodies, and substrate degradation activity of wild type kernels stayed at very low levels in both tissues throughout the maturation period (<50 nmoles of fatty acid/gFW/min). Aleurone tissue of *vp* mutants showed high autolytic activity at 16 and 25 DAP, but activity had decreased by 35 DAP. Substrate-degradation activity of *vp* was highest at 16 DAP and diminished over time. In contrast, *vp5* mutants showed continuously increasing activity with or without substrate over time. Autolytic activity in *vp5* at 25 DAP was similar to that of *vp* at 16 DAP.

Table 1. Total fatty acid (nmol FA/gFW/min) synthesized in each tissue homogenate.

Genotype and Age (DAP)		Aleurone		Scutellum	
		-substrate	+substrate	-substrate	+substrate
vp1	16	74.31	171.37	66.96	116.88
	25	115.27	157.7	1318.58	4234.89
	35	34.79	143.17	2034.04	4810.65
vp5	16	43.84	79.8	29.01	57.08
	25	78.53	100.61	35.41	76.33
	35	134.2	356.03	898.21	1812.61
wild type	16	16.84	30.58	N.D. (<20)	N.D.
	25	22.05	50.14	25.4	28.58
	35	N.D.	30.49	55.36	29.1

High lipase activity occurred earlier in aleurone than scutellum tissue in both mutants, and activity in *vp* was more pronounced at an earlier developmental stage than in *vp5*. This is consistent with our observations that axis elongation occurs earlier in *vp* and that *vp* expression is less affected by environmental factors than *vp5*.

The *vp* mutant is ABA-insensitive, and ABA levels in kernel tissues are comparable to those in normal tissue. Homozygous *vp5* kernels are ABA-deficient. They do not synthesize ABA within the kernel, but they contain ABA translocated from the heterozygous maternal plant. ABA levels in these kernels vary with age, but they are usually in the range of 25 to 50% of the ABA found in wild type kernels segregating on the same ear. Both in situ synthesis and translocation of ABA can be inhibited if normal kernel blocks are cultured in vitro on medium containing fluridone. ABA levels in these kernels are about 1% of those found in wild type kernels cultured on standard medium.

Although we did not have fluridone cultured materials at the same ages available, we assayed limited amounts of aleurone and scutellum tissue from 13 DAP kernel blocks cultured on fluridone. Lipase activity in these was similar to that observed in *vp* tissues at 16 DAP (169 nmFA/gFW/min).

The suppression of lipase during normal kernel development requires both the *Vp* gene product (presumably a regulatory protein) and the phytohormone abscisic acid. Degree of suppression appears to depend upon the concentration of ABA. Since *Vp* is always present in normal seed, ABA appears to be the effective regulator. Suppression of lipase may be one of the early control points in a cascade of metabolic events which induce embryo dormancy.

Regulation of anthocyanin synthesis in aleurone by *Vp* and abscisic acid

--J.D. Smith and B.G. Cobb

The inhibition of anthocyanin synthesis by *vp/vp* in *A C R* aleurone tissue is well known. Other known functions of *vp/vp*, e.g., failure to induce embryo dormancy, suppress lipase, suppress peroxidases, etc., are mimicked by the abscisic acid (ABA)-deficient mutants, e.g., *vp2*, *vp5*, etc., and both ABA and the *Vp* gene product are required to regulate these events during normal kernel development. However, all homozygous ABA-deficient mutants accumulate anthocyanins in *A C R* aleurone tissue. Thus, *Vp* regulation of anthocyanin synthesis has been presumed to be independent of ABA.

ABA-deficient mutants, except *vp8*, block carotenoid synthesis which facilitates early identification of homozygous kernels on segregating ears. Since ABA is derived from an unidentified xanthophyll, these kernels are not able to synthesize ABA, but they do contain ABA translocated from the heterozygous maternal plant. At 15 days after pollination (DAP), ABA-deficient embryos contain about 50% as much ABA as normal ones (MNL 61:39).

Since the regulation of lipase activity by ABA is concentration dependent (MNL 62:43), it seemed possible that induction of anthocyanin synthesis might be similarly regulated. If so, the ABA threshold level must be lower than the maternal ABA contribution. To test this, we harvested homozygous *A C R* ears at 5 DAP and cultured kernel blocks on media ± ABA ± fluridone (a chemical inhibitor of carotenoid and ABA synthesis).

Figure 1 shows kernel blocks at different ages cultured on the 4 media. Rows 1 to 4 were cultured on normal medium, + fluridone, +ABA and +fluridone +ABA, respectively. The columns show blocks at different ages, starting at 15 DAP on the left and increasing at 2 day intervals to 23 DAP on the right.

Although the differences in anthocyanin pigmentation are more dramatic in color, Fig. 1 (row 1) shows that *A C R* kernels on normal medium are colorless at 15 DAP, but

Figure 1.

color development increases with age. If ABA is added to the medium (row 3), anthocyanin accumulation is enhanced at all ages.

ABA levels in embryos cultured on media containing fluridone (100mg/l) are negligible (ca. 6 fmol/embryo at 15 DAP), and these levels decrease with age as the source of this ABA appears to be preformed carotenoids in the cob tissue of the kernel block at the time the ear was harvested (MNL 61:39). Fig. 1 (row 2) shows that kernels are white for the entire age sequence, indicating that both xanthophyll and anthocyanin synthesis are inhibited in kernels cultured with fluridone.

If both fluridone and ABA (10^{-4}) mol) are added to the medium (Fig. 1 row 4), the kernels are white due to inhibition of carotenogenesis, but anthocyanins accumulate at essentially the same rate as on medium +ABA (row 2) at all ages. This indicates that ABA is essential for the induction of anthocyanin synthesis.

Figure 2 illustrates the importance of serendipity to science. These *vp*/+ ears were self-pollinated at Homestead, FL, on Nov. 19, harvested without husking on Dec. 14 and

received in College Station, TX on Dec. 16 at 27 DAP. Surprisingly, these ears were colorless when husked, since *vp*/*vp* colorless kernels can be identified at 13 to 15 DAP in segregating ears developing at College Station in June. In Fig. 2, the dark colored ear was husked at 11:30 am, Dec. 16, the middle ear was husked at 11:30 pm, Dec. 16, and the colorless ear was husked at 11:30 am, Dec. 17. The ears were left on a lab bench after husking, and this photograph was taken shortly after the colorless ear was husked. Thus, these ears were allowed to desiccate at room temperature (72 F) for 0, 12 and 24 hours after husking. That desiccation occurred is illustrated by the noticeable denting of kernels 24 hours after husking.

Our interpretation of this is questionable, since we have not yet assayed these ears for ABA. However, we do know that ABA is stress induced and that ABA levels increase about 10-fold in maize seedling leaf tissue treated in the same way for one hour. Thus, we presume that mild temperatures in December and irrigation every third day created a non-stressful environment in the Florida nursery which resulted in abnormally low ABA levels in the plants and developing kernels, and these ABA levels were too low to induce anthocyanin synthesis. We further presume that ABA levels in aleurone tissue increased at a relatively rapid, though as yet undetermined, rate, since faint anthocyanin pigmentation was observed 5 hours after ears were husked.

Based upon these data, and similar data related to lipase, peroxidases and other enzymes, we have developed a working model which depicts how *Vp* and ABA may regulate events that occur during maize kernel development. This assumes that *Vp* codes for a soluble protein (VP) which may bind to upstream regions of specific genes. VP acts like a digital-type (on/off) transcriptional regulator. However, VP fails to function in the absence of ABA. Thus, ABA appears to function as an analog-type (concentration dependent) regulator of VP functions. ABA levels, in turn, appear to be regulated by external environmental conditions.

This may represent an example of environmental regulation of phytohormone levels (ABA) which regulate the functions of a regulatory protein (VP) which regulates transcription of specific genes during maize kernel development.

Anaerobic respiration and ethanol production in root tips from wild type and *Adh1-null* seedlings acclimated or not acclimated to low oxygen

--D.J. Hole, P.S. Hole, M.C. Drew and B.G. Cobb

Wild type root tips from the inbred line Tx5855 and from *Adh1-null* seedlings (seeds were originally provided by R. Ferl, Univ. of Florida and had been backcrossed 3 times into Tx5855) were hypoxically pretreated (HPT, 4.0% O_2 for 16 hrs.) and non-hypoxically pretreated (NHPT, 40.0% O_2 at 25 C) then placed under anoxic (100% N_2) conditions. Evolution of CO_2 and ethanol were monitored for 5 hours (Table). In both wild type and *Adh1-null* root tips, HPT tissue consistently metabolized more glucose and produced

CO_2 Production (µl CO_2 g FW^{-1} h^{-1})

	NHPT	HPT
Tx5855	298	397
Adh1-Tx5855	314	384

Ethanol production (nmol EtOH g FW^{-1} h^{-1})

	NHPT	HPT
Tx5855	5.65	9.18
Adh1-Tx5855	4.16	8.31

more ethanol and CO_2 than NHPT root tips indicating that the low oxygen treatment is necessary to permit acclimatization of the tissue to anoxic conditions. Interestingly, there were no noticeable differences in the production of ethanol or CO_2 between WT and *Adh1-null* root tips in any treatment. ADH activity was assayed using the method of Rumpho and Kennedy (Plant Phys. 68:165-168), and although ADH activity was detected in wild type root tips, no activity was detected in the *Adh1-null* root tips. The rate of production of ethanol was the same for both tissues indicating that the in vitro assay does not equally detect both ADH1 and ADH2. It is important to note that HPT *Adh1-null* root tips carried out anaerobic respiration at the same rate as HPT wild type tips suggesting that *Adh2* may play a more important role in anaerobic metabolism in cells acclimated to low oxygen levels than we have previously thought.

COLOGNE, WEST GERMANY
University of Cologne

Transcription of *Ac* and deleted derivatives of *Ac* in tobacco

--Siegfried Feldmar, Reinhard Kunze and Peter Starlinger

In order to study the influence of *Ac* deletions on transcription of *Ac* derivatives in transgenic tobacco, independently transformed tobacco plants were investigated by Northern blot analysis. The tobacco plants were transformed with Ti-plasmid vectors containing *Ac* or the derivatives integrated into the untranslated leader of the neomycin phosphotransferase II (NPTII) gene of plasmid pKU2 as described by B. Baker (EMBO J. 6:1547, 1987). The insertions are located about 50bp downstream from the transcription initiation site of the 1' promoter.

The sequences deleted from the *Ac* derivatives are within the 5' untranscribed region of *Ac* (pKU19), the untranslated region of the *Ac* transcript (pKU33) or within the long open reading frame of the *Ac* sequence (pKU4). By Southern analysis the structural integrity of the *Ac* derivatives and the flanking T-DNA was confirmed. All *Ac* derivatives tested so far were integrated in the plant genome at their original position in the T-DNA.

Northern hybridisation with short *Ac* homologous probes was performed on ten independently transformed tobacco plants that had been selected for resistance to hygromycin. From previous experiments with kanamycin-resistant plants it is known that the intact *Ac* is transcribed after excision from the T-DNA in form of the 3.5kb mRNA also found in maize.

All *Ac* derivatives still integrated in the T-DNA are transcribed in a complex manner. In every case more than one *Ac*-homologous transcript spanning the whole transcription unit of *Ac* could be detected on the Northern blots. In some cases the autonomous *Ac* transcript starting at the *Ac* promoter and terminating at the polyadenylation signal near the 3' end of *Ac* could be detected (pKU19, pKU33), but most of the *Ac* homologous RNAs are readthrough transcripts of various sizes (2.0kb - 13kb) initiated outside of *Ac*, as was shown by hybridisation with a single stranded probe from the normally untranscribed 5' end of *Ac*. It is unclear whether readthrough transcripts starting at the 1' promoter or farther upstream on the T-DNA are able to produce the *Ac* transposase if the long open reading frame is intact and the element is inserted in sense orientation with respect to the 1' promoter sequence like in pKU19 or pKU33. B. Baker et al. showed that frequency of excision of the intact *Ac* is independent of the element's orientation. In this case readthrough transcription of *Ac* is not necessary for excision from the T-DNA.

Binding of *Ac*- encoded protein to *Ac*- DNA

--Reinhard Kunze and Peter Starlinger

Crude nuclear protein extracts from insect cells infected with baculovirus containing the *Ac* coding sequence or from cells infected with wildtype baculovirus (Fusswinkel et al., MNL 62:47, 1988) were tested for DNA binding activity with various DNA fragments by a mobility shift assay. Fragments containing the terminal 181bp of the *Ac* 5'-end (the end from which transcription starts) and 147bp from the 3'-end, respectively, were incubated with the protein extracts. Both fragments were retarded during electrophoresis after incubation with the recombinant, but not wildtype extracts. Three other DNA fragments not derived from the *Ac* ends were not retarded by either extract. The *Ac* fragment-protein complexes did not enter into 3.5 % polyacrylamide gels, but only in agarose gels, and formed rather a smear than a uniform band.

In order to determine the DNA sequence which is recognized by the *Ac* protein the *Ac* 5'-end-fragment was cleaved with *Pvu*I, yielding two fragments containing the outermost 75bp (5'o) and the 106 inner nucleotides (5'i) of the *Ac* 5'-end fragment: In the mobility shift assay the 5'i-fragment was more efficiently retarded than the 5'o-fragment, which contains the 11bp inverted terminal repeat of *Ac*.

Since DNase I footprinting experiments have been unsuccessful so far, we examined the ends of *Ac* for peculiar sequence motifs. The hexamer motif AAACGG is repeated 6 times in the 5'-end and 2 times in the 3' end of *Ac*. Synthetic oligodeoxynucleotides with this sequence were ligated and cloned into pUC19. Fragments containing this motif behaved undistinguishably from the *Ac* 5'- or 3'-end fragments in the gel retardation experiment. However, DNA-fragments containing an AAAGGG motif were not retarded at all.

We conclude that the *Ac* protein expressed in the baculovirus system is capable of binding specifically to the ends of *Ac*. In particular, it can bind to an AAACGG motif which is repeated several times in the terminal sequences of *Ac*, but it does not recognize an AAAGGG motif. However, the AAACGG motif cannot be the only recognition sequence, because the 5'o fragment of *Ac* contains no complete copy of this motif but is retarded in the mobility shift assay. Also, the *Ds1* element does not contain this motif.

The *Ac* element can be inactivated by methylation. In this context it is conspicuous that the AAACGG motif contains a potential methylation site.

Analysis of *Ac* sequences required for transposition

--George Coupland, Christiane Plum and Peter Starlinger

We have shown that sequences internal to the 11bp inverted repeats are necessary for the transposition in *Ac* or *Ds* elements, even if the product of the *Ac* gene is provided in trans (MNL 62:46, 1988). We have extended these studies and have now seen that the internal sequences are not necessary for an all or none effect. A series of deletions extending from an internal position progressively towards the ends begins to decrease transposition frequencies at position 220 (marginally) and 195 (unmistakenly) from the 5'-end. No transposition is detected when the deletions extend to position 120 from the 5'-end. A deletion extending to position 170 shows an intermediate value. A similar pattern is seen at the 3'-end, where a decrease in transposition frequency is seen at position 170 from this end, while transposition is reduced to (near) zero, if the deletions extend to position 130 from the 3'-end. These results suggest that several functions (protein DNA interactions?) are necessary for optimal transposition. In connection with the results by R.Kunze et al. (see their report), the possibility arises that

several molecules of the *Ac* gene product must bind simultaneously.

Analysis of the N-terminus of *Ac* transposase in transgenic tobacco plants

--Min-gang Li and Peter Starlinger

We have used the phenotypic assay system developed in this laboratory (Baker et al., EMBO J. 6:1547, 1987) to study the 5' coding region of transposable element *Ac* in *Nicotiana tabacum*. In this system both *Ac* and *Ac* derivatives were inserted into the leader sequence of neomycin phosphotransferase gene (NPTII) and introduced into tobacco protoplasts via *Agrobacterium tumefaciens*. Excision or transposition of *Ac* allows us to detect Km-resistant calli.

There are several AUGs located 3' to the long untranslated leader of the *Ac* transcript. AUGs 1, 2 and 10 are located in the longest open reading frame (ORFa) which encodes an 807-amino acid protein initiated from AUG1. AUGs 7, 8 and 9 are completely covered by the ORFa sequence, but are located in a different open reading frame (ORFb) which potentially encodes a 102-amino acid protein and is not detected by Western analysis (Kunze et al., EMBO J: 6:1555, 1987). We altered all AUGs 7, 8 and 9 to AAGs by using oligonucleotide site-directed mutagenesis, to eliminate initiation of ORFb. In addition, we have inserted an amber codon into ORFb that causes the reading frame to stop 38 amino acids earlier, but without altering the amino acid sequence encoded by ORFa. The phenotypic assay showed that in both cases mutant *Ac* elements can still excise or transpose efficiently. No difference in transposition frequency between mutant *Ac* elements and wildtype *Ac* elements can be detected. These results indicate that the protein encoded by ORFb does not affect *Ac* transposition.

Furthermore, in order to find out whether a truncated protein starting from AUG 10 still has a biological function, we shifted the reading frame initiated at AUGs 1 and 2, deleted the fragment containing AUGs 3 to 6 and destroyed AUGs 7 to 9 by oligonucleotide site-directed mutagenesis, which causes the large protein to be translated only from AUG 10, producing a protein lacking 101 amino acids at the N-terminus. This *Ac* derivative gives rise to a similar number of Km-resistant calli as does wildtype *Ac*, indicating that the truncated protein is sufficient for *Ac* transposition.

Km-resistant calli from all assays mentioned above were regenerated into plants. Mutant constructs of *Ac* in transgenic tobacco plants were confirmed by Southern analysis of DNA isolated from these plants and/or by direct sequencing of DNA amplified by the polymerase chain reaction. Southern analysis also detected the DNA fragment left after correct excision of *Ac* by hybridization to either a NPTII probe or to a probe from the 1'-promoter.

Transcription of the *Bz2* locus

--Gregor Schmitz, Nikolaus Theres and Peter Starlinger

The *Bz2* gene is required for the development of full purple pigmentation in various tissues of the maize plant. Recessive mutations in the *Bz2* gene lead to a bronze pigmentation of the aleurone layer and modify purple plant colour to reddish-brown. Inter-tissue complementation studies provide evidence that the *Bz2* gene is involved in one of the last steps of the anthocyanin pathway but so far the gene product has not been characterized.

After cloning a DNA fragment from the *bz2-m* allele and a homologous fragment from a wild-type *Bz2* line, we have used *Bz2* specific probes to analyze the transcription pattern of this gene. Using different DNA fragments close to or spanning the *Ds* insertion site we were able to detect at least three different transcripts in RNAs from deeply pigmented leaf sheath and husk tissue (*R B Pl* plants). A transcript of about 1kb is detected with a single strand-specific probe located close to the *Ds* insertion site. When we use the opposite strand of the same DNA fragment as a probe, two larger transcripts of about 1.2kb and 2.7kb are detected. By comparing the strength of the hybridisation signals to known amounts of single stranded DNA we have estimated the relative abundance of the transcripts. The 1kb transcript was found to be about 100 times more abundant than the others.

A preliminary experiment suggests that the transcription of these RNAs in kernels depends on the action of the *C1* locus (collaboration with Udo Wienand, Max-Planck-Institut fuer Zuechtungsforschung, Koeln). High amounts of all three transcripts were found in a line carrying a *C1-s* allele, whereas they are absent in RNA of a *c1-m1* line without *Ac*.

The results of the Northern experiments could be confirmed by the analysis of cDNA clones. Most of the cDNA clones which were isolated from cDNA libraries prepared from leaf sheath and kernel tissue, are derived from the abundant 1kb transcript, two others are derived from a transcript of the opposite DNA strand. Sequence analysis revealed that the cDNA clones derived from the antisense transcripts partially overlap with the abundant 1kb transcript. From the sequence analysis we also conclude that the 1kb transcript codes for a protein; the function of the antisense transcripts is not known.

Chromosome breakage at the *Ds*-induced *sh-m6233-Dip* allele

--Detlef Piatkowski and Hans-Peter Döring

The *sh-m6233-Dip* allele arose in one of our maize cultures carrying *Ac* plus the *sh-m6233* allele isolated by B. McClintock. Maize plants carrying the *sh-m6233-Dip* allele or the original *sh-m6233* progenitor allele display *Ac*-dependent chromosome breakage. To analyse the chromosome breakage pattern, both strains which also carried *Yg2* and *C* were crossed as male to a strain carrying *yg2 c sh wx*. Chromosome breakage was scored by the loss of *C* in the endosperm tissue and by the loss of *Yg2* in the leaf tissue.

Chromosome breakage in the endosperm occurs very early during development, as the uncoloured sectors are very large and usually cover a larger area than the coloured tissue. In several cases there was no colour formation in the aleurone at all. This is indicative that chromosome breakage occurred during the last postmeiotic mitosis of the microspores or upon formation of the primary endosperm nucleus. Chromosome breakage in the leaves was displayed by the appearance of very many small *yg2* streaks. This variegation pattern shows that the *Ac* acts with a high frequency but late during the development of the sporophyte. Only in three plants out of approx. 350 plants, we found larger *yg2* sectors which comprised 1/6 of the leaf width and extended over the whole leaf length.

We examined the biochemical structure of the *sh-m6233-Dip* allele by Southern analysis. The authentic *sh-m6233* allele carries a 4 kb double Ds structure in the first intron of the sucrose synthase gene (Weck et al., EMBO J. 3:1713-16, 1984). The *sh-m6233-Dip* allele had suffered a rearrangement at the double *Ds* structure, whereas *sh* sequences on either side of the double *Ds* structure remained unaltered with respect to their restriction map. The results of our Southern hybridizations indicate that the breakpoint of the rearrangement in the *sh-m6233* allele is at the junction site between the double *Ds* structure and the 5' *sh* region or alternatively, at one of the junctions between the two *Ds* elements in the 4kb double *Ds* structure. For the *sh-m6233-Dip* allele it is very likely that at least a 3kb double *Ds* structure comprising one half and one complete *Ds* element is still linked to the 3' sequences of the *sh* gene. We have preliminary evidence that the missing other half *Ds* element is linked to the 5' *sh* region. To determine the nature of the rearrangement (inversion, insertion?) we are presently cloning the *sh-m6233-Dip* allele.

Upon analysis of approx. 20 progenitor cultures of the pedigree of the *sh-m6233-Dip/sh-m6233* alleles, we found another two independent rearrangements at the *sh-m6233* allele in the presence of *Ac* . Apparently, the *sh-m6233* allele is highly unstable in the presence of *Ac*.

COLUMBIA, MISSOURI
University of Missouri

Accumulation of indole in orange pericarp (*orp*) plants.

--Allen D. Wright

The accumulation of a UV-light stimulated blue fluorescent compound has been observed in the seedlings of orange pericarp, a mutation caused by the duplicate recessive genes *orp1* and *orp2*. This compound was identified as a glycoside of anthranilic acid (Wright and Neuffer, J. Hered. in press). For this reason, the mutant seedlings might have been expected to have a fragrance similar to that reported for *Bf/Bf* (Coe, MNL 59:39-40). Instead of the characteristic pleasing odor of anthranilic acid, however, seedlings of the duplicate homozygote had a less pleasing odor which was identified as that of indole. The odor can not be detected in plants that are homozygous recessive for only one of the *orp* factors. Anthranilate synthetase appears to be the control point for tryptophan biosynthesis in higher plants (e.g. see Singh and Widholm, Physiol Plant 32:240, 1974). The accumulation of anthranilate might therefore be related to the inability of this mutant to synthesize tryptophan from indole.

Indole was extracted from the above-ground portion of 12 day old greenhouse-grown seedlings by partitioning a methanol extract (2ml) with chloroform (2ml) and water (1ml). An aliquot of the chloroform layer was reacted with Ehrlich's reagent (p-dimethylamino benzaldehyde in ethanolic HCl) for the colorimetric determination of indole (Yanofsky, Meth. Enzymol. 2:233, 1955), correcting for background absorbance using a sample prepared in a similar manner without p-dimethylamino benzaldehyde. The mutant leaf tissue had an indole concentration (mean of six reps ± SE) of 935±330µg/gfw while that of the normal sibs was 67±12µg/gfw. The material for the above study was obtained from a self pollination which segregated 3:1, indicating homozygosity for one of the factors and heterozygosity for the other. It is conceivable that a lack of all recessive factors could further lower the concentration of indole. At present the lack of near-isogenic stocks makes quantitative assessment of dosage effects difficult.

An interesting feature of this mutant is the orange pigmentation associated with the duplicate homozygous recessive kernels of a segregating ear. Because the pericarp is maternal tissue, a segregation for pericarp color could only be expected to result from direct interaction with the underlying filial tissue. Indole evidently plays a direct role in the formation of this color. Non-mutant kernels or isolated pericarps will turn orange in a few days if covered with an aqueous solution of indole. This reaction is non-enzymatic, as similar results were produced using heat-treated pericarps. Oxygen appears to be required, as the orange coloration develops first in pericarps nearest to the surface of the indole solution of non-shaken vessels. At least one of the orange compounds produced in this manner has properties in TLC (with three solvent systems) and UV visible spectra properties identical to an orange compound isolated from mutant pericarps. This fortunate circumstance has enabled the production and isolation by preparative TLC of a quantity of the compound sufficient for identification (in progress).

The substance which reacts with indole to produce the orange compound is not unique to the pericarp. The orange compound has also been isolated from the scutella of mutant immature embryos, which are normal in appearance but turn orange (presumably due to indole accumulation plus exposure to air) during callus culture attempts.

Culture attempts on tryptophan-free media have failed. A slow-growing callus has been successfully initiated from the mutant on tryptophan-supplemented media, which should provide useful material for further biochemical assessment.

Chromosome 8 Mapping Data, 1989

--Dan England and M. G. Neuffer

Recessive *wlu3* is closely linked to *v16*; however, due presumably to gene interaction, data separation was a problem.

Two dominant mutants linked: *Bif1* - 20.3 + 2.9 - *Sdw1*.

Chromosome-Breaking *Ds* Sites

--M. G. Neuffer

In the 1986 News Letter (60:55), we described efforts to obtain chromosome *Ds*-transposition sites with appropriate markers on 16 chromosome arms. Two arms (6S and 8S) did not have good markers, and two arms (9S and 10L) already had such identified sites. We can now report considerable success as indicated by the entries listed below.

Symbol and Designated Marker	Chrom. Arm
A Sh2 Ds-1	03L
A Sh2 Ds-2	03L
a2-m	05S
A2 Ds-1	05S
A2 Ds-2	05S
b-m1:Peru	02S
b-m2:Peru	02S
B-Peru Ds-1	02S
B-Peru Ds-2	02S

Symbol and Designated Marker	Chrom. Arm
Bt1 Ds-1	05L
Bt1 Ds-2	05L
Bt1 Ds-3	05L
bz2 Ds-1	01L
bz2-m2	01L
bz2-m3	01L
bz2-m4	01L
bz2-m5	01L
bz2-m6	01L
C2 Ds-1	04L
C2 Ds-2	04L
C2 Ds-3	04L
C2 Ds-4	04L
C2 Ds-5	04L
C2 Ds-6	04L
C2 Ds-7	04L
Cl1 Ds-1	03S
Dek1 Ds-1	01S
Dek1 Ds-2	01S
Dek1 Ds-3	01S
Dek1 Ds-4	01S
Cp-1419 Ds-1	02S
Cp-76B Ds-1	07L
Cp-76B Ds-2	07L
Dek13 Ds-1	09L
Dek13 Ds-2	09L
Oy Ds-1	10S
oy-m1	10S
oy-m2	10S
oy-m3	10S
Pr Ds-1	05L
Pro Ds-1	08L
Vp9 Ds-1	07S
W3 Ds-1	02L

It is not possible at this time to verify all the entries listed. Some have peculiar behavior, which complicates their analysis and prevents proper verification.

id mutants from EMS treatment

--M. G. Neuffer and M. T. Chang[1]
[1]Garst/ICI Seeds, Slater, IA

Two recessive mutants with indeterminate growth habit have been found in the M2 from treatment of pollen with EMS. The first, found in a B73 M2 (produced by Ming-Tang Chang), failed to flower in long days of the summer field and continued to produce leaves until #32, when shorter days induced flowering. The plants resemble those of *id1*, described by Galinat & Naylor (Amer. J. Bot. 38:38, 1951), including the presence of plantlets with roots and many small ears in the base of the tassel. Since this mutant may be an allele of *id1*, we have assigned a lab designation of *id*-A972*.

The second mutant is a small dwarf-like type which produces leaves at the same rate as its normal sibs even though in early growth it is less than 1/4 normal height. As the N sibs mature at 17 leaves and dry down for harvest the mutants continue to produce new leaves and remain green. At 60 days from planting the mutants had 29-31 leaves, were 3/4 of their sibs' height, and still had no tassel. We have not been able to induce flowering by planting in Hawaii or to date with 10-hour short days. We have assigned this mutant a lab designation of indeterminate dwarf *idd*-2286.*

Designation of four recessive mutants

--M. G. Neuffer

sr4 (*stp*-65A*): Location 6L (confirmed by J.B. Beckett). Phenotype: Comes up pale luteus and changes to striped; mature plant is like *sr1*.

wlu5 (*wl*-266A*): Location 1L (confirmed by P. Sisco); not allelic to *lw* (Sisco); linkage: 26 - *bz2*; also near *br1*? but not *gs1*, not allelic to *w18* (*w*-495A* or *w18-571C*) (see Sisco, MNL 61:114).

w18 (*w*-495A*; allelic to *w*-571C*): Location 1L (confirmed by P. Sisco); not allelic to *lw* (Sisco), see notes on *wlu5* (above).

gl22 (*gl*-478C*): Location is not known; second member of a duplicate gene system with *gl21* (Sprague, personal communication).

Designation of four dominant mutants

--M. G. Neuffer

Fbr1 (Few-branched-1602) is an EMS-induced dominant mutant whose phenotype is expressed in the tassel only as a reduced number of tassel branches (0 to 3). The second tassel branch from the base is often replaced by a small leaf bract. In some plants irregularly formed awns appear on the tips of the glumes. The plant is otherwise quite normal. Homozygotes are slightly more extreme. Location is still unknown but since the phenotype is unique we are designating it *Fbr1*.

Blh1 (Bleached-1593) is an EMS-induced dominant mutant whose phenotype is expressed just before tassel emergence as bleaching of the lower 1/3 of those leaves from the 1st ear node to the top. The bleaching appears as a diminishing of chlorophyll to give a pale green appearance in the midvein regions of the leaf blade and progresses until some areas are almost white. Plant vigor is not affected until onset of mutant phenotype at which time growth is clearly reduced as compared to normal sibs. Homozygotes are viable but more extreme than heterozygotes. T *wx* linkage data listed below (for chromosome 1 only) show 22cM from *wx* T1-9c(1S.48); 42cM from *wx* T1-9(4995) (1L.19) and no linkage to *wx* T1-9(8389) (1L.74) or to any of the other 14 *wx* translocations tested. These data indicate that this mutant, which we have designated *Blh1*, is located on the short arm of chromosome 1, 22cM distal to the T1-9c breakpoint.

	M *Wx*	N *Wx*	M *wx*	N *wx*	χ^2	p
T1-9c	40	11	9	31	28.59	22
T1-9(4995)	67	39	50	57	7.85	42
T1-9(8389)	20	25	24	22	0.64	54

Ws4 (Pale green-1589) is an EMS-induced dominant mutant whose phenotype is first expressed in the late seedling stage as a lighter green whitish-appearing leaf sheath, which becomes more pronounced towards flowering. Leaves are near normal green and plants are vigorous. Homozygous mutant plants are indistinguishable from heterozygotes. T *wx* linkage data listed below (for chromosome 1 only) show closest linkage (14cM) to *wx* T1-9(4995) (1L.19); clear but less close (30cM) to *wx* T1-9c (1S.48) and suggestive linkage (45cM) to *wx* T1-9(8389) (1L.74). There was no significant linkage with any of 15 other *wx* translocations. This indicates location between T1-9c and T1-9(4995), which would place it near the centromere.

	M *Wx*	N *Wx*	M *wx*	N *wx*	χ^2	p
T1-9c	41	19	15	40	19.43	30
T1-9(4995)	51	9	8	51	60.74	14
T1-9(8389)	30	27	24	32	1.30	45

Wi2 (Wilted-1540) is an EMS-induced dominant mutant whose phenotype is expressed beginning at the 5-leaf stage as wilting of the top leaves whenever subjected to drought conditions (i.e., reduced moisture and high temperatures). Under low stress conditions the mutant grows well and is not distinguishable from normal sibs. Homozygotes are viable but more extreme than heterozygotes. Linkage data for *wx* T3-9c only show 11cM from the T3-9c breakpoint (3L.09) and no linkage for the other 16 translocations. This would place the mutant on chromosome 3.

	M *Wx*	N *Wx*	M *wx*	N *wx*	p
T3-9c	29	2	4	19	11

8L Linkage Data: *Bif1, Sdw1*

--M. G. Neuffer

The dominant tassel mutant *Bif1* (MNL 58:76) is linked with a dominant mature plant trait, *Sdw1* (MNL 59:42). Additional tests with other markers on 8L will be necessary to place these mutants precisely.

\+ + x *Bif1* +/+ *Sdw1*

Families	Parental		Recombinant	
	Bif +	+ Sdw	Bif Sdw	+ +
45:855	41	29	11	11
45:856	42	37	5	11
Totals	149		38	

% Recombination (N = 187) = 20.3 ± 2.9

Tester stocks for B-A translocations

--J. B. Beckett

Both seedling and endosperm recessive traits are useful for identifying male parents that carry intact B-A translocations (i.e., plants that carry both the A-B and B-A chromosomes, and thus can support nondisjunction of the B-A chromosome; an exception, TB-10L18, is discussed below). In a tester stock, the presence of endosperm and seedling traits in coupling is advantageous because, in testcrosses, 1) most good males can be identified immediately by the "segregation" of the recessive endosperm phenotype, 2) ears with scant seed set can be tested further by observing for recessive seedling phenotype in the sandbench and 3) because accidental contamination, usually selfing and sibbing within the tester stock, can be detected more readily (since testcross kernels that have the recessive endosperm phenotype should never produce seedlings with the recessive seedling phenotype of the other traits involved).

Loci used to detect the presence of a B-A translocation obviously need to be beyond the translocation breakpoint. If more than one translocation on a chromosome arm is being tested, at least one locus must be beyond the most distal translocation. In general, recessive alleles of seedling and endosperm traits should be in coupling. If not viable, two markers in repulsion can be selected to be as close together as possible so that they can be maintained as a balanced stock by selfing and eliminating the recessives each generation.

Even when recessive alleles are in coupling and are fully viable, a single stock is inadequate because vigor declines until the stock is not dependable as a female parent. Introgressing the recessive genes into two backgrounds allows the production of vigorous F1 testers.

If two such stocks are not available, suitable testers can be produced by pollinating inbred lines or appropriate F1 standards by the tester stock. Although the apparent frequency of nondisjunction will be cut in half, the likelihood of determining whether the male parent is valid or not is much greater when the tester stock is hybrid and vigorous.

When both of the recessives are lethal and relatively close together, balanced lethal tester stocks (carrying the two genes in repulsion) are excellent except for the fact that, again, the stocks become inbred and make poor seed parents. Outcrossing balanced lethals to inbred lines or standards generates usable testers, although each tester plant carries only one of the two recessives. Obviously, if the loci are too far apart, neither the balanced lethal stock nor its outcrosses will be fully dependable as testers.

Below are listed some of the testers that are in current use in my laboratory: (Symbols used: std = standard, such as a hybrid between two inbreds. An appropriate inbred can be substituted. *Y* std = yellow standard; W std = white standard; *R-sc* std = *C R-sc* or *C R-scm* standard; njW23 = *A C R-nj* version of W23. cl Ps = colorless endosperm, colored embryo. Parenthetical numbers, such as W23(4), indicate the number of times the tester stock has been crossed, not backcrossed, to the inbred. Stocks with unknown or complex backgrounds are designated "unk". Bcgd = background. m/c = may carry.)

TB-1Sb std x [(+ *dek1*)/(*nec2* +)]

Male is a fairly stable balanced lethal that is maintained by selfing. Bcgd unk. If the std is *C R-sc*, or if the TB parent carries *C* and *R-sc*, at least some of the *dek* kernels produced from crossing by TB-1Sb will be cl Ps.

TB-1L's *bz2* x *bz2*

bz2 x *an1-6923* (*an-6923* gives *bz* kernels)

R-sc std x *bz2*

The hypoploid seedling phenotype is so obvious that a seedling trait is unnecessary.

Bcgds: *bz2 C R* stocks: W23(4)(from E. H. Coe); K55(4)(from Coe).

+/*an1-6923* x *an1-6923*, *C R* (or *R-sc*) 2 stocks, unk and njW23(3), respectively.

TB-2Sb and TB-3La-2S6270 *Y* std x [*al1*, (+ +)/(*lg1 gl2*)]

The *al* was selected from [(+ + +)/(*al1 lg2 gl2*)] selfed. A (+/*al1*, *lg1 gl2*) x *al1 lg1 gl2* stock would be better. Craig Echt's TB-2Sa, which carries *B-Peru* on the B-2S chromosome, is still being confirmed. If it is a good translocation, it will give cl Ps kernels when crossed onto *b b C C r r* tester, preferably carrying *gl2* or *d5*. Bcgd of *al* stock unk.

TB-2L's std x [(*v4*, +/*w3*, +/*spt1*) selfed]

w3 and *spt1* are needed for TB-1Sb-2Lc, but they are too far apart to produce a stable balanced lethal stock. Bcgd unk.

TB-3Sb *Y* std x *d1 cl1 Clm-4*

Select dwarfs from a +/*d1*, *cl1 Clm-4* x *d1 cl1 Clm-4* stock. Bcgd unk.

TB-3L's Various F1 and 3-way crosses involving the stocks listed below, and sometimes *R-sc* std, are used.

Stocks and bcgds: *a1-m C R-scm2* (unk)(from J. Birchler and K. Newton)

gl6 lg2 a1 et Dt1 C R (unk); *gl6 lg2 a1 Dt1 C R-sc* (86.3% L289)

TB-4Sa std (or *su1*) x (+ *hcf23*)/(*su1* +)
std (or *su1*) x (*su1* +)/(+ *bt2*)
The *hcf/su* balanced tester stock is maintained by selfing and discarding *su*; *hcf23* is a near-white lethal. The *su/bt* balanced stock is maintained by selfing and discarding *su* and bt grains. Bcgds: *hcf/su* (unk); *su/bt* (unk). A +/*su1* x *su* stock (98% L289) is available.

TB-4L's *R-sc* std x *gl3 c2* (and preferably *dp1*) *C1 R-sc*
R-sc std x *gl3 c2 C1 sh1 R-sc*
F1's involving this *sh1* stock make good testers for TB-9Sb-4L6222 and TB-9Sb-4L6504.
Bcgds: (*gl3 c2*, +/*dp1*, *C1 R-sc*) selfed (75% W23)
(*gl3 c2 C1*, +/*sh1*, *R-sc*) selfed (25% W23, 12.5% L289)

TB-5S's *R-sc* std x *gl17 a2*
R-sc std x *a2*
Y std x (+ *ps1*)/(*vp2* +)
Stocks and bcgds: [(+ *ps1*)/(*vp2* +)] selfed (unk)
gl17 a2, +/*bt1* x *bt* sib, prob *R-sc* (74% L289) m/c *pr*
gl17 a2, +/*bt1*, +/*v2* x *bt*, +/*v2* sib (75% njW23) m/c *pr*
[(*a2* + + + *pr*)/(*a2 bm1 bt1 bv1 pr*); *C R*] x *a2 bm bt bv* sib (unk)
The (*ps/vp*) selfed stock is a balanced lethal; it can be used with TB-1La-5S8041 but not TB-5Sc. *bm*, an adult plant trait, is uncovered by TB-1La-5S8041 but not by TB-5Sc. (See 5L testers below).

TB-5L's *pr1 gl8 C R-sc* W23(2) x *pr1 gl8 C R-sc* L289(2)
R-sc std x *pr1 gl8 C R-sc*
R-sc std x [*a2 bm1 pr v2 C R* (25% N6)]
bt is not uncovered by any TB-5L; *bv* is an adult plant trait. (See 5S testers above).

TB-6Sa W or *Y* std x *hcf26 y1*
Most TB-6La stocks carry *y1* on the 6-B chromosome, so the presence of *y* in the tester is useful. A (+ *dek28*)/(*hcf26* +) balanced tester is being developed.
Bcgds: (+/*hcf26 y1*)/(*hcf y*) x *hcf y* (12.5% W23, 22% 38-11); *gl8 gl8*
(+ *Y*)/(*hcf26 y1*) x *hcf y*
4 stocks, all 6% W23 and 11% 38-11, in 50% Mo17Ht, A632Ht, B73Ht and L317 bcgds, resp. All seg *gl8*.
(+ *Y*)/(*hcf26 y1*)] selfed (30% W23, 11% 38-11) Seg *gl8*
(+ *Y*)/(*hcf26 y1*)] selfed (56% W23) Seg *gl8*
hcf26 Y1 selfed (25% 38-11, 25% WF9)

TB-6L's W std x *y py* (or *Y py*)
W std x yellow kernels from [(+ *Y*)/(*l12 y1*)] selfed
The white kernels of W std x (+ *Y*)/(*l12 y*) produce the best testers.
Use std x *py* to test TB-6Lb.
Bcgds: *l12* stock (unk)
y1 pl1 sm1? *py1* selfed (44% WF9) WF9 gives unusually tall *py* plants
(*Y Pl sm1 py1*)/(*Y Pl sm py*)/(*Py*) x *py* sib (56% Oh51A); *P-RR*
The + allele of *py* is carried on a B-6L chromosome from TB-6Lb

TB-7Sc *Y* std x *vp9/vp9/+*
The *vp* stock, which is 25% L289, is maintained by selfing, since *vp9* is lethal. The + allele of *vp9* is carried on an apparent telocentric chromosome. The telosome, which is transmitted poorly, depresses kernel size. *o2*, which has been hard to recognize in my stocks, has been ignored. A balanced stock, (*v5* +)/(+ *vp9*), could be maintained readily by selfing because adult *v5* plants are striped and thus can be eliminated. Unfortunately, *v5* is not a dependable sandbench trait.

TB-7Lb *Y* std x *o5 gl1*
The *o5* stock maintained as +/*o5*, *gl1* x *o5 gl1* sib (50% L289).

TB-8Lc std x normal grains from (*pro/pro/*+)selfed
std x normal from (*pro* +)/(+ *v21*)/(+ +)]selfed
A B-8 chromosome from TB-8Lc carries both + alleles. The stock is not stable because *pro* and *v21* are not closely linked; a [(*pro v*)/(*pro v*)/(+ *v*)] selfed derivative, if viable, would be better. Bcgd unk.

TB-8La and TB-8Lb std x *v21*
The tester is usually carried as +/*v21* x *v21*. Bcgd unk.

TB-9S's *R-sc* std (or *c1 R-sc* std) x *yg2 c1 sh1 wx R* (or *R-sc*)
Bcgds: One *yg2* stock is 75% njW23; another is 25% W22, 25% W23
The seedling trait *d3* is excellent but hard to use because dwarf plants shed little pollen. Neither *wx* nor *d3* is uncovered by TB-9Sb.

TB-9L's *Y* std is the tester for homozygous TB-9Lc (*Wc Wc*)
Hypoploid endosperms are yellow and somewhat smaller than normal. The homozygous TB-9Lc stock is in undefined background.
Std x *c1 wx v1 gl15 Bf1 bm4*
Bcgd of *gl15* tester: 12.5% Oh45, 12.5% Oh51A
Screen for *gl15* at the 3 to 4-leaf stage; *v1* can usually be recognized in a cold sandbench. Only *Bf1* is uncovered by TB-9La; use an appropriate ultraviolet light and goggles.

TB-10Sc *Y* std x *y9*
Std x *oy*
The *y9* stocks are [(*sr3* +)/(+ *y9*)] selfed and (+/*sr3*, *y9*) selfed, but the *sr3* plants usually die before flowering. The *oy* stock is maintained as *oy* selfed or +/*oy* x *oy*.
Unk bcgd for all testers.

TB-10L's *g1 r1 C* std x *g1 r1 C*
C r std (or *R-sc* std) x *g1 r1 C*
Three *g1 r1 C* stocks are available; the 3-way cross is rather vigorous. The TB parent should carry *R-sc* so that cl Ps kernels will be produced. I use these testers for all TB's on 10L; the presence of cl Ps kernels on testcross ears is generally diagnostic. For several of the more proximal TB's, cl Ps kernels are often smaller than normal, thus helping confirm the presence of intact translocations. For TB-10L18, with the B chromosome breakpoint in the short arm, testcrosses should be checked for golden hypoploids in the sandbench, or cl Ps kernels on testcross ears must be clearly smaller than normal; otherwise, the 10-B chromosome will soon be lost. To classify for golden in the sandbench, cut seedlings off just above the coleoptilar node and examine the cut ends for green vs. golden. Striate-2 is not a dependable seedling marker.
Stocks and bcgds: (*C* + *r-r*)/(*C g1 r-g*) x *C g1 r-g* (L289(4))
C g1 r-g (81% njW23)
C g1 r-r (?% W22)

Stocks used to make up standards, etc.:
C R-sc L289(10) *y1 c1* L289(8)

C R-scm L289(8)
C r-r L289(10)
c1 R-sc L289(10)
*c1 R-scm*3 L289(8)
y1 C-I L289(6)
y1 Hy2(4)
y1 38-11(5)

C R-nj W23 (= njW23) Originally obtained from Devender Nanda; the number of backcrosses to W23 is unknown; the stock is much like W23 but the pericarp is transparent instead of dull.

C R-sc njW23(11) The hybrid with *R-sc* or *R-scm* L289 = *R-sc* std.

C R-scm njW23(4)

c1, +/sh1, R-sc x *sh* sib (L289(9))

C r Y inbreds: FR35, N28Ht, H100, 38-11

C r y1 inbreds: K44, K61, Mo16W, Ky27, Ky228 Some or all of these may carry Wc.

Seed of the stocks described above is available in small quantities upon request.

Preliminary data on *Mu* tagging of *ij*

--Chang-deok Han and Ed Coe

To explore the establishment of cell fate (position-dependent) and its maintenance (path-dependent) during initial leaf ontogenesis, we are studying striping mutants (*ij, j1, j2* and *sr2*) and *Isr*. To be able to carry the studies to the molecular level, we have begun transposon tagging experiments on the striping genes with *Mu* for *ij, j1*, and *j2* and with *Ac* for *sr2*. Based on genetic criteria, we have obtained tentative *Mu*-tagged *ij* and *j2* mutants. Here we report preliminary genetic data on *ij* mutants derived from Robertson's *Mu* stocks.

We screened around 30,000 F1 seedlings from the cross of *Mu* stock (female) x *ij-ref*. Four independent striped seedlings, and one ear that was segregating for striped seedlings, were found. But 2 of the 4 seedlings, and the plants from the latter ear, had other than *ij* phenotype, displaying pale green stripes extending over whole leaves. Progeny tests from the ear revealed the following interesting genetic behavior: 1) there was no co-expression of pale stripes and *ij* among selfed progeny of the F1 pale striped plants; 2) when the F1 plants were crossed as female with normal plants (*Ij Ij*), pale striped seedlings were obtained, which indicates that the phenotype of pale green stripes may be maternally inherited; 3) when the F1 plants were used as male parent, all progeny showed normal, which eliminates the possibility of a dominant, male-transmissible factor for the pale striping phenotype. Further genetic tests are being undertaken to examine whether this mutant is a new allele of *ij*.

Two of the 4 plants selected as seedlings from the original screening showed *ij*-like striped patterns. Both plants grown in the greenhouse failed to set seeds, which frequently happens in the winter greenhouse. But we succeeded in propagating the plants as pollen parent by crosses to Ky21 and W23 inbreds. These backcross progeny were obtained in winter, 1986. Genotypes of the progeny will be *ij-Mu/+* and *ij-ref/+* if *Ij* is tagged by *Mu*.

Based on two genetic criteria, one of the two plants has been identified as a new *ij* mutant: 1) All the selfed progeny of the backcrosses, as expected, segregated for *ij* phenotype. 2) the backcross progeny showed 1:1 segregation of RFLP markers from the *Mu* and the *ij-ref* parents. By using RFLP markers, we have constructed a linkage map of *ij*. With one marker 6 m.u. proximal to the iojap locus as a probe, the backcross progeny showed 7 of the 14 plants with the RFLP alleles of the *Mu* and the backcross female parent, and 7 with the RFLPs of *ij-ref* and the backcross parent.

Genetic evidence that the new *ij* mutant is allelic to *ij-ref* comes from an allelism test. In summer 1988, normal plants (*ij-Mu/+* or *+/+*) among selfed progeny from the backcross plants were selfed and crossed to heterozygous *ij-ref* tester plants of 6 different inbred backgrounds (Oh51a, Tr, Mo17, Ky21, K55, and Wg). Our preliminary sandbench progeny tests confirmed that the new *ij* gene from *Mu* background is allelic to *ij-ref* from all the inbreds.

By using an internal sequence (pA/B5) of *Mu1* as a probe, we also examined whether the plants have a high copy number of *Mu*. All plants have 30-60 copies of *Mu*, while *ij-ref* and Ky21 inbred (female parent of the backcross) showed 2-3 hybridizing bands on the *Eco*RI-digested DNA blot.

A new *j2* mutant from Robertson's *Mu* background is being subjected to similar genetic tests.

Apparent non-random chromosome locations of mutant loci recovered from a Mutator-mutagenesis

--Bill Cook and Don Miles

A Mutator mutagenesis experiment was conducted with the purpose of isolating *Mu*-induced high chlorophyll fluorescent (*hcf*) photosynthesis mutants. Twenty colorless kernels obtained from D. Robertson (DR 78-79-8091/7092-8) were grown as male mutagenic parents and crossed to 1087 ears of Mo17 or W23. Thirteen *hcf* mutants were isolated and characterized photochemically and biochemically. Several other mutations with phenotypes detectable at the seedling stage were isolated as well.

Ten of the *hcf* mutant stocks as well as one blue fluorescent and one glossy mutant were crossed to 19 B-A translocation stocks (obtained from M. G. Neuffer) in order to determine their chromosome arm locations. Seven of the mutant loci were mapped in this way. Two of the mapped loci (*hcf103* and *gl*-1258*) were allelic to other mutations which were isolated independently in the current experiment (*hcf114* and *gl*-1253-6*). The chromosome locations of two other loci, *hcf3-Mu* and *bf2-Mu*, were deduced on the basis of their allelism to previously mapped loci, *hcf3* and *bf2*.

Among the 11 mutant loci which were mapped to chromosome arms, 7 were located on 7L (uncovered by 7Lb). 31 EMS-induced *hcf* loci were previously mapped using B-A translocation stocks (Miles et al., in: Molecular Biology of the Photosynthetic Apparatus, Cold Spring Harbor, 1985).

Table 1. Chromosome arm locations of Mutator-derived mutations.

Mutant	Phenotype	Chromosome Arm	Translocation Stock
hcf102	cytochrome b/f-deficient	8L	TB-8Lc
hcf103	photosystem II-deficient	7L	TB-7Lb
hcf114	photosystem II-deficient	7L	(allelic to *hcf103*)
hcf104	photosystem I-deficient	7L	TB-7Lb
hcf108	ATPase-deficient	5S	TB-5Sb
hcf111	cytochrome b/f-deficient	7L	TB-7Lb
hcf113	electron transport defective	9S	TB-9Sb
hcf3-Mu	photosystem II-deficient	1S	(allelic to *hcf3*)
1258*-gl	seedling foliage glossy	7L	TB-7Lb
1253-6*-gl	seedling foliage glossy	7L	(allelic to 1258*-gl)
bf2-Mu	blue fluorescent	10L	(allelic to bf_2)

[1]Cook *et al*, 1987, MNL 61:44

Seven of the 31 EMS-induced loci are located on 1L and four are on 6L. The remaining 20 EMS-induced loci are scattered among all of the other maize chromosome arms except those of chromosome 8 (one of the mutations in the current experiment has been mapped to 8L; Cook et al., MNL 61:44, 1987). It has been assumed, based on these data, that *hcf* loci are not clustered predominantly on one or several chromosome arms but are scattered randomly about the genome. Therefore, we tentatively interpret the high percentage of loci located on 7L in the present experiment to result from characteristics of the mutagen rather than clustering of loci on 7L.

There is evidence that both *Ac* and *Spm* transpose to physically linked chromosomal locations at a higher frequency than they transpose to unlinked positions (Peterson, Theor. Appl. Genet. 40:367, 1970; Greenblatt et al., Genet 108:471, 1984). To our knowledge, no published information demonstrating similar behavior by *Mu* elements is available. However, one explanation of the unexpected clustering of loci on 7L in this experiment is that a single *Mu* element, located on 7L, is responsible for all or most of the mutations located on 7L. The remaining loci mapped in this experiment (located on 1S, 5S, 8L, 9S and 10L) may have been tagged by the same element or by other, less active, elements which are located on those arms.

While we believe that these results may shed light on an interesting aspect of *Mu* transposition, we draw no firm conclusions from them. The mutagenesis and screening which yielded these new mutants were not designed to evaluate *Mu* transposition. Furthermore, the size of the experiment was not sufficient to either support or refute any particular hypothesis. Our limited knowledge of the mechanism(s) of *Mu* transposition combined with our expanding appreciation of the complexity of the Mutator transposon system(s?) (Talbert et al., MNL 62:59, 1988) continues to limit the usefulness of Mutator as a transposon tagging system. It is our hope that these results might be useful to those involved in the analysis of Mutator activity.

An interesting leaf development mutant from a Mutator active line which causes a loss of leaf blade

--Donald Miles

A variety of mutants have arisen in Mutator active lines which are being used at the University of Missouri to select photosynthesis (*hcf*) mutants. One such very interesting mutant has appeared in the second generation after outcross of Robertson Mutator stocks. This mutant segregates as a normal single recessive gene. Its primary phenotype is a loss (non-development) of leaf blade tissue. The absence of leaf blade due to this mutant gene can vary from a complete absence of leaf blade leaving only the midrib to a 10% loss of tissue from the extreme margins of the leaves. The gene is now being named leaf bladeless *(lbl)* for the most obvious phenotype.

It appears that normal development is expressed in the first 3 to 5 leaves rather than the *lbl* phenotype. Leaf tissue first appears not to developed normally from this juvenile stage to maturity in a temperature sensitive manner. The mutant gene *(lbl)* is expressed at temperature above approximately 24 C, however, near-normal leaf blade development occurs at temperatures below about 21 C. In our experiments plants show near-normal leaf development when grown in the winter greenhouse, but *lbl* is maximally expressed in summer field culture in Missouri. Summer plants produce only leaf midrib tissue but no leaf blade. This tissue becomes trapped inside the older tissue and often produces a tangled mass which is unable to elongate unencumbered. This results in a plant no more than 20 to 30cm high. Plants grown at less than 21 C in the winter greenhouse often produce an ear shoot and a small tassel. However we have not yet been able to collect viable seed from *lbl/lbl* plants.

The expression of *lbl* in plants in the 21 to 24 C range shows a range of expression in development of blade tissue. Five levels have been designated for non-development of leaf blade tissue in the leaves of these plants. All of these different stages can be observed in one individual plant.

Stage 1. In the most extreme case of *lbl* expression there is nothing more than the midrib of the leaf, which results in a cylindrical structure from 3 to 10mm in diameter (Figure 1B). This cylindrical structure has well-developed vascular tissue in the central location of the structure with a layer of parenchyma surrounding it. In the parenchyma there are 10 to 20 leaf vascular bundles complete with vascular tissue and a green bundle sheath cell layer. The midrib cylinder is surrounded by apparently normal epidermal tissue with normal appearing green guard cells. There is no chlorophyll in any of the other tissue of this structure other than the bundle sheath. The green vascular bundle strands have well-developed lamellar chloroplasts on a clear white parenchyma background. The vascular bundles appear to be separated by about 1mm. This provides an interesting, well developed tissue which contains only green bundle sheath cells, and only bundle sheath chloroplasts with no

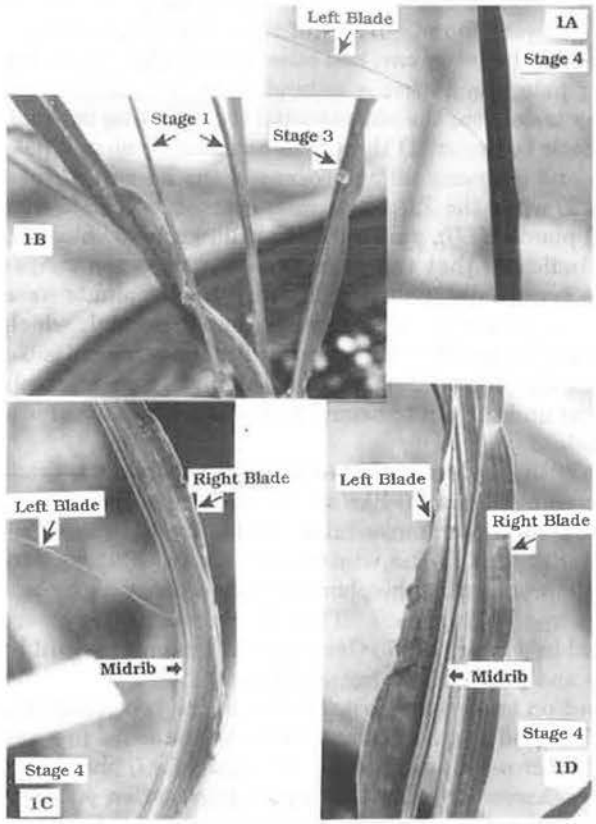


Figure 1. Expression of the leaf blade-less mutant gene (*lbl*) in growth of maize leaves at 24 C.

contamination with mesophyll chloroplasts. This could represent an important experimental tissue for C4 photosynthesis.

Stage 2. In the second level of expression we see the midrib with small amount of leaf blade tissue (less than 1.0cm wide) attached (not shown). This generally takes two forms which are classified as (2a), the blade tissue developed for the full length of the leaf or (2b), the blade tissue developed only on the basal 25% of the midrib tissue. This small amount of blade tissue has normal mesophyll cells and mesophyll chloroplasts.

In both stage 1 and 2 we always see a demarcation of the sheath location with a ligule. In stage 1 the ligule may be the only non-midrib tissue which develops.

Stage 3. In this stage the midrib is accompanied by a near normal half-leaf blade (Fig. 1B). That is, we observe leaf blade tissue on one side of the midrib but none on the other side. In this case the blade tissue is normally attached to the midrib throughout the length of the organ. Stages 1, 2, and to some extent, stage 3 occur at temperatures of 24 C or higher.

Stage 4. In stage four of expression of *lbl*, the midrib is separated from the blade tissue throughout the distal 1/3 to 1/2 of the length of the leaf. There is a clear gap of tissue which did not develop between the vascular tissue and the blade allowing the separation of the leaf into 2 or 3 different linear structures. The development of the two lateral halves of the leaf is usually not correlated. This is shown by the leaves in Fig. 1A, C, and D. In Fig. 1D, there are three distinct structures, the midrib, the left blade half and the right blade half. The gap between the foliar structures appears to be a lack of tissue development rather than abnormal tissue development. At the point the detached leaf blade halves meet the midrib there appears to be a small knot of tissue.

Stage 5. In stage five the leaf appears near normal except that as much as 10% of the lateral margins is missing or abnormally developed (not shown). Often this appears as undulating, abnormally expanding leaf blade tissue at the margins.

It appears clear that the normal function of the *lbl* gene and the site for *lbl*/*lbl* is at the shoot meristem in the early stages of leaf primordia development or development of the intercalary meristem leading to the development of the leaf tissue. The mutant gene expression only becomes apparent after the seedling leaves have developed. It could be suggested that the parent plant provides a diffusible factor to override *lbl*/*lbl* and allow normal leaf development for up to the first five leaves. When the embryo germinates this factor is no longer available and incomplete leaf development is observed provided the seedling is exposed to an environmental temperature of 24 C or higher.

The development of leaf tissues and the final shape of the leaf result from the system of cell division and elongation from the meristem. When leaf initiation occurs from the apical shoot meristem, the leaf develops from a meristematic cell mass which gives rise to the basal intercalary meristem (Sharman, B. C., Ann. Bot. 6:245-284, 1942). Clonal analysis indicated that leaf cells come from at least two layers (LI and LII) in the shoot meristem (Poethig, S., in Contemporary Problems in Plant Anatomy, White and Dickison, eds, 1984). LI (protoderm) produces the leaf epidermis complete with guard cells. This layer must be fully active in even the most extreme stages of *lbl* expression because a normal epidermis is always present. The LII layer gives rise to the remainder of the leaf including the vascular tissue, the bundle sheath and the mesophyll. Dengler et al. (Amer. J. Bot. 72:284-302, 1985) have shown that the bundle sheath cells and the mesophyll develop from different cell lineages, the bundle sheath and the vascular bundle originating from the procambium and the mesophyll from the ground meristem. Further, Langdale et al. (Genes and Develop. 2:106-115, 1988) have presented data supporting an independent development of mesophyll and bundle sheath cells.

These observations mesh well with the developmental leaf patterns observed with *lbl* plants. In stage 1 of development the protoderm and the procambium yield a cylindrical structure completely devoid of mesophyll tissue. In later stages the ground meristem becomes active providing from a very small amount (stage 2) to a near normal amount (stage 5) of mesophyll tissue. In stage 3 the ground meristem is active on only one side of the procambium (Figure 1B) and in stage 4, there are three points of activity, one at the procambium and two in the ground meristem (Figure 1 D) which later join to form a complete leaf for the basal portion.

Moreover, *lbl* regulates the activity of the ground meristem in a temperature sensitive manner. These explanations are a suggested starting point for future analysis of this mutant.

A further interesting feature of the mutant stock is that it was induced in a Mutator line. Therefore, it is possible that it was Mutator induced and potentially the gene could be cloned using the Mutator probe.

dek1 interferes with aleurone differentiation

--Karen C. Cone, Emily B. Frisch and Thomas E. Phillips

Anthocyanin synthesis in the single-cell aleurone layer of the maize kernel requires a complex interaction between many genes. A number of genetic, biochemical, and molecular studies have established that *a1*, *a2*, *bz1*, *bz2*, and *c2* encode enzymes in the biosynthetic pathway. The *c1* and *r* genes coordinately affect the expression of the biosynthetic genes, and thus are assumed to play regulatory roles. Mutations in other genes, e.g., *vp1*, *dek1*, and *anl1*, result in an anthocyaninless phenotype, but also cause abnormal development of endosperm and/or embryo. The pleiotropic nature of mutations in these three genes suggests that their colorless phenotypes may be secondary to their effects on kernel development.

In at least one case, that of *dek1*, this idea is substantiated by the morphology of mutant kernels. Kernels homozygous for *dek1* apparently arrest early in development. Mutant embryos develop only to the proembryo stage, and mutant endosperms are small, colorless, and floury (hence the early designation *clf*) with no anthocyanin or carotenoids present (Neuffer and Sheridan, Genetics 95:929-944, 1980). In non-mutant kernels, the proembryo stage is normally attained about 8-11 days after pollination (Sheridan and Clark, Trends in Genet. 3:3-6, 1987), and the anthocyanin-producing aleurone layer differentiates from the epidermis of the endosperm when periclinal wall formation ceases approximately 20-22 DAP (Randolph, J. Ag. Research 53:881-916, 1936). Therefore, if *dek1* embryo

and endosperm arrest simultaneously 8-11 days after pollination, then the anthocyaninless phenotype might be explained simply by the lack of a differentiated aleurone layer.

In fact, Gavazzi and colleagues have reported that *dek1* kernels do not have an aleurone (MNL 61:73-74; 62:91-92). To confirm this observation, we examined kernels that were mosaic for *dek1*. We obtained from G. Neuffer a self-pollinated ear (30 DAP) from a plant with the following markers on chromosome 1S: *P-WW dek1/P-VV (Ac) Dek1 Ds-4*. Approximately 3/4 of the kernels were mosaic for anthocyanin production; colorless sectors resulted from somatic loss of *Dek1*, mediated by *Ac/Ds*-induced chromosome breaks. A number of mosaic kernels were fixed, embedded, and prepared for light microscopy. The accompanying figure shows a cross-section through the pericarp and endosperm of a portion of a mosaic kernel. Purple sectors

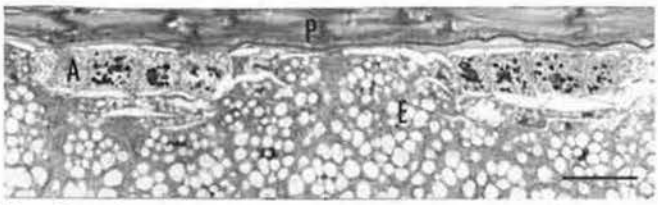


Figure Legend:
Cross-section through pericarp, aleurone, and endosperm of a kernel mosaic for *dek1* showing colorless regions missing aleurone cells. P: pericarp. A: aleurone cell. E: endosperm. Kernels were harvested 30 days after pollination and cut into small cubes. The tissue was fixed in 2% glutaraldehyde containing 1 M sucrose, 30 mM HEPES, 70 mM NaCl, 5 mM CaCl2, pH 7.2 and then treated with 1% OsO4 in 30 mM HEPES, 70 mM NaCl, pH 7.2. Samples were dehydrated in ethanol and embedded in Spurr's resin. Semithin (0.5um) sections were stained with 1% toluidine blue in 1 % sodium borate, and photographed with a Zeiss Axiophot microscope. Bar = 100um.

(*Dek1*) had normal aleurone cells, but colorless areas (*dek1*) were devoid of recognizable aleurone cells. A striking feature of the endosperm tissue underlying the aleurone is the lack of the cambium-like organization usually associated with the sub-aleurone tissue in non-mutant kernels. It is possible that this organization was disrupted by lateral expansion of non-mutant endosperm cells to fill "gaps" that resulted from *dek1*-mediated deficiencies in cell division/expansion. However, without other assayable markers on 1S to distinguish mutant from non-mutant endosperm, it is difficult to say what the origin of the underlying tissue is. The fact that the colorless sectors lack aleurone cells, however, is consistent with earlier observations.

Yet another rapid plant DNA prep

--Karen Cone

Last year, during a mutant hunt I was faced with the prospect of doing hundreds of DNA preps for the purpose of isolating enough DNA for one or two Southerns. I modified a large-scale method we had been using for leaf DNA preps. There are two main advantages to this method. All centrifugations are performed in microfuge tubes, thus facilitating the handling of large numbers of samples in a short period of time. The inclusion of a phenol/chloroform extraction eliminates most nuclease activity, and therefore the DNA is stable for fairly long periods at -20 C.

10 X lysis buffer: 3.5M NaCl; 0.01M Tris-HCl, pH 7.6; 0.01M EDTA.

1X final lysis buffer is 1X lysis buffer, 7M urea, 2% sarkosyl, 50mM EDTA.

For 100ml, 10ml 10 X lysis buffer, 42g urea (ultrapure), 10ml 20% sarkosyl, 10ml 0.5M EDTA, pH 8.0.

1. Harvest piece of leaf from seedling (usually at the 3-4 leaf stage). Leaf piece should weigh not more than 0.3g (about 3" x 3/4 " is right size). Roll up and put into 15ml polypropylene tube.

2. Pour liquid nitrogen into tube and push leaf to bottom to freeze. Grind to fine powder with glass rod. Keep on dry ice till all samples are processed.

3. Add 0.6ml lysis buffer. Warm in 42 C bath till thawed.

4. Shake in 37 C bath for 10 min.

5. Add 0.5ml phenol:chloroform:isoamyl alcohol (100:100:1). Vortex gently 30 sec. Shake at 37 C for 10 min.

6. Transfer tube contents to 1.5ml microfuge tube. Spin 5 min in microfuge.

7. Remove 500ul supernatant to new microfuge tube. Add 50ul 3M NaAc, pH 5 and 600ul isopropanol. Invert several times to mix.

8. Spin in microfuge for 1 min. (no longer or pellet will be hard to dissolve). Remove supernatant with drawn-out pasteur pipette.

9. Add 500ul 70% ethanol. Spin 30 sec. Remove supernatant with drawn-out pipette.

10. Resuspend pellet in 100ul TE. Vortex gently till dissolved--should take about 30 min with intermittent vortexing.

11. Store at -20 C.

Use 5-10ul per restriction digest. Addition of spermidine (to a final concentration of 4mM) to restriction digests facilitates complete digestion. When loaded in 2.5mm wells, blotted to nitrocellulose, and probed with single-copy hybridization probes, the time required for proper exposure is 1-2 days with Lightning-Plus intensifying screen and XAR film at -80 C.

Multiple fertility restorer genes for EP (Z.p.) cytoplasm

--K. J. Newton and E. H. Coe, Jr.

When cytoplasm from perennial teosinte, *Zea perennis*, was introduced into the A619 maize inbred line, by using A619 as the male parent in a recurrent backcross program, the resulting plants were found to be male sterile (Gracen and Grogan, Agron. J. 66:654, 1974). This type of cytoplasmic male sterility was called cms-EP for *Euchlaena perennis* (the old taxonomic designation for *Zea perennis*). Kermicle and Lonnquist (MNL 47:409, 1973) have reported on restorer factors for defective kernels with EP cytoplasm, different from and independent of the restorers of male fertility that are the subject of this note. EP cytoplasm has been found to be male sterile only in A619 or other inbred lines derived from Oh43 (Gracen and Grogan, 1974; Laughnan and Gabay-Laughnan, Ann. Rev. Genet. 17:27, 1983). All other lines tested restore fertility to cms-EP. Restoration is dominant and the mode of restoration is sporophytic; i.e., all the pollen grains of heterozygous, fertility-restored plants appear to be functional.

We have made some observations in an initial attempt to determine the number of nuclear genes involved in restoring cms-EP to fertility. The original cms-EP materials were generously provided by Jerry Kermicle. Apparently, mul-

tiple *Rf* genes for cms-EP exist. The estimates vary depending on which inbred line is analyzed. This is not unexpected because different restorer loci may be involved in the different inbred lines used for comparisons.

For example, cms-EP in A619 (male sterile) was crossed by WF9. All F1 plants were fertile. F2 progeny from self-pollinated F1 hybrids were scored for their fertility status. 19/86 plants were unambiguously male sterile (they were checked several times to ascertain that they did not show "delayed" pollen shedding). Thus, 22.1% of the F2 plants were male sterile and WF9 appears to carry a single dominant *Rf* gene. B73 also appears to carry a single dominant *Rf* gene for cms-EP. EP-A619/B73 fertile F1 plants were backcrossed by A619 as the pollen parent. 11/24 backcross progeny plants (45.8%) were scored as male sterile.

In contrast, dominant alleles of multiple restorer genes appear to be carried by the inbred line, W23. Casual observations over two seasons suggested that sterile F2 plants from EP-A619 x W23 hybrids were rare. However, when the fertile F1 plants were backcrossed by A619, male sterile plants were recovered. At least 4/43 backcross progeny were scored as steriles. This suggests that there are a minimum of three loci involved. The simplest hypothesis is that W23 carries dominant alleles for these fertility restoration genes and that the *Rf* genes act in a duplicate, rather than a complementary, fashion. According to this model, WF9 and B73 would carry homozygous dominant *Rf* alleles at only one locus. We do not yet know whether the *Rf* allele is at the same locus in both, nor whether either or both correspond to any of the loci identified in W23. The existence of multiple loci, any one of which can restore fertility, may help account for the rarity of the non-restoring genotype among inbred lines.

Effect of maize nuclear background on cytochrome c oxidase transcripts in teosinte mitochondria

--Pam Cooper, Ed Butler and Kathy Newton

One means of studying the influence of nuclear genes on mitochondrial gene expression is through the use of interspecific hybrids. Maternally transmitted variations in mitochondrial gene expression may arise because of an "incompatibility" between nuclear and mitochondrial genomes which have experienced separate selective pressures.

We are interested in determining whether nuclear background influences the production of transcripts coming from known mitochondrial genes. For our molecular analyses of nuclear-cytoplasmic interactions, we have been using teosinte-maize hybrids. The cytoplasms from the teosintes *Zea luxurians* (Z.l.), *Zea perennis* (Z.p.), and *Zea diploperennis* (Z.d.) were introduced into maize nuclear backgrounds by recurrent backcrosses with the maize inbreds W23 or A619. These materials were generously provided to us by Jerry Kermicle. The inbreds were used as pollen parents 6-9 times before the plants were analyzed.

We have examined the effect of nuclear background on transcript levels for the three mitochondrially encoded subunits of the cytochrome c oxidase complex, subunits 1,2,and 3. Mitochondria were purified from immature ear shoots (cobs) and the RNA extracted (Stern and Newton, Methods Enzymol. 118:488). The RNA was separated on 1.2% agarose-formaldehyde gels and then blotted onto nylon. The Northern blots were then probed with ^{32}P-labelled, cloned cytochrome c oxidase subunit genes, *cox1* (Isaac et al., EMBO J 4:1617), *cox2* (Fox and Leaver, Cell 26:315), or *cox3* (Hiesel et al., EMBO J. 6:29).

Nuclear background influences both the size and number of transcripts from the *cox2* gene when Z.p. and Z.d. are the cytoplasm sources. In the A619 background, three major transcripts of 2.2, 1.7, and 1.4kb are detectable, whereas in the W23 background, two transcripts of 2.2 and 2.0kb are visible. No effect of nuclear background is observed in plants possessing Z.l. cytoplasm. In contrast, no differences between nuclear backgrounds were detectable for transcripts from the *cox1* and *cox3* genes for any of the teosinte cytoplasms.

We conducted genetic analyses to characterize the nuclear gene(s) causing the *cox2* transcript differences. The F1 hybrids Z.p.-A619 X W23 and Z.p.-W23 X A619 both possess the three transcripts found in Z.p.-A619. The same is true if Z.d. is the cytoplasm source. When Z.p.-A619 X W23 was self pollinated, 4/13 (30.7%) of F2 individuals tested expressed two transcripts of the sizes found in Z.p.-W23. Thus A619 carries a single nuclear gene responsible for the dominant three-transcript pattern.

Southern analysis of mitochondrial DNA from the teosinte-maize hybrids revealed no differences between the two nuclear backgrounds when probed with *cox2*, suggesting that all the transcripts originate from a single mitochondrial gene. When Northern blots were probed with different regions of the cloned *cox2* gene, both exons of the gene were present in all the major transcripts. The intron of the gene was not present in any of the transcripts; thus intron splicing cannot account for the differences. We are currently exploring whether the differences might arise because of: A) differential processing of regions 5' and/or 3' to the gene, or B) multiple transcription initiation sites for the gene.

The use of teosinte-maize hybrids has enabled us to identify a nuclear gene which regulates *cox2* transcript production in the mitochondrion. This gene is specific for *cox2*, as we could identify no transcript differences for the other two mitochondrial *cox* genes.

Effect of maize nuclear background on mitochondrial protein synthesis in *Zea luxurians* mitochondria

--Pam Cooper and Kathy Newton

Another aspect of our work on nuclear-cytoplasmic interactions in teosinte-maize hybrids is the effect of nuclear background on mitochondrial protein synthesis. Mitochondria were isolated from *Zea luxurians*-maize plants generated by the crossing program described in the previous report, and in organello protein synthesis was carried out by the method of Newton and Walbot (PNAS 82:6879). Mitochondria were pelleted, the proteins were solubilized in boiling Laemmli sample buffer, and then separated on 12-18% linear acrylamide SDS gels. Newly synthesized proteins were visualized by fluorography.

When *Zea luxurians* (Z.l.) cytoplasm is placed in an A619 nuclear background (Z.l.-A619), a novel 22kD polypeptide is synthesized by the mitochondria. This protein is not synthesized when Z.l. is in a W23 background. Both the F1 hybrids Z.l.-A619 X W23 and Z.l.-W23 X A619 do not synthesize the polypeptide. However, when Z.l.-A619 X W23 was

backcrossed by A619, synthesis of the polypeptide was observed in 10 of 15 individual offspring analysed. Taken together, these results suggest that the A619 line possesses a recessive nuclear gene that allows the expression of the protein. We are currently analyzing more individuals from this backcross.

Other inbred lines also possess nuclear genes that control the synthesis of the 22kD polypeptide. The F1 hybrid Z.l.-A619 X B73 does not exhibit the synthesis of the protein, whereas the F1 Z.l.-A619 X Mo17 does.

The functional identity of the polypeptide is unknown. However, we have conducted submitochondrial localization studies using a modification of the method of Day, et al. (Aust. J. Plant Physiol. 12:219), and found the protein to be associated exclusively with the membrane fraction of the mitochondria.

The synthesis of a novel polypeptide by maize plants possessing a particular nuclear-cytoplasmic constitution is reminiscent of the synthesis of a 13kD polypeptide by cms-T (Dewey, et al. PNAS 84:5374.). However, we cannot correlate the presence of the 22kD polypeptide with male sterility in our interspecific hybrids. Although Z.l.-A619 plants shed less pollen than do Z.l.-W23 or inbred A619 plants with their original N cytoplasm, the pollen that is shed is functional.

Molecular analysis of the NCS3 mitochondrial mutant

--M. D. Hunt and K. J. Newton

NCS3, one of the nonchromosomal stripe mutations, arose in a WF9 line carrying cms-T mitochondria. It has a restriction enzyme profile which distinguishes it from other NCS mutants and from "normal" cms-T mitochondria. This mutation has been removed from the WF9 background, and thus stabilized, by repeated outcrossings with pollen from other inbred lines. We would like to determine what sort of DNA rearrangement has taken place, and this, in turn, should shed some light upon the mechanism by which NCS mutations occur. A potential use for these mutants lies in defining functional genes which have not yet been identified in the plant mitochondrial genome.

We have found that a new 20 kb *Xho*I fragment which appears in NCS3 (Newton and Coe, PNAS 82:6879) is the result of a rare recombination event between two regions of the cms-T genome, one contained within a 14kb and the other within a 16kb *Xho*I fragment. These two progenitor fragments have been cloned, and hybridization studies have indicated that the two regions share no apparent homology. By sequencing through the junction point of both progenitor fragments and the mutant fragment, however, we have found a 12-base reiterated sequence at the point of recombination. This situation is very similar to several described in *Oenothera* mitochondria (Manna and Brennicke, Mol. Gen. Genet. 203:377) and it may indicate that, under some circumstances, a very limited region of homology may be sufficient to initiate a recombination event in plant mtDNA. Because we have found no evidence of a reciprocal recombination product in the mutant and because part of one of the progenitor fragments seems to be missing from the mutant genome, we suspect that this event has given rise to a deletion involving at least one functional mitochondrial gene. The extent of the deletion is not yet known.

Using probes taken from the recombination junction of the 14 and 16kb *Xho*I fragments of cms-T DNA, we have identified two transcripts which are missing or severely reduced in NCS3. These RNAs are not homologous to any previously identified plant mitochondrial genes. We are currently analyzing these regions of the genome in an effort to identify a function for the affected transcripts.

Molecular analyses of the NCS2 mutant

--Deborah L. Roussell and Kathleen J. Newton

The NCS2 phenotype is characterized by reduced growth and vigor, sectors of pale green tissue on the leaf and sectors of small kernels on the ear. Our previous studies have demonstrated an absolute correlation between the mutant phenotype and a specific mitochondrial DNA rearrangement (Newton and Coe,PNAS 82:6879-83, 1986). Additionally, one mitochondrial protein is produced at reduced levels during in organellar protein synthesis assays (Feiler and Newton, EMBO J. 6:1535-39, 1987). The NCS2- defined DNA sequences are transcribed in both the mutant and progenitor mitochondrial genomes. There are at least three distinct transcription units within this region, one of which is characterized by multiple RNAs. In the mutant mitochondria, however, this transcription pattern is altered; large molecular-weight transcripts accumulate. We are currently examining whether these multiple transcripts result from multiple initiation or processing events and whether these processes have been disrupted in mutant mitochondria.

Because the NCS2 phenotype is distinguished by pale green stripes on the leaf, we predicted that the mitochondrial DNA rearrangement and resulting loss of mitochondrial function must exert a pleiotropic effect on the chloroplast. Indeed, electron micrographs of the pale green leaf tissue reveal that both the mitochondria and chloroplasts have markedly reduced inner membrane and thylakoid membrane structures respectively (Thompson and Newton, MNL 61:46, 1987). Further analyses (low temperature fluorescence spectra, fluorescence induction kinetics, and PAGE of thylakoid proteins) suggest that the chloroplasts isolated from pale-green sectors are functionally as well as morphologically distinct from those in dark-green sectors.

DIMBOA glucosyltransferase does not glucosylate quercetin

--B. A. Bailey and R. L. Larson

Recent attempts at purifying UDPG:2,4-dihydroxy-7-methoxy-1,4-benzoxazin-3-one (DIMBOA) glucosyltransferase from maize extracts identified two peaks of activity by Q-Sepharose gel chromatography. Enzyme eluting in the initial peak of activity also had a low level of activity on the flavonoid quercetin with the first thought being that UDPG:Flavonol 3-O-glucosyltransferase (UFGT) was eluting concurrently with peak 1 of the DIMBOA glucosyltransferase. It was also considered possible that UFGT and the initial DIMBOA-glucosylating peak were the same enzyme.

To try to distinguish between UFGT and the DIMBOA glucosyltransferase, enzyme was extracted from aleurone (23 dap) of genetic stocks dominant and recessive for *Bz1*, the structural gene producing UFGT. Fifty aleurones were

ground in a mortar with the addition of 30ml of Hepes buffer (50mM, 5mM DTE, pH 7.5). The debris was removed by filtering through Pellon followed by centrifugation (26,000g, 10min). A 30 to 60% ammonium sulfate precipitate was applied to a Sephadex G-200 superfine column and the protein eluted with 20mM Hepes, pH 7.5. Active fractions were pooled and applied to a Q-Sepharose ion exchange column and eluted with a 0.1 to 0.3M KCl gradient in Hepes buffer (20mM, pH 7.5) with fractions being assayed as described below.

The assay mixture included approximately 2µg of protein, 1mM UDPG and Hepes buffer (50mM, 5mM DTE, pH 8.2) in a volume of 200µl. Samples were incubated 10min at 37 C and included 1mM DIMBOA or 83M quercetin. The reactions were terminated by the addition of 0.8ml of a 2:1 chloroform:methanol solution (1% HCl). DIMBOA glucoside and isoquercetin were identified by HPLC methods and quantitated using standard curves based on peak height.

Assay of the fractions in the Q-Sepharose elution profile for the fully dominant *Bz1* aleurone preparation identified 3 peaks of glucosyltransferase activity (Fig. 1). The initial peak of activity on DIMBOA reaches a maximum one fraction prior to the maximum for glucosylation of quercetin. The elution profiles for peak 1 of DIMBOA glucosyltransferase and UFGT overlap partially indicating UFGT is carried through as a contaminant when attempting to purify DIMBOA glucosyltransferase. Both DIMBOA glucosyltransferase peaks are observed in the recessive *bz1* aleurone (Fig. 2) although activity on quercetin is completely absent further distinguishing DIMBOA glucosyltransferase from UFGT. These results clearly demonstrate that the activity observed on quercetin when attempting to purify DIMBOA glucosyltransferase is due to contamination by UFGT, and DIMBOA glucosyltransferase peak 1 does not glucosylate quercetin.

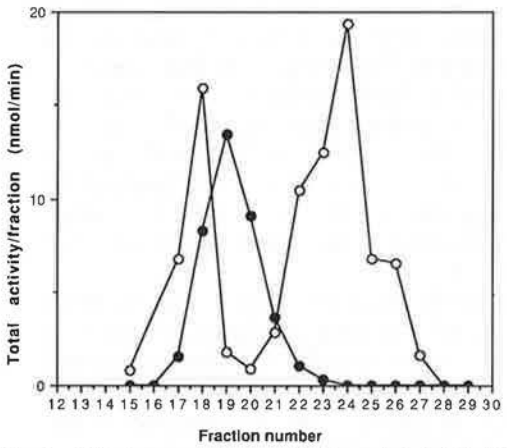


Fig. 1. Q-Sepharose elution profile for protein isolated from dominant *Bz1* aleurone. DIMBOA glucosyltransferase activity (o—o); UFGT activity (•—•).

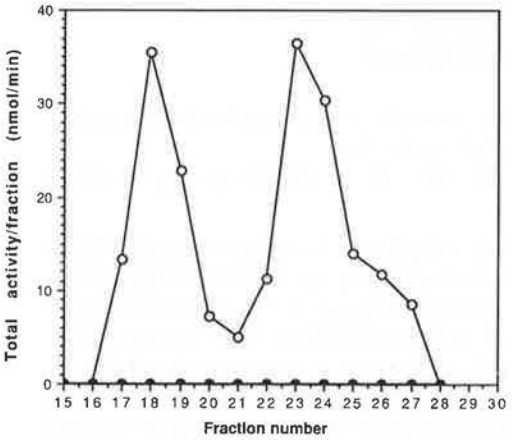


Fig. 2. Q-Sepharose elution profile for protein isolated from recessive *bz1* aleurone. DIMBOA glucosyltransferase activity (o--o); UFGT activity (•—•).

COLUMBIA, MISSOURI
USDA-ARS and University of Missouri
BOMBAY, INDIA
Tata Institute of Fundamental Research

Expression of fine stripe, japonica-1, striate and virescent-5

--M. M. Johri and E. H. Coe

We are examining the pattern of sectors in plants with the following factors affecting the chloroplasts: *f* (fine stripe); *j1* (japonica-1); *sr1*, *sr2*, *sr3* (striate-1, 2 and 3 respectively) and *v5* (virescent-5). At the time of examination in August 1988, the pedigrees of the test material varied considerably (Table 1) and these factors are being crossed to ensure uniform background. Though the top 12 or 13 leaves have been examined, the plants exhibiting the sectors were identifiable early at the seedling stage.

The expression of sectors at different nodes for various factors is shown in the Figure. Whereas fine stripe sectors were absent from the top 2 or 3 leaves, sectors for other factors were usually present in the top 12 or 13 leaves. In most cases, characteristically, the widest sectors were located along the edges of the blade, especially in the upper leaves. In the case of *f*, *sr3* and *v5*, sectors were also present in the lamina. In *sr1* plants, most of the sectors, including those along the edges of the blade, were narrow (1/16-1/32). A similar pattern is present in *sr2* plants also, but in all cases

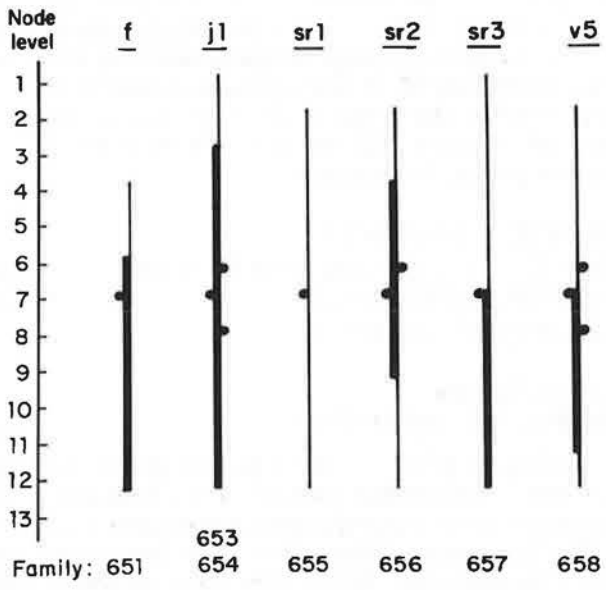


Figure. Extent of striping at different node levels, counting from the top. Thick line represents maximum expression for that factor.

Table 1. Pattern of striping in several mutant types.

Family	Gene	Back-ground	Extent (Nodes from Top)	Culm	Leaf	Sheath
R651	f		4-12	Sectors not obvious in lower internodes.	Lvs 1-3 no sectors; 4-7 on one or both edges 1/16-1/32; 1f 8 edges 1/8-1/16. Sectors in lvs 4-8 in lower 1/2-1/3 of blade. Lvs 9-12 show sectors throughout blade. Displacements of LII by LI not observed (naked eye).	Sectored, but not so prominent (1/16-1/32) as in the blade.
R653, R654	j1	Oh51a	1-12	Several parallel, thin, 1/32-1/64 sectors in lower internodes.	Strongest expression along edges, 1/4-1/16; elsewhere 1/16-1/32. LII displacements by LI on abaxial surface.	Not so obvious in top sheaths. 6th from top showed 1/4-1/8 along edges and 1/16 elsewhere.
R655	sr1	Oh51a (50%)	2-11/13	Several parallel, thin, 1/32-1/64 sectors in lower internodes.	Narrow 1/32-1/64 sectors throughout lvs	Clearest along edges, 1/32-1/64, but present in other locations.
R656	sr2	W22 (50%)	2-11/13	Sectors not obvious in lower internodes.	Narrow sectors 1/16-1/32, mostly along edges. Strongest expression in lvs 4-9.	Sectors wider than in leaf blades, 1/8-1/16. Could represent more than one clone.
R657	sr3	B37 (50%)	1-11/13	Several parallel, thin 1/16-1/32 seen in lower (9-12) internodes. In one plant, entered tassel.	Upper lvs show sectors only along edges. Lvs 7-11/12 show throughout blade. Edges 1/4-1/8. Abaxial surface shows many LII displacements by LI.	Both edges of all sheaths sectored 1/4; along midrib 1/16-1/32. Edges could represented more than one clone.
R658	v5	Oh51a (50%)	2-11/12	Several thin parallel sectors 1/16-1/32 seen in internodes 9-11.	Expression strongest in lvs 7-11. Ear sectored. Sectors in lower half of blade 1/16-1/64. Abaxial surfaces show LII displacements by LI.	Sectors along edges or in submarginal location 1/16-1/32. Mostly thin, parallel sectors.

the sectors along the edges in the leaf sheath were wider than those in the leaf blade. The extents of lateral proliferation of a lineage along the edges of sheath and blade show no relationship and these must be regulated by independent factors or genes. Since at least one cell lineage from sheath (along the margin) is known not to be represented in the leaf blade, the wide sectors at the margin of the sheath in *sr2* could be derived from more than one clone.

The abaxial surface of leaves in *j1*, *sr3*, and *v5* plants was pale green indicating a displacement of LII-derived layers by LI. The overall pattern of sectors suggests that the shoot apex of plants (at least in *j1*, *sr3* and *v5*) is chimeric for LI. Whether the exclusive expression of these factors in LI depends on the specific genes or on the background of the material is yet to be established. During embryogenesis in corn, the shoot meristem is derived from the derivatives of at least two cell layers (Poethig et al., Dev. Biol. 117:392, 1986). The two cell layers of the shoot apex maintain more or less their distinctiveness as most of the cell divisions are anticlinal. The periclinal divisions occur during organogenesis and since such divisions occur randomly in space and time, different types of variegation patterns are formed in plants with a chimeric LI. It is of considerable interest to determine if genetic factors regulate the time and location of periclinal cell divisions. The presence of wide or small sectors could be genetically regulated.

COLUMBIA, MISSOURI
USDA-ARS and University of Missouri
MADISON, WISCONSIN
University of Wisconsin

Pedigree compilations

--Ed Coe, Fritz Behr and Jim Coors

We are making an effort to compile parentage data on inbred lines and other breeding germplasm in a form suitable for computer searching and analysis, and have been entering simple database information from two main sources into computer files. At this time Behr and Coors have compiled pedigrees and citations from the North Central Technical Committee reports (NCR2) 1935 to 1961, and Coe has input pedigrees from the Maize Research and Breeders Manuals VIII (1976) and IX (1980) that Clarion Henderson, of Illinois Foundation Seeds, has compiled previously. These combined sources include the great majority of public-germplasm lines with North Central adaptations, and contain overlaps. We are currently checking for typographical accuracy and for consistent information among the sources. The specific format still needs to be developed and to be made consistent. Our intention is that these files should be checked and authenticated as thoroughly as possible, after which they can be merged and made available for general use when they are sufficiently refined. Among authentications we most wish to have are confirmations by persons who have experience with the materials or special knowledge, in addition to written release statements that are still to be assembled.

We would be pleased to have the help and advice of cooperators who might offer to work over an electronic copy or hard copy of part or all of these files, on the understanding that they are in quite preliminary form and that explicit feedback is expected. If you have special knowledge, interests, or experience that would help, please contact us.

COLUMBIA, MISSOURI
University of Missouri
URBANA, ILLINOIS
University of Illinois

Abnormal growth phenotype correlated with a mitochondrial gene (*cox2*) deletion

--K. J. Newton, C. Knudsen, S. Gabay-Laughnan and J. Laughnan

The maternally inherited nonchromosomal stripe (NCS) mutants are characterized by variable poor growth, abnormal morphologies and leaf striping. NCS plants arise most frequently in inbred or hybrid WF9 or WF9-related lines; therefore, nuclear genotype plays a role in the generation or selection of these mutations. The previously described NCS2 and NCS3 defective phenotypes are each correlated with a specific mitochondrial DNA rearrangement (Newton and Coe, PNAS 82:6879, 1986; Feiler and Newton EMBO J.

6:1535, 1987). Both NCS2 and NCS3 arose in plants with cms-T cytoplasm. We are analyzing several other NCS mutants that have been found in plants carrying cms-S and S-revertant cytoplasms. These new NCS mutants also have altered mitochondrial DNAs and they arose in the WF9 nuclear background. We have suggested that the NCS phenotypes are due to lesions involving essential mitochondrial genes and that NCS plants carry both mutant and normal mtDNAs (Newton and Coe, 1986). Somatic segregation of the mixed organelles would lead to sectors of defective and normal growth.

In support of our hypothesis, we have found that one of the new NCS mutants carries a deleted form of the cytochrome oxidase subunit II gene, in addition to reduced levels of the normal form. This NCS mutant was found among plants descended from a fertile revertant of the ML cytoplasm, a member of the cms-S group. The phenotype is typical of NCS in that maternally inherited variable stunting and striping is seen. However, the stripes are yellow-green, distinct from the pale-green stripes observed for NCS2 and the necrotic striations of NCS3 plants. The mtDNA from the revertant striped plants was similar to the mtDNA of revertant, nonstriped relatives, as assayed by restriction enzyme analysis. Use of several cloned gene probes led to the finding that the only *consistent* striped/nonstriped difference involved the cytochrome oxidase subunit II gene (*cox2*). The whole cloned pZmE1 probe (Fox and Leaver, Cell 26:315, 1981) hybridized to a 5.5kb *Xho*I fragment in mtDNAs from both sterile and fertile-revertant ML nonstriped plants. However, in mtDNAs from the striped, ML-fertile plants, the amount of 5.5kb *Xho*I hybridizing fragment was reduced and an additional 8.3kb restriction fragment hybridized strongly. The maize *cox2* gene consists of two exons separated by a 794bp intron (Fox and Leaver, 1981). We prepared probes that were specific for (a) exon1, (b) the intron or (c) exon2 plus 3' flanking regions. Hybridization of the 2nd exon probe (c) showed the same pattern of hybridization as did the whole *cox2* probe. However, the intron probe hybridized much less strongly to the 8.3kb *Xho*I fragment and the exon1-specific probe did not hybridize at all to the 8.3kb restriction fragment. The 5'-flanking region, the first exon and a portion of the intron of the *cox2* gene have apparently been deleted from this NCS mutant mtDNA. Cloning and detailed restriction mapping of the relevant clones suggest that the molecular origin of this mutation was a recombination event followed by a deletion. The two "parental" restriction fragments apparently lack long regions of homology to one another; however, the presence of very small repeats, such as the one seen at the site of an NCS3 rearrangement, has not yet been eliminated.

Transcripts corresponding to the *cox2* gene are detected at reduced levels in the plants carrying the *cox2* partially deleted gene. They are apparently specifically reduced because transcripts for cytochrome oxidase subunits I and III, as well as ATPase subunit 9, appear to be present at relatively normal levels. This finding of a quantitative reduction, rather than of aberrant transcripts, suggests that the partially deleted *cox2* gene is not transcriptionally active. The *cox2* transcripts in the striped plants would derive from the normal gene which is present in reduced amounts.

COLUMBIA, SOUTH CAROLINA
University of South Carolina

Variants with altered kernel oil composition

--Robin F. Keith[1] and Anthony H.C. Huang[2]
Present addresses: [1]Dept. Agronomy, Univ. Minnesota, St. Paul; [2]Dept. Bot. Plant Sci., Univ. California, Riverside

Maize kernel oils are triacylglycerols which contain linoleic acid (ca. 50%) and oleic acid (30%) as the major fatty acid constituents. We attempted to identify genetic variants which possess altered acylglycerol and fatty acid components. Kernels of M2 generation were kindly provided by M. G. Neuffer of the University of Missouri. Mutation was induced by treatment of pollen with ethylmethane sulfonate in paraffin oil, and the average induction frequency of recessive mutations per locus was estimated to be about 10^{-3} (M. G. Neuffer and W. F. Sheridan, Genetics 95:929, 1980).

The M2 kernels were screened by analyzing scutellar slices of eight kernels from each of 1000 different M2 generation lines. Biometric calculation shows that the analyses will provide a 90% chance of detecting a homozygous recessive mutant in the M2 generation (E. H. Coe, in Maize for Biological Research, W. F. Sheridan, ed., 1982). Scutellar slices of 1-3mg were carefully dissected from the kernels such that the embryonic axes remained intact, and the lipids were extracted with boiling hexane. Half of the lipid extract was applied to thin layer chromatography plates to determine lipid composition. The other half of the extract was transesterified with methanol and boron trifluoride to produce fatty acid methyl esters, which were identified by gas liquid chromatography.

Of the M2 lines tested, 2.6% have kernels showing dramatic decreases in triacylglycerols with concomitant increases in free fatty acids (Table 1). In these variants, accompanying increases in mono- and diacylglycerol content were slight or non-observable. Other variant lines, 3.6% of the M2 lines surveyed, have kernels showing dramatic decreases in linoleic acid with a concomitant increase in oleic acid (Table 1). In this second group of variants, the amount of stearic acid and palmitic acid did not appear to be affected.

Table 1. Changes in acylglycerol and fatty acid composition of kernel oils in several representative M2 maize lines.

Seed Identity	Ratio of linoleic/oleic	Ratio of free fatty acid/triacylglycerol
Mo17 (control)	5.67	0.02
M 387-3a	1.38	--
M 495-1b	0.82	--
M 496-1a	0.67	--
M 660-1b	0.69	--
M 4-4a	--	4.0
M 625-1a	--	2.0
M 855-1c	--	3.3
M 966-2a	--	4.0

--Denotes values not appreciably different from the control.

Attempts by both our laboratory and Pioneer HiBred International have failed to produce germination from any of the detected variant kernels. Presumably, the age of the kernels (more than 6 years old), the damage to the scutella, and the genetic alterations contribute to the non-viability.

From the variant lines which have kernels with altered lipid composition, we successfully germinated some unanalyzed (scutella undamaged) kernels. The seedlings have been grown to maturity, and the plants were self fertilized. The resulting M3 kernels will be analyzed in an attempt to determine if the variations are heritable.

We thank M. G. Neuffer for his generous supply of the M2 lines and his valuable suggestions, and Rongda Qu for scientific advice.

CORVALLIS, OREGON
Oregon State University

Polymorphisms involving the Mutator transposon terminal repeat in somaclonal variants

--Carol Rivin and Douglas Underwood

Normal lines contain several types of sequences with homology to Robertson's Mutator transposons. We are interested in the possibility that *Mu*-homologous DNA may play a role in generating genomic diversity in non-Mutator plants. To investigate this question, we have looked for polymorphisms involving *Mu*-homologous DNA sequences in the genomes of somaclonal variants. We are working with the inbred W182BN and regenerated stocks given to us by Elizabeth Earle. As we reported here last year, some variation is observed when DNA from these plants is probed with internal sequences of *Mu1* and *Mu2*. We now find that probing at lower stringency with the *Mu1* terminal repeat sequence reveals a very high level of restriction band polymorphism.

Nineteen bands hybridizing to the terminus probe can be distinguished on an *Eco*R1-*Hin*dIII digest of W182BN DNA. The banding pattern and the relative band intensities are the same among individuals of this line. Among the somaclones, however, variation was observed for 16 of the 19 bands. Eight somaclonal lines, each having lost one to six of the *Mu* terminus bands, were also found to have new bands and large increases in the intensity of individual bands. In contrast, only a small amount of heterogeneity was observed using *Mu* internal sequences or genic sequences as probes.

Many of the *Mu*-homologous sequences of maize consist of inverted terminal repeats surrounding DNA unrelated to Mutator transposons. Several examples have been cloned and sequenced (*Mu4*, *Mu5*, *Mu6*, *Mu7*, Talbert et al., MNL 1988, and submitted), but have not been shown to transpose. Other sequences with this general structure have been found to transpose in Mutator lines (*Mu3*, Chen et al., Genetics 116:469, 1987; and an element from *wx-mum5* isolated by Sue Wessler). We are testing whether these types of elements are associated with the rearrangements observed in the non-Mutator somaclones.

To date we have compared the inbred and somaclone banding patterns for the internal sequences of *Mu3*, *Mu4*, *Mu5* and *Mu6*. Clones were provided by Karen Oishi and Vicki Chandler. No polymorphisms were observed with *Mu5*. Some band loss, but no new bands were found with the *Mu3* probe. *Mu6* showed some new bands and a greatly intensified band. *Mu4* was the most variable. Seven of the eight polymorphic lines showed evidence of band loss, new bands and band intensification with this probe.

We do not know the molecular nature of the polymorphisms we observe in these lines. Experiments are in progress examining the DNA flanking the novel *Mu* elements to look for evidence of transposition, deletion, amplification, recombination between dispersed repeats or other rearrangements. We are also asking if other transposable element families show a similar level of variability.

Coordination of embryo maturation events by ABA

--Carol Rivin and Timothy Grudt

Abscisic acid (ABA) plays a dual role in embryo development, inhibiting germination and stimulating the accumulation of maturation proteins. Using viviparous mutants, we have been investigating the relationship of these two effects. Proteins from W22 embryos at developmental stages 1 through 6 were compared by 2D SDS-PAGE. About 40 polypeptides identified as characterizing embryo maturation were shown to be modulated by ABA. These patterns were then compared with those of *vp1* and *vp5* mutant embryos (obtained from Don Robertson in a W22 background). Proteins were extracted from mutant embryos and their wildtype sibs developing in planta, in culture with or without exogenous ABA, or in culture with a high level of osmoticum to block precocious germination.

The *vp5* and *vp1* mutants germinate precociously on the ear and we found that they didn't make the set of maturation proteins in planta. Wildtype embryos and *vp5* mutant embryos cultured with 10^{-5} M ABA are inhibited from germination and make a normal set of maturation proteins. The *vp1* mutants are not inhibited from germination at physiological levels of ABA, and while their protein profile changes slightly in response to the hormone, they do not make a normal developmental response.

Blocking germination by growth in a high osmoticum prevented germination of wildtype and both mutant embryos and it was sufficient, by itself, to induce a large fraction of the maturation protein set in *vp5* mutants and prematuration wildtype embryos. Interestingly, the *vp1* mutants did not show this response.

Notably absent from the proteins induced by growth in high osmoticum are the major embryo storage globulins. This subset of the maturation proteins must require ABA itself and the *vp1* gene product to initiate synthesis. However, ABA does not seem to be required for the continued synthesis and accumulation of these polypeptides. As long as germination was suppressed, embryos that had initiated globulin synthesis continued to accumulate them in the absence of ABA. Precocious germination of these embryos (with or without prior desiccation) led to a gradual decline in globulin synthesis after 12 hours. The accumulated globulins degraded rapidly beginning about 24 hours after the start of germination.

Our data suggest that ABA regulates maturation-phase polypeptides by two general pathways, both of which involve the *vp1* gene product. A peak of ABA early in development appears to initiate the synthesis of the storage globulins and it also limits the embryo's capacity to take up water. This event seems to then stimulate accumulation of a second class of prominent proteins which can also be induced by growth in high osmoticum. The osmoticum treatment does not result in measurable increases in ABA levels in wildtype embryos and it is effective in *vp5* mutant

embryos which are deficient in ABA synthesis. Blocking germination also prevents degradation activity and keeps the synthesis rate high.

The *vp1* mutant embryos show some changes in protein profile in response to high osmoticum and to exogenous ABA, but neither class of normal maturation phase polypeptides is produced, even when germination is inhibited.

Characterization of a cell wall protein in various tissues

--Connie S. Bozarth and Carol Rivin

Changes in cell wall proteins have been correlated with pathogen attack and adaptation to environmental stress, as well as with normal growth and development. We have recently begun to examine changes in cell wall proteins of several lines at different stages of development.

Cell walls were isolated, extracted with 1M CaCl (Bozarth et al., Pl. Physiol. 8:261, 1987) and separated by SDS-PAGE. Western blots were probed using a polyclonal antibody produced against a 28kd soybean cell wall protein (Bozarth and Boyer, submitted). A 50kd cross reacting cell wall protein was seen in each of the varieties examined (B37, Ky21, W22, Gaspe Flint, Wilbur Knobless Flint). Unlike the soybean 28kd protein, no cross-reacting protein band was found in the soluble fraction.

The 50kd wall protein occurs in extracts from roots and shoots of embryos, but not in the scutellum. It is present in immature husk and ears. In seedling roots and shoots, the protein is found primarily in young, actively growing tissue, and there are varietal differences in the quantity of protein present. Early experiments indicate an increase in extractable 50kd protein from seedling roots and shoots which have been subjected to low water potentials.

In immature husk tissue a second cross-reactive protein of 28kd is observed. Using a 19-mer oligonucleotide synthesized on the basis of the soybean nucleotide sequence (Mason et al., J. Mol. Biol, in press), we detected two transcripts of approximately 2.5 and 1.6kb on Northern blots of RNA from immature husks.

DAEJON, KOREA
Chungnam National University

Tiller angles of maize with tillers

--B. H. Choe, K. K. Kang, W. K. Lee and H. B. Lee

We have been interested in possible use of tillers for grain and silage production for the past seven years (MNL 56:62; 58:85; 62:54). The lines we have used were mostly from Korean local ones, and from our previous studies we have found that a few hybrids made from a limited number of inbred lines had two to three effective tillers, and the grain and silage yields of such hybrids were comparable to or higher than the check hybrids which do not have tillers.

During our past investigations on the tillers, we have found that tillers could be classified into a few distinctive types according to the angles between tillers and main stem. The first type may be called type A, which has virtually no angles between tillers and main stem (Fig. 1-a). The second type may be called type B which has less than 45 degrees

Fig. 1. Three types of tiller angles observed. 1-a, Type A; 1-b, Type B; 1-c, Type C (refer text).

between tillers and main stem (Fig.1-b). The last type, type C, has greater than 45 degrees between tillers and main stem (Fig. 1-c). The types mentioned above are applicable only to maize which has tillers from underground nodal bases of the main stem. The maize with tillers on the aboveground nodes of the main stem like *Tlr* (Neuffer) were variable in angles. The three types classified according to the angles maintained this throughout the full growing season, even though the photos were all taken during the beginning of the season.

The root systems of maize with tillers

--B. H. Choe, Y. W. Seo and H. B. Lee

The root systems of maize with tillers have not been fully understood. We assumed that the maize with tillers should have different root systems from the ordinary non-tillering maize. Dr. Wellhausen (personal com.) has also mentioned

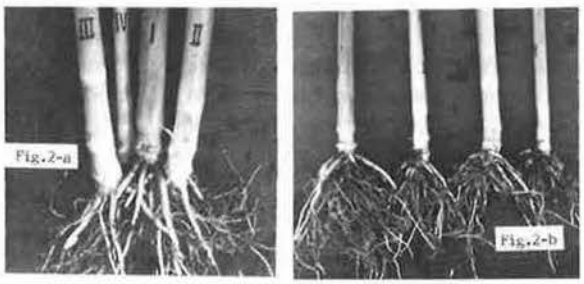


Fig. 2. Tillered maize showing root systems. 2-a : Before tillers were removed. 2-b : After tillers were removed.

the possibly different root systems of maize with tillers. In order to find out if the maize with tillers has different root systems, we dug out the roots of tillered maize, and found that each tiller of tillered maize has its own root system as shown in Figure 2-a and 2-b.

A new recessive mutant - rind absence

--B. H. Choe, K. K. Kang, W. K. Lee and H. B. Lee

During our field observation we have found one phenotype shown as in Fig.3. The mutant plants show a portion of internodes lacking rind longitudinally. The lines where the mutant was found were all inbred lines developed from Korean local lines for earliness. We believe that the lines have no relationship to teosinte. The expressivity was 100%, but the penetrance varied with internodes of the stem. We found that the upper internodes showed a greater portion of rind lacking. Because of lack of rind, the plants tend to break easily at the upper internodes. Sometimes the

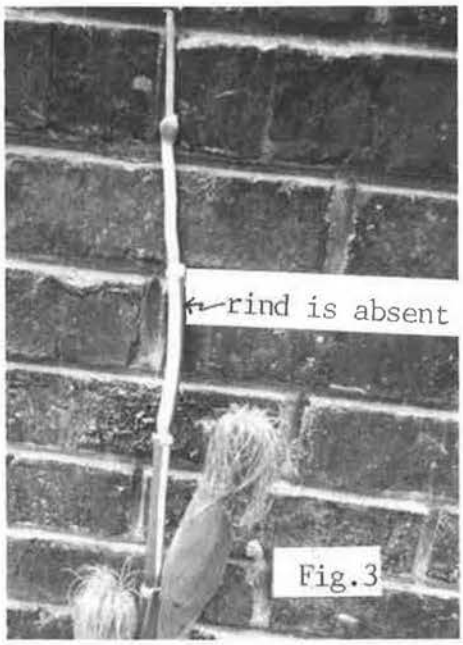


Fig. 3. Plant showing rind absence. White portion along the green (dark) internodes is the naked parts of pith without rind.

rind was found to be lacking in the rachis. We couldn't compare our materials with the materials reported by Dr. Walton C. Galinat (MNL 49:100-102). Our materials were different in a few respects from the materials described by Dr. Galinat. The first difference was that the gene involved in our materials was recessive rather than dominant (*Ri1*). The second difference was that the phenotype is so clear that it can be easily observed without any treatment as is required for *Ri1* material. The third difference was that the mutant phenotype was observed only in the rind of upper internodes, not in the cob as in *Ri1* materials. We need further information on the materials.

DURHAM, NEW HAMPSHIRE
University of New Hampshire

An improved method for the high-performance liquid chromatographic separation of quercetin and isoquercitrin.

--Michael Dowe, Carol Macomber and Anita S. Klein

Published methods (Gerats et al., Biochem. Genet. 22:1161, 1984) for the assay of UDP-glucose 3-O-flavonol glucosyl transferase, the Bronze-1 gene product, require the separation of quercetin and isoquercetrin by high-performance liquid chromatography. While obtaining rapid turnaround times (5-7 min per sample), these procedures require high solvent flow rates and generate column pressures in excess of the manufacturer's specifications. These conditions result in decreased column life and relatively high expenses for solvent components.

A new chromatographic method has been developed which employs a shorter cartridge column and a two pump system. This method allows a turnaround time of 6.5 min per sample with low column pressure and relatively low solvent flow rates. This method is comparable in sensitivity and resolution to published separation methods. The lower limit of detection was 50 picomoles of isoquercetrin.

Sample Preparation: Samples were prepared according to the method of Gerats et al.

Column: Pecosphere 33mm C18 cartridge column and column holder were obtained from Perkin Elmer, Norwalk, CT.

Instrumentation: A Beckman (Fullerton, CA) Model 110A dual pump HPLC system was used with an Altex (Danbury, CT) Model 420 controller. Sample loop size was 100ul. Absorbance was monitored at 355.5nm with an Altex model 155 UV/vis variable wavelength detector. Peak areas were calculated with a Hewlett-Packard (Avondale, PA) Model 3390A integrator.

Chromatographic Conditions: The mobile phase consisted of either solvent A, water:methanol:acetic acid (55:35:10) or solvent B, water:acetic acid (4:1). Samples were eluted with solvent A at a flow rate of 0.5ml/min for 2.5 min. The column was then stripped with solvent B at a flow rate of 2ml/min for one minute. The column was then re-equilibrated with solvent A at a flow rate of 2ml/min for three minutes. Under these conditions, the retention times were 1.7 min. for isoquercetrin and 2.9 min for quercetin.

Chemicals: HPLC grade methanol and reagent A.C.S. grade acetic acid were obtained from Fisher, Pittsburgh, PA.

Alkaline phosphatase activity in the aleurone and embryo of maize

--Anita S. Klein and Carol M. O'Brien

Cassab and Varner developed an elegant cytochemical method called tissue-printing to examine localization of the glycoprotein extensin (J. Cell Biol. 105:2581, 1987). We attempted to adapt this technique to localize the Bronze protein and by chance discovered endogenous alkaline phosphatase activity in the embryo and aleurone of maize kernels.

In Cassab and Varner's tissue-printing method, freehand tissue sections are blotted onto nitrocellulose. The 'print' is dried and extensin is detected as in a typical Western blot procedure: the nitrocellulose is blocked with gelatin or bovine serum albumin, incubated with rabbit anti-extensin antibodies and subsequently alkaline phosphatase [AP] conjugated goat-anti-rabbit IgG. Chromogenic substrates for AP are used to visualize the antibody binding sites on the blot. Cassab and Varner report that the soybean tissues in their study lacked endogenous AP activity. Controls in which the blots were not treated with primary antisera or those treated with preimmune sera did not stain positive for AP activity. Given the availability of antibodies to other plant proteins, tissue-printing should have general applicability to immunolocalization of those proteins.

We have prepared an anti-peptide antiserum against the N-terminal 12 amino acids of UDPglucose flavonol glucosyl transferase (UFGT), the Bronze gene product (Klein, Miller, Labonte and Laudano, unpublished data). The peptide was conjugated to bovine serum albumin [BSA] to facilitate recognition of the antigen; therefore the antiserum also reacts strongly with BSA.

Mature kernels (*sh Bz-McC, R-r*) were hydrated for 10-30 minutes in distilled deionized water and sectioned in both horizontal and longitudinal directions. Tissue prints were prepared according to the method of Cassab and Varner and processed by the Bio-Rad immuno-blot method. AP-

conjugated goat anti-rabbit IgG was purchased from Bio-Rad Laboratories. Binding of the second antibody was detected using Fast Red/Naphthol Phosphate (FR/NP) color development as specified by the manufacturer. Dot blots of a dilution series of BSA (25ug to 25pg) were treated in a similar fashion to monitor the sensitivity of the assay. The antisera reacted strongly with the BSA, for which the most dilute dot (25pg) stained a pink-red color.

In preliminary experiments with antisera treated blots, both the embryo and aleurone layer stained positive for AP. However control blots treated with preimmune sera also stained positively for alkaline phosphatase. New prints were prepared, blocked with gelatin and incubated directly with the FR/NP reagents. Again the embryo and the aleurone stained positive for AP activity. In other experiments prints were prepared from nonshrunken kernels, *Sh*, blocked and treated directly with the FR/NP reagents. In these prints the *Sh* endosperm also stained positive, albeit faintly, for AP activity.

An attempt was made to inactivate the endogenous AP activity so that the goat-anti-rabbit alkaline phosphatase IgG could be used with the tissue-printing method. Control prints were autoclaved to denature protein and treated directly with the FR/NP reagent. Autoclaving reduced but did not eliminate endogenous AP activity on the control prints. Autoclaving did, however, substantially reduce the sensitivity of detection of BSA in the dot blot assay.

We have observed that both the embryo and aleurone of maize kernels contain alkaline phosphatase activity and this AP is stable to the processes of seed desiccation and maturation and is heat-resistant. For kernels, another type of enzyme-linked second antibody for immuno-blot development may be more suitable for immunolocalization via the tissue-printing method.

EUGENE, OREGON
University of Oregon

Cloning *B* utilizing *R* genomic sequences

--Vicki L. Chandler, Devon Turks, and Pablo Radicella

The synthesis of anthocyanin pigments in maize plant tissues or seeds requires the products of numerous genes, both structural and regulatory (Coe and Neuffer, Corn and Corn Improvement, 1977). The *R* locus (on chromosome 10) regulates the formation and distribution of anthocyanin pigments in certain tissues of the plant and seed. *B* (on chromosome 2) controls anthocyanin formation in plant tissues independent of *R*, and several *B* alleles can substitute for *R* function in the seed. For example, *B-Peru* can substitute for *R-g* function in the seed, and only one functional allele at either locus is required for pigment synthesis. Experiments have demonstrated that both genes act on the same biosynthetic pathway in that a functional *R* or *B* allele is required for the presence of the structural enzyme encoded by the *Bz1* gene (Dooner, Mol. Gen. Genet. 189:136, 1983; Gerats et al., Biochem. Genet. 22:1161, 1984).

We reasoned that the fact that certain *B* and *R* alleles are functionally duplicate may reflect some DNA sequence conservation between the two genes that might be detectable using Southern blot hybridization. To investigate this we have used the cloned *R* gene as a hybridization probe to screen Southern blots containing DNA samples prepared from various *B* alleles for restriction fragment length polymorphisms.

The 5.0kb *R* clone corresponding to the seed component (S) of *R* was obtained from S. Dellaporta. This is an *R* allele that is functionally duplicate to *B-Peru*. The clone obtained from Dellaporta contained a sequence highly repeated in the maize genome, so subcloning was required to obtain a relatively unique copy region to use on Southern blots. A variety of restriction enzymes and hybridization conditions were then utilized to detect a restriction fragment polymorphism among our various *B* alleles.

An example of one Southern blot showing a *Bgl*II restriction fragment polymorphism between several *B* alleles is shown in Figure 1A. The *R* allele (*r-g*) is constant in

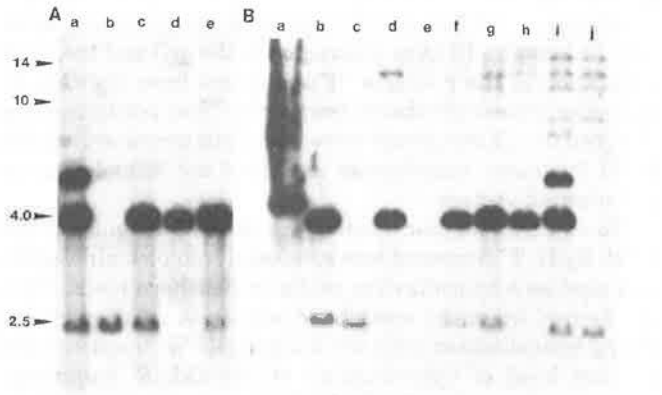


Figure 1. Southern blots on DNA from *B* and *R* stocks hybridized with the *R* probe. DNA samples were digested with *Bgl*II, electrophoresed on 0.5% agarose, transferred to nylon membrane (MSI), and hybridized with a 1.9kb *R* clone labelled by random hexamer priming. A: lanes a-e, DNA samples from maize stocks with the designated *B* and *R* alleles: a) *b* (null) *R-g*; b) *b R* deletion (DNA sample courtesy of Mary Alleman); c) *b r-g*; d) *B-Peru, r-g*; e) *b-Perumu5/b, r-g*. B: lane a) reconstruction containing ~1 copy per haploid genome of the ~5.0kb *R* clone (courtesy of S. Dellaporta); b) *b r-g* stock; c) size- fractionated DNA from *b r-g* stock; d) *B-Peru r-g*; e) size-fractionated DNA from *B-Peru r-g* stock; f) *b-Perumu5/b r-g* plant, g) *b-Perumu5/b r-g* plant; h) *b-Perumu5/b-Perumu5 r-g* plant; i) *b R-g* plant; j) *b R* deletion.

all of our *B* stocks and generates a 4.0kb *Bgl*II fragment. Our standard *b* tester stock contains a weakly hybridizing 2.5kb *Bgl*II fragment (lane c) which is not in the progenitor *B-Peru* stock (lane d). Instead we see a large ~14kb *Bgl*II fragment in the *B-Peru* stock. This ~14kb *Bgl*II fragment is missing in one of our *B-Peru* mutants (*b-Perumu5*) that contains a transposable element insertion into the *B-Peru* gene, and a ~10kb *Bgl*II restriction fragment is observed (lane e). (The *b-Perumu5* individual with the insertion mutation is heterozygous for the *b* tester allele and contains the 2.5kb *Bgl*II fragment.) This is the pattern that would be expected if the fragments hybridizing to the *R* probe were linked to the various *B* alleles.

To further test this hypothesis additional Southern blots were performed on DNA samples from plants segregating the various *B* alleles. The larger *Bgl*II fragments observed in the *B-Peru* stock and the insertion mutant were difficult to reproducibly detect on the Southern blots, presumably due to the weak signal and poor transfer of large fragments on blots. We also frequently observed additional restriction fragments hybridizing to the *R* probe in this region of the gel, masking the fragments we were trying to follow (Figure 1B). Therefore, we decided to address whether the 2.5kb *Bgl*II fragment segregated with the *b* allele. We

prepared DNA from plants that had been previously shown to be either *b/b*, *b-Perumu5/b*, or *b-Perumu5/b-Perumu5*. The ten individuals that were *b/b* or *b-Perumu5/b* all contained the 2.5kb *Bgl*II fragment, and none of the twenty-seven individuals homozygous for *b-Perumu5* contained the 2.5kb *Bgl*II fragment. This result demonstrated that the 2.5kb *Bgl*II fragment is linked to the *b* allele. An example of several individuals is shown in Figure 1A, lanes c and e, and Figure 1B, lanes f-h.

The 2.5kb *Bgl*II fragment was cloned from the standard *b* tester stock, by size fractionating the DNA, cloning in a lambda vector that will carry 0-12kb *Bgl*II fragments (*Bv2*, obtained from Noreen Murray), and screening the resulting phage with the *R* probe using the conditions established with the Southern blots. An example of the size fractionated DNA is shown in Figure 1B, lane c. The size fractionation resulted in ~200 fold purification of the fragment (there is very little maize DNA in this region of the gel) and the clean separation of the putative "b" sequences from the *R* gene and other cross-hybridizing fragments. Two positives were obtained in ~17,000 phage screened. Both contained a 2.5kb *Bgl*II fragment homologous to *R* and not homologous to any plasmid vectors.

To confirm we had cloned the desired fragment, the 2.5kb *Bgl*II "*b*" fragment was subcloned into plasmid vectors and used as a hybridization probe on Southern blots. That the desired fragment was cloned was demonstrated by the strong hybridization with the 2.5kb *Bgl*II "*b*" fragment and the low level of hybridization of the 4kb *R* fragment. However, the 2.5kb *Bgl*II "*b*" fragment contained some repeated sequences, and further subcloning was required to obtain a relatively unique sequence. Using an ~800 bp *BglI/Hind*III subclone as a hybridization probe (see Figure 3), we have tested whether it recognizes the functional *B* gene. As described last year, we have isolated four independent transposable element insertion mutations into the *B-Peru* allele and revertant derivatives of each. These independent insertion mutants have been compared to the progenitor *B-Peru* allele utilizing Southern blots hybridized with the putative "*b*" sequences. An example of one Southern blot is shown in Figure 2. Each DNA sample was digested with *BclI*, which produces a 4kb fragment in the *b* tester and the progenitor *B-Peru* allele. Each of the mutants (*b-Perumu5*, *b-Perumu216*, *b-Perumu218*, and *b-Perumu220*) contain restriction fragment differences relative to the wild type *B-Peru* allele, suggesting that each mutant contains an alteration within this 4kb fragment. The "new" restriction fragments are indicated by arrows on the autoradiograph shown in Figure 2. Most importantly, in the *b-Perumu216*- revertant (lane g), the restriction fragment characteristic of *b-Perumu216* is missing and only the progenitor 4kb fragment is observed. These results definitively demonstrate that the sequences we have cloned recognize the functional *B-Peru* gene. Further restriction mapping indicates that the restriction fragment variation between the different mutant alleles reflects a combination of where the element is inserted within the gene and different *BclI* sites within the various elements. Each insertion contains at least one *BclI* site. The 4kb *BclI* fragment is from the *b* allele, heterozygous in each mutant plant. The *b-Perumu5* allele contains an insertion within the region spanned by the 800 bp

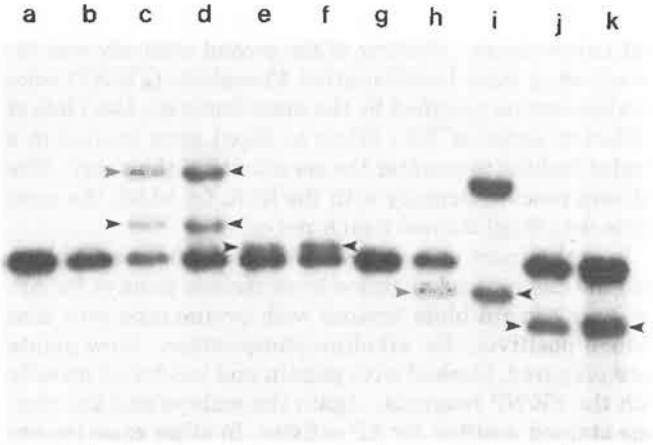


Figure 2. Southern blot on DNA samples from *B* and *R* stocks hybridized with the 800bp *b* sequences. DNA samples (~4ug) were digested with *BclI*, electrophoresed in 0.5% agarose, transferred to nylon membrane (MSI), and hybridized with the 800bp *b* fragment labeled by random hexamer priming. Lanes a-k, DNA from maize plants containing the designated *B* and *R* alleles: a) *b r-g*; b) *B-Peru r-g*; c) *b-Perumu5/b r-g*; d) *b-Perumu5/b r-g*; e) *b-Perumu216/b r-g*; f) *b-Perumu216/b r-g*; g) *b-Perumu216-revertant/b r-g*; h) *b-Perumu218/b r-g*; i) *b-Perumu218/b r-g*, the *b* allele in this stock is polymorphic relative to the other *b* allele shown; j) *b-Perumu220/b r-g*; k) *b-Perumu220/b r-g*. The *b-Perumu5*, *b-Perumu216*, *b-Perumu218* and *b-Perumu220* alleles are independent *Mu*-induced insertions into the *B-Peru* gene.

probe, so that both halves of the element are observed. The other alleles contain insertions to the left of the 800 bp probe (as drawn on the map in Figure 3), so only one-half of each element is observed in this hybridization.

Currently, the *b* sequences are being used to clone the progenitor *B-Peru* allele and each insertion mutant allele, and to generate more extensive restriction maps of the various alleles. A restriction map summarizing our current knowledge of the *B-Peru* allele and where the insertions have occurred is shown in Figure 3. Our best estimate for insertion size within the various alleles is: *b-Perumu5*, 6.5kb; *b-Perumu216*, 3.0kb; *b-Perumu218*, 7.5kb; and *b-Perumu220*, undetermined. Various restriction fragments of the *b* and *B-Peru* alleles have been used on Northern blots to identify an ~2.2kb mRNA. The restriction fragments hybridizing to this transcript are indicated in Figure 3.

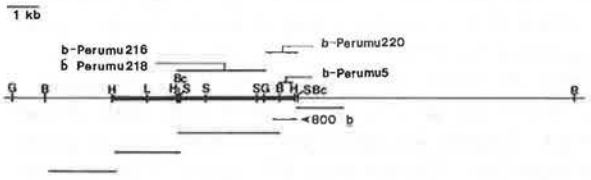


Figure 3. Restriction map of *B-Peru* allele, showing regions transcribed and sites of insertion. The restriction map was generated from a combination of genomic Southern blots, and digestions with cloned DNA sequences. The fragments indicated below the map are subclones used as hybridization probes to generate the restriction map and analyze transcripts on Northern blots. Abbreviations: G, *Bgl*II; B, *Bam*HI; H, *Hind*III; Bc, *Bcl*I; L, *Sal*I; S, *Sac*I. The thickened restriction fragments hybridize to an ~2.2kb mRNA in *B-Peru* and *B* containing stocks. The various *Mu*-induced alleles contain insertions in the fragments, as indicated above the map.

The laboratories of Ben Burr and Steve Dellaporta have independently cloned the *B* genomic sequences using a similar strategy, and our results are consistent with the results of those laboratories.

Isolation and molecular analysis of *Mu*-induced *bz1* and *sh1* mutants

--Kristine Hardeman, Susan Belcher and Vicki Chandler

To establish what types of transposable elements were contributing to the mutation rate in our Mutator stocks, experiments were undertaken to trap elements capable of transposing into genes for which molecular probes were available. Three different Mutator stocks were used in this experiment. One, which was the original Mutator stock used to generate *B-Peru* mutants in this lab, had a typically high number (30-50) of *Mu1*-hybridizing sequences. To increase the probability of isolating non-*Mu1* elements, two other stocks were used that contained *b-Peru* mutable alleles and had been screened for low (1-4) *Mu1* copy number (Chandler and Turks, MNL 62:58-59).

These three *Bz1 Sh1* Mutator stocks were crossed with *bz1 sh1* tester stocks and the progeny were scored for *bz1* and *sh1* mutants (Table 1). Only the progeny from the *B-Peru* Mutator source were fully scored for *sh1* mutants be-

Table 1. Crosses to generate *bz1* and *sh1* mutants.

	female parent		male parent
B-Peru Mu:	*Mu, B-P, Bz1 Sh1, r-g* purple, plump	X	*b, bz1 sh1, R-g* bronze, shrunken
b-Perumu5:	*b, bz1 sh1, R-g* bronze, shrunken	X	*b-Perumu5/b, Bz1 Sh1, r-g* colorless with purple sectors, plump
b-Perumu218:	*b, bz1 sh1, R-g* bronze, shrunken	X	*b-Perumu218/b, Bz1 Sh1, r-g* colorless with purple sectors, plump

The F1 progeny from each cross should be purple, plump unless a mutation event occurred: stable *bz1* mutations would be bronze and plump, unstable *bz1* mutations would be bronze with purple sectors and plump, and *sh1* mutations would be purple and shrunken.

The *b-Perumu5* and *b-Perumu218* stocks had to be used as male onto *bz1 sh1* ears because of the *R* alleles they contained. The *bz1 sh1* tester stocks contained the *R-g* allele and the *b-Perumu5* and *b-Perumu218* stocks contained the colorless *r-g* allele. If *R-g* pollen is crossed onto *r-g* ears, the resulting kernels would be mottled due to incomplete expression of *R-g*, which would have obscured the mutant phenotype we were searching for. However, if *r-g* pollen was crossed onto *R-g* ears, solid purple kernels would be obtained which could be scored for exceptional bronze kernels representing insertions into the *bz1* gene. Self contaminants were recognized as bronze, shrunken kernels and discarded.

cause the other stocks contained dent, which made scoring for *sh1* mutants difficult. However, a small number of ears from the *b-Peru* mutable Mutator sources were sufficiently plump to score for *sh1* mutants and 1 mutant was recovered. All putative *bz1* and *sh1* mutants obtained were again crossed to the *bz1 sh1* tester, and only those that transmitted the mutant phenotype were scored as mutants.

Table 2 lists the total number of kernels scored, the number of mutants isolated, and the frequency at which the mutants were recovered. All of the mutants were due to independent events. Despite the large difference in the *Mu1* copy number in the three Mutator stocks used, the frequency at which mutations were recovered at a single gene (*bz1*) was not significantly different in the three stocks.

Table 2. Number of mutants isolated.

		Total Scored		Mutants		
Mu Source	*Mu1* copy #	#ears	#kernels	*bz1*	*sh1*	Mutation Frequency
B-Peru Mu	30-50	587	76,313	5	8	*Bz1*: 6.6×10^{-5} *Sh1*: 1.0×10^{-4}
b-Perumu5	1-4	535	137,044	8	-	*Bz1*: 5.8×10^{-5}
b-Perumu218	1-4	646	215,355	7	1	*Bz1*: 3.3×10^{-5}

Several of the *Mu*-induced *bz1* and *sh1* mutants recovered have been molecularly analyzed. Table 3 lists the Mutator source and apparent sizes of the inserts found in the *Bz1* and *Sh1* genes as deduced from Southern blots. The results show that, although similar Mutator stocks were used to generate the *bz1* and *sh1* mutants, very different types of elements, based on size, inserted into the two genes.

Table 3:. Molecular results of insertion sizes of mutant.

Mutator Source	Size of insert in *Bz1*	Size of Insert in *Sh1*
B-Peru	2 mutants: 1.2-1.4kb	4 mutants: ~1.4kb ~4.0kb ~5.0kb undetermined
b-Perumu5	5 mutants: 800bp	none
b-Perumu218	1 mutant: 800bp	1 mutant: ~3.0kb

One of the *bz1* mutants derived from the *b-Perumu5* Mutator source has been cloned. The insert is ~800bp and hybridizes to both *Mu1* terminal and *Mu1* internal probes. The *Mu1* hybridization, the insert size, and restriction pattern suggest that the element is a deletion derivative of *Mu1*.

Numerous insertions into *bz1* have been isolated from Mutator stocks and molecularly characterized in other laboratories. Interestingly, almost all of the inserts have been *Mu1*, or the *Mu1*-related element *Mu1.7* (W.E. Brown, D.S. Robertson and J.L. Bennetzen, personal communication; L.P. Taylor, V.L.Chandler, V. Walbot, Maydica 31, 1986). The only exception we are aware of is the *bz1-rcy* allele which contains a *Mu* related element referred to as *r-cy:Mu7* that only shares the 220bp *Mu* termini with *Mu1* (P. Schnable, personal communication). The insertion in the *bz1* mutant that we have cloned appears to be a deletion derivative of *Mu1*. The remaining insertions in the *bz1* mutants that we have molecularly analyzed also appear to be either *Mu1* or the *Mu1*-deletion described above. The Mutator stocks which were used to generate these mutants resulted in *sh1* mutants with insertions that, based on size, hybridization, and restriction mapping, appear to be different from *Mu1*. This finding, as well as the result from other labs of almost every *Mu*-induced *bz1* mutant being due to the insertion of a *Mu1* or *Mu1.7* element, suggests that the *bz1* gene may contain a hotspot for *Mu1* insertion.

Further molecular studies of the *Mu*-induced *bz1* and *sh1* mutants are in progress. We are particularly interested in determining if the elements inserted into these alleles contain *Mu* termini, and if so, if they represent previously uncharacterized *Mu* elements. Alternatively, the insertions may represent other families of transposable elements.

HYDERABAD, INDIA
Osmania University

Hydroxylation pattern of certain intermediates in anthocyanin biosynthesis

--P. Suprasanna, K. V. Rao and G. M. Reddy

In maize, the *Pr* locus controls the hydroxylation pattern of the B-ring of anthocyanin, but the exact position in the known gene action sequence is not clear. Mutants recessive for *pr* in combination with other anthocyanin genes like *a* and *a2* have been studied in an attempt to understand the hydroxylation pattern of intermediates in anthocyanin biosynthesis in maize. It is not clear whether the 3' hydrox-

ylation occurs at the 9 carbon or 15 carbon level after closure of the ring. In the present report, aleurone extracts from homozygous double recessive mutants *c2 pr*, *bz pr* and single recessive mutants *c2 Pr* and *bz Pr* were analyzed and the accumulated compounds were isolated and characterized by spectrophotometric and chromatographic methods.

Homozygous double recessive *c2 pr* accumulated p-coumaric acid, and recessive *c2 Pr* cinnamic acid, which differ in the hydroxylation groups (Fig. 1). Aleurone extracts of *bz Pr* contained luteolinidin and double recessive

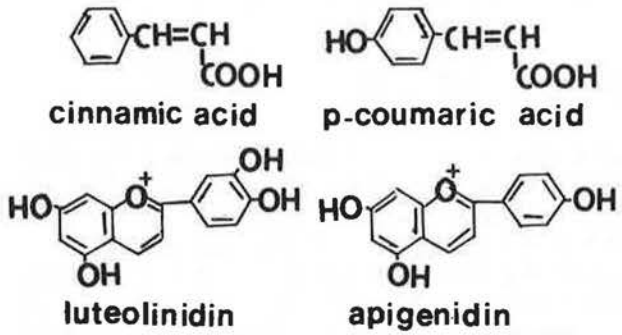


Figure 1.

bz pr contained apigenidin, suggesting that hydroxylation occurs prior to *Bz* in the gene action sequence proposed by Reddy and Coe (1962). The presence of cinnamic acid in *c2 Pr* and p-coumaric acid in *c2 pr* suggest that hydroxylation may occur after *C2* in the gene action sequence. These observations further offer evidence on the nature of hydroxylation pattern of the B-ring. Enzymatic studies with specific genotypes may provide further insight in the hydroxylation of the anthocyanin molecule in the biosynthetic pathway in maize.

Genotypic differences and effect of amino acids on somatic embryogenesis in immature embryo calli

--K. V. Rao, P. Suprasanna and G. M. Reddy

Most of the cereal tissues cultured in vitro regenerate plants through somatic embryogenesis (Vasil, 1987). In maize, embryogenesis has been reported from inbreds and hybrids. Previously we have reported on callus induction, somatic embryogenesis and plant regeneration from glume calli (MNL 1983, 1986). The present report deals with the screening of local germplasm and the effect of certain amino acids on embryogenesis in immature embryo cultures. Immature embryos of inbreds CM117, CM119, CM120, CM400, CM111 and a hybrid (DHM-1) (obtained from Maize Research Station, Amberpet, Hyderabad), and sweet corn were tested for efficient callusing and embryogenesis. Of these, DHM-1 exhibited a high frequency of somatic embryogenesis (52%) compared to other genotypes (10-38%), on MS medium supplemented with 2mg/l 2,4-D + 3% sucrose. Amino acids like L-proline, glutamic acid, asparagine and tryptophan were also used to see their effect on the frequency of embryogenesis at concentrations of 5-25mM in MS + 2,4-D medium. L-proline (10-15mM) was found to be effective in enhancing the frequency of embryogenesis by about 15% over that of controls without proline. Higher concentrations (20 and 25mM), however, decreased the embryogenic potentiality of the calli. Asparagine and glutamic acid did not have any effect, whereas tryptophan was found to be inhibitory. Plants were regenerated from the embryogenic cultures of sweet corn and DHM-1 and were transferred to vermiculite pots, surviving only for about a week, in mist chamber. Different methods of acclimatization are being used for further growth of these plants.

SEM and TEM characterization of embryogenic calli

--K. V. Rao, P. Suprasanna and G. M. Reddy

Within a week of culturing of immature embryos, embryogenic cells were formed as few sectors, after which the callus proliferated from the embryos. Numerous torpedo shaped embryoids could be seen on the embryogenic calli, under a dissecting microscope. At this stage, the embryogenic calli were separated and grown on MS medium supplemented with 2,4-D. The level of 2,4-D in the medium was crucial in maintaining the embryogenic competence of the calli. The embryogenic cells appeared small, and rich in cytoplasm, compared to non-embryogenic cells.

One-month-old subcultured embryogenic and non-embryogenic calli (originally separated from the primary calli that were initiated from the embryos), were characterized by scanning and transmission electron microscopy, to see the differences in cell types and organization between these calli. SEM studies revealed the presence of numerous globular structures besides exhibiting characteristic features of scutellum and coleoptile. The TEM studies revealed clear differences between embryogenic and non-embryogenic calli. Embryogenic cells were characterized by thin cell walls with uniformly distributed cytoplasmic material. On the other hand, the non-embryogenic calli showed thick cell walls with meagre cytoplasm along the cell wall.

IOWA CITY, IOWA
University of Iowa

Evolution of the B chromosome

--Wayne Carlson

Ostergren (Bot. Notiser, 1945) proposed that B chromosomes are parasitic entities. If that hypothesis is correct, a relatively simple scheme for evolution of the maize B chromosome can be proposed using existing evidence. Steps in the sequence are given below:

a. A fragment chromosome developed from one of the maize chromosomes. The mechanism for its origin is unknown, but Peeters and Wilkes (MNL 57:19, 1983) give one hypothesis.

b. A specific gene on the fragment produced a beneficial effect in extra dosage, allowing selection of the chromosome. Brewbaker and Natarajan (Genetics, 1960) constructed a fragment chromosome in *Petunia* with a gene that confers a dosage-dependent selective advantage.

c. Most genes on the nascent B chromosome gave a negative dosage effect, as expected. Harmful effects of partial trisomy occurred in the sporophyte. Also, transmission of the fragment chromosome was limited to the female, due to genetic imbalance in the pollen. As a result, most genes on the B were inactivated through random mutation or heterochromatinization plus selection.

d. Due to its low frequency, the early B chromosome was usually found in plants as a single extra chromosome. During meiosis, univalent B chromosomes had difficulty disjoining in anaphase I and were frequently lost. Meiotic loss caused selective pressure for evolution of a system which allows regular migration of univalent B's to one pole. Two regions have been identified on the B which suppress meiotic loss, apparently by enhancing polar movement of univalents (Carlson, Crit. Rev. Pl. Sci. 1986; Carlson, MNL, 1988).

e. The nondisjunctional mechanism of the B resulted from activation of the system which suppresses meiotic loss at the second pollen mitosis. The migration of a chromosome (with two chromatids) to one pole in meiosis is identical to the process of mitotic nondisjunction. Consequently, a single genetic system was able to suppress losses at meiosis and produce nondisjunction at the second pollen mitosis.

f. Nondisjunction at the second pollen mitosis initially served as another means for circumventing meiotic loss. In crosses of 0B x 1B plants, many of the progeny receive two B's due to nondisjunction. The frequency of univalent B chromosomes in a population was thereby reduced.

g. The completion of an accumulation mechanism, through addition of preferential fertilization, occurred later. It was selected in order to maximize the frequency of B chromosomes in populations.

h. Over time, the beneficial function of B chromosome trisomy may have become unimportant. Perhaps it was superseded by duplication of the relevant gene within the standard genome. In any case, the beneficial function was lost by random mutation. The B chromosome was established as a parasitic entity.

Several variations on this proposal are feasible. For example, the modern B chromosome may retain a beneficial function and not be truly parasitic. Also, preferential fertilization may not have required a separate evolutionary step. Preferential fertilization may be an inherent property of maize pollen which occurs when the two sperm have a differential chromosome content. Despite these uncertainties, the proposal does provide a logical evolutionary sequence for origin of the maize B chromosome. Some of the ideas discussed should be applicable to evolution of B chromosomes in other organisms.

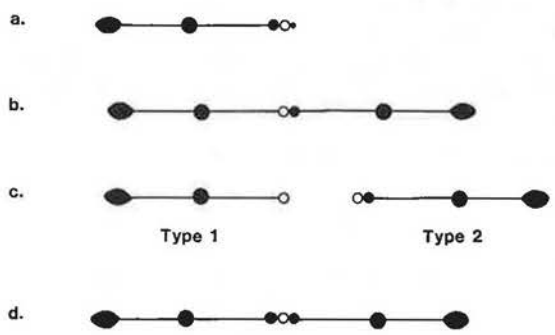
Region 4 on the B chromosome: evidence for a unique function

--Wayne Carlson

Studies by Lin (Genetics, 1979) and Carlson (Ann. Rev. Genetics, 1978) suggest that the B chromosome is not telocentric. They found that rearrangements at the B centromere which should delete an adjacent short arm have a genetic effect: they reduce the rate of nondisjunction at the second pollen mitosis. The findings provide some evidence for the existence of a B short arm. However, another explanation is possible. Perhaps part of the B centromere itself can be removed without disrupting the basic functioning of the centromere. This component of the centromere affects the rate of mitotic nondisjunction. To accommodate both ideas, the B centromere plus the hypothetical short arm are referred to as region 4. Within this region, there is a site(s) which affects the rate of nondisjunction.

One explanation for the effect of region 4 is to assume that the active site is heterochromatin in the B short arm. The heterochromatin is similar to that in region 3, adjacent to the centromere in the long arm. Region 3 heterochromatin controls nondisjunction through non-division at the second pollen mitosis (Rhoades and Dempsey, Genetics, 1972; Carlson and Chou, Genetics, 1981). Perhaps maximal nondisjunctional rates require that the B centromere be flanked on both sides by non-dividing heterochromatin.

A test can be made for equivalency of region 3 and region 4. In the accompanying diagram, misdivision products of the B^9 chromosome from TB-9Sb are shown. The following are depicted: a) standard B^9, b) pseudoisochromosome derivative of B^9, c) type 1 and type 2 derivatives of the pseudoisochromosome, d) type 2 isochromosome derivative of the type 2 telocentric.



The type 2 isochromosome contains two doses of region 3, flanking the centromere. It should give nondisjunctional rates similar to the standard B^9, if region 3 is equivalent to region 4. An ear was constructed which segregated two types of progeny: 9^B (*Wx*) 9^B (*Wx*) type 2-iso B^9 (*Yg2 Bz*) and 9 (*wx*) 9^B (*Wx*) standard B^9 (*Yg2 Bz*). Seven plants of each type were crossed as male to a tester of B^9 nondisjunction: *yg2/+ bz bz wx wx* (only *Wx* kernels were used in the analysis, because they contain TB-9Sb).

Nondisjunctional rates were calculated as *bz Yg* plus 2 X *Bz yg2* phenotypes (the *yg2* totals must be doubled due to heterozygosity of the tester). Rates of nondisjunction for two ears per cross are given below:

9^B 9^B type 2-iso B	
Plant No.	Rate Nondisjunction
6072-1	46% (292/638)
-10	74% (532/718)
-28	43% (280/652)
-45	54% (302/559)
-50	49% (285/587)
-56	55% (399/723)
-70	57% (330/582)
9 9^B std B^9	
6073-3	92% (338/369)
-6	92% (416/453)
-17	94% (361/385)
-21	90% (316/353)
-22	91% (364/399)
-23	99% (463/466)
-24	95% (362/382)

There is a large, nonoverlapping difference in rates of nondisjunction between the two groups. It is concluded that region 4 contains a unique site of nondisjunctional control which cannot be replaced by nondividing heterochromatin.

IRKUTSK, USSR
Siberian Inst. Plant Physiol. Biochem.

Transcription as dependent on respiration and phosphorylation in isolated mitochondria

--Yuri Konstantinov, Galina Lutsenko and Vladimir Podsosonny

To explain the interrelationship between two main enzyme systems of maize mitochondria, the genetic system and oxidative phosphorylation (OP), the effect of different inhibitors of OP and electron transport chain on kinetics of RNA synthesis was studied in vitro in intact maize mitochondria. The characteristics of nucleotide organization of the maize mitochondrial genome and mtRNA processing suggest that transcription may be related to OP at the level of adenine nucleotide translocation, an initial step of OP, which is performed by a special protein carrier.

The inhibition of adenine nucleotide translocase by carboxyatractyloside (1μM) caused an almost two-fold decrease in the rate of ^{3}H-UTP incorporation into the mitochondrial acid-insoluble fraction (Table 1). The inhibition of

Table 1. The effect of inhibitors of respiration and phosphorylation on kinetics of RNA synthesis in mitochondria of maize hybrid Krasnodarsky 303 TV (W64A x Sg25).
The reaction mixture contained 50 mM KCl, 2 mM $MgCl_2$, 40 mM KH_2PO_4 (pH 7.2), 4 mM succinate, 2 mM glutamate, 12 mM 2-mercaptoethanol, 2 mM ATP, 0.5 mM CTP and GTP each and 0.15 mM ^{3}H-UTP. Specific activity of ^{3}H-UTP was 550 TBq/mol.

Conditions	^{3}H-UTP incorporation in acid-insoluble mitochondrial fraction, counts (mg protein)$^{-1}$ min^{-1}			
	5 min	10 min	15 min	20 min
Control	9229±709	13876±734	20297±982	33737±1223
+carboxyatractyloside	3472±133	7378±324	-	-
+KCN	2173±121	3951±608	8447±872	9004±802
+oligomycine	7995±835	9005±570	13510±618	25241±1686

the mitochondrial electron transport chain with KCN resulted in more pronounced inhibition of RNA synthesis. The decrease in the rate of RNA synthesis in mitochondria in response to the addition of a classic OP inhibitor, oligomycin, was not so significant, amounting to about 30%. In the presence of OP uncoupler, carbonyl cyanide-m-chlorophenylhydrazone, decrease of the transcription rate was also observed.

The level of DNA-dependent synthesis of DNA did not change, but even increased when ADP was used instead of ATP as a precursor (Table 2). The transcription was

Table 2. Kinetics of RNA synthesis in mitochondria of maize hybrid Krasnodarsky 303 TV when ATP was substituted for ADP in the reaction mixture.

Conditions	^{3}H-UTP incorporation in acid-insoluble mitochondrial fraction, counts (mg protein)$^{-1}$ min^{-1}			
	5 min	10 min	15 min	20 min
Control	6069±445	10763±863	19239±2045	44288±1108
-ATP+ADP	10013±1246	16338±885	31042±757	49787±847
-ATP+ADP+ oligomycine	460±36	5951±168	17290±687	19876±1940

strongly inhibited in this case by oligomycin indicating the direct involvement of extramitochondrial ADP after its phosphorylation into the process of RNA biosynthesis. These experimental data also suggest possible functional coupling of OP and RNA synthesis on the basis of additional microcompartments in the inner mitochondrial space.

The effect of membrane active agent, sodium cholate, on mitochondrial transcription depended on the concentrations used: low concentrations of the detergent (100μM) produced significant activation of the RNA synthesis, while higher concentrations (500μM and 1mM) showed strong inhibition effect.

It may be inferred that DNA transcription in maize mitochondria strongly depends on respiration and oxidative phosphorylation. It is assumed that the regulation mechanism of mitochondrial genome transcription may involve conformational changes in DNA-membrane complex during energy transformation in mitochondria

The role of RNA synthesis in the maintenance of functional activity of isolated mitochondria

--Yuri Konstantinov, Vladimir Podsosonny and Galina Lutsenko

Little is known about the interrelationship between oxidative phosphorylation and the genetic system of plant mitochondria during their functional activity. The study of the effect of mitochondrial transcription inhibitors in vitro on the ability of isolated mitochondria to transform energy may give a deeper insight into this problem. It has been attempted here to study in vitro the role of mitochondrial RNA (mtRNA) synthesis in the maintenance of functional activity of isolated seedling mitochondria. The effect of mitochondrial transcription inhibitors (ethidium bromide and actinomycin D) on the parameters of phosphorylative activity of mitochondria has been examined.

The mitochondria were isolated from 3-day-old etiolated seedlings of W64AxSg25 by a conventional method of differential centrifugation. The activity of mtRNA synthesis was estimated by the incorporation of (^{3}H-UTP) into acid insoluble mitochondrial material. To inhibit DNA transcription in the mitochondria, ethidium bromide (2μg/ml) and actinomycin D (40μg/ml) were used. The mitochondrial respiration was analysed polarographically with a closed Clark electrode. The rate of phosphorylative oxidation was measured in terms of oligomycin-sensitive component of respiration. The protein was determined by the Lowry method with bovine serum albumin as a standard.

The kinetics showed that both inhibitors caused a significant decrease in the rate of mtDNA transcription, ethidium bromide exhibiting greater effect (Fig.). Furthermore, in the presence of ethidium bromide mtRNA synthesis quickly reached its stationary level.

Mitochondrial parameters of oxidation and phosphorylation revealed that mitochondria exposed to the inhibitors

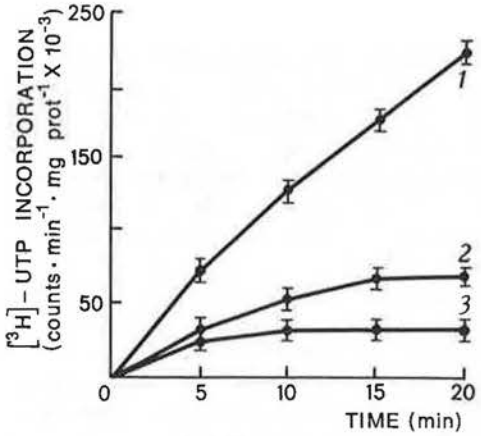


Figure. Kinetics of mtRNA synthesis inhibition in isolated mitochondria of maize hybrid W64AxSg25. 1, control; 2, actinomycin D; 3, ethidium bromide.

of transcription yielded a significant increase of the respiration rate in the 4th metabolic state and concomitant decrease in the value of respiration control after Lardy-Wellman (Table). No significant changes in the 3d metabolic state were observed in this case, which may indicate maintenance of the activity of enzymes of oxidative phosphorylation. It can be seen that of the two

Table. The in vitro effect of transcription inhibitors on mitochondrial respiration of hybrid W64AxSg25. Succinate is used as an oxidative substrate.

Time of mitochondria exposure to inhibitors (min)	Parameters of mitochondrial respiration (% of control)					
	Rate of mitochondrial respiration in MS4		Rate of mitochondrial respiration in MS3		Respiratory control	
	ActD	EB	ActD	EB	ActD	EB
5	108	90	107	111	99	123
40	191	239	101	103	53	43
100	170	251	100	109	59	43

Notes. ActD, actinomycine D; EB, ethidium bromide; MS3 and MS4, 3d and 4th metabolic states of mitochondria after Chance. The mitochondria were incubated with the transcription inhibitors under the temperature of melting ice. Mean values of three experiments with four replicates are given.

inhibitors, ethidium bromide had greater effect in the 4th metabolic state. The reduction in the phosphorylation activity of mitochondria in the presence of ethidium bromide and actinomycin D is likely to be due to a failure of the inner mitochondrial membrane to retain electrochemical potential of H+-ions causing uncoupling of oxidation and phosphorylation. The synthesis of mtRNA coding protein components of the inner mitochondrial membrane in isolated maize mitochondria appears to be necessary to maintain the energy transformation capacity of the organelles in such conditions. Dewey et al. (Plant Physiol. 79:914, 1985, and PNAS 82:1015, 1985) showed that the three genes encoding ATPase subunits in maize mitochondria were most actively transcribed. However, it is quite possible that the changes in membrane permeability may be associated with certain disturbances in the genetic system at the translation level resulting from the inhibition of ribosomal and transport RNA gene transcription. The data obtained suggest that the disturbances of mtDNA transcription in maize mitochondria in vivo may result in variations of the H^+-permeability of the inner mitochondrial membrane. Thus it appears to be the main reason of the mitochondria failing to synthesize ATP.

IRKUTSK, USSR
Siberian Inst. Plant Physiol. Biochem.
KRASNODAR, USSR
Krasnodar Research Institute of Agriculture

The low level of RNA synthesis in mitochondria of heterotic hybrids

--Yuri Konstantinov, A. S. Mashnenkov, Galina Lutsenko and Vladimir Podsosonny

According to our hypothesis that the level of organism adaptation is negatively associated with the level of mtDNA transcription (MNL 61:61, 1987), a low rate of mtRNA synthesis was expected in hybrid mitochondria. The assumption was strongly confirmed by the rate of mtRNA synthesis determined in 10 inbred lines and 17 hybrids. The conditions for the reaction of RNA synthesis to occur in intact mitochondria were essentially similar to those described elsewhere (J. Carlson et al., Curr. Genetics 11:151, 1986). The kinetics of mtRNA synthesis in organello was registered by ^{3}H-UTP (370 TBq.mol^{-1}). The kinetic data on transcription activity of mtDNA in each line and hybrid were obtained from 6-8 (3-4 independent) experiments. The data were analysed by conventional statistic methods and are given in the table.

Source of mitochondria	Incorporation of ^{3}H-UTP into acid insoluble mitochondrial fraction, counts/min mg protein			
	5 min	10 min	15 min	20 min
Lines (10)	6909±1436	11701±1170	23774±4829	38823±11092
Hybrids (17)	6958±897	9764±879	12330±1148	18211±1674

By the 5th min, the average rate of mtRNA synthesis was similar in mitochondria of the lines and hybrids. However the rate further increased with different acceleration. By the 20th min, the rate showed 5.6-fold increase in the mitochondria of the lines and only 2.5-fold increase in hybrid mitochondria. The high rate of mtDNA transcription in inbred lines may be partially accounted for by stimulating effect of recessive alleles of the nucleus (MNL 62:67, 1988).

As a whole, the data reported here and elsewhere (MNL 61:61, 1987) could also indicate the existence of negative nuclear control of transcriptive activity of maize mitochondrial DNA.

JOHNSTON, IOWA
Pioneer Hi-Bred International

Reliability of the protein extraction procedure used to prepare seedling samples for two-dimensional electrophoresis

--J. W. Higginbotham* and J. S. C. Smith
*Also affiliated with Cold Spring Harbor Laboratory, NY.

As part of the collaboration with Cold Spring Harbor Lab. and Pioneer, two-dimensional electrophoretic profiles of corn seedling proteins are being computer analyzed with the software developed by Garrels (J. Biol. Chem. 254:7961, 1979). Because computer analysis discerns quantitative differences among proteins with unprecedented precision, it is extremely important to be aware of those factors which may cause quantitative differences in proteins among gels or distortion of the profile and to minimize the differences or distortion due to laboratory manipulation. With these objectives, three components of the corn seedling protein extraction procedure used at Pioneer were tested.

Protein extraction followed Damerval (Electrophoresis 7:52, 1986) with a few exceptions. Embryos were homogenized with a Brinkman polytron directly in cold acetone, the TCA precipitation step being omitted. Protamine sulfate was added to the extraction solution, but ampholytes were not included.

The TCA-precipitable radioactivity in the acetone supernatant was assayed to determine how much lipophilic protein was being lost in the initial extraction. The TCA-pre-

cipitable radioactivity of the sample is routinely assayed. Repeated assays revealed 1000-fold less labeled protein in the acetone than in the sample. Samples often contained 100,000cpm/ul of TCA-precipitable radioactivity. Proportional amounts of acetone supernatants contain an average of 100cpm.

Protamine sulfate (PS) is reported to precipitate nucleic acids (Mayer et al., Plant Cell Rep. 6:77, 1987), but its effectiveness in a high molar urea solution was unknown. Samples with and without PS in the extraction solution were electrophoresed at Johnston, and the gels silver stained to determine the effectiveness of this chemical in removing nucleic acids. In those without PS, numerous horizontal gray streaks extended across the gels. Samples prepared with PS had a much clearer background due to the almost complete absence of gray streaks.

Lastly, the effectiveness of the procedure in preventing degradation due to proteinase activity was investigated in three ways. Two samples were divided into two aliquots each. One aliquot of each sample was incubated at 37 C for 3 hrs prior to electrophoresis (at Johnston). The other aliquots were warmed to 37 C immediately prior to electrophoresis. Gels were subsequently silver-stained to determine if high-molecular weight proteins had been preferentially lost and if there was visible degradation of the protein profiles in the aliquots that had been kept at 37 C for 3 hrs.

Proteinase activity was assayed using Azocoll dye-bound collagen as the substrate. Proteinase K and trypsin were used as positive controls. One assay was performed using protein extraction solution instead of buffer in the reaction mixtures. Other assays were performed using .1M sodium phosphate buffer. For the latter assay, embryos were homogenized in cold acetone, the pellet dried under vacuum, and resuspended in .1M phosphate buffer. All reaction mixtures and blanks were read at 520nm.

The extraction procedure appeared to be effective in preventing degradation due to proteinase activity. The pairs of silver stained protein profiles were essentially identical with no preferential loss of high molecular weight proteins or visible degradation of the protein profiles.

No proteinase activity in the samples was detected using Azocoll substrate even when the reaction was allowed to proceed 30 min at 37 C. When protein extraction solution was used instead of phosphate buffer, no proteinase activity was detected in any of the positive controls either.

In summary, the results presented here indicate that the protein extraction procedure used routinely in our lab is a reliable, reproducible method for extracting corn seedling proteins. The variability generated due to quantitative loss of protein into the acetone supernatant during protein extraction appears to be very minimal. The removal of nucleic acids by protamine sulfate precludes distortion of the profile due to their presence. There appears to be little if any variability generated by the action of proteinases in situ. Not only does the extraction solution prevent proteinase activity, the acetone extraction appears to be an effective inhibitor of activity, too. Furthermore, samples appear to be stable at 37 C for at least 3 hrs. Samples can be stored frozen and warmed for reuse many times without risk of degradation.

The effect of different seed lots versus genotypes on 2-D protein profiles of seedlings

--J. W. Higginbotham*, J. S. C. Smith, and O. S. Smith
*Also affiliated with Cold Spring Harbor Lab.

Before two-dimensional protein profiles are routinely used to describe inbred genotypes and to determine genetic associations between inbreds, the contribution of environmental factors needs to be understood to insure that results are reproducible and reliable.

This experiment was designed to examine the variation among protein profiles due to different environmental sources of kernels relative to the variation due to different genotypes. One lot of public inbred line B73 (seed source Johnston, 1986) and two lots of Pioneer Hi-Bred proprietary inbred line 207 (seed source Johnston, 1982 and 1986) were used.

Kernels from line B73 were placed on moist filter paper and incubated at 27-30 C until most of the kernels had germinated (27 hrs). Embryos were excised from eight germinated kernels, washed, dried, and weighed. Each embryo was placed in a spot well with 35-S methionine solution and labeled for 17 hrs. Subsequently, they were washed twice, dried, and reweighed. Total pre-label mass averaged 53mg per embryo, while total post-label mass averaged 92mg per embryo. Increase in mass was accompanied by elongation of all embryonic axes. Total elapsed time including imbibition and labeling was 45 hrs. Extraction of protein from the seedlings followed Damerval (Electrophoresis 7:52, 1986) and Higginbotham and Smith (in this Newsletter).

Kernels from the two lots of line 207 were treated as described for B73 except that total imbibition time was 43.5 hrs. This was due to slightly faster germination by 207. Embryos were labeled for 17 hrs. For lot 207-82, pre-label mass averaged 56mg per embryo, while post-label mass averaged 84mg per embryo. For lot 207-86, pre-label mass averaged 54mg per embryo, while post-label mass averaged 93mg per embryo.

The two-dimensional gel electrophoresis, fluorography, and computer analysis were performed at the QUEST facility of the Cold Spring Harbor Laboratory under the supervision of Heidi Sacco and Cecile Chang. Work with the QUEST facility was funded by Pioneer Hi-Bred International, Inc. through a cooperative agreement between Pioneer Hi-Bred and the Cold Spring Harbor Laboratory. A pH gradient of 4-8 was used in the first dimension, and a 12.5% SDS-polyacrylamide gel was used in the second dimension. Each of the eight gels that were run (2 B73, 3 207-82, and 3 207-86) were exposed to film for three periods of time. This generated a light, medium, and dark exposure for each gel.

The data set consisted of 1961 spots matched across all eight gels. Of these, nine spots had zero density values for 7 of the 8 gels and were deleted from the data set. Spot densities are in normalized units (ppm).

In order to determine the influence of environmental effects relative to genotypic effects, nested analysis of variance was performed on each spot (1952 nested analyses of variance). Two sets of spots were generated. The first set included those spots where at least 80% of their variation was partitioned between the two inbreds. The second set included those spots where at least 80% of their variation was partitioned between the two lots of 207. Based on this

method of selecting spots, 14.7% (287 spots) showed large differences between the two lines, while less than 1.0% (16 spots) showed equally large differences between the two lots of 207. Other methods of spot selection and analysis gave similar results.

Relative to the influence of different genotypes on protein profiles, the influence of different environmental sources appears to be very minimal. The effect of different genotypes on the protein profiles is large and easily extracted from the data set. It does not appear that major genotypic effects are confounded to any extent with environmental effects. Those spots which do show a putative environmental effect are also easily extracted. Whether or not these conclusions are true for other inbreds and other environments awaits further study.

The effect of slightly varying imbibition periods versus genotypes on 2-D protein profiles of seedlings

–J. W. Higginbotham*, J. S. C. Smith and O. S. Smith
*Also affiliated with Cold Spring Harbor Lab.

Before two-dimensional corn seedling protein profiles are routinely used to describe inbred genotypes and to determine genetic associations between inbreds, the contribution of slight differences in the development of seedlings to the variation present in the protein profiles needs to be understood to insure adequate stability of the profile.

This experiment was designed to test the impact of slightly varying total imbibition periods on the protein profile relative to the impact of different genotypes. One lot each of Pioneer Hi-Bred proprietary inbred lines G39 and G80 was used.

Sample preparation and protein extraction were as described above (Higginbotham and Smith in this Newsletter), but with the following exceptions. Three samples of G39 were prepared with total imbibition times of 37-38, 45-46, and 49-50 hrs. Four samples of G80 were prepared with total imbibition times of 37-38, 41-42, 45-46, and 49-50 hrs. All embryos were labeled during the last 17 hrs of their imbibition period. Each of the seven samples consisted of three embryos. The average mass of the embryos before and after labeling is given in Table 1.

Table 1. Average mass of embryos before and after labeling with 35-S methionine.

	G39		G80	
	Before	After	Before	After
49-50 hrs	66 mg	103 mg	70 mg	115 mg
45-46 hrs	61 mg	99 mg	61 mg	112 mg
41-42 hrs	-----	------	53 mg	96 mg
37-38 hrs	53 mg	87 mg	42 mg	85 mg

The laboratory parameters were as described above (Higginbotham, Smith, and Smith in this Newsletter), but with the following exceptions. A 10.0% SDS-polyacrylamide gel was used in the second dimension. Each of the seven gels that were run (3 G39 and 4 G80) were exposed to film for two periods of time. This generated a light and medium exposure for each gel. The sample of B73 used in the previous study (Higginbotham, Smith, and Smith in this Newsletter) was also run under these conditions and all eight gels were analyzed together.

The data set consisted of 729 spots matched across all eight gels. Of these, 112 spots had zero density values for at least six gels and were deleted from the data set.

The data were then subjected to principal component analysis with no further spot selection. Figure 1 shows the placement of the eight gels on the first three vectors. The first three vectors encompassed 27%, 25%, and 16% of the variation respectively. Even with slightly varying imbibition periods, principal component analysis succeeded in distinguishing gels of the different inbreds.

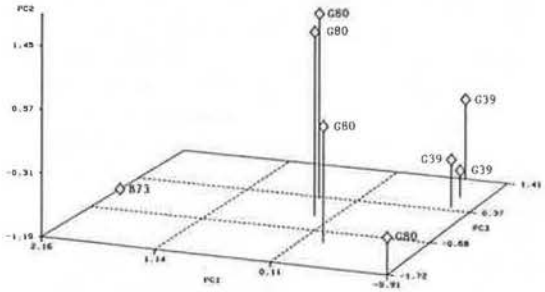


Figure 1. Placement of eight gels on the first three vectors of a principal component analysis.

A set of spots was generated which included only those spots where at least 80% of their variation was partitioned between the three inbreds. This set contained 82 spots (13.3%). Another set was generated which included only those spots which had a pattern of variation indicating they may be under regulatory control during this time interval. This set included 40 spots (6.5%). The two subsets shared 10 spots.

The set of 82 spots was subsequently subjected to cluster analysis to ascertain the relative similarity of the inbred protein profiles. G39 and G80 are more similar to each other than either is to B73. The same relative similarity between these three inbreds is obtained with pedigree data.

Among the causes of variability studied in our lab slight differences in the relative development of the seedlings seem to introduce more variation than any other factor save differences in genotype. Moreover some spots which are regulated during this stage of the life cycle may also vary among genotypes. It appears, though, that the set of proteins which are informative of genetic associations will always be large enough to delete those which appear to be developmentally regulated provided those proteins are known.

Finally, it appears that two-dimensional protein profiles will be useful in determining genetic associations among inbred genotypes. There are compelling reasons for using this technique in genetic and numerical taxonomic studies. Given good quality gels and fluorographs these complex molecular profiles appear extraordinarily stable and reproducible. The effects of different genotypes on the profile are obvious and measurable. This technique samples a greater portion of the total genome than any other technique currently available with the possible exception of the restriction fragment length polymorphism technique. Not only are polymorphisms at structural loci revealed, polymorphisms at regulatory loci are also revealed (through quantitative differences). The data appear amenable to traditional methods of numerical taxonomic analysis. Additional studies utilizing two-dimensional protein profiles are in progress in Johnston and Cold Spring Harbor.

RFLP's revealed among elite inbred lines and associations among lines revealed by multivariate analysis

--J. S. C. Smith, S. L. Bowen, R. A. Tenborg, R. Feazel, O. S. Smith and D. Grant

We have screened 261 maize DNA probes against 12 elite inbred lines with genomic DNA restricted separately with *Bam*HI, *Eco*RI, and *Hin*dIII. Eighty-three probes were made at Pioneer Hi-Bred International, Inc., 67 were provided by Ben Burr (Brookhaven, NY) and 111 were provided by Dave Hoisington (University of Missouri at Columbia). Two hundred forty-five of the probes have been mapped, to date, in maize; all chromosome arms were represented among the probes. The 12 inbred lines encompassed a range of germplasm diversity that is currently used in the U.S. corn belt.

Percent relationships between the lines according to pedigrees ranged from 0% to 38% for 11 of the lines; one pair was more closely related at 98% by pedigree. Of the 261 probes, 190 gave polymorphic banding patterns that were either single or double banded for one or more of the restriction enzyme treatments and thus, their simplicity of banding would make them amenable to genetic analysis of the variants. For the 12 lines, the 190 probes revealed 772 electrophoretic variants. Individual probes revealed up to 9 variants across the 12 lines; frequently 2 individual restriction enzyme treatments were necessary to reveal 5 or more variants for each of the probes. Principal component and cluster analysis of the RFLP data showed associations among lines that agreed with those that would be expected on the basis of pedigree.

RFLP's provide a wealth of variants that should be useful in describing inbred lines and hybrids, corroborating pedigrees, and revealing germplasm associations among lines.

Associations among inbred lines revealed by RFLP data and correlations with F1 yield and heterosis

--J. S. C. Smith, O. S. Smith, S. L. Bowen, R. A. Tenborg, and D. Grant

Ten elite inbred lines of maize involving 2 sets of 5 lines, one primarily Iodent and the other mainly Iowa Stiff Stalk Synthetic, with pairs of lines within each set related from 52% to 95% by pedigree, have been profiled for 85 mapped RFLP probes. Associations among lines on the basis of RFLP and pedigree data were revealed by principal component and cluster analysis. F1 yield and heterosis data, which were calculated on the basis of inbreeding depression incurred between the F1 and F2 generations, were collected during 1987 and 1988 at 5 locations with 3 replicates per location, 60 plants per replicate. All generations of seed were made simultaneously in the same year and location. Interline distances calculated from RFLP data showed correlations with distances calculated from pedigree records of (Malecot's coefficient of kinship) r = 0.97, with F1 yield r = 0.76, and with heterosis r = 0.56. Pedigree data resulted in correlations with F1 yield of r = 0.62 and with heterosis r = 0.66. In comparison, HPLC zein data gave correlations with F1 yield of r = 0.34 and heterosis of r = 0.54. These data show that associations on the basis of RFLP data among these relatively closely related lines agree well with those that would be expected on the basis of known pedigree.

For the first time we have found a laboratory derived data base (RFLP's) that provides a more accurate prediction of F1 yield than do pedigree data. We expect that this is due to the large number of marker "loci" and the abundance of variants that, therefore, allow chromosomal regions to be tracked from parents to progeny with a degree of detail that was hitherto unafforded by isozymic or zein protein data. We suspect that the relatively low correlation between distances measured by RFLP data and heterosis compared to that shown between RFLP and F1 yield data may be due to the reduced ability to accurately measure heterosis between the relatively closely related inbred lines used in this study. In these lines, the average performance of the F2 generation would likely be similar to that of the inbred line per se and the relatively poor performance of the inbred compared to the F1 generation can result in biased estimates of heterosis.

We are continuing on from these preliminary analyses by looking at associations among a larger set of less closely related inbred lines estimating correlation between field, pedigree, and laboratory data. This set of data also includes the immense power of analysis afforded by the technique of two-dimensional gel electrophoresis which reveals in qualitative and quantitative fashion on the order of 1,000 individual protein products for each inbred line (see Higginbotham, Smith, and Smith in this Newsletter).

Comparison of heterosis among hybrids as a measure of hybrid relatedness with that to be expected on the basis of pedigree

--J. S. C. Smith and O. S. Smith

The use of heterosis data to measure germplasm associations among hybrids was proposed by Troyer et al. (Crop Sci. 28:481-485, 1988). Comparisons of the associations between hybrids using heterosis, isozymic, and zein HPLC data have been shown to give broad agreement (Smith et al., Proc. Ann. Corn Sorghum Ind. Res. Conf. 42:187-203, 1987). However, no comparison has been reported for the associations that would be shown by heterosis data with those that would be expected on the basis of known pedigree. This comparison is important to make in order to test the validity and accuracy of the assumptions upon which the use of heterosis to estimate relatedness among hybrids is founded.

In this study, we measured distances among 10 commercial hybrids currently released by Pioneer Hi-Bred International, Inc. Relationships on the basis of pedigree between pairs of hybrids ranged from 99% (Wright's coefficient) or 50% (Malecot's coefficient) to 6% (Wright) or 4% (Malecot). Yield data were collected at 3 reps per location (60 plants per rep) with 7 locations during 1988; all generations of seed were made simultaneously at Johnston in 1987. Distances between hybrids on the basis of heterosis were calculated according to the formula proposed by Troyer et al. (Crop Sci. 28:481-485, 1988). A correlation of r^2 = 0.81 was found between inter-hybrid distances calculated from the heterosis and pedigree data. These preliminary data (a second round of heterosis measures will be made in 1989) suggest that heterosis could be utilized to estimate germplasm and pedigree similarity among hybrids. However, RFLP data (reported by us in this Newsletter) provide estimates of relatedness that are more expedi-

tiously arrived at and which can show a high degree of correlation both with pedigree and with agronomic performance (F1 yield); the correlation between F1 yield and RFLP distance data being greater than that between F1 yield and pedigree distance data. Thus, RFLP or 2-dimensional gel electrophoresis data (see Higginbotham, Smith, and Smith in this Newsletter) may be more useful and reliable means to characterize germplasm as aids in breeding and agriculture.

KRASNODAR, USSR
Krasnodar Research Institute of Agriculture

Effect of genotypes on seed set frequency in maize-*Tripsacum* crosses

--E. P. Erygina and A. S. Mashnenkov

Maize ears with the silks shortened in advance were pollinated in 1988 with a mixture of *Tripsacum dactyloides* (2n=72) and *Zea mays* pollen in a ratio of 10:1 according to Mangelsdorf and Reeves. In four lines seed set of hybrid kernels varied from 10.3% to 39.8% of the total number of ovaries in the zone of kernel development (Table).

Table 1. Results of Tripsacum dactyloides (2n=72) X Zea mays L. hybridization in 1988 (Krasnodar, USSR)

Cross	Number of pollinated ears	Total number of kernel initiation	Hybrid kernels			
			Total		Well-developed endosperm	
			Number	%	Number	%
T22 x Tr.dact.	7	1290	0	0	0	0
Gk26 x Tr.dact.	9	2310	0	0	0	0
A344 x Tr.dact.	7	1868	730	39.1	108	5.8
PLS61 x Tr.dact.	5	368	88	23.9	34	9.2
Gb834 x Tr.dact.	3	528	210	39.8	28	5.3
F_2 x Tr.dact.	7	1230	127	10.3	8	0.7

PLS61 produced a relatively high percent of hybrid kernels characterized with well-developed endosperms. In two lines hybrid kernels did not develop. This fact cannot be attributed to some occasional reasons or insufficient value of sampling size. In three years 108 ears of T22 inbred were pollinated. However, all attempts to obtain viable hybrid kernels in this inbred were not successful.

LONDON, ONTARIO, CANADA
University of Western Ontario

Ras-related transcripts

--R. B. Zabulionis and D. B. Walden

Retroviral oncogenes were derived from cellular genes during successive infections of the parental viruses into their respective animal hosts (Swanstrom et al., PNAS USA 80:2519, 1983). These oncogenes, unlike their cellular counterparts, do not have introns and consequently are often used as radiolabelled probes in Southern hybridizations when searching for similar sequences. They have been used successfully to detect not only their parental sequence in their respective animal host but also homologous genes in many other species, some of which (e.g. *Drosophila*, yeast) are quite removed in evolutionary time from the original animal host.

We decided to use a similar strategy (i.e., using viral oncogenes as probes) to detect homologous sequences in maize. It is difficult to obtain hybridization signals above the background when heterologous probes are used on plant genomes. This is particularly evident with maize due to the size and complexity of its genome as well as the abundance of repeated sequences in its genome. These problems can be overcome with the use of several technique modifications and controls (Zabulionis et al., Genome 30:820, 1988). To date we have reported maize sequences homologous to the following animal oncogenes: Ha-ras, Ki-ras, src, myc, myb, and abl (MNL 60:91, 61:72, 62:87).

Last year we reported the detection of maize mRNA transcripts homologous to Ha-ras using Northern blot hybridizations. At that time, "relaxed" hybridization conditions were used (30% formamide, 5.5X SSPE, 56 C) and detected transcripts of 2.8 and 3.2 kilobases.

Since then, more stringent conditions have been used (40% formamide, 3X SSPE, 56 C) in the detection of ras-related transcripts in 5-day-old plumules. The viral Ha-ras probe detected 2 transcripts, 1.6 and 2.0 kilobases in size. The smaller transcripts initially went undetected because of the "relaxed" hybridization conditions and their relative scarcity in comparison to the larger transcripts.

Using the more stringent Northern hybridization conditions, two transcripts in 5-day-old plumules were found to be homologous to Ki-ras. The transcripts are approximately 6kb in size. At the "relaxed" hybridization conditions, Ki-ras also detected the more abundant transcripts (2.8 and 3.2kb) that were detected by Ha-ras.

Sequencing has begun on maize ras-related clones isolated from a cDNA library made from mRNA of 5-day-old plumules.

Recovery of plantlets from apical meristem culture

--V. R. Bommineni, D. B. Walden and R. I. Greyson

The axillary bud procedure (Raman et al., Ann. Bot. 45:183-189, 1980) has recently been employed by us to propagate and maintain a single seedling sweet corn (cv. Seneca-60) genotype through 13 generations. Irish and Nelson (Planta 175:9-12, 1988) reported recently the recovery of plants through shoot tip culture from greenhouse grown plants. However, these two procedures, i.e. axillary bud culture and seedling shoot tip culture, appear to have limitations of genotype specificity; the number of recovered plants which are fertile is very low; and/or the meristems are incapable of surviving further manipulation.

In an attempt to circumvent these difficulties, and provide a suitable plant regeneration culture system, we have developed a medium to recover the plants from the shoot apical meristems (0.5-1.0mm long) from immature embryos or 72h imbibed seeds.

Plants were grown in the glasshouse and pollinations were made during the spring of 1988. Ears were harvested after 12-14 days of pollination and surface sterilized for 25-30 min with 10% 'Javex' after removing the husk leaves. Each embryo (1.0-2.5mm long) was removed from the caryopsis, the embryonic axis was separated from the scutellum and the coleoptile primordium was removed to expose the apical meristem. The final size of meristems ranged from 0.5-1.5mm. The excised meristems were placed onto a medium which consisted of Murashige and

Skoog salts plus 500mg/l L- proline, 100mg/l casein hydrolysate, 30g/l sucrose, and 8% agar. Ten to fifteen explants were placed in a 150x25mm petri dish. The explants were incubated under white fluorescent lights for two weeks (Bommineni and Greyson, MNL 60:94-95, 1986).

In two weeks each explant developed as an individual plantlet with roots and leaves. The data are summarized in Table 1. The recovery of plantlets was high in all the genotypes cultured. The plantlets were transferred to small

Table 1. The number of plants recovered in MS medium from shoot apices cultured from immature embryos of different genotypes.

(a)	(b)	(c)	(d)	(e)
Genotype	Shoot tips cultured	Plants potted	Plants producing inflor.	Percentage*
Oh43	63	62	62	98
M14	13	11	11	85
Oh43 x W23	30	30	30	100
W23 x M14	26	26	25	96

inflor. = inflorescences
*(e) = (b) / (d) x 100

plastic pots with glasshouse soil mixture. After 2-4 weeks, the plants were transferred to large pots and grown to maturity. Pollinations were made on these plants, and fertile seeds were recovered.

Morphological study of male sterile-silky (*ms-si* or *si1*)

--D. W. Dales, R. I. Greyson and D. B.Walden

The gene "male sterile-silky" (*ms-si* or *si1*) is located twenty centimorgans from *Adh1* on the long arm of chromosome six (MNL 62:143, 1988). The phenotype was described by A. C. Fraser (J. Hered. 24:41, 1933) as "ears bearing silks in great profusion and a small number of thin, wiry silks developing from the tassel. The tassel normally produces no, or an extremely small quantity of pollen." As part of a broader study of this trait we have discovered a number of items to add to this brief description. These include:

A) Tassel:

1) No filament elongation or anther extrusion.

2) The protrusion of wiry silks or "silk-like structures" (SLS) varied among *ms-si* plants and among spikelets of the same tassel. There was a range from zero to three SLS which emerged from a single spikelet. In those spikelets with no SLS emerging, SLS were frequently enclosed within the glumes.

3) The length of the SLS varied and ranged from 0-5cm.

4) The upper floret of each *ms-si* spikelet contained three abnormal stamens while the lower floret aborted. The extent of stamen development varied between spikelets on the same tassel and between plants, and ranged from no development to a structurally normal, but non-functional, stamen.

5) *ms-si* differed from other male sterile (genic or cytoplasmic) maize due to a failure in stamen development rather than a failure of the tapetum and/or microsporogenesis.

6) SLS developed from the tip of the connective cells of the abnormal stamen.

B) Ears:

1) The number of silks on the *ms-si* ear was approximately four times that found on a *ms-si*/+ ear.

2) An isolated wild type spikelet had only one silk, but a *ms-si* had SLS in addition to the one normal silk attached to the upper floret ovary. The number of SLS per *ms-si* spikelet varied and ranged from two to six, with three being most common.

3) SLS in ears emerged from "supernumerary ovaries" (SO) which developed, in addition to the central gynoecium, in each spikelet. One SLS was associated with each SO.

4) SEM analysis revealed that the SO developed from the organs that we normally identify as the aborted stamens of the ear spikelet. In addition the lower floret developed to some degree.

5) SO development varied. Generally the two SO located opposite each other in the upper floret and one from the lower floret were larger at maturity than the others.

6) The *ms-si* gynoecium, after fertilization, developed into a mature seed (kernel) while the SO became dried remnants and remained attached to the kernel.

7) After de-husking a mature *ms-si* ear, the SLS remained attached to the dried remnants of the SO while the silk of the gynoecium abscised.

In summary, *ms-si* is a recessive trait which is expressed in the early stages of flower development. It is developmentally distinct from other genic maize mutants with its restricted aberration of stamen development and development of SO in the ear spikelet and SLS that protrude from the abnormal stamens and SO. Because of the variation in expression, we hope that the *ms-si* is open to environmental manipulation and possible chemical reversion. Various plant growth regulators (PGR) are being used in these studies.

Seeds used in our study were from UWO stocks obtained originally from Maize Genetics Cooperation Stock Center.

LUDHIANA, INDIA
Punjab Agricultural University

Cytoplasmic male-sterile (cms) mutants

--V. K. Chaudhary and J. L. Minocha

This investigation was designed to ascertain whether ethyl methane sulphonate (EMS), ethidium bromide (EB) and streptomycin (SM) can create cms mutants in maize. Seeds of inbred CM400 were soaked in a water solution of 0.1 and 0.2 percent EMS, EB and SM for 24, 48, and 72h at 5±1 C. Treatments of EMS (0.2%) and EB (0.3%) for 8h at 25±1 C were also used. Three male-sterile mutants were observed in M1 generation, i.e., one in the EB (0.2%) for 48h at 5 C and two in SM (0.1%) for 48h at 5 C treatments. These mutagens along with EMS (0.1% and 0.2%) also induced such male-sterile mutants in M2. Non-stainability of male-sterile pollen grains ranged from 85.7 to 99.2 percent as compared to the control (7.8%). It was difficult to distinguish visually between fertile (F) and sterile (S) plants of the same inbred line before flowering. The frequency of male-sterile mutants ranged from 7.5 to 26.6 (EMS), 1.2 to 23.2 (EB) and 2.8 to 44.5 (SM) on a 1000 plant basis for the M2 population of approximately 25,000 plants. To find the

nature of the mutation induced, the male-sterile mutants were crossed with pollen from untreated inbred parent. The progenies of these mutants segregated in the subsequent generations up to M4 producing sterile as well as fertile plants. One of the progenies from EB treatment segregated to give male-sterile plants even in M5 generation. Occurrence of male-sterile mutants in some of the progenies right from M1/M2 up to M4/M5 generations clearly demonstrate that this trait is not controlled exclusively by nuclear genes (genetic). This conclusion further gets support from the study of segregation behaviour of (i) selfed male-fertile sibs of male-sterile progenies and (ii) male-fertile sibs in reciprocal crosses with the control. Some of these selfed and reciprocally crossed plants gave male-sterile mutants, giving an indication of the involvement of nuclear genes, and in some of the cases no male-sterile plants were observed, giving the indication that nuclear genes were not involved in the production of male-sterile mutants. There seems to be differential expression of the character under study.

In the past such attempts have also been made by Briggs (MNL 45:13-16, 47:35-37, 50:28-29, 51:5-6, 52:18-19) and Burnham (MNL 47:127-128), using different concentrations of EMS, EB and SM.

Male sterility induced in these progenies cannot be genetic because in such cases where the F1 of male-sterile x control (fertile) should possess plants with normal pollen fertility, several crosses segregating for male-sterility traits were found.

In this study it appears that the male-sterility induced could be attributed to the interaction of both cytoplasmic and nuclear genes. Since induced male-steriles and their fertile sibs segregated giving male-sterile as well as fertile individuals, it can be assumed that the genome carries some sort of partial restorer genes or modifiers. An attempt now may be made in the subsequent generations to try a number of inbreds for the purpose of maintaining this male-sterility. According to Singh and Laughnan (Genetics 71:607-620, 1972) it also appears at least equally plausible that the male-fertility encountered in the male-sterile progenies may be the result of an occasional contribution of normal cytoplasm by the male-fertile maintainer parents which are employed routinely as pollinator in crosses with male-sterile mutants. This needs to be meticulously investigated.

Morphological characterization. Mutants were characterized morphologically for spike characteristics, anther dehiscence and anther size.

Spike characteristics - The inflorescence and the individual florets of male-sterile plants were rather smooth in touch and appearance. Maybe this is due to the non-plump nature of the anthers. The florets had unextruded anthers. Anthers when examined were observed to be thin, papery, small and pressed in shape and size and had different colours like green, whitish, and whitish yellow (Table 1). The matured florets of CM400 (control) on the other hand had extruded anthers and were thick, plump, green and bigger than those of the male-sterile mutant. For testing the spikelet fertility a few of the male-sterile mutants when selfed gave no seed set whereas seed set was normal when crossed to the control.

Anther dehiscence - There was no dehiscence of anthers in any of the male-sterile mutants as compared to the control, where there was complete dehiscence. This characteristic was used as one of the criteria to pick up male-sterile mutants in field conditions.

Table 1. Morphology, colour and size of anthers of male-sterile mutants and control.

Line and Plant No.	Anther length (mm)	Anther width (mm)	Anther morphology	Anther colour
1-2-3	4.00	0.536	Papery, thin	Whitish
2-4-2	3.50	0.580	Thin, pressed	Green
15-5-6	4.50	0.545	Thin, papery	Brownish
30-6-5	4.43	0.557	Thin, papery	Whitish yellow
5-84	4.75	0.565	Thin,pressed	Brownish
5-87	4.41	0.559	Thin,pressed	Brownish
9-161	4.50	0.563	Thin, pressed	Brownish
2-20	4.16	0.540	Thin,papery	Whitish yellow
Sib (Fertile)	5.23	0.791	Plumpy, thick	Green
Control (F)	6.14	0.783	Plumpy, thick	Green

Anther size - Anther length and width of male-sterile mutants were reduced as compared to the anthers of the control inbred parent (Table 1). The range of anther length and width was 3.50 to 4.75mm and 0.534 to 0.580mm respectively as compared to the control, where length ranged from 5.23 to 6.14mm and width from 0.783 to 0.791mm.

It was difficult to distinguish visually between fertile (F) and male-sterile (S) mutants of the same inbred line before flowering. However, at flowering tassels of male-sterile mutants and the control inbred differed in their degree of anther exsertion as discussed earlier.

Cytological characterization. Pollen stainability (in 0.2% I-KI and 1% acetocarmine stain) was very low (below 15%) in the case of male-sterile mutants as compared to the control (Above 90%) inbreds (Table 2). Using ocular and stage micrometer, reduction in the pollen grain size (at the maximum dimension) was also observed in the case of male-sterile mutants when compared with the control. This size varied from 19.10 to 60.58µm in male-sterile mutants as compared to the control inbreds (73.87 to 75.67µm). On the basis of dehisced pollen grains per anther, two types of male-sterile mutants were observed. In one category (Type 2) there were abundant dehisced pollen grains per anther (1312 to 1719) while in the other (Type 1) there were few (11.00 to 25.66) pollen grains per anther as compared to the control inbred (2700-2905). This study was done by crushing the individual anther of male-sterile and control (fertile) plants on separate glass slides and mounting in 0.2 I-KI (stain) solution. A count of dehisced pollen grains was made and calculated per anther. It was ensured that no pollen grains went out of the field covered by the coverslip. This could be done by using a very small judicious amount of I-KI solution. Generally ten anthers from different spikelets were sampled from the male-sterile and fertile plants. The meiotic behaviour of male-sterile mutants (Type 2) was studied. The PMC's were examined at diakinesis, metaphase I, anaphase I and anaphase II. At least 100 cells from each of the mutants were studied. All mutants showed regular bivalent formation at diakinesis and metaphase I. There was normal separation of chromosomes at anaphase I and anaphase II. These male-sterile mutants were examined in M2 and later generations for meiotic chromosomal behaviour and no such abnormality has been observed so far.

Table 2. Pollen grain size and pollen grains/anther of male-sterile mutant and fertile plants.

Line and plant no.	No. of pollen grains studied	Size (µs.)	No.of pollen grains/anther	Pollen stainability(%)
1-2-3	50	54.56	1690.00	3.2
2-4-2	50	51.49	1612.10	0.8
15-5-6	50	60.58	1312.00	2.0
30-6-5	50	58.10	1719.00	3.4
5-84	50	55.50	1715.15	10.3
5-87	50	58.00	1611.12	5.0
9-161	30	24.56	25.66	6.6
2-20	20	19.10	21.50	14.3
Control (F)	50	73.87	2720.50	92.2
Sib (F)	50	75.67	2905.00	90.6

Biochemical characterization. Quantitative as well as qualitative (isozyme) differences of some of the enzymes, viz. peroxidase, esterase, acid phosphatase and amylase (only isozyme) were studied. In the present study biochemical levels of soluble proteins, peroxidase, acid phosphatase and esterase were assayed in male-sterile mutants MS-1 and MS-3 (with few pollen grains in the anther), MS-2 (with many pollen grains), their fertile sibs (FS) and control (CF). All the assays were carried out from the mature anther extracts. In male-sterile mutants and their fertile sibs there was an observed decrease in the soluble protein level as compared to the control. The maximum reduction was in MS-1 followed by MS-3, MS-2 and fertile sibs. Specific activity of peroxidase was about nine and one half times more in MS-2 and fourteen times more in MS-1 and MS-3 as compared to the control. For acid phosphatase and esterase specific activities were less than the control in all the male-sterile mutants.

The isozyme patterns in mature anther extracts of male-sterile mutants obtained through starch gel electrophoretic studies were different from those of the control. In total, eleven isozymes of peroxidase (nine anodal and two cathodal), three of acid phosphatase (all anodal), six of esterase (all anodals) and five of amylase have been observed. The intensity and the number of bands of peroxidase isozyme were much higher in male-sterile mutant anther extracts than their fertile counterpart. However, isozymes of acid phosphatase and esterase showed higher intensity of bands in the control than in male-sterile mutant mature anther extracts. The results obtained from isozyme pattern clearly support the study of enzyme activities for peroxidase, esterase and acid phosphatase.

Isozyme pattern in the case of amylase showed more enhanced activity in extracts of mature anthers in male-sterile mutants than in the control.

The enzyme activities were calculated in terms of intensity scores from electrophoretic patterns of the enzymes. The results obtained were similar to the ones obtained by direct enzyme assay. This proved to be meaningful criteria for characterization of male-sterile mutants when comparing with fertile and normal plants.

These large numbers of isozymes for different enzyme systems indicate multiple gene control where there seems to be protein multiplicity in anthers of different male-sterile and fertile plants.

Histological characterization. Light microscopic comparisons of anther histology of fertile plants (control) and cms mutants at different stages of anther development revealed two distinct types of histological changes (Type 1 and Type 2) causing pollen abortion in cms mutants. The following disturbances were noted at different developmental stages in Type 1: (i) the development of vacuolate sporogenous cells was not normal as compared to the control; (ii) the tapetal layer was found to be separated from the sporogenous cells; (iii) due to non-secretion of callose, the sporogenous cells did not undergo meiosis to form tetrads and microspores, leading to the absence of pollen grain; and (iv) as a result, the entire locule bore an empty look with the remains of the sporogenous cells adhered to the internal wall of the locule in the streak form. Thus no pollen grains were formed. However, in Type 2, the development of the vacuolate sporogenous cells was normal at the callose stage but exhibited a gap between tapetum and endothecium contrary to the control. Splitting of sporogenous cells, formation of tetrads and microspores were normal. However, microspores released in the locule cavity were irregular in their outline due to the simultaneous breakdown of the tapetal layers, which have a nutritive role to play in the normal development of the pollen grains. The tapetal breakdown was further found to be accentuated in later stages, leading to the formation of disorganized, crumpled and irregular pollen grains thus causing pollen abortion. Thus, in Type 1 the disturbances in the pathway of anther development took place at callose stage due to non-splitting of sporogenous cells, whereas in Type 2 it started at the microspore stage leading to tapetal breakdown.

Effect of treatment temperature, duration and crop-season on the frequency of induced male-sterility. Eighteen treatments of EMS, EB and SM with 0.1 and 0.2 percent concentrations were used for 8, 24, 48 and 72 hrs. The subsequent generations were raised in summer (June-July) as well as winter (Oct.-Nov.) to advance the generations. Among these, only three (7th, 10th, and 13th) treatments were administered at 25±1 C and the rest at 5±1 C (Table 3).

Table 3. Mutation frequency in relation to treatment temperatures, duration and crop season.

Mutagen	Treatment Conc.(%)	Treatment No	Treatment Tem.(°C)	Treatment Duration	Frequency of male-streile mutants/1000 M_2 plants Winter	Summer
EMS	0.1	1	5 ± 1	24	7.5*	-
EMS	0.1	2	5 ± 1	48	10.2*	-
EMS	0.1	3	5 ± 1	72	10.0*	-
EB	0.1	4	5 ± 1	24	2.1*	-
EB	0.1	5	5 ± 1	48	1.2*	-
EB	0.1	6	5 ± 1	72	22.6*	-
EMS	0.2	7	25 ± 1	8	26.6*	8.4**
EMS	0.2	8	5 ± 1	48	0.0*	0.0**
EMS	0.2	9	5 ± 1	72	0.0 *	0.0**
EB	0.2	10	25 ± 1	8	13.2*	18.7**
EB	0.2	11	5 ± 1	48	-	10.5**
EB	0.2	12	5 ± 1	72	-	9.8**
EB	0.3	13	25 ± 1	8	4.4*	23.2**
EB	0.2	14	5 ± 1	48	-	9.4++
SM	0.1	15	5 ± 1	48	-	18.3++
SM	0.2	16	5 ± 1	48	-	20.6++
EB	0.2	17	5 ± 1	48	2.3++	15.3+
SM	0.1	18	5 ± 1	48	2.8+++	44.5+

* Winter 1982
** Summer 1983
+++ Winter 1983
++ Summer 1983
+ Summer 1984

These three treatments produced male-sterile mutants in the frequency of 26.6, 13.2, 4.4 respectively in winter (20±1 C) and 8.4, 18.7, 23.2 respectively in summer (30±1 C). The frequencies of 26.6 and 23.2 (Table 3) are apparently higher as compared to others in different treatments carried out at 5 C except that of SM (44.5). In the case of EMS (0.1%) there appears to be an increase in the frequency of male-sterile mutants up to 48 hrs of treatment but not thereafter. After EB treatment of 0.1% (4-6) there was no effect except the one given for 72 hrs. In EMS (0.2%) treatment (7-9) there were no male-sterile mutants observed except at 8 hrs in either season. An increase in mutation frequency was recorded up to 48 hrs in the treatment of EB (0.2%) (10-12) and a decrease thereafter. The frequency of male-sterile mutants in EMS treatment 7 (0.2%) was observed as 26.6 and 8.4 and in EB treatment 10 (0.2%) it was 13.2 and 18.7 in winter and summer respectively. There was no recovery of such mutants in the EMS 0.2% treatment (8-9) in either season. In the EB and SM treatment the frequency of male-sterile mutants recorded as low in winter and high in summer, whereas the trend was just the reverse in the case of EMS treatments. A similar pattern of results was obtained in winter and summer of 1984, 1985, 1986 , and 1987.

Studies of some induced morphological mutants

--V. K. Chaudhary and J. L. Minocha

Besides male-sterile mutants which have been reported elsewhere other morphological variants/mutants were obtained in M2 generation. These mutants were viable (pistillate, pygmy, multitillering and red cob) and non-viable (albino, staminate and vegetative). Their characteristics are given in Table 1. The EMS treatments of 0.1% for 24, 48 and 72 hrs produced more of such morphological mutants than the treatment of EMS, 0.2% concentration for a corresponding time of treatment. The number of pistillate variants was much higher in all three treatments of EMS in a 0.1% concentration. Ethidium bromide, which is considered a potent cytoplasmic mutagen, in a 0.1% concentration induced more mutants than the treatment of 0.2% given for 24, 48 and 72 hrs. This treatment produced a higher number of pygmy and pistillate mutants than other mutants. More multitillering mutants were obtained after the treatment with EB (0.2%) than any treatment of EMS. The appearance of these morphological mutants as well as albino seedlings gives an indication of EB being nuclear in its nature. Treatment with streptomycin at 0.1 and 0.2% concentrations for 48 hrs produced mostly multitillering mutants as compared to the other treatments.

Table 1. Characteristic features of some induced morphological mutants.

Types	Mutants	Characteristics
Viable	Pistillate	Plants with cob terminally located in upright position, no tassel, short statured.
	Multitillering	Plants with more than one tiller, short tassel, reduced height & internodes.
	Pigmy	Plants with erect, short, stiff leaves, reduced male inflorescence, leaves dark green, short statured.
Non viable	Albino	Seedling with complete white leaf.
	Staminate	Plants with only male inflorescence.
	Vegetative	Plants without tassel and cob.

Normal seed set was observed in these viable mutants. The non-viable mutants did not give seed set hence no further study. The albino seedlings in M2 could not survive and hence no further study. Their occurrence has been reported elsewhere. The viable morphological mutants were further analysed in M3 and subsequent generations. As pistillate and multitillering mutants were without male inflorescence, these were crossed to the control (CM400) as pollen parent. The resulting progeny in M3 generation were phenotypically normal looking like the control. Upon selfing such plants in M3 their progeny exhibited segregation in M4 generation. Out of 780 seeds sown in each case, in one, 552 normal and 180 pistillate mutants were recorded, whereas in another 534 normal and 192 multitillering mutants, providing an indication for these characters being monogenically controlled. Pygmy mutants bred true in M3 and subsequent generations. Male-sterile mutants could set seed when crossed with the control pollen and the result has been reported elsewhere.

Induced albino mutants

--V. K. Chaudhary and J. L. Minocha

While using various chemical mutagens (EMS, EB and SM), albino seedlings were observed in M1 generation. The frequency of such seedlings varied in different treatments (Table 1). Besides, streptomycin treatment (SM) also produced 5 and 23 such seedlings with their central leaves white out of which 3 and 19 seedlings respectively could not survive. The rest could recover and develop like a normal plant and did not give any chlorophyll deficiency symptoms in subsequent generations. This appearance of albino mutants was also observed in M2 and M3 generations in the progeny lines that gave albino mutants in M1 generation. The frequency of such seedlings was different in different treatments and crop seasons (Table 1). It is apparent that among all the treatments streptomycin produced the highest frequency of albino mutants in each generation, indicating its superiority over other mutagens used for creating such mutants. The frequency of these mutants was observed to be higher in winter (21±1 C) than in summer (30±1 C) (Table 1). This gives an indication that low atmospheric temperature may play some role in enhancing the expression of the albino phenotype in the presence of certain chemical mutagens used here that affect the chloroplast development or the factors in the chloroplast.

Table 1. Frequency of albino seedlings.

	Frequency of albino seedlings (%)				
	M 1		M 2		M 3
Treatment	Sum.	Sum.	Wint.	Sum.	Wint.
EMS 0.1% 24hr	0.8	12.5	15.7	11.9	13.8
EB 0.1% 48hr	0.4		- Not obtained -		
EB 0.2% 48hr	1.5	10.2	10.9	9.9	11.2
EB 0.2% 48hr	0.5	19.4	21.2	12.5	13.2
SM 0.1% 48hr	3.0	38.0	39.0	15.7	17.2
SM 0.2% 48hr	4.6	40.0	41.0	16.5	17.1
EB 0.2% 48hr	1.1	19.5	21.0	11.2	13.4
SM 0.1% 48hr	1.3	20.3	23.9	15.8	18.6
Control H20	NIL	NIL	NIL	NIL	NIL

EMS-induced red cob mutants

--V. K. Chaudhary and J. L. Minocha

Among the eighteen different treatments of EMS, EB and SM given, the treatment of EMS (0.1%) for 72 hrs at 5±1 C produced two red cob variants out of a total of 299 plants in M2 generation. This was further analysed in later generations. After selfing in M2, it was observed as almost breeding true in M3 and subsequent generations. Out of 500 seeds sown in the field, 460 surviving plants produced red cob mutants in the next generation. In the later generations when tested, this mutant bred true, giving red cob characteristics. The mutant has red cob and the tip of the grain had a red coloured tinge. There was purple lining at the base of each floret in its inflorescence. This characteristic of purple lining at the base of each floret was of use in identifying the plants with the red cob characteristic quite in advance. Such mutants can be used as genetic markers in genetic studies. Results indicated that the purple lining at the base of each floret in the tassel is associated with the red cob colour. The red cob colour has been earlier reported to be associated with brown tassel colour (Coe, MNL 57:33-34).

MADISON, WISCONSIN
University of Wisconsin

Unusual behavior of the *R-g:15* allele in paramutation tests

--Andrew Harrison and Jerry Kermicle

Analysis of testcross data from the summer of 1987 has revealed that the *R-g:15* allele is unusually sensitive to the paramutagenic action of *R-st*. A noncrossover derivative of *R-g:15* governs anthocyanin production in the aleurone but not the various plant tissues pigmented by *R-r:std* (coleoptile, roots and anthers). The allele was included in a study designed to compare the behavior of several groups of paramutable alleles following different paramutation treatments.

Association with *R-st* in a heterozygote heritably alters the expression of many *R* alleles at high frequency. Brink called this allelic interaction paramutation and described those alleles susceptible to change as paramutable and those inciting change as paramutagenic (Annu. Rev. Genet. 7:129-152, 1973). When extracted from the heterozygote by testcrossing onto a W23 *r-g* tester (*r-g* is a null allele of *R* that is neither paramutable nor paramutagenic), paramutable *R* alleles that normally give a phenotype of darkly mottled aleurone in single dose exhibit reduced capacity for such pigmentation.

125-kernel samples having the genotype of interest were removed from testcross ears and prepared for analysis in the Agtron M-31-A, a device that records the spectral reflectance of a sample on a scale of 0-100 relative to calibration standards. Higher numbers denote greater reflectivity and thus lighter sample color. The average reading from four measurements was designated the Agtron score for a given sample; each Agtron score was converted to a quantity designated % full color (% fc.) to facilitate subsequent comparisons. Family averages for the *r-g:Stadler* and *R-sc:n656* alleles constituted the colorless and full colored controls, respectively.

R-r:std is very sensitive to the paramutagenic action of *R-st* and thus serves as a convenient reference allele. Although *R-r:std* and *R-g:15* exhibit similar levels of single dose nonparamutant pigmentation (mean % fc. scores of 86.9 and 83.8, respectively), the pigmenting potential of *R-g:15* is driven essentially to zero by a single generation of heterozygosity with *R-st*. *R-r:std*, on the other hand, retains about 17% of nonparamutant pigmenting capacity after the same treatment (see Table 1). This is in sharp contrast to the results of Brown (Genetics 54:899-910, 1966), who observed no significant differences among 9 independently isolated *R-g* derivatives and *R-r:std* with respect to paramutational sensitivity.

Table 1. Testcross data.

Allele	Family(no. plants)	Agtron score	% fc.	% nonpar	% rel. chg.
R-g:15[a]	11(5)	63.3	-3.5	-4.2	
	12(4)	64.5	-5.6	-6.7	
				-5.4 ± 1.9	
R-r:std[a]	1(8)	52.3	15.2	17.4	
	2(5)	57.6	6.1	7.0	
	17(5)	50.9	17.6	20.2	
	18(7)	50.7	17.8	20.5	
				16.8 ± 1.6	
R-g:15[b]	47(5)	39.3	37.3		47.3
	48(5)	29.3	54.3		66.6
					55.4 ± 4.2
R-r:std[b]	37(5)	39.5	37.0		31.0
	38(5)	28.4	55.8		57.0
	53(7)	31.0	51.3		50.8
	54(5)	23.9	63.4		67.5
					51.5 ± 3.6
R-r:std[c]	71(5)	50.1	18.9		18.7
	72(5)	56.4	8.2		5.9
					12.3 ± 3.6
R-sc:n656	81(6)	2.4	100		
r-g:Stadler	82(6)	61.2	0		

[a]Heterozygous with *R-st* for one generation.

[b]Heterozygous with *r-g:Stadler* for one generation following extraction from the *R-st* heterozygote.

[c]Heterozygous with *r-g Stadler* for one generation following two generations of heterozygosity with *R-st*.

The negative values listed for paramutant *R-g:15* testcross families deserve comment. The Agtron scores for *R-g:15* kernel samples were higher on average than those from the *r-g:Stadler* control family, and therefore the corresponding % fc. scores were negative. Why this should be the case is not known, perhaps variation in the level of carotenoid pigmentation contributes to the difference. Visual inspection of kernel samples from *R-g:15* testcross ears revealed that most kernels retained a few small flecks of anthocyanin pigmentation. The Agtron is relatively insensitive to small differences in weakly pigmented samples, and evidently this very light mottling passed undetected. In any event the mean % fc. score for *R-g:15* did not differ significantly from that of the colorless control.

Pairing a sensitive allele (paramutant or nonparamutant form) with an *r-r* or *r-g* allele tends to enhance the pigmenting action of that allele relative to controls maintained as homozygotes (Styles and Brink, Genetics 61:801-811, 1969). Paramutant forms of the *R-r:std* and *R-g:15* alleles were compared with respect to this property of reversion. Both alleles were found to regain about 50% of the pigmenting potential lost in a prior generation of heterozygosity with *R-st* by experiencing a generation of pairing with *r-g:Stadler* (see Table 1). By this criterion, the mutational

event that generated *R-g:15* apparently increased its paramutational sensitivity without affecting its capacity for reversion. In contrast, after two generations of heterozygosity with *R-st*, the *R-r:std* allele (exhibiting a mean % fc. score not significantly different from that of *R-g:15* following one generation of exposure to *R-st* action) was only restored by approximately 12% with similar treatment. *R-g:15* has yet to be tested in this manner. The contrasting reversion behaviors of *R-g:15* and *R-r:std* alleles reduced to similar levels of pigmenting action by interaction with *R-st* might reflect structural differences between the two alleles, or perhaps the effect of increasing generations of exposure to *R-st* is generally to reduce the capacity of a paramutant allele to revert towards a higher level of expression.

Diversity and relationships of historically important sweet corn inbred lines

--J. T. Gerdes and W. F. Tracy

A study was conducted to determine the diversity of 43 historically important sweet corn inbred lines. The 43 sweet corn inbreds, along with three dent inbreds (A635, W64A, and W182E), were subjected to RFLP analysis. Seventy-one probes distributed throughout the genome were used. In addition, the inbreds were scored for 22 morphological traits in a replicated field trial. Both data sets were analyzed using a hierarchical clustering technique. These results were compared to the known pedigrees of the inbreds.

Three major clusters were seen in the RFLP cluster analysis. The three dent inbreds formed the smallest and most distinct group. The second and largest cluster contained most of the yellow sweet corn inbreds. This agrees with the pedigrees of these inbreds in that nearly all of the yellow inbreds contain some Golden Bantam germplasm. The third cluster contained the Evergreen and Country Gentleman inbreds. This was unexpected in that the pedigrees of these varieties show no common ancestors. Several inbreds showed affinity to both sweet corn clusters. This agreed with their pedigrees which show that these inbreds were the result of crosses between varieties represented in the two clusters. In addition, some of the inbreds did not fit into any of the clusters. Most of these inbreds contain diverse or unknown germplasm, and some have been important in sweet corn breeding.

The morphological clustering analysis showed less distinct clusters. There was a general gradient of tall, late maturing inbreds to short, early maturing inbreds. A few of the associations seen in the RFLP analysis were also seen in the morphological analysis particularly with the most closely related inbreds. (The authors wish to thank the Agrigenetics RFLP Mapping Group for their assistance in RFLP data collection and analysis. Agrigenetics Corporation, Madison, WI.)

RFLP diversity among P39 and four derivatives

--J. T. Gerdes and W. F. Tracy

Purdue 39 (P39) was the most widely used sweet corn inbred line in the 1930's and 1940's. This inbred is reported to be highly mutable, and a number of strains of P39 were previously identified. These strains are thought to be single gene mutations. Four such strains are C30 (a small mutant of P39 and the source of the *rd* gene), P39M94, P39M96, and IP39 (narrow grained mutant). These inbred strains all closely resemble P39 morphologically, even C30 which looks like a small P39 plant. All four of these inbred strains gained use in hybrid sweet corn production.

P39 and these four strains were subjected to RFLP analysis. Seventy-seven markers spread throughout the genome were scored. Thirty-two of the 77 markers showed differences from P39 for at least one strain. At 14 of these markers, two to all four strains differed from P39. Furthermore, three different alleles were seen at 7 markers among the inbreds. The four strains were compared to P39 and each other for similarity. The differences of the strains from P39 ranged from 9 markers (11.7%) for IP39 to 18 (23.4%) for P39M96 (Table 1). The strains differed from each other by as few as 5 markers (6.5%) for IP39-P39M94 to as many as 28 (36.4%) for C30-P39M96.

Table 1. Number of differences observed among P39 and four inbred strains derived from P39 at 77 RFLP markers.

	P39	C30	P39M94	P39M96	IP39
P39	--	17	14	18	9
C30		--	14	28	11
P39M94			--	23	5
P39M96				--	21

The exact cause of these differences is not known. Outcrossing is an unlikely explanation because of the strong morphological resemblance of these inbreds along with the historical references of instability. Also residual heterozygosity is not a likely candidate because as many as three alleles were seen at a number of markers. These results raise an interesting question on the origin of variability in maize. (The authors wish to thank the Agrigenetics RFLP Mapping Group for their assistance in RFLP data collection and analysis. Agrigenetics Corporation, Madison, WI.)

Effect of corngrass on cell wall components

--W. F. Tracy and R. D. Hatfield

In addition to its manifold effects on morphology and development, the corngrass gene *Cg* alters cell wall composition. In preliminary studies we examined the neutral sugar composition, uronic acid levels and lignin in leaves, sheaths, and stems of *Cg* plants and +/+ segregates. The greatest differences were detected in the leaf tissue, with smaller differences in the sheaths and then in the stems. The direction of the difference was consistent in all 3 tissues.

Glucose and uronic acid levels were significantly higher in the *Cg* leaves than in the normal segregates. The amount of xylose in normal leaves was nearly double that found in *Cg* leaves. Lignin levels were not different. These differences may affect cell wall structure and thus forage quality, pest resistance, and biomass production.

Lindsey-Meyers blue sweet corn

--W. F. Tracy

In the fall of 1987 I received a call from a Mrs. Meyers of Pewaukee, Wisconsin. She explained that her family had been maintaining an open-pollinated variety of blue sweet corn for over 40 years but was no longer able to do so. Her family had received it from the Lindsey family of the Milwaukee area and she believed that they had been maintaining it for at least 30-40 years prior to her family receiving it.

Mrs. Meyers supplied me with approximately one pound of seed. It was planted in Madison, Wisconsin in 1988, sib-pollinated by hand, and was harvested. Notes on its appearance were taken. It is most probably a strain of Black Mexican Sweet. The plants are heavily tillered, 4-6 feet tall and have thin main stalks. The dried ears are 5-7 inches long. Most ears have 8 rows of deep purple-black sugary kernels. A few 10-12 rowed ears are present and approximately 3% of the ears segregated purple/nonpurple kernels. Seed of this variety will be provided to the Plant Introduction Station at Ames, Iowa.

Andromonoecious maize

--W. F. Tracy

Anthers were observed at the base of the kernels in an inbred of unknown tropical background. The anthers were fully formed and dry at the time of dry seed harvest. It is not known if they produced pollen. Unlike anther ear (*an*) the plants are normal in appearance with fully functional tassels.

Testcrosses indicate that the andromonoecious trait is recessive. Of over 100 F1 plants and 200 F1-backcross to outcross parent plants none had anthers in the ear. Andromonoecy did segregate in the F2 and in the backcross to the andromonoecious parent. The F2 data fit (240 normal:18 andromonoecious) a duplicate dominant (15:1) hypothesis ($p>0.5$). The backcross to the andromonoecious parent does not support this hypothesis ($p<0.01$). These generations will again be grown in 1989 in attempt to sort out the inheritance of this trait.

MARTONVASAR, HUNGARY
Agric. Res. Inst. Hungarian Acad. Sci.

Cold tolerance of S2 lines and testcrosses at germination

--P. D. Quang and T. Szundy

The cold tolerance of 18 S2 lines developed from the hybrid Pioneer 3925 and their testcrosses on 2 non-related testers (T1 and T2) were studied at the phytotron in Martonvasar. 10-10 seeds per genotype were planted per replication in 40x60cm wooden boxes at a row and plant distance of 3cm. The experiment was set up in a randomized block design with 4 replications. The temperature of the E 15 chambers was 8 C for 10 days and 13.5 C for 20 days. Soil from a several year maize monoculture and sand at a ratio of 2:1, saturated with water to 70% water capacity,was used for the studies. Until emergence the boxes were covered with plastic. The emergence was observed every day until the 30th day after planting. The number of emerging plants was expressed as a percentage of the seeds planted.

Table 1 presents the emergence percentage of the S2 lines and their testcrosses. The data reveal that the average emergence percentage of the S2 lines was poorer than that of the test hybrids.

The emergence of hybrids developed using the T2 tester was the best in every combination. The behaviour of hybrids developed using T1 tester was not consequent. In 2 cases (7284 and 7303) the emergence percentage of the T1xS2 hybrids was significantly poorer than that of even the S2 lines. Four T1xS2 hybrids (7292, 7304, 7314 and 7315) emerged significantly better than the corresponding S2 lines, whereas in the other cases there was no significant difference between the emergence percentage of the S2 lines and their hybrids developed on T1 testers.

Table 1. Emergence percentage of maize S2 lines and their testcrosses.

S_2 lines		Test hybrids		Mean of combinations
Code number	Emergence %	$T_1 \times S_2$ Emergence %	$T_2 \times S_2$ Emergence %	
7280	40.0	42.5	97.5	70.0
7283	25.0	25.0	72.5	48.8
7284	67.5	37.5	87.5	62.5
7287	67.5	55.0	87.5	71.3
7288	37.5	32.5	85.0	58.8
7289	72.5	62.5	90.0	76.3
7291	70.0	62.5	87.5	75.0
7292	32.5	77.5	87.5	82.5
7294	52.5	52.5	77.5	65.0
7295	43.3	37.5	95.0	66.3
7303	67.5	40.0	92.5	66.3
7304	20.0	60.0	95.0	77.5
7305	65.0	60.0	90.0	75.0
7309	55.0	45.0	92.5	68.8
7311	55.0	47.5	70.0	58.8
7312	50.0	55.0	82.5	68.8
7314	30.0	57.5	67.5	62.5
7315	40.0	62.5	70.0	66.3
Mean	49.5	50.7	84.9	

$LSD_{0.05\%}$ between any two combinations = 18.3

$LSD_{0.05\%}$ between the means of combinations = 10.6

$LSD_{0.05\%}$ between means of S_2 lines and their test crosses = 4.3

No significant relationship was found between the emergence percentage of S2 lines and their hybrids and the hybrids developed on T1 and T2 testers.

Our data support our information so far that the role of the female partner is decisive in the emergence of seeds planted in cold, wet soil. The different behaviour of the hybrids developed on T1 and T2 testers draws attention to the fact that the choice of testers is also important in the study of the cold tolerance of maize hybrids. We are continuing our studies using further different testers.

MEXICO CITY, MEXICO
Internat. Maize and Wheat Impr. Center
COLUMBIA, MISSOURI
University of Missouri and USDA-ARS

Silk pH, nonbrowning silks, and resistance to corn earworm

--P. F. Byrne, E. H. Coe, L. L. Darrah and K. B. Simpson

While investigating resistance of maize to the corn earworm (CEW), *Heliothis zea* (Boddie), we noticed an association between a low level of CEW damage, relatively high silk pH, and silks which do not turn brown when crushed (Byrne et al., Environ. Entomol. in press). Coe and Han (MNL 59:40 and 60:50) presented evidence that the silk browning trait was controlled by the *P* locus, and Miranda (MNL 56:30) suggested that this same locus was among the loci that were associated with resistance to CEW in the race Zapalote Chico.

We report here the results of an experiment that examined these three traits in crosses that were expected to segregate 1:1 for browning and nonbrowning silks. Plants were grown in the field in a split-plot design in which whole plots were designated either for measurement of pH and the browning reaction, or for artificial infestation with CEW eggs. Subplots were progeny of the crosses listed in the ta-

bles which follow. pH was measured on six-to-eight-day-old silks using a Corning portable pH meter equipped with a pair of microelectrodes (Lazar Research Labs, Inc., Los Angeles, CA 90046). Evaluations of resistance were obtained by measuring the length of CEW ear tunnels 14 days after infestation.

In all crosses, nonbrowning segregates had significantly higher mean pH values than segregates with browning silks (Table 1). Because we have found discrepancies between silk pH values obtained with the Corning/Lazar

Table 1. Mean pH values of browning and nonbrowning silk classes.

	Browning silks		Nonbrowning silks		LSD(0.05) between
Cross	n	Mean pH ± SE	n	Mean pH ± SE	classes
(W23 x K55) x K41	32	4.46 ± 0.04	18	5.22 ± 0.07	0.19
(W23 x K55) x K302	29	4.47 ± 0.05	31	5.23 ± 0.06	0.17
(W23 x K55) x FR29	18	4.87 ± 0.09	42	5.43 ± 0.04	0.18

equipment and those obtained with a standard laboratory Beckman pH meter, the pH values reported here may not indicate solely the hydrogen ion concentration of silk tissue. Ceska et al. (MNL 54:118) reported that plants with the WW allele (nonbrowning silks) at the *P* locus accumulate flavonol glycosides due to a blocked flavone pathway. Perhaps the Lazar electrodes were sensitive to the increased concentration of these compounds in nonbrowning silks.

Mean tunnel length of ears with nonbrowning silks was significantly lower than that of ears with browning silks in progeny of the cross (W23 x K55) x K41 (Table 2). For the

Table 2. Mean CEW tunnel lengths in ears having browning and nonbrowning silks.

	Browning silks		Nonbrowning silks		
Cross	n	Mean tunnel length ± SE (cm)	n	Mean tunnel length ± SE (cm)	LSD(0.05) between classes
(W23 x K55) x K41	31	5.3 ± 0.9	44	3.5 ± 0.5	1.7
(W23 x K55) x K302	34	4.0 ± 0.4	42	3.4 ± 0.4	1.7
(W23 x K55) x FR29	70	3.9 ± 0.3	61	4.3 ± 0.5	1.3

other two crosses, however, significant differences among browning and nonbrowning silk classes were not detected.

Our data indicate that the nonbrowning silk trait is associated with chemical changes detectable with the equipment we used. While this trait might be useful as a selection criterion for CEW resistance in some materials, it would not be a generally applicable criterion for such resistance.

MILAN, ITALY
University of Milan

Preliminary characterization of the heat-shock response in developing kernels

--Carla Frova

The heat-shock response of developing kernels (7, 14, 21 days after pollination) was investigated in two genotypes. In order to optimize a procedure for the analysis, different protocols were used for both lines:

a) A first set of kernels was excised from the cob and singularly incubated at 42 C over a drop of distilled water containing 20 uCi of 35S Methionine.

b) A second set of kernels from the same cob was injected without excision with an equal amount of labelled amino acid into the endosperm, and the whole cob put at 42 C.

After 3 hrs of treatment kernels from both sets were dissected into scutellum+embryo, endosperm and pericarp. The tissues were extracted and analyzed separately by 1 D SDS PAGE.

Incorporation of the label into proteins was good and more or less the same in the case of both procedures in all tissues from 7 DAP kernels. However, in 14 and 21 DAP embryo and endosperm from excised kernels, incorporation, although sufficient for the analysis, was significantly lower than in those directly injected. This discrepancy was not found in the pericarp.

The hsp pattern showed marked differences depending on the procedure adopted. Excised heat-shocked kernels synthesize the typical set of HSPs in all tissues considered, even though at a very reduced level in the endosperm as compared with scutellum and pericarp. No significant differences in the HSPs synthesized were detected between genotypes or developmental stages. In injected kernels, however, scutellar (+embryo) tissues show a very similar pattern of protein synthesis at 25 and 42 C. Surprisingly, in the early stages of development (7 DAP) this common pattern is typically that of non-shocked tissues, while by 14 DAP it appears to switch to that of heat-shocked tissues. At 3 weeks (21 DAP) the typical heat-shock response is induced by high temperature. Pericarp tissues exhibit the same heat-shock response as those from excised kernels.

The first approach (excised kernels) appears more reliable and will be adopted for further studies. However, the unusual behaviour of injected kernels is under investigation.

Post-pollination gene expression: a methodological approach and preliminary results

--Carla Frova and Gloria Padoani

Gene expression in the male gametophytic phase has been detected and analysed essentially at or before anthesis. Post-anthesis stages, i. e. germination, tube growth and fertilization, have been characterized to a limited extent for two main reasons: (a) even the best in vitro germination procedures currently available do not promote tube elongation to the extent to which it occurs in vivo (few mm versus 20-25cm); moreover in the in vitro system pollen-style interactions are excluded. (b) in vivo analysis of isolated pollen tubes is not feasible since they cannot be separated from the stylar tissues they are growing through.

To overcome these limitations we developed an indirect approach to the problem, based on the comparative analysis of pollinated and non-pollinated silks. This method allows the expression of those genes which code for an easily identifiable gene product to be detected. By choosing appropriate genotypes as female and pollen source, the pollen tube and stylar contribution to the gene products found in pollinated versus non-pollinated silks can be discriminated. The approach appears susceptible to further applications also for the study of pollen-style interactions.

Silks are cut 0.5cm above emergence from the husks and heavily pollinated; 3.5 hours are then left for germination and tube growth. After this time it is expected that tube tips have reached a distance of 2-2.5cm under the silk emergence level. This region of the silks is called "base". The

portion of the silks where the pollen grains are deposited is called "tip" and is analysed separately.

In this study we considered 4 enzymatic systems: ADH, GOT, CAT and B-GLU. For all of them a pronounced enzymatic activity was detected in mature non-germinating pollen and in the early stages of germination (in vitro and tip region of pollinated silks), while the elongating tubes (base region of pollinated silks) showed activity only in the case of GOT-1.

In the course of this analysis we found additional enzymatic activities, expressed in pollinated and non-pollinated silks but not in mature germinating pollen or in other sporophytic tissues: ADH-2, whose gene is usually transcribed only under anaerobiosis, and an as yet unidentified CAT. The nature of this catalase isozyme and the possible role of these additional activities in pollen-style interactions are currently under investigation.

Temporal and tissue specificity of enzyme coding genes in the developing kernel

--Carla Frova and Gloria Padoani

Data in the literature indicate that in maize several enzyme coding genes are developmentally regulated during the male gametophytic phase (Frova et al., 1987, in Curr. Topics Biol. Med. Res., vol. 15), seed germination (Scandalios et al., Dev. Gen. 4:281-293, 1984) and in the endosperm of immature kernels (Chandlee and Scandalios, Dev. Gen. 4:99-115, 1983). Here we report data on gene expression in different tissues of the developing kernel at 7, 14, 21 and 25 days after pollination (DAP). Four tissues--scutellum+embryo, endosperm, aleurone, pericarp--were analyzed in two genotypes: F198 and W22Rsc. The latter (kindly provided by Prof. G. Gavazzi) is characterized by a strong pigmentation in the aleurone layer, so that separation of this tissue is easy.

The following enzymatic systems were considered: ADH, GOT, CAT, B-GLU, for a total of 6 isozymes. Extracts from each sample were analyzed by native PAGE followed by specific staining for each enzyme. The results allow the identification of three patterns of expression:

1) Genes showing both tissue and temporal specificity. *Glu1* belongs to this group. This enzymatic activity was found only in scutella up to 14 DAP. A residual activity was present at 21 DAP but not in later stages.

2) Genes which reveal only quantitative variations between tissues and stages. This is the case for *Cat1*. The strongest activity is expressed by the aleurone, the weakest by the endosperm, scutellum and pericarp showing an intermediate level. In all tissues the activity increases with development.

3) Genes which show quantitative variations between tissues but not developmental stages. In this preliminary survey this was the most represented group. *Adh1*, *Got1*, *Got2* and *Got3* show this type of regulation. In all of them enzymatic activity was lowest in the pericarp. Differences between enzymes were found with regard to the other tissues. While ADH-1 and GOT-1 are mainly expressed in scutella and endosperms, and to a lesser extent in the aleurone, GOT-2 activity is stronger in scutella than in triploid tissues (endosperm and aleurone); the reverse is found for GOT-3. This difference in the level of activity between GOT-2 and GOT-3 is not found in other sporophytic tissues, including the scutellum of germinating seeds (24 hrs of imbibition).

We are now extending the analysis of these enzymatic systems to very early (0-7 DAP) and late (21-40 DAP) kernel development.

Preliminary results on induction of *Alu*I-bands in maize chromosomes

--Silvana Faccio Dolfini

Recent work (Miller et al., Science 209:395, 1983; Mezzanotte et al., Cytogenet. Cell Genet. 36:562, 1983) has proved that exposure of fixed chromosomes in cytological preparations to restriction endonucleases results in cleavage of DNA at specific base sequence targets.

The enzyme most frequently used is *Alu*I, which selectively digests DNA (restriction sequence AGCT), producing a linear differentiation along a chromosome, consisting of alternating regions where DNA has been cleaved (and consequently extracted) and regions not affected by the enzyme (*Alu*I-bands). In human, great ape, mouse and *Drosophila* chromosomes an overlapping between *Alu*I-bands and localization of satellite DNAs has been demonstrated. Recent data from *Vicia faba* chromosomes (Frediani et al., TAG 75:46, 1987) favour the hypothesis that the induction of *Alu*I-bands depends not only on the presence of a specific DNA base sequence, but also on a suitable configuration of chromatin.

In this note I report some preliminary attempts to induce *Alu*I-bands on maize chromosomes from two inbred lines (H84 and W117). Permanent root tip preparations were digested by placing enzyme solution (15 units of *Alu*I in 100ul of the appropriate buffer) over the slides, covering with a coverslip and incubating overnight at 37 C. The chromosomes were then stained with 3% Giemsa.

*Alu*I treatment on H84 chromosomes induces discrete bands on precise sites of metaphase (Fig.1c) and prophase (Fig.1b) chromosomes and intensely stained dots on interphase nuclei (Fig.1a). Different results in terms of number, size and position of the bands are observed on W117 chromosomes.

Fig. 1. *Alu*I digestion of interphase nuclei (a, x2000), prophase (b, x1600) and metaphase (c, x2400) chromosomes from H84 inbred line.

These preliminary observations open the way to a series of investigations on the homology between the bands revealed by *Alu*I treatment and the heterochromatic knobs visible in pachytene and the relationships between *Alu*I-bands and knobs distinguishable by C-banding in mitotic chromosomes.

Finally, maize chromosomes, characterized by the presence of different classes of heterochromatin (centromeric, knob, B-chromosomes, NOR), may help to elucidate the mechanism of *Alu*I-bands appearance.

Light regulated and tissue specific expression of *Sn*

--Gabriella Consonni and Chiara Tonelli

We reported (MNL 62:93-94, 1988) that *Sn* shares molecular similarity to the *R-nj* gene and several *Sn* accessions have been characterized at the DNA level using a *R-nj* DNA probe (S. Dellaporta et al., Chrom. Struct. and Funct., Plenum, 263-281, 1988).

The expression of *Sn* is tissue specific and light dependent. Tissues generally devoid of pigment but developing colour in the presence of *Sn* are the scutellar node, mesocotyl, leaf base and midrib, and ovary integuments. To study the expression of this gene we compared two different *Sn* accessions: *Sn-Coop*, which is strictly light dependent, and *Sn-Bol3*, which is partially constitutive in its expression, conditioning a weak pigmentation in dark growing seedlings. Total RNA was extracted from scutellar nodes, mesocotyls and leaves of seedlings grown either in dark or in light, analyzed by Northern blot according to Koes et al. (EMBO J. 7:1257-1263, 1988) and probed with *R-nj:1* fragment.

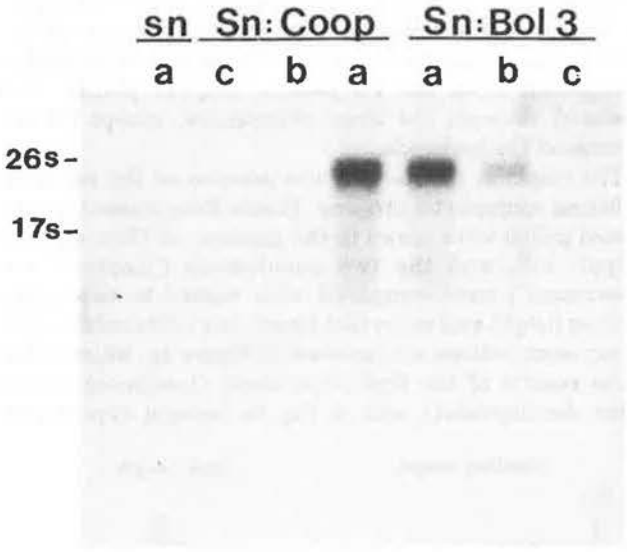


Figure 1. Expression of *Sn-Bol3* and *Sn-Coop* analyzed by Northern blot. Each lane contains 10µg of total RNA extracted from: scutellar nodes and mesocotyls of light grown seedlings (a), scutellar nodes and mesocotyls of dark grown seedlings (b), and leaves from light grown seedlings (c). The relative positions of the maize 26s and 17s ribosomal subunits are indicated on the left.

The results (Fig. 1) can be summarized as follows:

1) A transcript of about 3.5Kb is found only in the colored seedling tissues (scutellar nodes and mesocotyls and not leaves).

2) The transcript is found only in genotypes that carry *Sn* and not in *sn*.

3) The expression of *Sn* is light regulated. In fact no transcript is found in scutellar nodes of *Sn-Coop* grown in darkness, while for the partially constitutive allele *Sn-Bol3* the mRNA is found, at lower levels as expected, and also in the scutellar nodes of dark growing seedlings. These results confirm the tissue specificity and light dependency of *Sn* expression.

MILAN, ITALY
C.N.R. - Istituto Biosintesi Vegetali

Transient expression of fragments from the 5' flanking region of a zein gene in electroporated protoplasts

--G. Giovinazzo, I. Coraggio, A. Viotti and L. A. Manzocchi

The analysis of cloned zein genes (Brown et al., Eur. J. Cell. Biol. 42:161, 1986; Boronat et al., Plant Sci. 47:95, 1986) has led to the identification of a unique promoter arrangement, containing two promoters (zP1 and zP2) lying approximately 1000 bases apart, and, between them, a group of short highly conserved sequences which may play a role in the control of the highly regulated zein expression; some of them have been recognized as specific binding sites for nuclear proteins (Maier et al., Mol. Gen. Genet. 212:241, 1988). In the aim to identify specific regulatory sequences, we have inserted in chimaeric plasmids, upstream from the reporter gene GUS (β-glucuronidase: Jefferson et al., EMBO J. 6:3901, 1987), the first 1415bp of the 5' flanking region of the genomic zein clone E19. Fragments from this region have been inserted in similar constructs: fragment 1 (290bp) comprises the zein transcription start point and the zP2 promoter; fragment 2 (125bp) contains the so called "-300" box (Maier et al., MNL 61:55, 1987); fragment 3 comprises the zP1 promoter. The plasmids were inserted by electroporation (1500 V/cm; 100msec; square wave) in protoplasts obtained from leaves of *N. tabacum* SR1, from long term suspension cultures of Black Mexican Sweet maize cells (of scutellar origin) and from a recently established suspension culture of A69Y maize endosperm cells. The promoter activity of the fragments has been monitored as transient expression of the enzyme coded by the reporter gene (Jefferson et al., EMBO J. 6:3901, 1987); the usual (Fromm et al., Nature 319:791, 1986) and a modification of the 35S CaMV promoter (as suggested by Pierce et al., in Plant Gene Systems and Their Biology, p. 301, 1987) have been used as constitutive promoter controls.

The expression of GUS activity (as pmoles 4-MU formed / hour / 10^5 protoplasts) is summarized in the following table:

PROTOPLASTS FROM:	SR1 mesophyll	BMS scut.cells	A69Y endosp.cells
PROMOTERS			
pCaGUS	382	-	-
DP33GUS	-	94	4032
1,2,3	74	4	0
1,2 (A)	48	0.8	0
1,2 (B)	26	0	0
1 (A)	n.d.	10	0
3 (A)	7	32	49
3 (B)	0	12	0

A69Y endosperm cultured cells were used with the aim to employ a homologous maize protoplast system. Endosperm cultures have been shown to maintain some tissue-specific features (Sarawitz and Boyer, TAG 73:489, 1987; Felker, Am. J. Bot. 74:1912, 1987) and zein synthesis (although at reduced levels) has been detected in our endosperm cell cultures (Manzocchi et al., Plant Cell Rep. 1989, in press).

From our preliminary data we can observe that the entire zein promoter region drives the expression of the reporter gene in a heterologous system such as tobacco mesophyll protoplasts, while it appears inactive in both types of maize cultured cells. Promoter activity, on the contrary, is observed in maize cells for fragment 3, which is not active in tobacco protoplasts. A more detailed analysis of zein transcription in endosperm cultured cells will enable us to interpret the unexpected failure of activity of the entire zein promoter region, and the functional activity of fragment 3.

Characterization of cDNA clones for glutelin polypeptides

--F. Sparvoli, F. Quattrocchio, M. W. Bianchi, L. Bernard, N. E. Pogna*, A. Viotti
*Ist. Sper. Cerealicolt., S. Angelo Lodigiano

Two cDNA clones, pcMY21 and pBFLO, coding for glutelin polypeptides were isolated from a cDNA library of polyA-RNA of endosperm polysomes.

Nucleotide sequence comparison of pcMY21 and pBFLO shows a difference of about 10% in the 3' coding region. The clones code for polypeptides rich in proline and cysteine and also share a high degree of homology to the glutelin clones recently isolated by Prat et al. (Gene , 52:41, 1987). In hybrid-selected translation experiments pcMY21 selects a mRNA coding for a 16Kd (apparent molecular weight) polypeptide, while pBFLO selects a mRNA coding for a 28Kd polypeptide. However, both cDNA clones also select a mRNA coding for a polypeptide of about 21Kd.

In order to clarify the genomic organization of these glutelin sequences, Southern blot experiments and copy number evaluation were carried out. DNAs from different inbred maize lines (A69Y, W64A and W22) were digested with different restriction enzymes (*Xba*I, *Bam*HI, *Eco*RI, *Hin*dIII) and then hybridized with the pcMY21 fragment or pBFLO fragment. The resulting hybridization patterns indicate restriction fragment polymorphism among the lines and about 8-12 copies per haploid complement. These results are quite different from those reported by Gallardo et al. (Plant Sci. 54:211, 1988) who evaluated one or two copies per haploid genome.

By in situ hybridization experiments on different maize cytogenetic stocks carrying heterologous translocations, the pcMY21 sequence was localized only on the short arm of chromosome seven close to the cluster of the zein genes previously localized in the same chromosomal region (Viotti et al., EMBO J. 1:53, 1982).

MILAN, ITALY
University of Milan
BOLOGNA, ITALY
University of Bologna

Gametophytic selection for Chlorsulfuron tolerance

--M. Sari Gorla, E. Ottaviano, E. Frascaroli and P. Landi

The purpose of this research was to prove the possibility of obtaining in the sporophytic generation a response to selection, applied at the gametophytic level, for specific resistance factors. Gametophytic selection can be particularly useful to select very rare mutants or allelic combinations, which is the case of resistance to herbicide molecules: owing to the peculiar characteristics of pollen (haploidy and large population size), the procedure can be much more effective than selection on the sporophyte, provided that the genes controlling resistance (or tolerance) are expressed both in the haploid and the diploid phase of the life cycle.

Chlorsulfuron was chosen on the basis of: i) its biological target (acetolactate synthase), which is expressed at the cellular level and so could be expressed in pollen too, and ii) its favourable characteristics as herbicide (low dosage, no toxicity for animals). However, it is degraded slowly in the soil, thus damaging susceptible crops sown after a resistant one. Maize is a susceptible plant species, but variability for tolerance has been described.

Selection was applied to pollen from heterozygous plants obtained from a tolerant (Va85) and a susceptible (B73) inbred, at different stages of the gametophytic phase: during microspore development or during pollen function. In the first experiment, tassels were detached about ten days before anthesis, put in liquid medium supplemented with the herbicide (20mg/l) for 24 hours and then grown in artificial conditions until flowering; the pollen produced was used to pollinate female plants of the sensitive genotype. In the second, the recipient plants, of the sensitive genotype, were treated by spraying their silks with a solution containing the chemical (200mg/l) after pollination with pollen from the F1 plants. Control plants were produced through the same procedures, except for the presence of the herbicide.

The response to selection was assayed on the resulting backcross sporophytic progeny. Plants from treated or non-treated pollen were grown in the presence of Chlorsulfuron (0.3ppb vol), and the two populations ("Control" and "Treatment") were compared with regard to two traits: seedling height and main root length (cm). The relative frequency distributions are reported in Figure 1a, which refers to the results of the first experiment (treatment during pollen development), and in Fig 1b (second experiment:

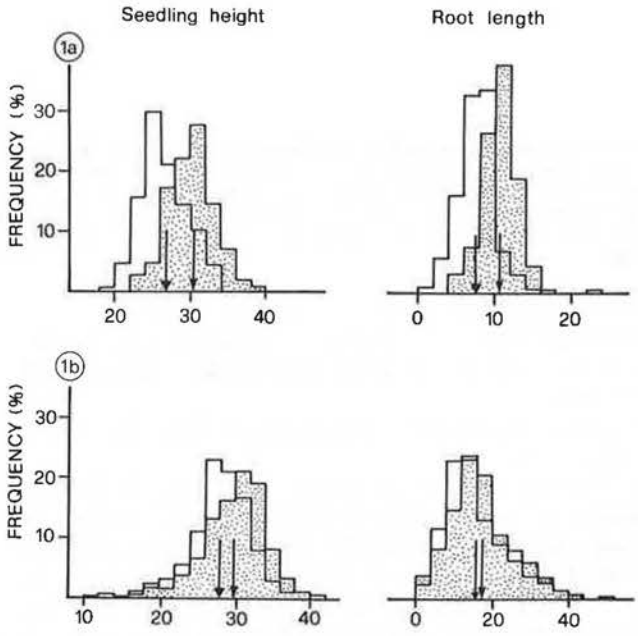


Figure 1. Frequency distribution of seedling height and root length of backcross populations obtained from treated (dotted) and non-treated (non-dotted) pollen. 1a: first experiment; 1b: second experiment.

treatment during pollen function); in each diagram the dotted area refers to the backcross progeny from treated pollen, the non-dotted to the backcross seedling from non-treated pollen; the arrows indicate the means. The results reveal a significant increase of the mean values for both characters in the treated populations when compared with the controls (F test); immature tassel treatment produced larger effects than silk treatment.

The response to selection, applied on pollen produced by single hybrid plants, in the resulting sporophytic progeny, demonstrates that genes controlling tolerance are expressed in pollen and are the same genes conferring tolerance to the plant. These data indicate the possibility of using male gametophytic selection for crop improvement: since the increase of tolerance has been obtained in only one generation of selection, it could prove to be a very good tool for increasing the efficiency of the traditional breeding methods.

NORMAL, ILLINOIS
Illinois State University

Identification of monosomic-5 plants
--David F. Weber and M. C. Schneerman

I (Weber) have worked with the *r-X1* system for nearly two decades, and had recovered, and genetically and cytologically confirmed, several plants that were monosomic for nine of the ten maize chromosomes in the first few years working with the system. However, my exhaustive efforts to recover plants monosomic for the last chromosome (chromosome 5) were unsuccessful until last summer.

Nearly all of the monosomics we have studied were produced by crossing plants containing the *r-X1* deficiency as female parents with a Mangelsdorf's tester inbred which was generously provided to our laboratory by Kante Satyanarayana. This Mangelsdorf's tester is quite weak, and although it usually sheds sufficient amounts of pollen for some crosses, it develops lesions on its leaves as it gets older and usually is in such poor shape that it cannot be successfully crossed as a female parent. For this reason, it is extremely difficult to self or sib these plants to maintain this line.

A different Mangelsdorf's tester was obtained from the Maize Genetics Coop Stock Center, Urbana, IL and used as a male parent in crosses onto *R/r-X1* female parents in the inbred W22. This Mangelsdorf's tester didn't develop the leaf lesions, and it was possible to cross many of these plants as female parents.

This past summer, progeny of crosses between *R/r-X1* plants in the inbred W22 x Satyanarayana's Mangelsdorf's tester and x the Coop Mangelsdorf's tester were planted. Six of 2012 plants produced by the cross where the male parent was the Coop Mangelsdorf's tester were identified which each had the same distinctive abnormal morphology. They were small plants (about 3 ft tall) with very narrow leaves. Plants with this distinctive morphology had been observed in previous years; however, they were too weak to be used in crosses. Three of these plants shed pollen, and all three were semisterile. It was possible to cross two of these plants with a *pr* tester, and all kernels produced were *pr* in phenotype as would be expected if they were monosomic-5 plants. RFLP loci were analyzed in DNAs from four of the plants with the distinctive abnormal morphology with RFLP probes which mapped near the end of each of the arms of chromosome 5 (NPI 409 and NPI 288). Three of the exceptional plants only contained the alleles from the male (Mangelsdorf's tester) parent; thus, these plants were monosomic for chromosome 5. The fourth exceptional plant contained the allele from the male parent on the long arm (288) and both alleles on the short arm (409); therefore, this plant carried a deficiency in the long arm. Thus, RFLP analysis has enabled us to identify the last possible monosomic type to complete the monosomic series in maize.

Three additional extremely small plants were observed in this material which had very narrow leaves; one was 1.5 ft tall and the other two less than a ft tall. Leaf samples were not analyzed from these plants. These plants may have been monosomic for chromosome 5 and simultaneously aneuploid for all or a part of another chromosome.

Progeny of the cross between *R/r-X1* x the Satyanarayana Mangelsdorf's tester were also planted, and none of 2162 plants produced by this cross had the distinctive abnormal morphology typical of the monosomic 5 plants. Thus, this Mangelsdorf's tester appears to be producing a far lower frequency of monosomic-5 plants or none at all.

The reason that the two Mangelsdorf's testers are producing monosomic-5 plants in greatly different frequencies is not known. It is possible that a recessive dosage-sensitive mutation(s) is located on chromosome 5 in the Satyanarayana Mangelsdorf's tester which in the hemizygous condition, renders the monosomic-5 plants inviable or too weak to survive. Alternatively, it may be that the male parent influences the mechanism of action of the *r-X1* deficiency such that monosomic-5 plants are produced at a higher frequency when the Coop Mangelsdorf's tester is utilized as a male parent.

In any case, monosomic-5 plants have now been recovered and verified, and all possible primary monosomic types in maize have been produced with the *r-X1* deficiency in this single cross.

Using selfs of monosomic plants for RFLP studies
--David F. Weber

Primary monosomics for each of the ten chromosomes have been recovered in maize. These monosomics were extremely useful in constructing the first RFLP map in maize (Helentjaris, Weber, and Wright, Proc. Nat. Acad. Sci. USA 83:6035-6039, 1986), and they are being used by others in their RFLP mapping (for example, Murray et al., MNL 62:89-91, 1988).

The monosomics are generated utilizing the *r-X1* deficiency, and female parents containing this deficiency undergo a high rate of nondisjunction during the second embryo sac division to produce some embryo sacs which have a chromosome missing in the egg nucleus. We typically cross an inbred male which is *r/r* and which carries a recessive mutation on each of its ten chromosomes (Mangelsdorf's tester) to a female parent in the inbred W22 which is *R/r-X1* and carries the corresponding dominant alleles. The colorless deficiency-bearing kernels are planted. Approximately 10-18% of the resultant plants are monosomics, and we have recovered all ten primary monosomic types from this cross between these two highly inbred lines. Each of the monosomic types can be recovered in a population of several thousand plants; however, this is a rather

massive undertaking which consumes a major portion of our work each summer.

For RFLP studies, plants monosomic for as many of the maize chromosomes as possible are compared with their parents and diploid siblings, and the monosomic type which contains the RFLP allele from the male parent, but lacks the RFLP allele from the female parent has lost the chromosome which contains the RFLP locus. This identifies the chromosome which contained the RFLP locus.

We realized that if a monosomic plant is selfed and leaf samples from several of the progeny are pooled and analyzed, the results would be exactly the same as with the original monosomic plant for this type of study. These plants will only contain the alleles from the male parent of the monosomic plant on the monosomic chromosome and lack the alleles on this chromosome from the female parent. Alleles on both maternal and paternal chromosomes will be present on all other chromosomes.

Alternatively, the monosomic plant could be backcrossed to the male parent (Mangelsdorf's tester), and the same results would be found. Thus, for these types of studies, analysis of pooled samples from several progeny of a self of a monosomic plant will give exactly the same result as analyzing the monosomic itself.

Chromosomes bearing RFLP alleles have also been identified on the basis of differences in signal intensity (Helentjaris et al., 1986). We found that in cases where the two parents contained the same RFLP allele, plants monosomic for the chromosome bearing the RFLP locus give a detectably weaker signal than their diploid siblings. Clearly, progeny of selfs or backcrosses of monosomics would not be useful for this type of evaluation.

Maize plants monosomic for many of the chromosomes produce viable gametes as described in the following article. I tried to self and outcross the various monosomic types the past two summers in an attempt to obtain populations of kernels which could then be grown out for future RFLP studies; however, the number of progeny produced by these crosses was quite small because the past two summers were poor for us. I will try again next summer.

Characteristics of maize monosomics

--David F. Weber

Because several researchers are now using the *r-X1* deficiency system to produce monosomics in maize, we felt that it might be useful to describe the features we use to identify the various monosomic types. We typically cross Mangelsdorf's tester (which is *bm2*; *lg1*; *a1*; *su1*; *pr1*; *y1*; *gl1*;*j1*; *wx1*; *g1* and *r1* on chromosomes 1 to 10 respectively) as a male onto inbred W22 female parents which are *R*/*r-X1* and carry the dominant alleles for the marker loci in Mangelsdorf's tester. The colorless kernels (*r*/*r*) from this cross are identified and planted, and 10-18% of the plants which germinate from these kernels are monosomics.

Five of the mutations are for traits expressed in the plant (*bm2*, *lg*, *gl*, *j*, and *g* located on chromosomes 1, 2, 7, 8, and 10 respectively), and most plants expressing one of these recessive phenotypes are monosomic for the chromosome carrying the locus. However, plants which have lost a portion of the chromosome arm carrying the marker locus are also found (Weber, pp. 351-378 in Swaminathan et al., Cytogenetics of Crop Plants, 1983; Lin, Genome 29:718-721, 1987). The other five mutations (*a*, *su*, *pr*, *y*, and *wx*) are expressed in the endosperm of kernels, and plants monosomic for chromosomes bearing these genes do not express the recessive phenotype for these mutations because the loss of a chromosome from the maternal parent in the embryo of a kernel is not accompanied by the loss of the same chromosome from the endosperm. The reason for this is the *r-X1* deficiency induces nondisjunction after meiosis is completed during second embryo sac division (Lin and Coe, Can. J. Genet. Cytol. 28:831-834, 1986; Simcox, Shadley, and Weber, Genome 29:782-785, 1987). We can detect these plants in the following way. Semisterile (with 50% or greater pollen abortion) plants of subnormal stature are identified as presumptive monosomics and testcrossed with a line that is *a*; *su*; *pr*;*y*; *wx*; and *R*. All kernels produced by this testcross of a monosomic 3, 4, 5, 6, or 9 plant will only express the recessive phenotype of the gene carried on the monosomic chromosome while diploids and all other monosomic types will give a 1:1 ratio for the gene. For example, plants monosomic for chromosome 4 will only produce sugary (*su*/*su*) kernels from this testcross while all other monosomic types and diploids produce a 1:1 ratio of plump (*Su*/*su*) to sugary (*su*/*su*) kernels. Monosomics for each of these chromosomes have distinctive morphologies which are described in the table.

Characteristics of maize monosomics produced by the *R*/*r-X1* x Mangelsdorf's tester cross.

Monosomic-1 plants express the brown-midrib phenotype, are less than 3 ft tall, are too small to be crossed, and have asynaptic microsporocytes.

Monosomic-2 plants express the liguleless phenotype, are highly variable in height but up to 6 ft tall, are male-sterile, and set seed as female parents.

Monosomic-3 plants have thick, leathery leaves which appear dark green and are slightly narrow and stiff, the plants are up to 4.5 ft tall and set seed, and may produce a little pollen. Plants hypoploid for TB-3La also have leathery, narrow, stiff leaves; therefore, the dosage-sensitive region responsible for this is on 3L and uncovered by this B-A translocation.

Monosomic-4 seedlings have leaves that are blue-green in color; however, they become normal colored as the plant matures. The upper leaves have wide mid-veins and the upper surface of the upper leaves is flat because the midvein is not recessed as in normal plants. They are up to 5 ft tall, the tassel typically is partially retained within the leaf whorl, and anthers are extruded irregularly at anthesis; however, some viable pollen is produced. The ears on these plants are very large, and set seed. Plants hypoploid for segments of 4L (TB-1La-4L4692, TB-9S-4L6504, and TB-7L-4L4698) also have blue-green leaves as seedlings; thus, the dosage-sensitive region responsible for this phenotype is on 4L and uncovered by these B-A translocations.

Monosomic-5 plants have extremely slender leaves, are about 3 ft tall, and are too small to be crossed as female parents; however, a small amount of viable pollen is produced.

Monosomic-6 plants have leaves that are more upright than in other types, the internodes near the top of the plant often are shorter, and they shed abundant pollen and set seed. They are up to 4.5 ft tall and typically are the slowest maturing monosomic type.

Monosomic-7 plants express the glossy phenotype, have leaves that are thin, soft, and wrinkled in mature plants, and are up to 6.5 ft tall. They shed some pollen and set a few seed. TB-7Sb hypoploids also have wrinkled leaves; therefore, the dosage-sensitive region responsible for this phenotype is uncovered by this B-A.

Monosomic-8 plants express the japonica phenotype, have stalks that are somewhat thin, are up to 5.5 ft tall, shed pollen and set seed, and typically have several tillers.

Monosomic-9 plants have leaves that are somewhat thin and stiff, and are up to 5.5 ft tall. They shed no pollen; however, essentially empty anthers (sticks) are extruded irregularly by the tassel, and an occasional seed is produced as a female parent. This is the most rapidly maturing monosomic type. TB-9Lc plants also have thin, indehiscent anthers and resemble monosomic-9 plants in several ways.

Monosomic-10 plants express the golden phenotype, are up to 5 ft tall, shed abundant pollen and set seed.

Microspore development in most of these monosomic types is described in a paper in press in Genome by Z.Y. Zhao and D.F. Weber.

PHILADELPHIA, PENNSYLVANIA
University of Pennsylvania

An interaction between *Tp1* and a region on chromosome 1

--Scott Poethig

Last year I reported that hyperploidy for TB-1La suppressed tiller formation in *Tp1*/+ plants. This effect may be due to an increase in the dose of the wild type teosinte branched allele (which is located at the distal end of 1L) because recessive mutations of this locus interact synergistically with *Tp1* to enhance tiller production. In order to test this hypothesis further, *Tp1*/+ plants carrying a duplication for the region around the *tb* locus were created using an overlapping translocation stock originally generated by Jim Birchler. This stock carries the translocations T1-3(5267) and T1-3(5242), and duplicates a region representing 18% of 1L. The results presented in Table 1 demonstrate that this

Table 1: The effect of a 1L duplication (.72--.90) on the expression of *Tp1*.

Families	# of tillers	
	+/+; *Tp1*/+	+/+/+; *Tp1*/+
D926A	3.6 (N=8)	2.2 (N=10)
D926B	1.8 (N=9)	0.6 (N=10)
D926C	2.9 (N=10)	1.0 (N=4)
D926D	3.5 (N=11)	0.6 (N=7)
Total	3.0 ± 0.25	1.2 ± 0.22

duplication has a significant suppressive effect on tiller formation in *Tp1*/+ plants. This result is therefore consistent with the hypothesis that the dosage effect of 1L is a function of an increase in the dose of the wild type *tb* allele. Conclusive proof of this hypothesis would be provided by the phenotypic equivalence of +/+/*tb*; *Tp1*/+ and +/+; *Tp1*/+ plants. These genotypes are currently being constructed.

Confirmation of the linkage between *Tp1* and *sl1*

--Scott Poethig

Tp1 has never been mapped relative to its closest neighbor, *sl* , in crosses involving both of these loci. Three point linkage data between *Tp1*, *gl1* and *sl1* indicate that *sl1* is 3.2cM distal to *Tp1* (Table 1). These data confirm the location of these loci on the current genetic map.

Table 1. + *Tp1* +/*gl1* + *sl1* x *gl1* + *sl1*

a) + Tp1 +	104
gl1 + sl1	81
b) gl1 Tp1 +	10
+ + sl1	13
c) + Tp1 sl1	6
gl1 + +	1
Total progeny:	215

% recombination
gl1--Tp1: (b) = 10.7 ± 2.1 %
gl1--sl1: (b + c) = 14.0 ± 2.4 %
Tp1--sl1: (c) = 3.2 ± 1.2 %

The location of *Wrk1*

--Scott Poethig

Wrk1 is a dominant, EMS-induced mutation that causes the endosperm to become deeply furrowed. Given its severe effect on endosperm development, it is interesting that this mutation has little or no seedling phenotype. *Wrk1* was mapped to the short arm of chromosome 3 by M.G. Neuffer. Three point testcrosses involving *Wrk1*, *Lg3*, and *Cg1* or *Tp1* (Tables 1 and 2) demonstrate that *Wrk1* is located 2.8 ± 0.9cM distal to *Lg3*. A two-point testcross between *Wrk*

Table 1. *Lg3* + *Cg1*/+ *Wrk1* + x +++

a) *Lg3* + *Cg1*	96
+ *Wrk1* +	85
b) *Lg3* + +	25
+ *Wrk1 Cg1*	23
c) *Lg3 Wrk1* +	0
+ + *Cg1*	4
d) *Lg3 Wrk1 Cg1*	1
+ + +	0
Total Progeny:	234

% Recombination
Wrk1--Cg1: (b + d) = 20.9 ± 2.6 ~
Lg3--Wrk1: (c + d) = 2.1 ± 0.9 %
Lg3--Cg1: (b + c) = 22.2 ± 2.7 %

Table 2. *Lg3* + *Tp3*/+ *Wrk1* + x +++

a) *Lg3* + *Tp3*	20
+ *Wrk1* +	41
b) *Lg3* + +	8
+ *Wrk1* Tp3	14
c) *Lg3 Wrk1* +	1
+ + *Tp3*	3
Total Progeny:	87

% Recombination
Wrk1--Tp3: (b) = 25.3 ± 4.7 %
Lg3--Wrk1: (c) = 4.6 ± 2.2 %
Lg3--Tp3: (b + c) = 29.9 ± 4.9%

and *Cl1* indicated that these genes are 1.9 ± 0.5cM apart (12/632). Only the non-wrinkled seeds were used in calculating this latter recombination frequency because *Cl* could not be reliably scored in *Wrk1* seeds. Since *cl1* is 5cM distal to *Lg3*, these data suggest the following map order: *Lg3*--3--*Wrk1*--2--*cl1*.

ST. PAUL, MINNESOTA
University of Minnesota

Sequence of the cDNA for a zein-associated-protein

--Keqin Chen, John Hunsperger, and Irwin Rubenstein

Maize endosperm protein bodies contain two types of prolamins: the zeins (about 85% of the total) and the zein associated proteins (ZAPs). Three genes encoding for ZAPs have been isolated and characterized: the genes for 10kD, 15kD, and 27kD proteins.

A cDNA clone encoding a new ZAP has been isolated from a cDNA library made from W22 protein body mRNAs (22 DAP). A clone (Z19) which weakly hybridized to a Z36 probe (coding for the 27kD ZAP) was isolated. Northern blots indicated a 1kb mRNA. Hybridization release - in vitro translation experiments produced a protein product with a relative molecular weight of 26kD. The sequence of the

cDNA clone indicated a 785bp insert with a 549bp open reading frame. The deduced amino acid sequence consists of 183 amino acid residues with an absolute molecular weight of 19,560kD. The DNA sequence is high in GC (about 67%).

The Z19 amino acid sequence in some ways resembles the Z36 sequence. The exact length of its signal peptide is unknown, but the amino acid sequence is similar to the Z36 signal peptide (Fig. 1). The basic residue near the N-terminal of the Z19 signal peptide, however, is Lys instead of Arg

```
CZ15A3  M-K-M-V-I-V-L-L-L-W-L-A-L-S-A-A-S-A-S-A
Z19     M-K---V-L-I-V-A-L-A-L-L-A-L-A-A-S-A-A(S)(S)?
Z36     M-R---V-L-L-V-A-L-A-L-L-A-L-A-A-S-A-T-S
```

Fig. 1. Comparison of the signal peptide sequences.

as in Z36. A hexapeptide (Pro-Pro-Pro-Phe-Tyr-X) near the N-terminal of the mature protein is repeated twice in Z19. In Z36, the hexapeptide (Pro-Pro-Pro-Val-His-Leu) is repeated 8 times with only one replacement amino acid residue in the seventh repeat. The N- terminal and the C-terminal amino acid sequences of the mature Z19 and Z36 proteins are conserved (Fig. 2, 3). An interesting difference

```
CZ15A3  S-Y-R-T-N-P-C-G-V-S-A-A-I-P-P-Y-Y
Z19     Q-Q-P-G-P-C-P-C-N-A-A-A-G-G-V-Y-Y
Z36     Q-Q-P-T-P-C-P-Y---A-A-A-G-G-V-P-H
```

Fig. 2. Comparison of the C-terminal region peptide sequences.

```
CZ15A3  M-Q-M-P-C-P-C-A-G-L-Q
Z19     (S)(S)?T-S-G-G-C-G-C-Q-T
Z36     T-H-T-S-G-G-C-G-C-Q-P
```

Fig. 3. Comparison of the N-terminal region peptide sequences.

is that the last 2 amino acid residues at the C- terminus of Z19 are identical to those of the 15kd ZAP. Southern genomic blots indicate only one copy of the Z19 gene in the inbred W22.

Characterization and complete sequence of a cDNA encoding for ubiquitin from W22

--Keqin Chen, John Hunsperger and Irwin Rubenstein

Ubiquitin is a small (76 amino acid residues) protein found in eukaryotic cells. Its amino acid sequence is highly conserved. The ubiquitin protein forms a covalent attachment to cytosolic proteins destined for degradation. It also is found conjugated to certain histones in chromatin and is involved in ribosomal RNA processing and cell surface receptor modification. The structure of genes coding for ubiquitin has been studied in yeast, chicken, *Xenopus*, pig, humans, *Drosophila*, barley, and *Arabidopsis*.

We have determined the DNA sequence of a cDNA clone coding the ubiquitin protein from W22 inbred. The clone UB14 was found in the cDNA library made from endosperm protein body. The UB14 sequence contains seven perfect ubiquitin (76 amino acid) repeats. The amino acid sequence deduced from the cDNA sequence is identical to the sequence of ubiquitin from barley, oats, and *Arabidopsis*. Plant ubiquitin differs from animal and yeast ubiquitin by only three amino acid substitutions. In addition, there is an additional Gln (Q) at the C-terminal end of the last 76 amino acid maize ubiquitin repeat (Fig. 1). The 3' untranslated region is about 170 nucleotides long. This cDNA clone includes a 5' untranslated region 65 nucleotides long.

```
(MQIFVKTLTGKTITLEVESSDTIDNVKAKIQDKEGIPPDQQRLIFAGKQ
LEDGRTLADYNIQKESTLHLVLRLRGG)6
-MQIFVKTLTGKTITLEVESSDTIDNVKAKIQDKEGIPPDQQRLIFAGKQ
LEDGRTLADYNIQKESTLHLVLRLRGGQ
```

Fig. 1. Amino acid sequence of maize polyubiquitin.

Total RNAs were isolated from different tissues of 4-day-old seedlings: coleoptile, scutellum, radicle, and endosperm. Northern blots of these RNAs indicated several ubiquitin transcripts of different lengths: 600 to >2000 nucleotides. The sizes of the transcripts found were specific to a given tissue. Some high molecular weight ubiquitin-related RNA sequences found in coleoptile and radicle total RNA are absent from scutellum and endosperm. Total RNAs were isolated from 4 day-old seedlings which had undergone a heat shock (1 hr, 42 C). Northern blots of this RNA indicated that the amount of ubiquitin RNAs is dramatically increased in all four tissues.

Southern blots of W22 genomic DNA show two intensive ubiquitin-specific hybridization bands and several faint bands. We estimate that there are two or more ubiquitin genes in W22 inbred.

Detection of amino acid variants

--Deborah A. Muenchrath and Ronald L. Phillips

Lysine, methionine, and threonine are synthesized in a common branched pathway, the aspartate pathway. The enzymes in the pathway are regulated by allosteric feedback inhibition by the end product amino acids. Exogenously added lysine and threonine (LT) synergistically inhibit root growth of germinating kernels. Phillips et al. (Crop Sci. 21:601, 1981) screened over 200 maize lines for root growth on medium supplemented with equimolar amounts of lysine plus threonine. Relative inhibition was determined by the ratio of root lengths of seedlings grown on LT medium (Treatment) to root lengths of seedlings grown on basal medium (Control), the T/C ratio. In the 1981 study, the T/C of B37 opaque-2 was less than the inbred B37 T/C. Whole kernel amino acid analyses indicated the *o2* version had a lower ratio of methionine to lysine (M/L), and to lysine-plus-threonine, than the wildtype. A relationship between the response to LT medium (T/C) and the M/L ratio was hypothesized. Based on this relationship, a screening system was proposed to detect high lysine amino acid variants by simply screening lines on LT-supplemented medium and searching for lines with low T/C ratios.

The objective of the current study is to test the hypothesis that the LT response is a function of the M/L ratio; i.e., high M/L results in resistance and low M/L leads to inhibition of root growth on LT medium. Nine B37 endosperm mutants, provided by F. Salamini, were evaluated for LT response and amino acid composition. The nine genotypes were tested in two groups to permit timely handling of the materials. The T/C and M/L data for each group are presented in Table 1.

A highly significant correlation between M/L and T/C (r=0.98) exists for group 1. A similar trend is observed for group 2 (Figure 1); however, the T/Cs of sugary-2 and the *o2:su2* double mutant are reduced. Without the *su2* lines,

Table 1: LT response and amino acid data of B37 endosperm mutants.

GENOTYPE	LT RESPONSE T/C	S.D.	AMINO ACID DATA M/L	S.D.
Group 1				
++	1.140	0.370	1.109	0.016
fl2 fl2	0.735	0.184	0.677	0.020
o2 o2	0.515	0.180	0.498	0.010
o2o2fl2fl2	0.691	0.181	0.543	0.026
Group 2				
++	0.838	0.115	1.152	0.033
fl fl	0.732	0.253	0.848	0.005
bt bt	0.560	0.177	0.719	0.048
sh sh	0.771	0.258	0.919	0.159
o2 o2	0.443	0.075	0.497	0.022
su2 su2	0.307	0.058	0.980	0.009
o2o2su2su2	0.207	0.058	0.513	0.018

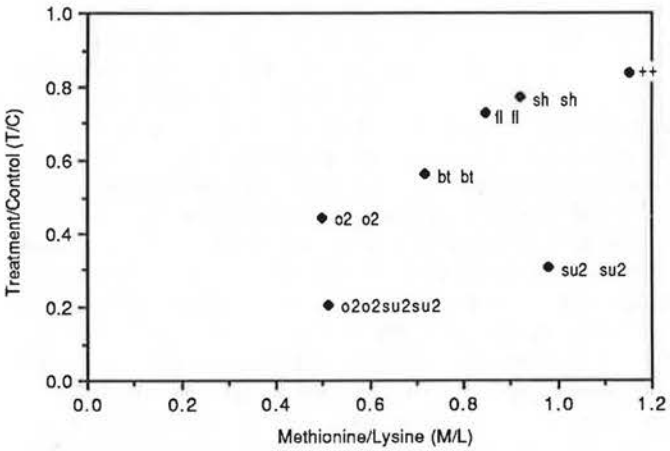


Figure 1: Relationship of LT response and amino acid composition.

the correlation coefficient between M/L and T/C in group 2 is increased from 0.63 to 0.97, consistent with the correlation for group 1. The reduced T/C of the *su2* mutants may be due to linkage of *su2* with a locus affecting seedling vigor, or related to endosperm starch or protein composition. Other endosperm mutants will be evaluated. Other inbred lines and their *o2* versions have been tested; in each case, the T/C and M/L are reduced in the *o2* version. Based on the correlation between M/L and T/C, seedling screening on LT-supplemented medium may effectively detect amino acid variants.

Activation of the *Spm* transposable element in a tissue culture-derived plant

--V. M. Peschke, R. L. Phillips, and L. Pritchard

As part of our studies on the activation of transposable elements in tissue culture, we have tested a large number of regenerated plants for *Spm* activity. We had previously observed that *Ac* activity could be detected in 3% of the tissue culture-derived plants tested, even though no active *Ac* elements were present in the explant sources (Science 238:804, 1987). Evola and coworkers (11th Ann. Aharon Katzir-Katchalsky Conference, 1984; 1st Intl. Cong. Plant Mol. Biol., 1985) have also reported the activation of both *Ac* and *Spm* in tissue culture-derived materials.

Approximately 500 R1 progeny of 144 regenerated plants were crossed as males onto a *c-m(r)* tester stock (kindly provided by P. Peterson). The regenerated plants were derived from 62 embryo cell lines, and had been obtained from C. L. Armstrong (Crop Sci. 28:363, 1988) and M. Lee (Genome 29:122, 1987; Genome 29:834, 1987). Based on the testcrosses, *Spm* activity was observed in two progeny of one regenerated plant, designated 283(1), from Armstrong's material. This plant had been regenerated from Type II (friable embryogenic) callus of an A188 X B73 genetic background after approximately eight months in culture.

As shown in Table 1, 20 tests of three other regenerated plants from the same embryo cell line were negative for

Table 1. Testcrosses of plants regenerated from cell line which produced *Spm* activity.

Regenerated plant	Progeny tests Positive:Negative
283 (1)	2:2
283(2) triplet 1	0:3
283 (3)	0:7
283(4) twin 2	0:10

Spm activity. This indicates that *Spm* activity arose during callus growth or plant regeneration and was not present in the embryo used to initiate the callus. Thirteen tests of the inbreds A188 and B73 were also negative for *Spm* activity.

Nearly all of the material tested in the present study had previously been tested for *Ac* activity. Plant 283(1) had been used directly in a testcross for *Ac* activity, which was negative. Likewise, progeny of seven regenerated plants which contained *Ac* activity were tested for *Spm* in the present study and in a previous smaller study (Peschke, M. S. thesis, 1986); all of these tests were negative as well. In addition, the *Ac* and *Spm* activities were detected in plants from different culture types (Type I vs. Type II), culture media (modified MS vs. N6 + proline), and genetic background (Oh43 X A188 vs. A188 X B73). This indicates that activation of transposable elements in tissue culture may occur under varying conditions and is not limited to a specific culture environment.

Relation of maturity and the 185bp DNA sequence in knob heterochromatin

--R. L. Phillips, J. Suresh and S. Kaeppler

In recent studies by Steffensen et al. (MNL 61:97-98, 1987) a relationship was reported between maturity and knob heterochromatin. This report represents an initial screening of seven inbreds differing in maturity (Table 1) for the level of a repetitive sequence found in maize heterochromatic knobs. The maize lines selected were ND101, CG 16, Mt42, Wilbur's Knobless Flint, A86-9, R225 and NC254.

Table 1. Maturity of seven maize inbreds used for slot blot hybridization.

	Inbreds	Maturity (Days to 50% pollen shed)
1	ND101	71
2	CG16	76
3	Mt 42	77
4	Wilbur's Knobless	90
5	A86-9	75
6	R225	118
7	NC254	115

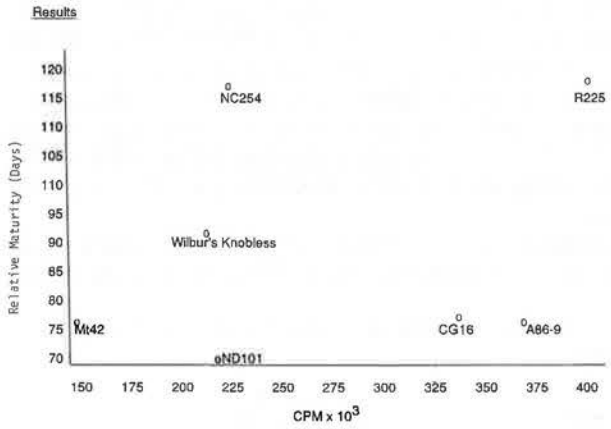


Figure 1. Hybridization data of 185bp sequence to DNA from seven inbreds.

DNA extracted from leaf tissue (Hoisington - personal communication, Missouri, 1988) and CsCl purified (Maniatis, Molecular Cloning, p. 93) was blotted (50-100ng) on nitrocellulose filter paper using a slot blotter (Schleicher and Schuell). The nitrocellulose filter was hybridized (procedure from Cuno Laboratories Connecticut) with a ^{32}P-labelled probe synthesized (A. Biochem. 137:266-267, 1984) from a 185bp sequence localized in heterochromatic knobs (Peacock et al. PNAS 70:4490-4494, 1981). Later each slot of the nitrocellulose filter was cut out and counted in a scintillation counter.

In these maize lines no obvious relationship was observed between maturity and proportion of the 185bp sequence (F = .015 NS and correlation coefficient = .17 NS).

Characterization of sethoxydim and haloxyfop tolerant mutants selected from tissue culture

--W.B. Parker, F.A. Keith, J.D. Burton, D.A. Somers, D.L. Wyse, J.W. Gronwald[1] and B.G. Gengenbach
[1]USDA-ARS

Sethoxydim is a postemergence herbicide that is toxic to most grass species, including corn. Haloxyfop is a herbicide from a different family with the same mechanism of herbicide action as sethoxydim. It also is toxic to grass species. Both herbicides have recently been shown to inhibit monocot Acetyl- Coenzyme A carboxylase (EC 6.4.1.2) (Burton et al., Biochem. Biophys. Res. Comm. 148:1039-1049, 1987). Corn hybrids tolerant to these herbicides would increase the herbicide options available for control of annual and perennial grasses in corn. The objectives of this research were to select sethoxydim- and haloxyfop-tolerant corn tissue culture lines, characterize the level and basis of tolerance, and determine if tolerance could be transmitted to regenerated plants and their progeny.

Initial experiments were conducted using "Black Mexican Sweet" (BMS) corn tissue cultures as a model system. Sethoxydim and haloxyfop were toxic to unselected BMS corn tissue cultures at 5 and 0.1μM concentrations, respectively. Three sethoxydim- tolerant BMS culture lines (B10S, B50S, and B100S) were selected. Sethoxydim concentrations reducing growth by 50% were 11-, 88-, and 40-fold higher in B10S, B50S, and B100S, respectively, than in BMS. Haloxyfop concentrations reducing growth by 50% were 9-, 64-, and 51-fold higher in B10S, B50S, and B100S, respectively, than in BMS. Tolerance was stable for one year when B10S was grown in the absence of sethoxydim indicating a genetic or epigenetic basis for tolerance. ACCase activity was 79, 167, and 88% higher in extracts from B10S, B50S and B100S, respectively, compared to BMS. Herbicide concentrations reducing ACCase activity by 50% in total protein extracts were not significantly different in selected lines compared to BMS. SDS-PAGE of total protein extracts probed with avidin-conjugated horseradish peroxidase indicated that levels of a biotin-containing protein, presumably ACCase, were increased in selected lines corresponding to their increase in ACCase activity. These results indicated that sethoxydim tolerance in selected BMS culture lines was conferred by increased ACCase activity likely due to elevated levels of the enzyme. Further investigations to determine the mechanism of increased enzyme expression are in progress.

Regenerable, friable, embryogenic callus cultures (A188 x B73) were selected for sethoxydim and haloxyfop tolerance. Two sethoxydim-tolerant (S1 and S2) and two haloxyfop-tolerant (H1 and H2) corn callus culture lines were obtained. S1 and S2 callus exhibited 100- and >100-fold increases in sethoxydim tolerance, respectively, compared to the unselected control callus. S1 and S2 also exhibited 4-and 30-fold cross-tolerance to haloxyfop, respectively. H1 and H2 callus exhibited no sethoxydim tolerance and 60- and 4-fold increases in haloxyfop tolerance compared to control callus, respectively. ACCase activity levels of the unselected control, S1, S2, and H1 were similar in the absence of herbicide. ACCase activity from H2 was 5-fold higher than the unselected control in the absence of herbicide. ACCase activity from S1 and S2 was inhibited 50% by sethoxydim concentrations that were 4- and 40-fold higher and haloxyfop concentrations 3- and 7-fold higher, respectively, than concentrations required for 50% inhibition of wildtype ACCase activity. ACCase activity from H1, H2, and the unselected control was inhibited 50% by similar sethoxydim concentrations. ACCase activity from H1 was inhibited 50% by a 6-fold greater haloxyfop concentration than required for the unselected control; whereas, ACCase activities of wildtype and H2 were inhibited by similar haloxyfop concentrations. These results indicated that herbicide tolerance was conferred either by elevated ACCase activity as in line H2 or by an altered ACCase activity that was less sensitive to herbicide inhibition.

Plants regenerated from unselected wildtype callus were killed by greenhouse applications of 0.05kg/ha sethoxydim and 0.01kg/ha haloxyfop. Plants regenerated from S1 and S2 callus lines were injured by 0.11 and 0.22kg/ha sethoxydim, respectively, but plants from both lines survived 0.44kg/ha sethoxydim, which is twice the field application rate of sethoxydim. S1, S2, and H1 plants were injured, but survived a 0.01kg/ha haloxyfop treatment; whereas, they were killed by 0.10kg/ha haloxyfop.

Progeny from reciprocal crosses of S1 and S2 plants x wildtype inbreds segregated 1:1 for tolerant:susceptible plants when treated with 0.44kg/ha sethoxydim. Progeny from self- pollinated S1 and S2 plants segregated 1:2:1 for uninjured:tolerant:susceptible plants when treated with 0.44kg/ha sethoxydim. Progeny from self-pollinated H1 regenerated plants segregated 1:2:1 for uninjured:tolerant:susceptible plants when treated with

0.01kg/ha haloxyfop. These results indicated that the S1, S2, and H1 plants regenerated from tissue culture were heterozygous for single dominant allele(s) which conferred sethoxydim tolerance. Putative homozygous plants that survived sethoxydim treatment have been grown to maturity and selfed. Seed set appears normal and selfed progeny will be evaluated for herbicide tolerance to determine if the trait can be maintained in a homozygous condition.

Threonine overproducing mutants exhibit aspartate kinases with reduced feedback inhibition

--S.B. Dotson, D.A. Somers and B.G. Gengenbach

Aspartate kinase is the first enzyme in the aspartate-derived amino acid biosynthesis pathway which leads to the production of lysine, methionine, threonine and isoleucine. Two lysine plus threonine (LT) resistant mutants previously selected from tissue culture overproduce free threonine compared to wildtype, implicating an alteration in the regulation of aspartate-derived amino acid synthesis (Hibberd et al., Proc. Natl. Acad. Sci. 79:559-563, 1982; T.J. Diedrick, Ph.D. Thesis, U of M, 1984). These mutants represent dominant alleles of two different genes designated *Ltr*-1* and *Ltr*-2* (Frisch and Gengenbach, MNL 60:115, 1986). The objectives of this research were to purify and characterize wildtype aspartate kinase to determine its regulatory characteristics and to determine whether the *Ltr*-1* and *Ltr*-2* mutations encode lysine-insensitive forms of AK that allow threonine overproduction.

Aspartate kinase was purified to greater than 1200-fold from wildtype 'Black Mexican Sweet' corn suspension culture cells (Table 1). Holoenzyme molecular weight of corn

Table 1. Purification of aspartate kinase from Black Mexican Sweet Corn cultures.

Step	Total Protein (mg)	Total[a] Activity (nktal)	Specific Activity (nktal/mg)	Fold Purif.	Percent Recovery
Crude Extract	1390	---	---	---	---
G-25	1500	21.1	0.014	1	100
Phenyl Sepharose	223	53.7	0.240	17	225
60% $(NH_4)_2SO_4$ Pellet	116	61.4	0.528	37	296
Gel Filtration	14.7	44.9	3.06	216	224
Anion Exchange-early	0.36	6.4	18.0	1273	33
Anion Exchange-late	0.62	11.7	18.9	1339	60

[a] Determined using hydroxamic acid assay. Paired control reactions were always run without aspartate and then adding the aspartate after the 60 min assay time just prior to developing the color with TCA and acid $FeCl_3$. Reactions were run at 30 C.

aspartate kinase determined by gel filtration was 255,000Mr. Separation by anion exchange resolved corn aspartate kinase activity into two isoforms. Both isoforms were similarly inhibited by lysine and lysine analogs. Threonine had no effect on corn aspartate kinase activity either alone or in combination with lysine. Aspartate kinase activity staining of native PAGE gels indicated that corn aspartate kinase disaggregated to ca. 120,000Mr species from the 255,000 Mr species originally detected by gel filtration. The aspartate-dependent aspartate kinase activity visualized on native gels was excised and rerun on SDS PAGE gels. Three bands of ca. 49-63 kDa were resolved from this highly purified preparation.

Characterization of LT resistant corn mutants has been somewhat limited by difficulties in deriving homozygous kernels that germinate and develop normally. This summer we isolated unpollinated immature ears from homozygous *Ltr*-2* plants and heterozygous *Ltr*-1* plants in A619 background. Aspartate kinase was purified more than 1000-fold from each mutant and from wildtype A619 using the procedure developed for corn cell suspension culture aspartate kinase. Only one peak of aspartate kinase activity was resolved by ion exchange chromatography for *Ltr*-2* and A619 (Fig. 1). Furthermore, the shapes of the ion exchange peak of aspartate kinase from A619 and *Ltr*-2* were

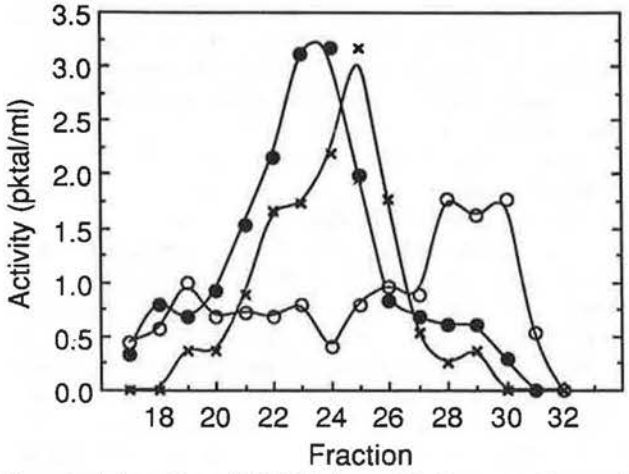


Figure 1. Anion exchange (FPLC) elution profiel of aspartate kinase activity of A619 (•), *Ltr*-1* (o), and *Ltr*-2* (x).

similar. Lysine inhibition of aspartate kinase activity from homozygous *Ltr*-2* was substantially different compared with A619 aspartate kinase. The activity in *Ltr*-2* required approximately 760μM lysine for 50% inhibition compared to approximately 10μM lysine for the wildtype, indicating about a 76-fold reduction in sensitivity to feedback regulation by lysine.

Analysis of aspartate kinase from heterozygous *Ltr*-1* plants was more complicated because of the presence of the wildtype and mutant alleles in this material. *Ltr*-1* aspartate kinase showed broader elution profile on ion exchange with the majority of activity eluting later than *Ltr*-2* and A619 AK. The *Ltr*-1* aspartate kinase preparation also exhibited reduced lysine feedback inhibition. Early and late eluting *Ltr*-1* aspartate kinase activity was inhibited 50% by 38μM and 25μM lysine, respectively, in contrast to 10μM lysine required for 50% inhibition of aspartate kinase from wildtype A619.

The aspartate kinase isoforms encoded by *Ltr*-1* and *Ltr*-2* were apparently copurified by our procedures. We assume that the lysine-insensitive aspartate kinase isoforms from *Ltr*-1* and *Ltr*-2* represent different aspartate kinase isozymes. It is also possible that the lysine-sensitive and lysine-insensitive subunits produced from alleles of the different genes assemble into functional but lysine-insensitive heterodimers, based on the lack of a detectable distinct anion exchange elution fraction with the wildtype level of lysine sensitivity. Regardless of the mechanism involved, relaxation of lysine feedback inhibition of corn aspartate kinase is associated with overproduction of free threonine in vegetative and kernel tissues.

Plastid transcript accumulation in developing endosperm

--Andrew McCullough and Burle Gengenbach

Amyloplasts in storage organs such as the endosperm of maize kernels are considered to be differentiated plastids

that do not carry out photosynthetic activities, but rather accumulate starch. This study was initiated to characterize the plastid genome in differentiated amyloplasts and to determine whether plastid genes are expressed specifically during amyloplast biogenesis in maize endosperm. We have previously reported (McCullough and Gengenbach, Genome 30, suppl. 1:315, 1988) that the plastid genome is present in total DNA prepared from maize endosperm tissue and that the abundance of plastid DNA increases relative to nuclear rDNA during early endosperm development. Nuclease protection assays with total endosperm RNA and cosmid clones of plastid DNA showed at least part of most plastid *Bam*HI fragments were protected by RNA isolated from 16 day post-pollination (dpp) endosperm.

Restriction fragments representing most of the plastid genome recently were used as probes in Northern hybridizations. We detected fewer RNA species homologous to plastid DNA in endosperm than in leaves. As a proportion of the total RNA, the abundance of most plastid transcripts was lower in endosperm than in leaves. We have, however, identified probes for which the corresponding transcripts accumulate to a higher abundance in 16 dpp endosperm than in shoots, leaves, or roots. One of these probes, *Bam*HI fragment 23, contains the ribosomal protein (rp) s12-s7 operon. This operon contains exon II, intron II and exon III of *rps12* and the uninterrupted *rps7* gene. Exon I and intron I of *rps12* map several kilobases from *Bam*HI 23 and maturation of *rps12* mRNA is thought to involve trans-splicing. In Northern analysis, the *Bam*HI 23 probe detected two preferentially accumulating transcripts in 16 dpp endosperm RNA. One of these transcripts was not detected in leaf, etiolated shoot, or root RNA. The second was much more abundant in the endosperm RNA than in RNA from other tissues; it also was detected in endosperm RNA by a probe containing exon I of *rps12* (*Bam*HI fragment 10) suggesting that trans-splicing occurs in endosperm plastids.

Probes specific for the *rps7* coding region and exon II and intron II of *rps12* were constructed. Subsequent Northern analyses revealed that the transcripts preferentially accumulating in 16 dpp endosperm contained homology to all three probes, suggesting that these transcripts were unprocessed, polycistronic RNA molecules. RNA isolated from 5, 8, 12, 16, and 20 dpp endosperms was probed with *Bam*HI 23 which revealed a progressive accumulation of these unprocessed transcripts over the 5-12 dpp period. The levels of other, but unrelated, plastid transcripts homologous to these probes remained constant during this period. Experiments are in progress to further characterize the structure and accumulation pattern of these and other transcripts which accumulate preferentially in endosperm plastids. Our results agree with those of Dang and Gruissem (EMBO J. 7:3301-8, 1988) which strongly suggest that post-transcriptional rather than transcriptional events play key roles in plastid gene regulation.

Purification of dihydrodipicolinate synthase from suspension cultures

--David A. Frisch, Burle G. Gengenbach and David A. Somers

Dihydrodipicolinate synthase (EC 4.2.1.52), which catalyzes the condensation of L-aspartate semialdehyde and pyruvate, is the branch point enzyme leading to lysine biosynthesis and is feed-back inhibited by the end-product lysine. Dihydrodipicolinate synthase (DHPS) from suspension cultures of Black Mexican Sweet was purified over 30,000 fold, relative to the crude desalted fraction, with a recovery of 25% (Table 1).

Table 1. Purification of dihydrodipicolinate synthase from Black Mexican Sweet Corn cultures.

	Total protein (mg)	Total activity	Specific activity	Fold purif.	Percent recovery
Crude homog.	7032	ND	ND	ND	ND
G-25 desalt	6522	814	0.13	1	100
Heat denaturation	397	ND	ND	ND	ND
Hydroxylapatite	9	1230	137	1096	151
Phenyl Sepharose	2.5	587	235	1880	72
Gel filtration	.15	586	3907	31256	72
Anion exchange	(ca.01)	202	ND	ND	25

ND: not determined

The purified preparation after anion exchange chromatography contained three detectable proteins as visualized on a silver-stained native gel. One of the proteins had an estimated molecular weight of 120,000Mr, which is similar to the estimated molecular weight of DHPS from wheat (Kumpaisel et al., Plant Physiol. 85:145-51, 1987). The purified DHPS had a pH optimum of 8.5 in a tris buffer.

Estimates of the Km values were 0.4mM for aspartate semialdehyde (ASA) and 5mM for pyruvate. Increasing L-ASA concentrations showed substrate inhibition which became less apparent as the concentration of pyruvate or L-lysine increased. The alleviating effect by pyruvate on ASA substrate inhibition indicates an ordered enzyme mechanism, either an ordered bi-bi or ping-pong reaction mechanism with pyruvate binding first.

Lysine inhibition was found to be of a mixed type with respect to L-ASA and of a noncompetitive type with respect to pyruvate. Lysine inhibition showed cooperativity with an I(.5) of 25μM and an estimated Hill number of 2.8, indicating at least three, or more likely four, interacting subunits.

In a survey of lysine analogues, lysine ethyl ester, threo-hydroxylysine, aminoethyl cysteine, and arginine inhibited DHPS 89, 83, 18, and 8%, respectively, at 100μM. Other analogues which did not show inhibition at 100μM were diaminopimelic acid, delta-hydroxylysine, epsilon-caproic acid, aspartate, norleucine and D-lysine. From these analogue studies, it appears that the alpha and epsilon amino groups and their stereochemistry are important in inhibitor binding; whereas, the carboxyl group is less important.

Enzyme preparations from the *Ltr*-1* and *Ltr*-2* mutants as described by Dotson et al. (MNL, this issue) also were analyzed for DHPS activity and inhibition by lysine. DHPS from the two mutants did not exhibit changes in kinetic parameters or lysine inhibition relative to the A619 wildtype control, indicating that control of the lysine-specific biosynthesis pathway was not altered in the mutants.

ST. PAUL, MINNESOTA
7 North Winthrop Street
AMES, IOWA
Iowa State University

***Zea diploperennis* B73 adapted to U. S. corn belt**

Lawrence A. Carlson and Steven C. Price

During 1985 in St. Paul, B73 was crossed with *Zea diploperennis* originating from Laventana, Jalisco, Mexico.

The female parent was B73. A large amount of F1 seed was obtained of which eight plants were grown in 1986 in St. Paul. The F1 plants were planted in May, and were short-dayed at the three leaf stage by covering them with 30 gallon trash barrels from 6 PM until 8 AM. The short-day treatment was discontinued after 24 days. Three of the plants tasseled eleven days later. The eight F1 plants were grown in isolation to obtain as much F2 seed as possible. Over 600 seeds were obtained.

In 1987 597 F2 plants were observed at the University of Minnesota. Seven plants flowered without the short-day treatment during the period July 28 through August 15. Sibs were made between these plants. The balance of the plants did not mature.

In 1988 about 35 F3 plants were grown in isolation without the short-day treatment in St. Paul. Open pollination with no selection was used and a reasonable amount of seed was saved. All of the plants set seed with a three week range in the time of maturity.

In summation: The plants have a very pronounced teosinte growth habit, with 1 to 10 tillers; 4 to 30 silking locations; 10 to 150 viable seeds per ear; numerous brace roots extending from the 1st to the 10th node; some plants regenerate from planted nodes with brace roots; new plant regeneration takes place when a tiller is held to the ground with the new roots growing from the node.

Seed from the 1988 adapted plants is available by contacting LAC.

Zea diploperennis-maize hybrid adapted to the U. S. corn belt

--Lawrence A. Carlson and Steven C. Price

A cross between *Zea diploperennis* No. 1190 as female parent, and Minhybrid 8201 (A641 x W182B) as male was made in 1979 in St. Paul (materials were furnished by John Doebley of the University of Wisconsin and Jon Geadelmann of the University of Minnesota). Approximately 6,000 F2 seeds were collected from an isolation plot of the F1 plants.

In 1982 380 seeds of the 1980 F2's were planted. Only two plants flowered at the normal (110-day maturity) time. These were observed and crossed with each other and a satisfactory quantity of good seed was saved.

Each year the resulting seed (17LD) has been planted and has bloomed and produced seed. A minority (less than 5% of the plants) continue to bloom too late in Minnesota to mature seed.

In summation: The plants have a very pronounced teosinte growth habit, with 3 to 30 tillers; 10 to 200 silking locations; 10 to 100 viable seeds per ear; numerous brace roots extending from the 1st to the 7th node; some plants regenerate from planted nodes with brace roots.

Seed is available on a limited basis from 1988 by contacting LAC.

ST. PAUL, MINNESOTA
University of Minnesota
PHOENIX, ARIZONA
Desert Botanical Garden

Further evidence regarding gene flow between maize and teosinte

--John Doebley and Gary Nabhan

Hybrids between maize and its nearest wild relatives, the teosintes, are frequently observed where these plants occur sympatrically in Mexico and Central America. For this and other reasons, it has often been assumed that there is "constant gene flow" between maize and some types of teosinte. The alternative view that selective forces act to prevent substantial gene flow, especially from maize into teosinte, has been supported by one of us (J.D.) on the basis of field observations and evidence garnered from allozyme and chloroplast DNA studies. To further examine this question, one of us (G.N.) collected seed of (1) a teosinte (*Z. mays* subsp. *mexicana*) population from the Nobogame valley in Chihuahua, Mexico, (2) a maize landrace sympatric to this teosinte population (from the same field), and (3) a similar maize landrace allopatric to teosinte (from the nearby village, Baborigame) but in similar environmental setting. Thirteen to 16 individuals of each of these three collections were analyzed for their allozyme constitutions in accordance with procedures we have used previously; however, three new enzyme assays (adenylate kinase, hexose kinase and shikimic acid dehydrogenase) were also employed. Figure 1 shows the Rogers' distances between the

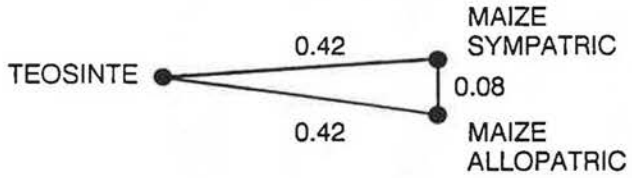


Figure 1. Rogers' distances between a Nobogame teosinte population and maize growing sympatrically and allopatrically with it.

collections. From this figure, it is clear that Nobogame teosinte is not genetically more similar to maize growing with it than it is to the same type of maize growing elsewhere, i.e., maize and teosinte growing in the same field and forming occasional hybrids do not become allozymically homogeneous. This conclusion is further supported by an examination of the actual allelic frequencies (Table 1), which show that teosinte maintains an allozymic constitution distinct from that of maize growing sympatrically with it. Of the 16 alleles that are found in only two of the three collections, 13 are shared by the two maize collections, two are shared by teosinte and the sympatric maize, and one is shared by teosinte and the allopatric maize.

These results are in agreement with previous studies of the allozymic constitution of sympatric maize and teosinte populations. Taken together, these studies provide convincing evidence that, despite the formation of hybrids, maize and teosinte are under strong disruptive selection that (1) prevents the free flow of germplasm between them and (2) enables them to maintain distinct genetic constitutions for loci other than those controlling the differences in the morphology of their ears.

Table 1. Isozyme allele frequencies for a collection of Nobogame teosinte, maize growing sympatrically with this teosinte and maize growing allopatrically to this teosinte but from the same general region.

Locus-allele[1]		Maize alone	Teosinte	Maize with Teosinte
Acp1-a	(2)	0.731	1.000	0.737
-b	(3 or 4)	0.269	0.000	0.263
Adh1-a	(4)	1.000	1.000	1.000
Adk1-a		0.808	1.000	0.868
-b		0.192	0.000	0.132
Enp1-a	(6 or 7)	0.961	1.000	0.947
-b	(8)	0.039	0.000	0.053
Glu1-a	(1)	0.125	0.000	0.053
-b	(?)	0.042	0.000	0.053
-c	(2)	0.125	0.063	0.237
-d	(3)	0.042	0.000	0.000
-e	(6)	0.292	0.126	0.263
-f	(7)	0.208	0.000	0.211
-g	(7.8)	0.000	0.000	0.026
-h	(8)	0.000	0.813	0.053
-i	(10)	0.083	0.000	0.026
-j	(12)	0.000	0.000	0.079
-k	(n)	0.083	0.000	0.000
Got1-a	(4)	0.731	0.031	0.842
-b	(5.8)	0.000	0.969	0.000
-c	(6)	0.269	0.000	0.158
Got2-a	(3)	0.000	0.344	0.000
-b	(4)	0.961	0.656	1.000
-c	(2)	0.039	0.000	0.000
Got3-a	(4)	1.000	1.000	1.000
Hex2-a		0.115	0.094	0.079
-b		0.885	0.906	0.921
Idh2-a	(4)	0.500	0.438	0.500
-b	(6)	0.500	0.562	0.500
Mdh1-a	(1)	0.000	0.000	0.079
-b	(6)	1.000	0.000	0.920
-c	(10.5)	0.000	1.000	0.000
Mdh2-a	(3)	0.077	0.031	0.290
-b	(3.5)	0.115	0.000	0.000
-c	(6)	0.808	0.969	0.710
Mdh3-a	(16)	0.885	1.000	0.974
-b	(18)	0.115	0.000	0.026
Mdh4-a	(12)	1.000	1.000	1.000
Mdh5-a	(12)	1.000	0.219	0.921
-b	(15)	0.000	0.781	0.079
Mmm1-M	(M)	1.000	1.000	1.000
Pgd1-a	(2)	0.462	0.000	0.500
-b	(3.8)	0.538	1.000	0.500
Pgd2-a	(5)	1.000	1.000	1.000
Pgm1-a	(9)	0.807	0.208	0.900
-b	(16)	0.077	0.792	0.100
-c	(?)	0.115	0.000	0.000
Pgm2-a	(3)	0.077	0.042	0.079
-b	(4)	0.923	0.958	0.921
Phi1-a	(2)	0.115	0.000	0.079
-b	(3)	0.039	0.031	0.158
-c	(4)	0.846	0.969	0.763
Sad1-a		0.077	0.000	0.053
-b		0.923	0.937	0.947
-c		0.000	0.063	0.000

[1]The parenthetical allelic designations are those used by C. Stuber and coworkers and are tentatively assigned.

ST. PAUL, MINNESOTA
University of Minnesota
RALEIGH, NORTH CAROLINA
North Carolina State University

On the origin of the maize male sterile cytoplasms: its completely unimportant, that's why its so interesting

--John Doebley and Paul H. Sisco

During the course of a survey of variation in the chloroplast genome of maize and the teosintes, we examined the chloroplast genomes of the maize male sterile cytoplasms cms-C, S and T. The question in mind was whether (1) these cytoplasms were similar to the fertile cytoplasms of maize for their chloroplast genome or (2) they were foreign cytoplasms (probably teosinte cytoplasms) which have become incorporated into a maize nuclear background. If the former is the case, then it is probable that the male sterile trait arose within maize, most likely as a result of mutations in the mitochondrial genome (Model 1). If the latter is true, then male sterility may have originated because of incompatibility between the foreign cytoplasm and the maize nuclear genome (Model 2).

Chloroplast DNA or total cellular DNA preparations of maize and teosinte were restricted with 21 restriction enzymes, electrophoresed, blotted and probed with cloned portions of the chloroplast genome representing 87% of its length. The analyses included 86 accessions of maize, mostly U.S. and Latin American landraces, and 74 accessions of teosinte. Eight cpDNA types were defined by the presence/absence of restriction sites or insertion/deletion events. Four of these eight types were very distinct from maize (14 to 16 restriction site differences) and were restricted to the teosintes, *Zea diploperennis*, *Z. perennis* and *Z. luxurians*. The remaining five types were quite similar to one another (only 1 or 2 site differences) and were found in maize and the Mexican annual teosintes, *Z. mays* subsp. *mexicana* and subsp. *parviglumis* (Table 1). The chloroplast genome types of cms-C and T were the same as those found in the majority of the maize accessions (fertile cytoplasms) and subsp. *parviglumis*, the teosinte that cytological and isozymic data indicate was the progenitor of maize (Table 1, type E). This implies that the origin of the male sterility of these cytoplasms fits Model 1 (above). The chloroplast genome type of cms-S (Table 1, type A), however, was not found in any other type of maize but was present in three collections of *Z. mays* subsp. *mexicana* (Central Plateau Teosinte) from Copandiro, Michoacan, Mexico. This chloroplast genome type is distinguished from the common type of maize by its lack of both an 80bp deletion and a single *Eco*RI site typical of normal maize (type E). The 80bp deletion had been previously observed by Pring and Levings (Genetics 89:121-136, 1978). To confirm that this teosinte and cms-S actually contain the same cytoplasm, we isolated mitochondrial DNA from this teosinte and observed that it contains the S-1 and S-2 plasmids typical of cms-S. Restriction fragment banding patterns of mi-

Table 1. Numbers of accessions of maize and Mexican annual teosinte possessing each of five distinct chloroplast DNA genotypes.

Taxon	cpDNA Genotypes*				
	A	B	C	D	E
subsp. *mexicana*	3	4	24	-	-
subsp. *parviglumis*	-	1	16	12	3
subsp. *mays*					
landraces	-	32	8	-	45
cms-C	-	-	-	-	1
cms-S	1	-	-	-	-
cms-T	-	-	-	-	1

* Using *Zea diploperennis* as an outgroup, cpDNA type A would be judged most primitive (the first to diverge from the others). Type B is the next derived form, distinguished by a deletion mutation (LM-3). Types C, D and E are the most advanced, each possessing LM-3 plus additional restriction site loss/gains. Type C has an unique *Eco*RI site; Type D has an unique *Cfo*I site; Type E has another unique *Eco*RI site.

tochondrial DNA revealed that Copandiro teosinte belongs to cms-S subgroup CA as defined by Sisco et al. (Theor. Appl. Genet. 71:5-15, 1985). This is the most common of the cms-S subgroups. Thus, there seems to be little question that this teosinte and cms-S share the same cytoplasm.

There are two possible explanations for the presence of the same cytoplasm in cms-S and Copandiro teosinte: (1) cms-S represents a domestication from Copandiro teosinte (subsp. *mexicana*), while the fertile cytoplasm types represent a separate domestication from subsp. *parviglumis*; or (2) introgression was involved. Of these alternatives, introgression seems more plausible as it is consistent with other data which suggest that maize was domesticated only once and that subsp. *parviglumis* (not subsp. *mexicana*) was the ancestral teosinte (see Doebley, Goodman & Stuber, Econ. Bot. 41:234-246, 1987). Accepting the introgression hypothesis, the next question is did the cms-S cytoplasm originate in maize and subsequently become transferred into Copandiro teosinte or the reverse. Again, if one accepts the evidence (1) that teosinte existed before maize, (2) that maize represents only a recent branchlet in the evolution of *Zea*, and (3) that subsp. *parviglumis* was the progenitor of maize (see Doebley, Goodman & Stuber, Econ. Bot. 41:234-246, 1987), then the direction of introgression must have been from Copandiro teosinte into maize because the original domesticated maize would have had the cytoplasm type of subsp. *parviglumis*. cms-S cytoplasm is also found in race Cónico Norteño maize of the Mexican Central Plateau (Weissinger et al., Genetics 104:365-379, 1983). This race might have been the link between Copandiro teosinte and the cms-S cytoplasms of the U.S. A scenario for the origin of cms-S cytoplasm is graphically summarized in Fig. 1.

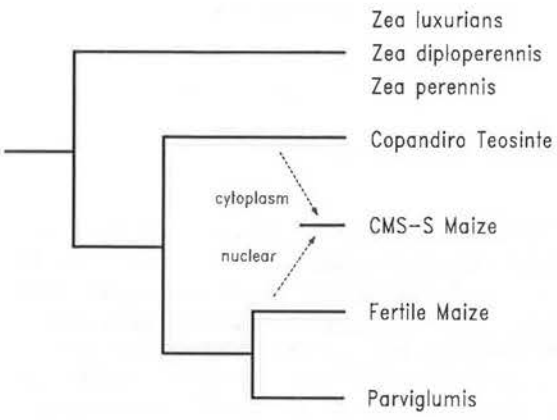


Figure 1. A phylogeny showing the presumed origin of cms-S maize by the introgression of Copandiro teosinte cytoplasm (=cms-S cytoplasm) into the maize nuclear background.

This interpretation of the facts suggests that the origin of male sterility in cms-S maize follows model 2 (above). If this is true, Copandiro teosinte may possess restorer genes and may even have been the source of the restorer genes for cms-S that are found in maize. A question we do not know the answer to is whether Copandiro teosinte exhibits the male sterile trait, although this seems unlikely as that would inhibit or even prevent its survival as a wild plant.

SALT LAKE CITY, UTAH
NPI

Use of PCR and direct sequencing to compare DNA sequences from homologous regions for several cultivars

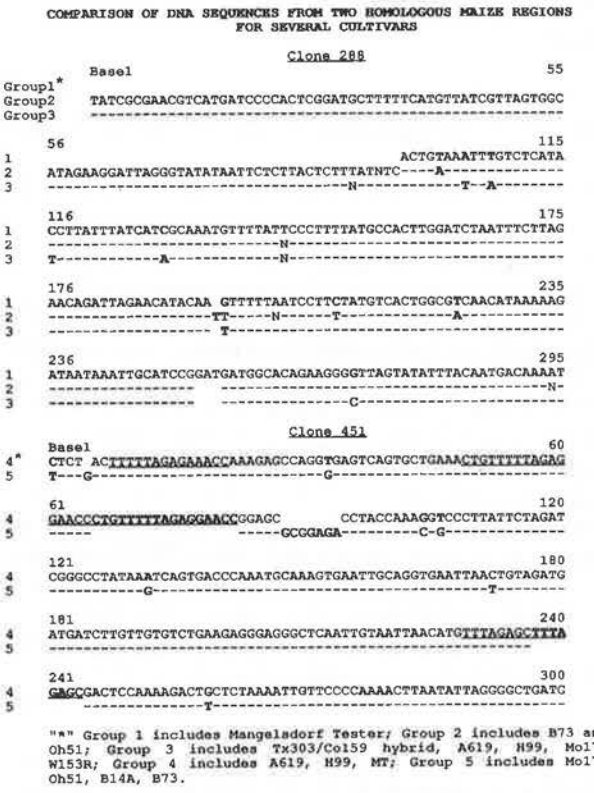
--Tim Helentjaris, Donna Shattuck-Eidens and Russell Bell

We have had the opportunity to produce genetic linkage maps based upon restriction fragment length polymorphisms (RFLPs) in several species. We are intrigued by the relatively high rate of polymorphism exhibited in maize and *Brassica* when compared to other species such as tomato and melon. Virtually any unique sequence clone in maize can reveal multiple polymorphisms amongst a selection of domesticated maize inbreds using only a few restriction enzymes. In tomato the occurrence of RFLPs is much lower; less than 5% of the clones tested reveal any polymorphism and many more enzymes were tested. Since RFLP analysis simply reflects the DNA sequence variation between individuals, we reasoned that an examination at the nucleotide sequence level of species exhibiting RFLPs at differing rates, would yield a better explanation of this difference. Previously available technologies made it impractical to economically approach this problem.

With the development of the polymerase chain reaction (PCR, Saiki et al., Science 230:1350-1354, 1985), it has become possible to amplify homologous regions from many different individuals and sequence them directly to compare any DNA sequence variation in the amplified regions. Inserts from clones used previously to detect RFLPs amongst maize cultivars (Helentjaris, Trends Genet. 3:217-221, 1987) were sequenced completely. Pairs of oligonucleotide primers were synthesized from each end of the inserts, approximately 500 to 1000bp apart, so that their 5' to 3' orientations were directed towards each other. Using standard conditions from the original papers, these primers were used to amplify homologous regions from 7 to 9 maize cultivars. The PCR products were then purified from agarose gels and sequenced using a Sequenase kit.

Some sample data for maize are shown in the accompanying table. It can be seen for these two regions that there are multiple DNA sequence variations between these cultivars. We estimate from our current data that there are 30 to 50 alterations per 1000bp. Both base pair changes and sequence rearrangements of various sizes are seen. Interestingly, a tandem duplication of 17bp in the 451 region of group 4 (underlined) is followed by a very similar 8bp tandem duplication less than 200bp away. In the group 5 lines neither of these sequences is duplicated. A large insertion of several hundred base pairs was seen in the 288 region in B14A (not shown) but was not found to be related to any of the transposable element sequences in the Genbank database. We are currently expanding both the amount of sequence information on specific regions and the number of individuals evaluated.

Using this type of analysis, we feel that it will now be much more practical to compare homologous regions of numerous individuals at the DNA sequence level. It is also interesting that in melon we have found by similar analysis that the rate of sequence variation is about tenfold less than



that seen for maize. Since short tandem duplications and rearrangements can be generated by transposable element activity, it is tempting to speculate that the high level of polymorphism observed in maize is the result of transposable element activity. To date no natural transposable elements have been identified in tomato and melon, and both exhibit little polymorphism. It should also be noted that the species which exhibit higher levels of RFLP variation reproduce primarily by outcrossing whereas the less polymorphic species are primarily self-pollinated. The different modes of reproduction may affect the level of transposable element activity itself or it may be that a high mutation frequency resulting from transposable element activity or any other mechanism can not be tolerated in a self-pollinated species and is therefore selected against. At this point we remain at the speculation stage but the PCR method of analyzing sequences will allow us to examine the question "Why do different species exhibit different levels of RFLP variation?".

A survey of Iowa Stiff Stalk Synthetic parents, derived inbreds and BSSS(HT)C5 using RFLP analysis

--Susan L. Neuhausen

The Iowa Stiff Stalk Synthetic (BSSS) has been a source of successful inbreds and derivative lines. B14 and B37, from the initial cycle, and B73, from the fifth cycle, have proven useful as parents in commercial hybrids and as breeding germplasm in the development of recycled lines. Restriction fragment length polymorphisms (RFLPs) may be a useful tool to characterize this population at a genetic level. RFLP analysis was performed on the BSSS parents; the 4 inbreds comprising IA13, the tester for BSSS(HT); 12 inbreds released from BSSS cycles; 5 B14-derived inbreds; 2 B37-derived inbreds; 2 B73-derived inbreds; 2 testers for BSSS inbreds; 3 parents of some of the derived inbreds; and 124 individuals from the BSSS(HT)C5. The 170 lines were examined with 42 clones, and the 46 inbreds were examined with an additional 28 clones, spaced throughout the genome.

Conservation of chromosomal segments among derived inbreds and contributions from donor parents can be readily observed and followed using RFLPs. In Figure 1, alleles

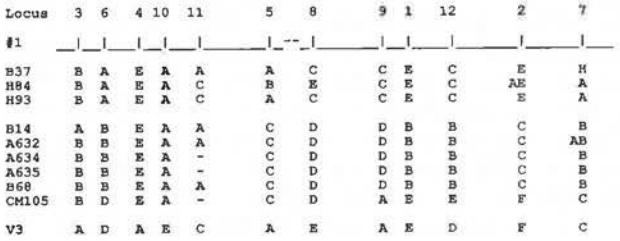


Figure 1. Genotypes of inbreds for chromosome 1.

of inbreds are shown for loci on chromosome 1. H84 and H93 were highly conserved for B37 alleles except around the centromere and at the end of the short arm. There was strong conservation of B14 on chromosome 1 for all of the derived inbreds except for CM105, which had many alleles from V3, the donor parent, on the long arm.

Changes in allelic frequencies from the original BSSS to the BSSS(HT)C5 may be due to selection for favorable alleles at those loci. In Table 1, two loci are shown as examples of little change in allelic frequencies from the

Table 1. Examples of little change in allelic frequencies from BSSS(HT)C0 to BSSS(HT)C5.

	Alleles of Locus 3				Alleles of Locus 26				
Line	1	2	3	4	1	2	3	4	5
	Allelic Frequency								
BSSS(HT)C0	.69	.25	.06	0	.65	.16	.12	0	.06
BSSS(HT)C5	.71	.29	0	0	.80	.20	0	0	0
B73 [from BSSS(HT)C5]	1.00					1.00			
B78 [from BSSS(HT)C6]		1.00			1.00				
B84 [from BSSS(HT)C7]	1.00				1.00				
IA13	.25	.50		.25	.50	.25	.25		

BSSS(HT)C0 to the BSSS(HT)C5. This suggests that there was no selection pressure or genetic drift for these loci. In contrast, in Table 2, two loci are shown which exhibited large changes in allelic frequencies probably resulting from selection. Allele 3 for locus 4 increased from 0.19 to 0.83 in 5 cycles, such that B73 and B84 have that allele. Allele 4 for locus 4 was probably an important allele for that locus because its initial gene frequency was very low, yet it was maintained and was found in an inbred released from the

Table 2. Examples of allelic frequency changes from BSSS(HT)C0 to BSSS(HT)C5 likely due to selection.

	Alleles of Locus 4									Alleles of Locus 42		
Line	1	2	3	4	5	6	7	8	9	1	2	3
BSSS(HT)C0	.06	.06	.19	.06	.31	.12	.06	.06	.06	.19	.75	.06
BSSS(HT)C5	.04		.83	.12						.50	.39	.10
B73			1.00							1.00		
B78				1.00						1.00		
B84			1.00							1.00		
IA13	.25		.50		.25						1.00	

sixth cycle. The allele was from CI.540. For locus 42, the allelic frequency of allele 1 increased and the three inbreds subsequently released contained that allele. Allele 2 for locus 42 decreased in the BSSS(HT) and was found in the tester. This is suggestive that there was a favorable heterotic response of allele 1 to the tester and no response for the additive effect of allele 2. On chromosome 7, gene frequencies changed from the BSSS(HT)C0 to the BSSS(HT)C5 such that all of the later cycle inbreds were of one allele type (Table 3), except for a locus at the end of the chromosome. This is a highly conserved chromosome for

Table 3. Loci on chromosome 7 at which one allele was conserved for inbreds released from later BSSS(HT) cycles.

	Chromosome 7 - Locus					
	49	50	45	51	47	48
	Allele					
	1	1	1	2	2	3
	----------Allelic Frequency----------					
BSSS Parents	.33	.81	.38	.57	.75	.31
BSSS(HT)C5	.88	-	.91	-	1.00	.84
B73	1.00	1.00	1.00	1.00	1.00	1.00
B78	1.00	1.00	1.00	1.00	1.00	1.00
B84	1.00	1.00	1.00	1.00	1.00	1.00
IA13	0	.75	.50	.67	.50	.50

these inbreds due to gene frequencies approaching fixation. These results are suggestive that there is a selective advantage for these alleles at these loci and that selection pressure was on the entire chromosome. There were large shifts in gene frequencies from BSSS(HT)C0 to BSSS(HT)C5 for some of the loci and were likely due to effects of selection. A field evaluation of the BSSS(HT)C5 for yield in relation to IA13 and for stalk quality, with subsequent analysis for identification of QTLs, is necessary in order to confirm that changes were due to selection.

It was difficult to assess whether individual BSSS parents were more important than others in the success of this synthetic. Of the 402 alleles examined for the 70 loci, only 91 alleles were represented by only one inbred. Therefore, in most instances, a determination of which parent contributed an allele was not possible. Only 28 of the 91 rare alleles were found in the BSSS(HT)C1 inbreds, the BSSS(HT)C5, B73, B78, or B84. Many of the rare alleles were probably lost due to drift. Of the 9 rare alleles which were in at least one of the three inbreds released in the fifth (B73), sixth (B78), or seventh (B84) cycles, two each were from CI.540, LE23, ILL.HY, Ia.OS420, and one from Ind.B2. This is suggestive that these parents were more important than others in the success of this synthetic.

SALT LAKE CITY, UTAH
Howard Hughes Medical Institute
University of Utah

Variation in the organization of the mitochondrial genome

--Christiane Fauron and Marie Havlik

The mitochondrial genome of different lines varies in size and organization. The physical map of the normal cytoplasm in a Wf9 or B37 nuclear background (Lonsdale et al., Nucl. Acid. Res. 16:9249, 1984, Fauron and Havlik, Nucl. Acid. Res. 16:10395, 1988) reveals a sequence complexity of 570kb. However, the physical map of the cytoplasmic male sterile type Texas (cms-T) in a Wf9 and B37 nuclear background reveals a sequence complexity of 540kb (Fauron et al., Mol. Gen. Genet., in press, 1989a, Fauron unpublished). For both genomes the entire sequence complexity can be represented on a master chromosome or alternatively as a multipartite structure via recombination at repeated sequences.

Using the three restriction enzymes *Bam*HI, *Xho*I, and *Sma*I, 350 and 339 sites have been located in the N and cms-T map respectively. A detailed comparison of N and cms-T mitochondrial genomes by hybridization studies (genotype B37, Fauron and Havlik, Curr. Genet., in press, 1989b) led to the following observations:

1. Because the repeated sequences are mostly different between N and cms-T, recombination generates a rather different population of submolar circular molecules.

2. A complex pattern of sequence permutations (involving sequences as small as 1kb, as large as 95kb) between the two genomes indicates that at least thirty events are needed to explain the sequence rearrangement.

3. The sequences not shared between the two genomes (70kb in N and 40kb in cms-T) can be accounted for by the presence of different repeated sequences, the absence of the integrated form of plasmids R1 and R2 in cms-T, the presence and absence of chloroplast sequences and a number of unidentified sequences that are specific to N or to cms-T.

We screened various N and cms-T mtDNAs in a variety of nuclear backgrounds with various probes to see if differences could be observed between those genomes (Fig. 1). some rare microheterogeneity (e.g. point mutations, small deletions/additions creating different restriction sites) could be observed between some N cytoplasms. However, a dis-

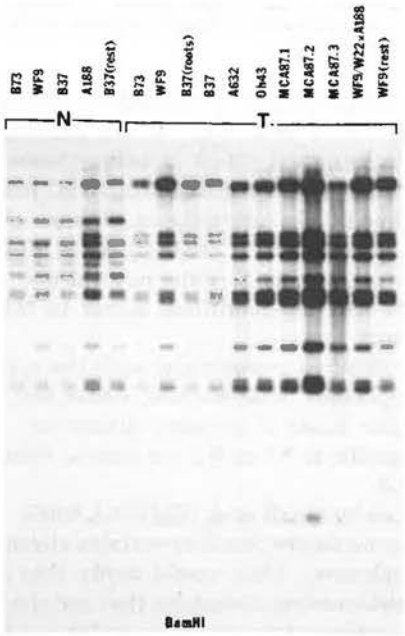


Figure 1. Hybridization of the cosmid clone Tu1E3 to a Southern blot containing *Bam*HI digested and mtDNA of N and cms-T in a variety of nuclear backgrounds as indicated on the top of the picture. NA188 is different from the other Ns. No consistent difference was observed between the T cytoplasms.

[rest] indicates the cytoplasms containing the dominant nuclear genes Rf1 and Rf2.

tinct second class of normal mtDNA organization could also be identified (genotype A188, Ky21, W182B) when most of the cosmid clones used as probes give an hybridization pattern rather different from the known N or cms-T mtDNA, suggesting that the physical map is different as is the sequence complexity. This second class of normal maize mitochondrial genome is called N2 versus the N1 notation that is going to be used for the already published N map (Wf9, B37). An example of the hybridization studies is given in Figure 2. Depending on the clone used as a probe, four different results were obtained: 1) N1 and N2 are identical, but different from cms-T (Fig. 2A). 2) N1 and N2 differ by more than one fragment which is unique to each genome and different from cms-T (Fig. 2B, 2D). 3) N1 and N2 differ from each other by the presence or absence of fragment also shared with cms-T (Fig. 2C). 4) N2 is different from N1 but identical to cms-T (data not shown). Also, N2 contains sequences not identified either in N1 or cms-T (data not shown).

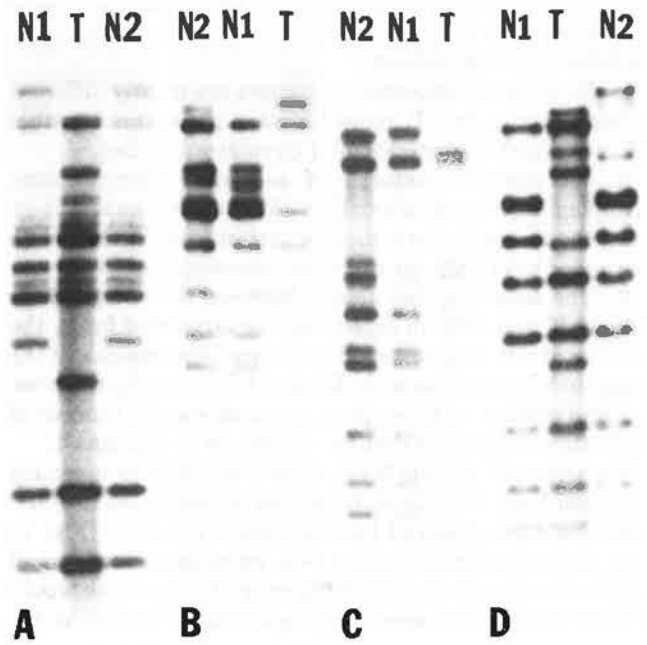


Figure 2. Hybridization of four different probes (A: N8B11, B: N7C9, C: N8A1, D: N5G8) to a Southern blot of *Bam*HI digested mtDNA from NB37 (N1), NA188 (N2) and TB37 (T).

More hybridization comparison with the mtDNA from the other cytoplasmic male steriles, cms-S and cms-C, revealed yet other kinds of genomic alteration. However, some bands specific to N1 or N2 can also be found in either cms-C or cms-S.

As postulated by Small et al. (EMBO J. 6:865, 1987) some restriction fragments are found in variable stoichiometry in different cytoplasms. This would imply that a common pool of substoichiometric molecules that are the product of infrequent recombination events is maintained at a very low level in all of the maize cytoplasms. A mechanism of differential amplification would determine which one of those substoichiometric molecules will be amplified in the various cytoplasms.

SAN JOSE, CALIFORNIA
Sungene Technologies Corporation

Identification of RFLP markers for the *Ht1* gene by comparison of inbreds and their *Ht1*-conversions

--Manju Gupta, F. Dale Park, Bradley Hoo, Matthew Frome, David Zaitlin, Yan-San Chyi, William D. Banks and Philip Filner

Fifteen RFLP (restriction fragment length polymorphism) probes which detect loci on chromosome 2L were used for comparing the occurrences of RFLPs in 8 inbred/*Ht1*-converted line pairs--A632, A635, B37, B73, N28, Mo17, A619 and Oh43. Five restriction enzymes were used: *Eco*RI, *Eco*RV, *Hin*dIII, *Bgl*II and *Bam*HI. Correlations between 3 RFLP markers and the *Ht1* gene for resistance to *Bipolaris turcicum* (Pass.) Shoemaker were detected. When DNA was digested with the restriction enzyme *Eco*RI, RFLPs were detected with the probe SGCR506 in 8 out of 8 cases. The RFLP patterns in the *Ht1*-converted lines fell into 2 classes, which is consistent with the use of 2 *Ht1* gene donors--Ladyfinger popcorn and GE440. Thus there was a 100% correlation between presence of *Ht1* and presence of an RFLP detected by SGCR506. With *Eco*RV and probe SGCR507, RFLPs were detected in 6 out of 8 cases. With *Bgl*II and probe SGCR25, RFLPs were detected in 6 out of 8 cases. The order of the loci detected by these RFLP probes is SGCR507 - SGCR506 - SGCR25, proceeding away from the centromere. The map distance between SGCR507 and SGCR25 is approximately 14cM, which indicates that a surprisingly large and distinct chromosomal segment, not merely a locus, has been transferred when *Ht1* has been introgressed. From these results, the *Ht1* gene is probably within the 14cM fragment, and in the vicinity of the RFLP locus detected by SGCR506. Data from segregating populations are being analyzed to determine the genetic linkages of these loci.

Identification of RFLP markers for the *rhm* gene

--Yan-San Chyi, Manju Gupta, Matthew J. Frome, F. Dale Park, Bradley Hoo, David Zaitlin, William D. Banks, James Perkins and Philip Filner

Correlations between 2 RFLP (restriction fragment length polymorphism) markers on chromosome 6 and the *rhm* gene for resistance to *Bipolaris maydis* (Nisik.) Shoemaker, race O were detected by comparing the occurrences of RFLPs in inbred and *rhm*-converted lines of B73, H95, Mo17, N28 and Va35. The marker SGCR148 detects RFLP's between inbred and *rhm*-converted line pairs in 5 out of 5 cases. The restriction fragment detected in all 5 *rhm* lines is the same size and was not observed in 45 other inbred lines lacking *rhm*. SGCR65 detects RFLPs in 2 out of 5 inbred vs. converted line pairs. Genetic linkage between *rhm* and the loci detected by SGCR148 and SGCR65 was established by analysis of an F2 population segregating for these loci. The order of the loci is *rhm* - SGCR148 - SGCR65. However, in contrast to the close linkage expected from the data on converted lines, the calculated map distances from the segregating population are 34.1cM for *rhm*-SGCR148 and 39.9cM for *rhm*-SGCR65. These map distances indicate that a chromosomal segment greater than 34cM was retained from the original *rhm* source line

during at least 6 backcrosses in each of 5 separate backcross programs designed to introgress *rhm*, despite the fact that no barrier to recombination between *rhm* and the locus detected by SGCR148 exists. We infer the existence of a second gene about 34-40cM from *rhm* which enhances expression of *rhm* sufficiently to be selected for consistently by breeders when introgressing *rhm*.

STANTON, MINNESOTA
The New Northrup King

RFLP analysis of a breeding population selected for yield

--Diana Beckman, Christine Bredenkamp, Ann Larkin, Ann Majerus, Doug Mead, Carol Wangen and Edward Weck

A number of researchers have attempted to establish statistical correlations with yield. It has been found that isozyme diversity among inbreds is correlated with hybrid vigor (Frei et al., Crop Science 26:37, 1986). More recently analysis of 1700 plants from a wide cross with 17 isozymes showed these isozymes to be effective in identifying and locating Quantitative Trait Loci (QTL) affecting grain yield and 24 component traits of yield (Stuber et al., Crop Science 27:639, 1987).

At the beginning of our search for QTLs we were curious to know if it would be possible to use a small population for QTL analysis. We identified a yield selected population "tail" for analysis which consisted of 17 S3 lines derived by single seed descent from the cross of E x F backcrossed once to E; E(2) x F S3. In addition, three other yield selected populations were selected for multiple population correlations with RFLP markers: G with 21 lines, H with 20 lines, and I with 34 lines.

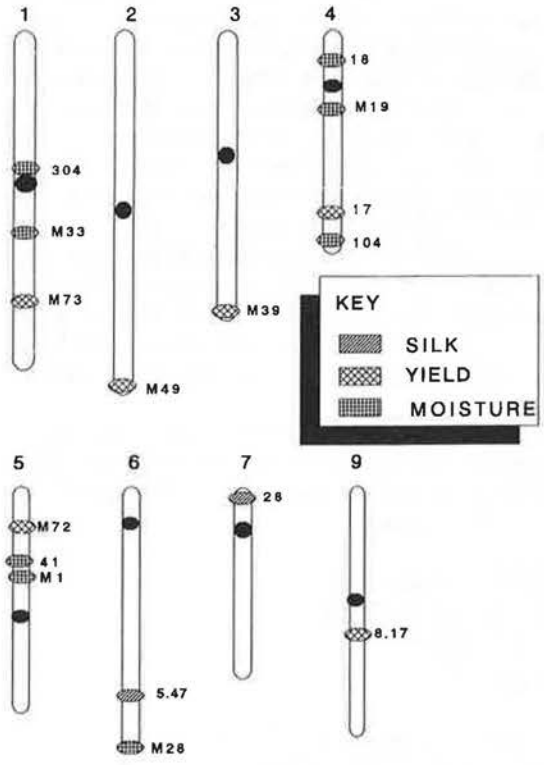


Figure 1. Agronomic trait correlations with p<0.01; E(2) X F S3, 228 loci, 207 probes.

The 17 lines derived from the E(2) x F cross were hybridized with 207 probes. Correlations (p<0.01) with yield, percent harvest moisture, and days to 50% silk emergence are shown in Figure 1. Correlating markers are randomly distributed along 8 of the ten chromosomes of maize (there were no correlations on chromosomes 8 or 10).

In order to determine if these correlations were valid in more than one population, 38 probes showing significant correlations at the p<0.05 level in E(2) x F were hybridized to the three additional populations. Table 1 shows the four markers that correlated with agronomic traits in at least two populations (p<0.05). One marker correlated with yield in 3 populations (p<0.05). No markers correlated at a higher statistical stringency (p<0.01) in more than one population.

Table 1. Number and chromosomal location of probes correlating in multiple populations.

	Trait			
Population	Yield	Moisture	Silk Time	p value
G x Tester 1	0	0	0	<0.05
H x Tester 2	1, 8L	1, 6L	0	<0.05
H x Tester 3	0	0	0	<0.05
I x Tester 2	0	1, 4L	1, 9L	<0.05
I x Tester 3	1, 8L	0	0	<0.05

The dearth of multiple population correlations suggests that generalizing about yield promoting regions will be difficult. A more fundamental understanding of heterosis at the physiological level will be required to allow identification of important yield influencing chromosomal regions.

TUCSON, ARIZONA
University of Arizona

sh4 is closely linked to *pr*

--Craig Echt

The cross *sh4 +/+ pr* X *sh4 +/sh4 pr* was made with one pollen parent onto three ears in the double heterozygote family. The frequencies of the four phenotypic classes of the resulting progeny are listed below.

	sh4 Pr	*Sh4 pr*	*Sh4 Pr*	*sh4 pr*
1	157	90	91	1
2	140	79	64	4
3	136	64	86	1
total	433	233	241	6
% total	47.4	25.5	26.4	0.66

The only crossover products that can be scored directly from this type of cross are the double recessives generated in the female parent. (The Chi squared value of the *Sh Pr* phenotypic class is 1.48 and does not significantly differ from expected.) An estimate of recombination between the *sh4* and *pr* loci of 1.3% is obtained if one assumes an equal number of the reciprocal crossover products (+ +).

Attempts to obtain a recombination value from an F2 or test cross were confounded by the very extreme shrunken phenotype of *sh4* in certain lines and in Tucson environments. In severely shriveled kernels red aleurones are indistinguishable from purple ones. In the present cross the *sh4 pr* kernels were easy enough to score but, nonetheless, the double mutant class may be slightly underrepresented due to misclassification of the pr phenotype.

If anyone has an *hcf18, A, C, R* or *hcf18 pr* stock from which they could spare some seed I would like to use it in a three point cross to try to place *sh4* and *hcf18* on the map with respect to *pr*.

TUCSON, ARIZONA
University of Arizona
COLUMBIA, MISSOURI
University of Missouri

Les1 expression alters pathogen development

--Craig Echt and Art Trese

The lesion mutations of maize were first described as "disease lesion mimic mutations" (Neuffer and Calvert, J. Hered. 66:265) because of the striking similarity of their phenotype to pathogen-induced disease lesions on leaves. This morphological similarity has prompted speculation about the role of the lesion mutant loci in plant disease but evidence for a functional connection between lesion mutant and disease expression has been lacking.

We have found that when certain fungal pathogens infect lines segregating *Les1* the *Les1* allele enhances hyphal growth immediately following infection. *Les1* does not, however, alter the basic compatibility (susceptibility) or incompatibility (resistance) interactions determined by the major resistance loci. If *Les1* is a mutation in a locus that normally functions in limiting pathogen growth early in the infection process then this function appears to act separately from the pathogen growth-limiting functions of the major resistance loci.

Inoculation of sibling seedlings from several *Les1* lines with *Bipolaris maydis* race O, *Cochliobolus heterostrophus* race 1 or 2, or *Exserohilum turcicum* race 1 or 2 showed that the *Les1* allele could change an incompatible interaction, giving small chlorotic or necrotic flecks, into a pseudocompatible interaction, giving moderate size necrotic lesions. The necrotic pathogen-induced lesions that formed on +/*Les1* plants appeared within 16h following inoculation and enlarged until about 24h following inoculation. For those line-isolate combinations that resulted in a fully compatible interaction on the +/+ sibs the interaction on the +/*Les1* sibs was identical after a period of time (several days to two weeks depending on the pathogen). The reaction types of the various line-isolate combinations tested are given in Table 1. Note that the increased growth of *C. heterostrophus* race 1 observed on *Les1* plants occurred even in the presence of a defined dominant resistance factor, *Hm1* (present in the *Pr1* line) but that there was no difference between *Les1* and normal plants in the compatible combination (with the *Pr* line). The same appears to be true for *E. turcicum* race 2 although the presence of the *Ht2* allele in the *Pr1* inbred has not been confirmed.

Table 1.

Effect of *Les1* on Reaction Types of Maize-Pathogen Interactions

Inbred genotype	Mo20W + *Les1*		W23 + *Les1*		Pr, *hm* + *Les1*		Pr1, *Hm* + *Les1*		N6J/W23 + *Les1*	
Fungal isolate										
C. heterostr. R1	1,2	2,3	1	3	3,4	3,4	1,2	3	N.D.	
C. heterostr. R2	1,2	2,3	1	3	1,2	3	1,2	3	N.D.	
B. maydis RO	3	3	1,2	3	N.D.		N.D.		N.D.	
E. turcicum R1	N.D.		N.D.		N.D.		1*	3*	N.D.	
E. turcicum R2	N.D.		N.D.		N.D.		1	3	1*	3*

Reaction types were scored five days after inoculation. Reaction types: 0=no reaction, 1=chlorotic fleck, 2=necrotic fleck (< 1mm), 3=necrotic lesion (1-4mm), 4=extensive necrosis, N.D.= not determined. Where two scores are given both reactions were observed on the same leaf.

All *Les1* vs normal comparisons were made between sibling progeny from ears segregating +/*Les1* : +/+ in a 1:1 ratio. Ten to 20 plants of each genotype were scored for each interaction. Inoculations were made with conidial suspensions of 25,000/ml either dropped into the whorl or sprayed in a mist in a humidity chamber.

*These reactions developed into long spreading necrotic lesions after about 2 weeks.

Two observations argue that the larger lesions on infected *Les1* plants are not simply due to wound induction of *Les1* expression. First, the enhanced disease reactions can occur on leaf sections which are too young to form genetic lesions and which are not yet responsive to wound induction. The second observation is the fluorescence staining of the fungal hyphal structures at the lesion sites showed that the larger disease lesions on +/*Les1* plants infected with *C. heterostrophus* race 1 were correlated with increased hyphal growth. For all disease lesions examined the hyphal growth extended to or just beyond the borders of the necrotic areas. The histology of lesions resulting from other pathogens is currently being examined.

Preliminary experiments with environmental conditions indicate that when conditions inhibitory to normal *Les1* lesion formation (30 C or darkness) are employed immediately following inoculation then the differences between +/+ and +/*Les1* sibs are not expressed. High temperatures prevent necrotic disease lesion formation while darkness results in increased pathogen growth and lesion size.

The necrotic lesion reactions observed in the *Les1-E. turcicum* combinations is a novel reaction type for *E. turcicum* infections on maize. All previously reported maize-*E. turcicum* interactions, whether ultimately compatible or incompatible, are initially expressed as chlorotic flecks within the first two days after inoculation (Hilu and Hooker, Phytopath. 55:189). Susceptible plants then give rise to long, spreading necrotic lesions about two weeks after inoculation.

Initial whorl inoculations with *Colletotrichum graminicola* did not elicit larger disease lesions on *Les1* plants. The effect of *Les1* on pathogen development may be specific to certain groups of pathogens.

TUCSON, ARIZONA
University of Arizona
SLATER, IOWA
Garst Seed Co.

Transmission and cytology of TB-2Sa

--Craig Echt and Ming-Tang Chang

TB-2Sa is a B-A translocation for part of the short arm of chromosome 2 created by gamma irradiation of mi-

crosporocytes containing multiple B chromosomes. It carries the dominant endosperm and scutellum color allele *B-Peru* and has its translocation breakpoint on 2S between the *wt* and *B* loci (Echt, MNL 61:94).

Early generations demonstrated low transmissibility of TB-2Sa, initially throwing some doubt on the actual nature of the translocation. We have been successful at obtaining higher transmission rates following three cycles of selection (Table 1). Cytological, as well as genetical, observations indicate TB-2Sa is indeed a simple B-A translocation involving approximately half of the 2S arm translocated to the distal heterochromatic region of a B chromosome.

TABLE 1. Color classes for the TB-2Sa pedigree from outcrosses of hyperploids onto a recessive tester; *r-g* X TB-2Sa(*B-p*/*B-p*)*b,r-g*. The ear with the highest transmission rate (bold type) from each generation was used for the next generations' crosses.

generation	TB-A family-plant #	endosperm colored	endosperm colorless	hyperploids
1	40:1072	178	45	**4 (est.3%)***
2	41:2396-1	125	179	**37 (12%)**
3	87:887-1	243	204	**28 (6%)**
	-2	166	283	21 (4%)
	-3	270	195	9 (2%)
	-4	256	212	12 (3%)
4	88:4245-1	199	149	**81 (19%)**
	-1	323	233	67 (11%)
	-11	136	114	14 (5%)
	-3	173	489	10 (1%)
	-6	102	252	25 (7%)
	-12	171	299	29 (6%)
	-12	245	333	57 (9%)

* The first outcross was to an *R/r* tester so the 3% is an estimate.

Actual nondisjunction rates (% hyperploid + % hypoploid) were not calculated because classifying the hypoploid class, kernels with colorless scutellum and colored endosperm, was difficult in some crosses. There appears to be genetic variability among our testers that affects the intensity of *B-Peru* expression in the scutellum and opacity of the pericarp covering the scutellum. Because of the greater contrast of a colored scutellum on a colorless endosperm we feel confident only about the classification of the hyperploid class in the crosses we have made so far. Hypoploids can of course also be scored in the field or in crosses with a distal 2S recessive marker but these data were not obtained.

We suspect that the presence of extra B chromosomes is responsible for the low transmission rates of this particular B-A translocation and that what we are selecting is for fewer B chromosomes in each generation. At least one individual in the fourth generation was carrying only one to two B chromosomes from the original multi-B parent (Table 2). A TB-2Sa stock expressing reasonable nondisjunction rates and devoid of B chromosomes should be available after next season. This stock will be sent to the Coop.

TABLE 2. Cytological observations of microsporocytes from one individual (not used in crosses) in the 88:4245 hyperploid TB-2Sa family.

Observed pairing	# of cells	Proposed chromosomal constitution
11 II (bivalents)	14	$9\,II + A^{B}A + B^{A}B^{A}$
11 II + 1 I	22	$9\,II + A^{B}A + B^{A}B^{A} + B$
11 II + 2 I	4	$9\,II + A^{B}A + B^{A}B^{A} + B + B$
12 II + 1 I	1	$9\,II + B^{A}B^{A} + A^{B}A\,B$
10 II + 1 III	6	$9\,II + B^{A}\cdot B^{A} + A^{B}A\,B$
9 II + 1 IV	8	$9\,II + A^{B}A\cdot B^{A}B^{A}$
9 II + 1 IV + 1 I	8	$9\,II + A^{B}A\,B^{A}\cdot B^{A} + B$

URBANA, ILLINOIS
University of Illinois

Isolation of cDNA and genomic clones corresponding to maize *Glb1* genes

--Faith C. Belanger, Nancy M. Houmard, Lee Stromberg and Alan L. Kriz

Maize embryos contain large amounts of saline-soluble, water-insoluble proteins called globulins. The most abundant globulin component, GLB1 (formerly PROT), is encoded by a single gene for which several protein size alleles and a null allele have been described (D. Schwartz, MGG 174:233; A.L. Kriz and D. Schwartz, Plant Physiol. 82:1069).

To further characterize the *Glb1* gene, we have isolated cDNA and genomic clones corresponding to this locus. An embryo-specific cDNA library was constructed in the expression vector LambdaZAP (Stratagene). The RNA used for cDNA synthesis was polyA+ RNA from 27 DAP embryos of the maize inbred line VA26, which is homozygous for the *Glb1-S* allele. The primary library contained 500,000 clones from an estimated 0.1ug of cDNA. Screening of 250,000 clones with antiserum specific for GLB1 yielded 10 positive clones with insert sizes ranging from 700bp to 1800bp. The 1800bp clone was chosen for further characterization and was subjected to nucleotide sequence analysis. Because of the high (68%) G+C content it was necessary to use the dGTP analogs 7-deaza dGTP or dITP in the dideoxy termination reactions to obtain unambiguous sequence data. The amino acid sequence deduced from the cDNA sequence is in good agreement with the amino acid composition determined for GLB1-S. A 300bp restriction fragment from the 5' end of the 1800bp clone was used as a probe to rescreen the cDNA library for a full-length clone. The size of the longest clone (2200bp) obtained from this secondary screen corresponds to the size of *Glb1*-specific transcripts detected by Northern blot analysis.

The 1800bp clone was used as a probe in Southern blot analysis of maize DNA and found to hybridize with an *Eco*R1 fragment of 3.4kb in plants carrying either the *L* (Large), *S* (Small), or *null Glb1* allele. We have obtained genomic clones for the *S* and *null* alleles by isolating sized *Eco*R1 fragments from an agarose gel and cloning into LambdaZAP. A genomic clone corresponding to the *L* allele was isolated by screening an EMBL3 phage library constructed from DNA of the inbred line W64A (this library was kindly provided by J.C. Wallace, Purdue University). Efforts are underway to determine the nucleotide sequence differences between the null and functional alleles.

The cDNA clones have also been used as probes to investigate *Glb1* transcript levels in various tissues by Northern blot analysis. Embryos homozygous for the null allele produce very low levels of *Glb1*-specific transcripts but these are of a different size than those encoded by the *L* or *S* alleles. *Glb1* transcripts have also been detected in developing W64A endosperm, at much lower levels than in the embryo, but not in unfertilized ears, immature tassels, or the leaves of 7-day-old seedlings.

Globulin gene expression during precocious germination in maize viviparous mutants

--Alan L. Kriz, Jana S. Holt and Renato Paiva

The availability of viviparous mutants in maize provides an excellent opportunity for the study of molecular mechanisms involved in the control of embryo maturation. Embryos homozygous for the recessive allele at any of the six *vp* loci apparently fail to undergo normal maturation but rather germinate precociously on the ear. As a first step in determining if gene expression in *vp* embryos is switched from a pattern of development/maturation to one of germination, we are investigating the expression of genes encoding seed globulins during precocious and normal germination. These genes are ideal markers for this type of study: both the Mr 63,000 product of the *Glb1* gene and the Mr 45,000 product of the *Glb2* gene accumulate to high levels during embryo development and are rapidly degraded during the early stages of seed germination. For these experiments, RNAs and proteins were extracted from developmentally staged (23 to 31 DAP) normal and precociously germinating sib embryos homozygous for the different *vp* alleles. Northern blot and immunoblot analyses were performed by using as probe either a *Glb1*-specific cDNA clone (see above note by Belanger et al.) or globulin-specific antisera, respectively.

The results of these experiments are presented in Table 1. Each of the *vp* mutants exhibits characteristics of normal

Table 1. Levels of *Glb* gene products in precociously germinating *vp/vp* embryos.

	Age	*Glb1*		*Glb2*
	(DAP)	Protein*	Transcript*	Protein
vp1	28	absent	absent	absent
vp2	28	reduced	normal	absent
vp5	30	reduced	reduced	absent
vp7	23	absent	reduced	absent
vp8	31	normal	normal	absent
vp9	23	reduced	normal	absent

*Amount relative to normal sib embryos

germinating embryos in that the *Glb2* protein is absent. With respect to *Glb1* expression, however, each of the mutants has a distinctive phenotype. For example, *vp7* exhibits the pattern of normally germinating kernels in that *Glb1* transcript levels are drastically reduced and the protein is absent; on the other hand, *vp2* and *vp9* both exhibit characteristics of normal embryo development (high levels of *Glb1* transcript) and normal germination (reduced levels, presumably through degradation, of *Glb1* protein).

These studies will be extended this summer to characterize globulin gene expression in *vp* mutants prior to the onset of precocious germination. This work may provide some insight as to the molecular basis of embryo maturation in maize.

Comparison of globulin gene expression in normal, opaque-2, and floury-2 kernels

--Jayne L. Puckett, Mark Wallace and Alan L. Kriz

Globulins are the most abundant proteins in maize embryos. Previous studies have demonstrated that globulins are also present in the endosperm and that the amounts of these proteins are increased in opaque-2 endosperm relative to normal endosperm (Dierks-Ventling and Ventling, FEBS Lett. 144:167). To determine if similar changes in globulin constitution occur in the embryo, and whether such changes are a result of zein deficiency, we investigated globulin gene expression in kernels of various genotypes. Specifically, we compared normal kernels with kernels homozygous for the opaque-2 and floury-2 mutations in each of W64A, Oh43, and W22 backgrounds. Total embryo and endosperm proteins from mature kernels were analyzed by SDS-PAGE and by immunoblot analysis in which antiserum specific to the *Glb1* proteins was used as probe. The amount of *Glb1*-specific proteins was two to four times higher in both the embryo and endosperm of opaque-2 kernels, relative to levels in the respective normal tissue, in each of the three backgrounds. Northern blot analysis of embryo RNA indicates the amount of *Glb1* transcript in opaque-2 embryos is at least twice that in normal embryos. No increases in amount of *Glb1* proteins were observed in floury-2 kernels. In addition, no changes in the level of the Mr 45,000 *Glb2* protein were found in any of the genotypes examined. These data suggest the following: 1) of genes encoding globulins, *Glb1* expression is specifically increased in opaque-2 kernels and this increase occurs at the transcriptional level; and 2) this increase does not appear to be due to decreased zein synthesis since the zein-deficient floury-2 kernels contain normal amounts of *Glb1* proteins. Since the *Glb1* proteins contain a significant amount of lysine (5.6 mole percent), the elevated lysine content in opaque-2 kernels may be due in part to increased amounts of these proteins.

Variation in the vigor of plants regenerated from a single petri dish of regenerable callus

--David Duncan and Jack Widholm

For studies of somaclonal variation using type I regenerable maize callus, it is useful to obtain from the callus as many regenerated plants as possible. This goal is desirable primarily because not all regenerated plants survive or set seed and a large number of plants are needed to have enough plant material to conduct a valid experiment. Not all regenerated plantlets, however, develop at the same rate. Consequently, regenerated plantlets may be harvested from a given Petri dish of regeneration medium over about a 30d period. Poor plant survival and a lack of synchrony to plant regeneration has prompted studies aimed at increasing the efficiency of plant regeneration from maize callus cultures.

Callus from the self-pollinated inbred Mo17 was initiated and maintained as described by Duncan, Williams, Zehr and Widholm (Planta 165:322). After maintenance for 205d, plants were regenerated from the callus as described by Duncan and Widholm (Plant Cell Reports 7:452). One half of the callus was placed in a 12h light/dark cycle and the other half was placed in continuous light. Plants were removed from regeneration medium at three 10-d intervals.

Six hundred five shoots were removed from regeneration medium and placed in individual culture tubes and from these, 326 plants were placed in the greenhouse. Many of the shoots in tubes that were not placed in the greenhouse developed so late and so slowly that it was futile to attempt to move them to the field. Plants were taken to the field after approximately two weeks in the greenhouse. Planting dates were June 10th, June 19th, and July 17th (1987).

Table 1. The number of regenerated plants moved to the field and pollinated for each planting date.

planting date	# of regenerants planted	# of regenerants pollinated
June 10th	59	54
June 19th	73	69
July 17th	194	100

Of the 336 plants taken to the greenhouse, 326 were transplanted to the field and 223 of these plants were pollinated (Table 1). Of the 103 plants not pollinated, 12.6% were from the first and second planting and 87.4% were from the third planting. More than 50% of plants from the third planting were pollinated and viable seed was obtained and used the following year. However, many of the plants in the third planting matured too late in the season to be pollinated. Whether this late maturity was due to somaclonal variation (such as has been previously documented; Zehr, Williams, Duncan and Widholm, 1987) or environmental stress could not be determined.

Forty-two plants were found with tassel ears and all of these were from the third planting. No tassel ears were found on control plants derived from seed that had been germinated in the greenhouse and transplanted to the field at the same time.

Three plants from the second planting and five plants from the third planting produced R1 seed with a shrunken phenotype but this was not found in seed from plants of the first planting. Germination of R1 seed decreased with planting date (third planting, 60%; second planting, 69%; first planting, 75%). This lowered germination may be the result of a shorter grain fill period for the later plantings.

The 12h light treatment delayed plant development by about 10d, which, because of the limited length of the growing season, resulted in fewer plants being moved to the field and fewer pollinations of these plants being made (Table 2).

Table 2. The effect of light on plant regeneration from regenerable maize callus.

treatment	# of plants regenerated	# of plants in field	# of plants pollinated
24 h light	274	171	138
12 h light	331	155	85

For instance, no plants from the 12h treatment were included in the first planting. Also, of the 42 plants with tassel ears 71.0% were from the 12h light treatment. These results suggest that the 12h light/dark cycle may be detrimental to effective regeneration of normal plants from maize callus cultures.

There are obvious environmental effects resulting from the different planting dates, for instance, small plants and poor grain fill seen in material planted later. These effects may also carry over to the progeny, for instance, poor germination and seedling vigor. However, the regenerated plants that were planted later also appeared more abnormal, as indicated by the prevalence of tassel ears in the last planting.

The last planted regenerants were so planted because they either differentiated later or slower, or they grew slower. To date we do not know if this variation in shoot development resulted from segregation in time of somaclonal variants (i.e., the most normal plants developing the fastest) or from changes with time in culture condition that adversely affect normal shoot development. We can conclude, however, that harvesting all of the regenerated shoots from a Petri dish of regeneration medium may not be the most efficient means of plant regeneration. Instead, using a large quantity of callus and only the first few regenerated plants found on regeneration medium may be the most efficient method of producing the most normal plant material.

Allelism tests of somaclonal variants

--Brent Zehr, David Duncan and Jack Widholm

As previously reported (Can. J. Bot. 65:491-499), we have regenerated plants from callus cultures of several inbreds including an experimental inbred line (EXL-1) and examined their progeny (R1 generation) for somaclonal variation. From 75 R1 EXL-1 families, one white cob, 18 male sterile, three dwarf, one brown midrib, four narrow leaf, one dark green, two "miniature", two albino, one viviparous, one indeterminant growth and five striated leaf somaclonal (SC) mutants were found. These are all nuclear, single gene recessive mutations.

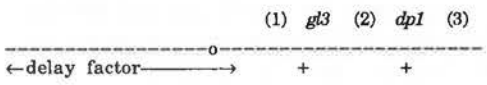
Since similar mutations have been previously described, we carried out allelism tests with known genetic stocks from the Maize Genetics Cooperation Stock Center and the viable R1 families that proved to be homozygous in the R2 generation or R1 families maintainable only in a heterozygous state. In summary, the SC viviparous mutant is allelic with *vp1*, the SC crinkled leaf mutant is allelic with *cr1*, the SC indeterminant growth mutant is allelic with *id1*, the SC white cob mutant is allelic with *P1*, and one of the SC dwarf mutants is allelic with *br1*. Another SC dwarf mutant was found not to be allelic with *br1*, *br2*, *na1*, or *na2*. Also, the SC brown midrib mutant does not appear to be allelic with any of the known brown midrib alleles (*bm1*, *bm2*, *bm3*, *bm4*). Additional work is being conducted to further characterize the latter two mutants as well as other mutants as yet not analyzed.

While the mechanism causing somaclonal variation is unknown and may be due to several phenomena, any change which would inactivate a gene such as an insertion, deletion, base change or rearrangement could lead to the recessive phenotypes described here. Thus, although the phenotypic mutations generated through somaclonal variation may not be unique, the molecular basis for these mutations may be unique and useful for studies of gene expression and regulation.

4L mapping and Mo17

--D. M. Steffensen

Using *dp1* and *gl3* in coupling, selections of recombinants were made of crossovers with chromosome 4 of Mo17:



All putative crossover types in this backcross were selfed to obtain marker genes coupled to the Mo17 strand of chromosome 4 as indicated above in regions 1, 2 and 3. The reason for doing these isolations is threefold: 1) To locate the Mo17 "delay factor" involved in hybrid vigor in the F1. 2) Since chromosome 4 of Mo17 is knobless and most corn-

belt strains have a knob on 4L, to map the 4L knob genetically. 3) Locate zein loci on the map using Mo17 null genotype and the existing information from Curtis Wilson and others. We will use Oh43 or Oh45 in further recombinant crosses because it has several zein genes and a 4L knob.

Reid Yellow Dent--knob genetics, maturity and yield

--D. M. Steffensen

Open pollinated Reid Yellow Dent has been maintained in isolation at Illinois by D. E. Alexander as have several other classic open pollinated varieties. According to Wallace and Brown (1956) Reid Yellow Dent (RYD) had its origin from the late-maturing Hopkins (a late maturing gourdseed from Virginia) crossed to "Little Yellow," an early flint grown by the Indians in the northeastern U.S. Subsequent selection of Reid contributed significantly to present day dent corn in the Midwest.

For the sake of simplicity let us assume that the RYD (O.P.) population from D. E. Alexander is polymorphic for all of the possible combinations of 4L and 7L knobs. We grew several hundred of these plants and selfed several every day from this "random" population over a thirty day period. The rest were left to open pollinate. However, *every plant was scored for the first day of pollen shedding.* When these data were plotted, four sharp discontinuous peaks were observed. This is consistent with our cytological observations that RYD has two knobs on 4L and 7L. Extensive analysis of knob genetics of inbred lines of known constitutions such as crosses with B37, B14, B73, Mo17, C103 and crosses to several knobless varieties (Chughtai, 1988, U. of I. thesis, and Steffensen, unpublished) has shown that the earliest plants to flower were knobless or heterozygotes. The next later group to flower were homozygous for one knob pair and knobless or heterozygous for the others and so on to later peaks with 2, 3 and 4 knobs homozygous.

This RYD population was studied to answer three questions: 1) Does homozygosity for knobs (0,1,2 and maybe 3) affect maturity? 2) Does the knob and its bracketing genes have an effect on hybrid vigor when heterozygous? 3) Are successful open pollinated lines maintained by selecting for balanced polymorphism of the knob-gene and knobless gene blocks?

The analysis, so far, indicates that the answers to these three questions will be unambiguous.

First of all, the largest ears are found *only* in the families with *four distinct maturity peaks*. Furthermore, the largest ears are only on plants that flower in the *earliest* peak, indicative of their being heterozygotes for all knob-loci regions.

Most of these and other families have been tested in crosses to known-knob inbred lines (B37, B14, B73, Mo17 and Wilbur's Knobless Flint) for analysis of maturity this spring. Additional crossing in the greenhouse and Molokai between selfed F3 and F4 RYD families will nail down our hybrid vigor conclusions in summer grow-outs.

Nuclear control over molecular characteristics of cms-S male-fertile cytoplasmic revertants

--G. Zabala, S. Gabay-Laughnan and J. R. Laughnan

The first studies of mitochondrial DNA (mtDNA) of male-fertile revertants from S-type male-sterile (cms-S) plants suggested the involvement of two episome-like double-stranded linear DNA molecules, S1 and S2 (Science 209:1021-1023, 1980; Molec. Appl. Genet. 2:161-171, 1983). Upon reversion, these free S-episomes disappear from the mitochondria and rearrangements involving S2 or S1 homologous sequences take place in the main mitochondrial genome. These results were consistent in revertants of cms-S plants with an M825 or 38-11 nuclear background. In a WF9 nuclear background however, cms-S revertants maintain the free S-episomes. Their homologous sequences in the main mitochondrial genome do not undergo rearrangement (Theor. Appl. Genet. 75:659-667,1988) but the σ-R and ψ-R integrated sequences that are targets for recombination with the free S-episomes are missing (Theor. Appl. Genet. 76:609-618, 1988). These results indicate that the nucleus has its influence on mtDNA organization either during or prior to reversion to fertility.

The influence of the nuclear background on mtDNA has been demonstrated further by the conversion of cms-S lines having nuclear backgrounds WF9 and 38-11 to the nuclear background of M825 by backcrossing with M825 as the recurrent male parent. In revertants recovered subsequent to these nuclear conversions, the pattern of reorganization of the mitochondrial genome follows the pattern unique to revertants with the M825 nuclear background. All revertants lose the free S1 and S2 episomes and the S2 sequences integrated in the main mitochondrial genome suffer disruption of the left unique end (Escote, Ph. D. Thesis U. of Ill).

Nuclear action on mtDNA organization is not exclusive to the reversion event but is also observed in the absence of reversion. The sterile F1 progeny from the cross RD-WF9 x M825 (RD is a subtype of S) have an mtDNA organization different from that of the original RD-WF9 female parent. Differences are detected as the loss, starting as early as the F1 generation, of some S1, S2 and cytochrome oxidase subunit I (COXI) DNA fragments present in the parental RD-WF9. In the progeny of the first backcross of RD-WF9 with M825, additional RD-WF9 parental bands disappear, after which the Southern blot pattern is stable through the fourth backcross (Escote et al. MNL 60:127).

To determine whether the change in mtDNA organization already observed in the sterile F1 hybrids has any effect on the mtDNA rearrangements which occur upon reversion, we have analyzed the rearrangements in newly arisen cytoplasmic revertants of an F1 generation resulting from a cross between RD-WF9 and M825. The F1 hybrid plants have the mitochondria of the female parent, cms-RD, and a hybrid nucleus: 50% WF9 and 50% M825.

In Table 1 we have listed the eleven groups of cytoplasmic revertants (cyto-revs) used for this study. Five of them (I,II,IV,VIII,X) were recovered from plants carrying hybrid nuclei of approximately 50% WF9 and 50% M825 and six groups (III,V,VI,VII,IX and XI) derived from plants with differing WF9:M825 nuclear dosages.

When undigested mtDNAs were analyzed (Table 1), the following patterns emerged: cyto-revs from groups I,II,VI,VII and X maintained the S1 and S2 episomes and those from groups III,IV,V,VIII,IX and XI were missing both episomes. Retention of episomes upon reversion is characteristic of reversion under WF9 nuclear control. Loss of episomes upon reversion is characteristic of reversion under M825 nuclear control.

TABLE I: Patterns of reversion in cytoplasmic revertants with hybrid nuclear backgrounds. Superscripts indicate number of times the seed parent has been crossed to the line carrying the superscript number. V50%: variable anthers, 50% pollen abortion.

GROUP	CROSS	CYTO-REV (# of cases)	Nuclear background WF9	Nuclear background M825	S1 & S2 Episomes
I	RD-WF9/M825	4	50%	50%	+
II	RD-WF9/M825(V50%)xM825	4	50%	50%	+
III	RD-WF9/M825^2	2	12.5%	87.5%	-
IV	RD-WF9/M825^{3}xWF9	9	56%	44%	-
V	RD-WF9/M825^{3}xWF9^2	1	78%	22%	-
VI	RD-WF9/M825^{3}xWF9^3	4	89%	11%	+
VII	RD-WF9/M825^{3}xWF9^3	2	97%	3%	+
VIII	RD-WF9/M825^{3}xWF9	1	51.6%	48.4%	-
IX	RD-WF9/M825^{3}xWF9^2	1	76%	24%	-
X	RD-WF9/M825^{10}xWF9	5	50%	50%	+
XI	RD-WF9/M825^{10}xWF9^3	3	87.5%	12.5%	-

These results, although puzzling at first, reveal some aspects of the nuclear effect on plasmid retention. Although the nuclear dosage at the time of reversion in groups I, II, IV, VIII and X was about 50% WF9 and 50% M825, the genetic crosses performed to obtain the above mentioned revertants differed in each group. Revertants of group I and II arose from a cross of an RD-WF9 sterile plant with an M825 maintainer line. However, revertants of groups IV, VIII and X were obtained after crossing RD-WF9 sterile plants to the M825 maintainer line three (group IV), five (group VIII) and ten (group X) times, respectively, and then back to UF9 once.

Mitochondrial DNA of group I, II, III, IV, V, VI, VIII and X revertants has been subjected to Southern hybridization analyses using various mtDNA probes (Table 2). The patterns of mtDNA rearrangements in revertants of group I,II and X (50% WF9-50% M825) are equivalent to those observed by Escote et al. (Theor. Appl. Genet. 75:659-667, 1988) for cyto-revs in a 100% WF9 nuclear background or those we have observed in cyto-revs of group VI (89% WF9 nuclear background). The patterns of mtDNA rearrangements in revertants of group IV (56% WF9-44% M825), V (78% WF9-22% M825) and VIII (51.6% WF9-48% M825) are equivalent to those obtained by Escote et al. for cyto-revs in a 100% M825 nuclear background or those we have observed in cyto-revs of group III (87.5% M825 nuclear background).

TABLE II: Southern hybridization analyses of cytoplasmic male fertile revertants. An "X" indicates that mtDNA from a particular group of revertants has been digested by the indicated enzyme, blotted, and probed with the clone at the head of the column.

GROUP I REVERTANTS:

		DNA Probe							
		pZmS4(S2)	pZmS21(S1)	COXI	ATP9	ATP6	ATPA	IR	IS1E5(R)
Restriction Enzyme	*XhoI*	X		X				X	
	HindIII	X		X		X			
	SalI	X							
	PstI	X	X						
	BamHI							X	X

GROUP IV REVERTANTS:

		DNA Probe							
		pZmS4(S2)	pZmS21(S1)	COXI	ATP9	ATP6	ATPA	IR	IS1E5(R)
Restriction Enzyme	*XhoI*	X		X	X		X	X	
	HindIII	X			X				
	SalI	X							
	PstI	X	X						
	BamHI								

GROUPS II, III, V, VI, VIII, X REVERTANTS:

		DNA Probe							
		pZmS4(S2)	pZmS21(S1)	COXI	ATP9	ATP6	ATPA	IR	IS1E5(R)
Restriction Enzyme	*XhoI*	X						X	
	HindIII			X		X			
	SalI	X							
	PstI	X							
	BamHI							X	X

Our results to date suggest that the nuclear effect on reversion is not a simple case of M825 dominance. At least one or possibly two backcrosses to M825 (see results of groups III and IV cyto-revs in Table 1) are required to change the WF9 reversion pattern to that of M825. Escote et al. (in preparation) have shown that, in the absence of reversion, mtDNA rearranges during the process of conversion of an RDWF9 sterile line to the M825 nuclear background. It is possible that a threshold level of these mtDNA rearrangements, mediated by exposure of the mitochondria to the M825 nucleus for two to three generations, needs to be attained before a reversion event can display the pattern of mtDNA reorganization characteristic of reversion in an M825 nuclear background. In other words, prior exposure of mitochondria to M825 nuclei alters their subsequent response to WF9 nuclei (see results of groups IV, IX and XI cyto-revs in Table 1). We have found an exception to this hypothesis in cyto-revs of group X. These revertants, obtained after crossing RD-WF9 sterile plants to the M825 maintainer line ten times and then back to WF9 once, retain the S1 and S2 episomes, a characteristic typical of reversion under WF9 nuclear control. Because of this nonconforming result obtained with group X cyto-revs, we have analyzed the hybridization patterns of several mtDNA probes to the sterile progenitor mtDNA from which revertants of group X were obtained (sterile RD-WF9/M825^{10}) and an unrelated source of the same genotype. Interestingly enough, the hybridization pattern of the group X sterile source differed from the unrelated sterile source with pZmS4 ATPase 9 and IS1E5(R) probes. The observed mtDNA rearrangements in the sterile progenitor of group X cyto-revs could possibly be responsible for the aberrant mtDNA organization of group X revertants. We plan to examine additional cyto-revs derived from sources with the same genetic constitution as the one from which the group X revertants were obtained but unrelated to it. If the mtDNA reorganization of these revertants follows the pattern of reversion in an M825 nuclear background, we plan to investigate further the abnormalities of the sterile source of group X revertants which obviously may have something to do with the retention of S-episomes and the other mtDNA rearrangements characteristic of reversion under the influence of WF9 nuclear background.

Analysis of group VI revertants which arose after crossing an RDWF9 sterile plant to an M825 line three times and then back to a WF9 line three times showed that these revertants retained the S episomes (characteristic behavior of reversion in a WF9 nuclear background). This contrasts with the results obtained with revertants of groups IV (RD-WF9/M825^{3}xWF9) and V (RD-WF9/M825^{3}xWF9^2), which lose the episomes, suggesting that effects such as the mtDNA rearrangements and possibly other changes imposed by exposure to the M825 nucleus are reversible.

Naturally occurring restorers of cms-S are located at various chromosomal sites in different inbred lines and appear to be transposable

J. R. Laughnan, S. Gabay-Laughnan and Janet M. Day

A number of inbred lines of maize carry a nuclear gene capable of restoring male fertility in plants carrying S male-sterile cytoplasm (cms-S). The cms-S restorer gene designated *Rf3* exhibits a gametophytic mode of restoration such that *Rf3 rf* heterozygotes produce 50 percent *Rf3*-carrying pollen grains that function normally in fertilization, and 50 percent *rf*-carrying grains that abort. Tests of allelism of the *Rf3* gene carried by ten inbred lines, including Ky21, CE1 and Tr, indicated that all are allelic (see Maize Breeding and Genetics, 427-446, 1978). Some of these tests involved crosses between two cms-S restoring inbred lines followed by testcrosses of the F1 plants with (S) *rf rf* male-sterile testers; with this protocol the absence of male-sterile plants in testcross progenies indicated either allelism or very close linkage of the cms-S restorers carried by the parental inbred lines. Another procedure involved crosses of a cms-S restoring inbred line as female parent with another restoring line carrying cms-S or normal cytoplasm; since the pollen of F1 plants was all normal, where 25 percent of grains of the shriveled type characteristic of abortion found in the cms-S system is expected if the restorers are nonallelic and segregate independently, it was concluded that the restorer genes carried by the F1 plants are either allelic or very closely linked. Another procedure, involving testcrosses of F1 plants heterozygous for an inbred-line restorer gene, Inversion 2a and the *B* allele, sufficed to assign the cms-S restorer(s) involved to chromosome 2, but was not useful for determination of allelism of inbred line restorers or for mapping their positions in chromosome 2.

In the final analysis the chromosomal location of *Rf3* in inbred lines CE1 and Tr was determined by testcrosses of cms-S *wx*-translocation heterozygotes, that were also heterozygous for an inbred line cms-S restorer, onto *wx wx* testers. For male parents carrying the CE1 restorer the testcross may be illustrated: (S) *rf rf wx wx* X (S) *Rf(CE1)* N *Wx/rf* T2-9d *wx*. Since pollen grains carrying the nonrestoring allele abort, only *Rf(CE1)* grains function so that the percentage of waxy kernels on these testcross ears provides a direct estimate of *Rf(CE1)-wx* recombination. We have extensive data on *Rf(CE1)-wx* recombination in T2-9d (breakpoint 2L 0.8) heterozygotes. Some of these are given in the second footnote of Table 1, where this recombination rate, with over 6000 kernels scored, is 8.3 percent. The cms-S restorer carried by inbred line Tr has most often given similar recombination rates in testcrosses involving *wx*-T2-9d heterozygotes, but at least one subline of Tr inbred line has an *Rf(Tr)-wx* recombination of 17 percent with a total of 3018 kernels scored.

Because of the apparent discrepancy indicated above we commenced experiments to determine *Rf-wx* recombination rates in T2-9d heterozygotes that carry cms-S restorers from a number of inbred lines. The results of testcrosses involving six such lines are given in Table 1. Since the crosses that produced the male parents here involved a cms-S inbred line as female parent and a highly inbred strain of homozygous *wx*-T2-9d as male parent, the testcrossed sibling F1 plants were isogenic. Surprisingly, the *Rf-wx* recombination rates for all six inbred line sources showed highly significant departure (at 0.005 level) from the *Rf(CE1)-wx* rate (8.3%). In fact, the recombination rates for restorers carried by inbred lines A634, C123 and C103 do not differ significantly from a theoretical 1:1 ratio, indicating that these restorers assort independently of the chromosome 2 *wx*-linked allele in the T2-9d heterozygote and may be presumed either not to be in chromosome 2 or to be located in chromosome 2 at some considerable distance from the *wx* locus in the translocation heterozygote. In both H95 and LH38, one tested sibling in each shows a highly significant departure from a 1:1 ratio, while the other is in good agreement with it. Since these F1 siblings are, in all other regards, presumed to be isogenic, these differences in *Rf-wx* recombination rates for sibling F1 plants indicate that *Rf(H95)* and *Rf(LH38)* can occupy different chromosomal sites in sibling plants within each inbred line. These results are most easily explained if it is assumed that these restorer elements have transposed in recent or not so recent generations. Note that the *Rf-wx* recombination rates for the two sibling H95 F1 plants show a significant difference in the contingency Chi-square analysis and that this further supports the transposition hypothesis. Both sibling parents carrying the R177 restoring allele, as well as one of the sibling plants from each of the inbreds H95 and LH38, exhibit recombination rates that differ significantly from the *Rf(CE1)-wx* rate as well as from a theoretical 1:1 expectation. The recombination rates (28.0%, 39.6%, 37.6%, and 35.4%) indicate *Rf* chromosomal sites linked with the *Rf(CE1)* site in chromosome 2. The contingency Chi-square analysis for the R177 siblings indicates a significant difference in *Rf(R177)-wx* recombination rates, suggesting that these siblings carry the restorer at different sites in chromosome 2. In view of the various chromosomal sites for cms-S restorers, the possibility that any one inbred line may carry two or more restorer genes should not be ignored. Although there is presently no evidence for this, it would explain why the test for allelism of *Rf* genes in two different cms-S restoring inbred lines would be positive even though these two lines might show significantly different *Rf wx* recom-

Table 1: Rf-wx recombination data from testcrosses of wx-Translocation heterozygotes involving six cms-S-restoring inbred lines.

		Progeny from testcross: (S) rfrf wxwx x (S) Rf N Wx/rf T2-9d wx						
Inbred line	Male parent plant number	Total kernels	Wx	wx	%wx*	92:8[b] Chi²	1:1[c] Chi²	Contingency[d] Chi²
R177	1172-1	175	126	49	28.0	***	***	*
	1173-7	235	142	93	39.6	***	***	
A634	1174-4	340	168	172	50.6	***		
	1175-2	180	79	101	56.1	***		
C123	1177-3	211	107	104	49.3	***		
H95	1178-7	325	175	150	46.2	***		*
	1179-7	274	171	103	37.6	***	***	
C103	1180-1	198	106	88	45.4	***		
	1181-1	291	142	149	51.2	***		
LH38	1182-2	195	126	69	35.4	***	***	
	1182-10	127	73	54	42.5	***		

*Since rf-carrying pollen grains abort, all functional pollen grains carry Rf; therefore the % wx kernels is a direct estimate of Rf-wx recombination.

[b]Chi-square test for goodness of fit of these Wx:wx progeny with the corresponding ratios obtained among testcross progeny where the heterozygous male parent carries the standard Rf of inbred line CE1: 28 plants testcrossed, total population 6,393; 5860 Wx:533wx; Rf(CE1) - wx recombination = 8.3%. Symbols *, ** and *** indicate a significant difference at the 0.05, 0.01 and 0.005 probability levels, respectively; no-entry indicates probability level greater than 0.05.

[c]Chi-square test for independence of Rf and wx; symbols have same meaning as indicated above.

[d]Contingency Chi-square attached to comparison of Wx and wx progeny from testcrosses of the two male parents within the same inbred line; symbols have same meaning as indicated above.

recombination rates in the *wx*-T2-9d testcross system; both lines could carry an *Rf* gene at the same site, e.g. *Rf(CE1)*, thus providing a positive test for allelism, but one of the lines might carry a second restorer at a site some distance from the first in chromosome 2 which, it can easily be shown, would increase the frequency of *Rf-wx* recombinant kernels on testcross ears. Given such a *wx*-T2-9d heterozygote in which the two *Rf* genes are at maximum distance the frequency of waxy kernels on testcross ears approaches a maximum of 33%, and the same is true if the second restorer is located in a chromosome other than 2. This means that in this testcross system a *Wx:wx* ratio of 1:1 indicates the presence of only 1 cms-S restorer at a site in chromosome 2 or elsewhere such that it shows independent assortment with *wx* in the *wx*-T2-9d testcross heterozygote.

Evidence for transposition of the naturally occurring cms-S restorer in inbred line CE1

--J. R. Laughnan, S. Gabay-Laughnan and Janet M. Day

Natural restorers of S-type male-sterile cytoplasm (cms-S) are found in a number of inbred lines of maize. As indicated in another article in this group such restorers, designated *Rf3*, act at the gametophytic level, meaning that cms-S *Rf3 rf* heterozygotes produce 50% *Rf3*-carrying pollen grains that function normally in fertilization, and 50% *rf*-carrying grains that abort (shriveled pollen). The *Rf3* gene carried in inbred line CE1 is located in the long arm of chromosome 2 where it gives from five to ten percent recombination with the *wx* allele in *wx*-T2-9d translocation heterozygotes. We have noted that (S) *Rf(CE1)* N *Wx/rf* T2-9d *wx* plants (N refers to a noninterchanged chromosome) crossed as male parents onto (S) *rf rf wx wx* male-sterile testers (see Table 1 legend) occasionally give strikingly higher *Rf-wx* recombination rates than usual. The male parents in this testcross are produced by crossing cms-S plants of inbred line CE1 with a highly inbred strain of *wx*-T2-9d. Since the parents are highly inbred the resulting F1 heterozygotes to be testcrossed are isogenic. Also, since the heterozygous male parents in the testcross have S-type cytoplasm, *rf3* (nonrestoring) pollen grains are aborted, so only *Rf(CE1)* pollen grains are functional. The testcrossed ears exhibit both starchy and waxy kernels and in this particular case the percentage of waxy kernels corresponds directly to the *Rf(CE1)-wx* recombination rate.

The data in Table 1 are arranged in three groups each derived from a separate cross of a cms-S inbred line plant with a *wx*-T2-9d pollen parent. Thus, the nine testcrossed plants in family 1064 all came from kernels on the same ear, those in family 1065 from kernels on another ear, and so forth. The Chi-square values for contingency homogeneity tests were not significant at the 0.05 level for the ten plants in family 1065, but the corresponding Chi-square values for the 1064 and 1066 groups were highly significant (0.001 level).

The basis for the highly significant Chi-square values for the contingency homogeneity tests of plants in families 1064 and 1066 is apparent when it is noted (Table 1) that certain male parents when testcrossed gave unusually high frequencies of waxy kernels, as indicated in the # footnote in Table 1. For example, the starchy:waxy testcross progeny from plant 1064-6, when compared with all other

Table 1: Rf-wx recombination data from testcrosses of wx-Translocation heterozygotes carrying the cms-S restorer from inbred line CE1.

Male parent plant number	Progeny from testcross: (S) rfrf wxwx X (S) Rf(CE1) N Wx/rf T2-9d wx — Total kernels	Wx	wx	%wx*
[a]1064- 1	263	245	18	6.8
2	239	227	12	5.0
3	380	367	13	3.4
4	290	267	23	7.9
5	304	291	13	4.3
6	197	174	23	11.7[#]
8	298	277	21	7.0
9	187	177	10	5.3
11	238	233	5	2.1
Totals	2396	2258	138	5.8
[a]1065- 1	59	58	1	1.7
3	190	180	10	5.3
4	159	146	13	8.2
6	210	188	22	10.5
7	314	279	35	11.1
8	369	331	38	10.3
9	148	141	7	4.7
10	282	259	23	8.2
11	362	327	35	9.7
12	86	79	7	8.1
Totals	2179	1988	191	8.8
[a]1066- 1	280	247	33	11.8[#]
2	167	125	42	25.1[#]
3	124	116	8	6.5
4	298	282	16	5.4
5	187	177	10	5.3
7	241	229	12	5.0
8	140	132	8	5.7
9	269	207	62	23.0[#]
10	112	99	13	11.6[#]
Totals	1818	1614	204	11.2

*Since rf-carrying pollen grains abort, all functional pollen grains carry Rf(CE1); therefore the % wx kernels is a direct estimate of Rf-wx recombination.

[a]Chi-square values for contingency homogeneity tests were not significant at the 0.05 level of probability for plants in family 1065; corresponding Chi-square values for plants in families 1064 and 1066 were highly significant (0.001 level).

[#]The contingency Chi-square value for plant 1064-6 (11.7% wx), when compared with all other plants in family 1064, is significant at the 0.001 level. In family 1066 the corresponding Chi-square values for 1066-1, -2 and -9 plants compared individually with the summed data from plants 1066-3, -4, -5, -7 and -8 are significant at the 0.001 level of probability; the corresponding Chi-square value for plant 1066-10 is significant at the 0.01 level.

plants in the family, gave a contingency Chi-square value significant at the 0.001 probability level. The same is true for testcross data from 1066-1, -2 and -9. Data for 1066-10 indicate a difference that is significant at the 0.01 level. These higher values for *Rf(CE1)-wx* ratios suggest that in these plants the *Rf* gene has transposed to a site more distant from the *wx* marker than in the controls. If the *Rf* gene were to transpose nonreplicatively to a site in a chromosome other than chromosome 2, or to a site in chromosome 2 far removed from *wx* in the translocation heterozygote, the *Wx:wx* ratio on testcross ears should be 1:1. Since the percentage of *Rf-wx* recombinants for the exceptional plants ranges from 11.7 to 25.1 it appears that these transpositions are to sites in chromosome 2 that are still linked with *wx* but at a sufficient distance from it to give elevated *Rf-wx* recombination values compared with the controls. If this is the case it should be noted that increased recombination rates will be produced whether or not the transposition event is replicative or nonreplicative. Moreover, if a replicative transposition of *Rf* to another chromosome occurs it can be shown that the *Rf-wx* recombination value should not exceed 33.3 percent in this testcross system.

Although the data in Table 1, and other data not presented here, suggest that the *Rf3* of inbred line CE1 is transposable it remains to be shown by tests for linkage with other marker genes that the transposed element occupies a new site in those plants that show significantly elevated *Rf-wx* recombination rates. This analysis is underway.

Behavior of cms-S pseudorestorer genes

--S. Gabay-Laughnan and J. R. Laughnan

Exceptional male-fertile plants which arise in S-type male-sterile (cms-S) maize result from changes at either the nuclear or cytoplasmic level. A class of nuclear revertants in which pollen carrying the newly arisen restorer of fertility (*Rf**) fails to function (pseudorestorer) has been previously reported (Maydica 28: 251-263). When ears of plants heterozygous for these *Rf** genes are crossed by their respective maintainers, male-sterile and phenotypically male-fertile (50% pollen abortion) plants segregate among the progeny. When cms-S male-sterile testers are crossed by these phenotypically male-fertile heterozygotes there is no seed set on the tester ears. To date, seven strains carrying this class of *Rf** gene have been identified in four inbred line-cytoplasm combinations: cms-ML WF9 (2), cms-RD Oh51A (3), cms-S M14 (1), and cms-S B37 (1). All seven strains exhibit 50 percent pollen abortion as expected if they are heterozygous for the nuclear restorer gene (*Rf* rf*). In two of these seven strains the remaining 50 percent of the pollen grains appear only partially filled. This pollen trait is inherited and it is not surprising that these two strains, when used as pollen parents in testcrosses, produce barren ears. In the remaining five strains, however, the pollen carrying the *Rf** gene appears to be normal; nevertheless, in each of these strains the normal-appearing pollen also fails to function.We believe this failure of pollen function results either from transposition and insertion of an *Rf* element into a gene that controls an indispensable male-gametophyte function, or from a defective *Rf* gene that codes for a product not fully functional in restoration. We suggest that such a nonfunctional restorer be designated *Rf-nf*.

In other spontaneous nuclear revertants in which the *Rf*-carrying pollen is functional, the newly-arisen *Rf* gene is most often homozygous lethal. These zygotic-lethal *Rf* genes are interpreted to result either from insertion of *Rf* into a gene whose function is indispensable for post-zygotic development, or from the deleterious effect of two doses of the *Rf* gene itself. We suggest that such restorers be designated *Rf-lz* (lethal zygote).

We have been interested in studying the allelic relationships between the newly arisen restorer genes, those with functional as well as those with nonfunctional pollen. To assist in the analysis the *Rf-nf* and *Rf-lz* strains were crossed with the inbred line M825 as pollen parent. This resulted in vigorous F1 plants with excellent tassels. The pollen-bearing F1 plants of the different *Rf-nf* and *Rf-lz* strains were intercrossed to test for allelism of their restorer genes. Some of the pollen-bearing F1 plants in the strains carrying *Rf-nf* genes were crossed onto cms-S male-sterile plants and were also self-pollinated. Surprisingly, these crosses and self-pollinations set seed. Even crosses and self-pollinations involving one of the two *Rf-nf* strains (the other is still being tested) with partially filled pollen grains, in which this phenotype is evident in the F1 plants, also set seed, although the sets were not always good.

The seven *Rf-nf* genes in the vigorous background are being further analyzed. These strains can now be tested for homozygous lethality and it appears that at least some are homozygous viable. They are also being converted to the inbred lines in which they arose to determine whether the *Rf-nf* genes return to the nonfunctional state. The *Rf-nf*-carrying plants are also being successively self-pollinated to determine whether the pollen viability of the F1 plants is due to hybrid vigor alone; if so, as the strains approach homozygosity the *Rf-nf* gene will again become nonfunctional. If the homozygous strains retain restorer function it would suggest that modifier genes introduced by M825 are responsible for F1 pollen viability, and that they were selectively retained in the inbreeding procedure. The effects of hybrid vigor resulting from crosses of the *Rf-nf* strains by inbred lines other than M825 are also being studied.

Nature of cms-S pseudorestorer genes

--J. R. Laughnan and S. Gabay-Laughnan

Among the many spontaneous nuclear revertants from S-type male-sterile (cms-S) maize that have been identified is a class we refer to as pseudorestorer. A plant heterozygous for one of these pseudorestorer genes is phenotypically male-fertile, having normal anther exsertion and exhibiting the 50% pollen abortion expected of gametophytic restorers of cms-S. However, when these "fertile" plants are crossed as male parents onto cms-S male sterile testers, or onto male-fertile isogenic maintainer plants with normal (N) cytoplasm, there is no seed set. The fact that this class of restorer gene produces nonfunctional pollen has led to the designation *Rf-nf* (see previous report).

Two alternative hypotheses have been proposed to explain the characteristics of the *Rf-nf* strains. The *Rf-nf* gene may code for a product that, while resulting in phenotypically male-fertile plants, does not lead to production of functional pollen. Alternatively, failure of pollen function may result from insertion of a newly arisen *Rf* element into a gene necessary for male-gametophyte function, thus in effect inducing a male gametophytic lethal mutation. A protocol has been developed that will differentiate between these two hypotheses. It involves crossing a strain heterozygous for an *Rf-nf* gene in cms-S by a strain carrying an unlinked *Rf* gene that is male functional. Initial experiments were invalid since we later learned that pollen grains carrying *Rf-nf* alleles can function in hybrid backgrounds. The crosses are now being repeated in the inbred-line backgrounds Oh51A, B37 and WF9 since it is only in these nuclear backgrounds that we have both functional *Rf* and *Rf-nf* genes.

The procedure which, by analogy with the behavior of normal and defective lambda particles in *E. coli*, we have called the "helper" experiment is as follows: a cms-S *Rf-nf rf* (50% pollen abortion) plant is crossed as female parent with a pollen parent carrying an unlinked functional *Rf* gene. Male-fertile plants among the offspring will be of two types: those with 50% aborted pollen, carrying only the functional gene from the male parent, and those with 25% aborted pollen, carrying both the functional *Rf* and the *Rf-nf* gene. Those plants with 25% pollen abortion are crossed as female parents by the appropriate nonrestoring maintainer inbred line. Plants with 50% pollen abortion are crossed as controls. The former cross should yield only 1/4 male-sterile plants while the later control cross will produce 1/2 male-sterile offspring. These crosses are made to confirm the pollen records of the female parents. The same plants that are tested as female parents are also crossed as pollen parents onto cms-S male-sterile testers. Testcrosses of the con-

trol plants (50% abortion) will produce only male-fertile plants with 50% pollen abortion. Testcrosses of plants carrying both the functional *Rf* and the *Rf-nf* (25% abortion) genes will give either of two results depending on which hypothesis is valid. If the *Rf-nf* gene codes for a defective restorer gene product, the testcrosses will yield both plants with 50% aborted pollen and plants with 25% aborted pollen since, on this hypothesis, pollen grains receiving both the functional *Rf* (helper gene) and the *Rf-nf* will be functional. These two types of offspring should occur in a 1:1 ratio if the two kinds of pollen grains, *Rf*; *rf-nf* and *Rf*; *Rf-nf*, function equally well. If, on the other hand, failure of *Rf-nf* pollen function results from insertion of a newly arisen *Rf* into a gene that is indispensable for male-gametophyte function, thus in effect producing a gametophytic lethal, pollen grains carrying an *Rf-nf* gene, even in the presence of a functional *Rf* gene, will fail to function and no plants with 25% aborted pollen should be observed in the testcross progeny.

The first tests of the two hypotheses concerning the nature of *Rf-nf* genes in the Oh51A inbred line background were carried out in the summer of 1988. The progeny of eight different plants exhibiting 25% pollen abortion and testcrossed as pollen parents onto cms-S male-sterile testers were examined. Of 78 plants, 77 were male-fertile and exhibited 50% pollen abortion. No plants with 25% abortion were observed. One plant was too late in maturity to be scored. Four control plants yielded 41 plants, all with 50% pollen abortion. These results indicate, at least for the two Oh51A *Rf-nf* strains tested, that nonfunctional pollen in pseudorestorer strains can result from disruption of a gene necessary for male-gametophyte function.

As indicated above, only two *Rf-nf* genes, of separate origin, have been tested by the helper procedure. The finding that in both cases disruption of a gene that is indispensable for male-gametophyte function is involved does not mean that other *Rf-nf* genes that fail in male-gametophyte function because they are defective versions of restorer genes will not be found. In any case, it now appears that, given numbers of spontaneously revertant *Rf-nf* genes to analyze by the helper procedure, those that involve lethal mutations, presumably brought about by insertion of *Rf* elements into genes that are vital for pollen function, provide a unique opportunity to identify, map and characterize them at the molecular level. Although pollen grains that carry *Rf-nf* in this category are lethal to the male gametophyte, they do not abort. In all but two such strains the pollen grains, upon microscopic examination, are normal in appearance. The two exceptions involve *Rf-nf* genes that arose spontaneously in cms-S versions of inbred lines M14 and WF9, but even in these strains the pollen grains are partially filled. This means that pollen from these mutant strains and from their isogenic maintainer and *Rf*-carrying strains with normal pollen function can be analyzed at the DNA, RNA and protein levels. Moreover, if these *Rf-nf* strains result from transposition and insertion of previously quiescent restorer genes, it is expected that such studies will lead to isolation and characterization of these restorer genes themselves, and following that to a better understanding of the molecular basis for S-type cytoplasmic male sterility. In this connection we note that *Rf-nf* mutants whose nonfunctional character is based on defective *rf* genes also afford the opportunity to isolate and characterize both normal and defective versions of the cms-S restorer.

How shall we proceed to obtain the relatively large numbers of *Rf-nf* revertants that will be required to establish a reasonably good map of male-gametophyte genes and an understanding of their functions? First, we now have in hand a total of seven such revertant strains. Second, we plan to intensify our search for spontaneous revertants from a variety of cms-S male-sterile inbred lines. Probably the most fruitful source of these revertants will come from the use of the *Rf-nf* strains themselves as male parents in crosses with cms-S male sterile tester strains. At an earlier point in this article it was stated that such crosses give no seed sets. Actually, some such crosses produce seeds at a very low frequency, most often one or two kernels per ear with most of the ears being barren. Since both the male-sterile female parent and the *Rf-nf* male parent are isogenic (same inbred-line background) contaminants are easily identified on the basis of the hybrid vigor exhibited by next generation offspring. Using this crossing procedure we have identified numbers of legitimate offspring from such crosses. In some cases, even though the male parents carried an *Rf-nf* gene, the new derivative carries a functional *Rf* gene. In other cases these offspring turn out, like their male parents, to carry *Rf-nf* genes. Since these might be rare cases of transmission through the male gametophyte they can not be regarded as different from the parental *Rf-nf* unless procedures such as mapping and allelism tests prove otherwise. The search for increased numbers of *Rf-nf* revertants, and their verification by these methods are underway at the present time.

VARANASI, U.P. INDIA
Banaras Hindu University

Maize breeding year round in the Indian subcontinent: combining ability pattern of inbreds in summer and winter maize

--S. V. Singh, N. J. M. Rao and R. M. Singh

Combining ability pattern of 10 elite inbreds and all possible F1 hybrids for grain yield and other prime traits was studied in 4 diverse environments (E), constituting both summer and winter seasons at Hyderabad (tropical) and Varanasi (subtropical) to characterise good combiners. The inbreds CM 202 and PhDMRF1a3H94 were found to be the best general combiners for grain yield and yield traits in the pooled analysis. Also among the best specific cross combiners, CM 202 ranked first for yield in 5 top combinations followed by PhDMRF1a3H94. Both general and specific combining ability (GCA and SCA) variances were significant, however, the magnitude of GCA variances was higher for all the traits in individual as well as in pooled analyses, thereby showing greater importance of additive and additive epistasis components. The interaction variances for GCA x E and SCA x E were significant, therefore, season and location specific breeding strategies would be more important. The study also revealed that integrated use of temperature and tropical germplasm would be most beneficial.

VICTORIA, BRITISH COLUMBIA, CANADA
University of Victoria

The *R* locus - regulator and regulated
--E. Derek Styles

Phenotypically at least, the *R* locus controls the concentration, tissue specificity, and the pattern of distribution within a tissue of anthocyanins and related 3-hydroxy flavonoids. This is demonstrated most conclusively when different *R* alleles are compared against a common genetic background. In this sense the *R* locus acts as a regulatory locus, and whether or not the locus is eventually assigned an enzymatic role, this regulatory function will still need to be explained. Comparisons against common genetic backgrounds have built-in limitations, however, because a specific genetic background may lack factors that allow certain allelic differences to be expressed. Obviously the genetic backgrounds against which different *R* alleles are compared need to carry the so-called complementary factors known to be required for *r* locus expression. Factors such as *C2*; *C* (if aleurone color is being studied); *A1*, *A2*, *Bz1*, *Bz2* (if anthocyanins are used as a phenotype) are usually considered essential to study *R* locus expression. Some factors, such as *Pr*, probably modify the substrate for the *R* locus, others (*A1*, *Bz1*) modify the product. Factors that enhance the expression of *R* controlled pigments (*in*, *Pl*, *a3*) are not so regularly incorporated in such comparison studies, though in theory at least, they should be required for maximum expression of the *R* locus potential, and, as with *Pl* and the 'cherry' alleles, they may also be essential in uncovering *R* allele differences.

We can report on a specific example of how the expression of a *R* allele is affected by both known and unknown factors in the genetic background. As with many of our studies that have turned out to be interesting, we did not plan on doing a detailed study on this particular *R* allele, and much of what we can report at this time comes from a retrospective study of our records. The *R* allele in question was carried in a 'demonstration' ear kept mostly because it had mosaic pericarp. Beyond this the ear has no pedigree. We were interested in the *R* allele because in the backcross generation to *r-g* it appeared to determine a very strong silk pigmentation. We initially classified the allele as '*R-g*' because it determined green anthers and colored aleurone. It also conditioned coleoptile and seedling root pigment, and thus grouped with Stadler's 'Group D' *R* alleles. We have not backcrossed this allele into an inbred line such as W22, but rather we have tried to identify the factors that modify (regulate?) its expression independently of other genetic background effects.

Although initially characterized as *R-g* this *R* allele consistently determines red anthers with *Pl*. With *a3* and *Pl* the anthers range into purple, and *a3* plants show the instability typically of Group D *R* alleles in the presence of *a3*, i.e., tissues that can range from green with small purple sectors to uniform diffuse purple. We have recently been trying to characterize a factor tentatively named '*Pth*', that with Group D *R* alleles, *a3*, and *Pl* determines a strong purple cob pith pigment. The *R* allele from the 'demonstration' ear does not require *a3* to express the pith pigment with *Pth* and *Pl*, but when *a3* is present together with *Pth* and *Pl*, the whole cob is purple, pith, pedicels, pericarp - the works! The one characteristic that we were originally interested in, the silk pigment, shows variability, presumably due to genetic background differences, but so far we have not been able to isolate specific factors that will assure a good silk color expression.

In summary, we have isolated an *R* allele that with *pl* is a typical Group D *R-g* allele. As with other *R* alleles of this group, with *a3* the plant phenotypes can range from green with small purple sectors to uniform diffuse purple. With *Pl* both *A3* and *a3* plants have red anthers, and *a3 Pl* plants have the potential to produce strong purple anthers. With *a3*, *Pl*, and a factor we have tentatively termed *Pth*, all parts of the plant can be purple. If the *R* locus is a 'regulatory' locus, clearly it, or at least some allelic forms of it, can also be regulated. The allele described above has the potential to determine pigment in all parts of the plant. Yet in a W22 background, with *pl* and *A3*, it would have been characterized as '*R-g*', with the inherent assumption that it determined (regulated?) the characteristic tissue specificities associated with *R-g* alleles, namely, colored aleurone but green anthers and plant. It is worth noting that Group D *R-g* alleles are paramutable, i.e., if maintained heterozygous with a paramutagenic allele such as *R-st*, the aleurone pigmenting potential can be markedly and progressively reduced to the point that even some *R R R* endosperms may be colorless or near colorless. Thus can the biter become bitten, the regulator become regulated!

WALTHAM, MASSACHUSETTS
University of Massachusetts

Recognition of interphase homologies by the vestigial glume gene (*Vg*)
--Walton C. Galinat

While my phytomer concept (Galinat, Bot. Mus. Leafl. Harvard Univ. 19:1-32, 1959) made it very clear as to the vegetative and floral homologies, examples of genetic recognition of these have been slow to accumulate. The first clue came from phase reversal in which male spikelets metamorphosed into tassel plantlets (Galinat and Naylor, Amer. J. Bot. 38:38-47, 1951).

Then the evidence for overlapping phases came from the phenotype of the mutants corngrass and teopod (Galinat, MNL 40:102-103, 1966). The evidence from a class of defective phase change genes (*Bif*: barren inflorescence, Neuffer; *isp*: interrupted spikelets, Sprague; H99: reduced tip, Cowen) indicates that the vegetative phase shuts down by independent gene action from the turning on of the floral phase genes. Normally the turning off of the vegetative phase is synchronized with the turning on of the floral phase. The symbol *is* that I used previously (MNL 62) to designate interrupted spikelets is not available in that I had also used it to refer to cupulate interspace (MNL 45:98).

While the ligule is not the only part of the leaf component of the phytomer, it is significant that the *Vg* gene reduces both ligule development during the vegetative phase and glume wing development during the floral phase and, thereby, demonstrating the interphase homologies of these two structures. In general, the effects of the *Vg* gene are stronger during the floral phase than in the vegetative phase. In the former, it also reduces the sheath part of the leaf (glume), the other floral bracts (lemma and palea) and

the rachilla. In the latter it has no obvious effects other than on the ligule.

Multiranking in the floral and vegetative phase

--Walton C. Galinat

In teosinte, the ancestor maize, as in most other grasses, the phyllotaxy of the floral and vegetative phase is the same, namely two-ranked. But when man domesticated teosinte, he selected for a multiranked phyllotaxy in the uppermost spike of both the tassel and ear clusters during the floral phase. At low levels of condensation, multiranking has a fragile penetrance that is normally confined to these points in space and time. Almost any form of stress during development such as disease, injury, heat or drought may cause multiranking to revert to two-ranking. The tassel branches, the secondary ears, the spikelets and florets all normally remain two-ranked in the floral phase. Therefore, if there is an epigenetic switch to multiranking during development of the uppermost ear and the central spike of the tassel, the reversion to the two-ranked state in preparation for the vegetative phase of the next generation usually is either unnecessary, as with pollen from the tassel branches, or is reverted within the two-ranked spikelets.

Most corn breeders have at some time observed individual corn plants that express multiranking in the vegetative phase (at a frequency of perhaps 1/100,000). On trying to propagate this condition, it has usually been found either not to be inherited or just a tendency within certain lines. We have discovered a more stable mutant form of vegetative multiranking in which the leaves occur in whorls of two, three or four.

The situation with the phytomers in the areas of multiranking is interesting. There has been a mirror-image repetition of phytomeric instructions such that rather than just a solo performance, it is played out as a duet with four ranking, a trio with six ranking or a quartet with eight ranking, etc., with the whorled phytomers sharing a common internode. The concept of expressing simultaneously different instructions is known to apply elsewhere in the case of overlapping phases in the corngrass and teopod mutants.

In the case of the mutant genes such as *Bif* and *isp* which I have described (MNL 62) as delaying the switching on of the floral phase after the vegetative phase is turned off, an alternative explanation is that the simultaneous mirror-image instructions for multiranking are temporarily cancelling each other out in the barren areas.

Our studies that are underway involve pollinations with pollen collected separately from the multiranked central spike of the tassel in comparison with pollinations by pollen from its two-ranked lateral branches. The pollen from the two sources has been placed on ears at different multiranking levels including two ranking in teosinte. Spikelet morphology of the vegetative multiranked lines is being examined in terms of possible multiranking within the spikelets.

Ear shape as a correlate of differences in rachilla and cupule development that trace to two independent domestications of two different teosintes

--Walton C. Galinat

A natural grouping of maize into two different ear shapes, pyramidal and cylindrical, was observed by Anderson and Cutler (Ann. Mo. Bot. Gard. 29:6988, 1942). These two shapes correlate with my findings on differences in rachilla and cupule development that trace to two different systems for kernel exposure by independent domestications of two different teosintes. The pyramidal corns have short sharply tapering ears and elongated kernels. They include Palomero Toluqueno, Conico, Pepitilla and Chalqueno as well as the U.S. Gourdseed corn. The ancestral system of kernel exposure during domestication of their teosinte progenitor was based on cupule reduction and to this day these races have short rachillae and reduced cupules.

When multiranking is added to cobs that have short rachillae and reduced cupules on the one hand and cobs with long rachillae and pronounced cupules on the other, the cob assumes two different shapes. The circumferential space necessary to accommodate multiranking in the presence of short rachillae and reduced cupules can only come from increases in pith diameter. Without the mechanical support of cupules, such cobs rapidly taper into a pyramidal shape except in the presence of fasciation which may be either terminal or subterminal.

In contrast, cobs with long rachillae and well-developed cupules have a cylindrical shape that has structural support from a rigid framework of these cupules. This cylindrical shape is most prevalent in modern corn as a result of selection by commercial corn breeders. It represents another natural grouping of the races of corn, probably tracing back to domestication from Guerrero teosinte.

Sexual feedback, internode elongation and perfect-flowered dwarfs

--Walton C. Galinat

At the time the floral primordia are laid down that will eventually reach either tassel or ear, they are perfect flowered (bisexual) and below them the vegetative phase is still in the juvenile stage with telescoped internodes and only partly developed leaves. Each type of sexual development has a different feedback effect on the degree of completion of the vegetative phase below.

In the inflorescence terminating a main stalk with strong apical dominance, the female primordia are inhibited, the male primordia develop slowly and a hormonal signal is sent down to the vegetative phase that stimulates internode elongation and completes leaf development by the growth of blades. Meanwhile, the reverse occurs in the lateral branches and their terminal inflorescences. The internodes of the branches remain short, and the leaf blades fail to develop as if suppressed by apical dominance of the main stalk. The male primordia are inhibited and the female primordia develop precociously. As a result, the ears become trapped within their own bud, the leaf sheaths become a jail of protective husks and the styles must elongate until they are at the summit where pollination is possible. Because of the artificially imposed time required for style emergences, maize has been falsely accused of being protandrous.

In the perfect-flowered dwarfs, the internodes of the main stalk can not elongate normally with the result that its apical dominance is weakened and lateral branches freed for male expression. Thus a better balance is achieved between male and female hormones. Development of the female primordia is no longer precocious and the primordial situation of perfect flowers advances onward to floral maturation.

The biphyletic hybrid vigor of the Corn Belt Dent

--Walton C. Galinat

I have described in last year's News Letter how the races of maize may be divided up into extreme differences in pronounced cupule with elongate rachilla development on the one hand and reduced cupule with short rachilla development on the other and how these cupule-rachilla associations probably stem from two systems for kernel exposure during two independent domestications from teosinte.

Hybridizations between members from the two domestic pathways have occurred repeatedly over time yielding productive new races such as Tuxpeno and the Corn Belt Dent. On inbreeding and/or backcrossing to one parent or the other, the hybrids yield varieties and inbreds which may be used to reconstruct the heterosis of the hybrid, such as Reids Yellow Dent/Southern Dent (inbreds Wf9, B73) crossed by Lancaster/Northern Flint (inbreds C103, Mo17).

Since my hypothesis was presented, I have rendered in a drawing of ear cross-sections, the highlights of 8000 years of evolution leading to biphyletic hybrid vigor in the Corn Belt Dent. This is presented here in 11 figures described as follows:

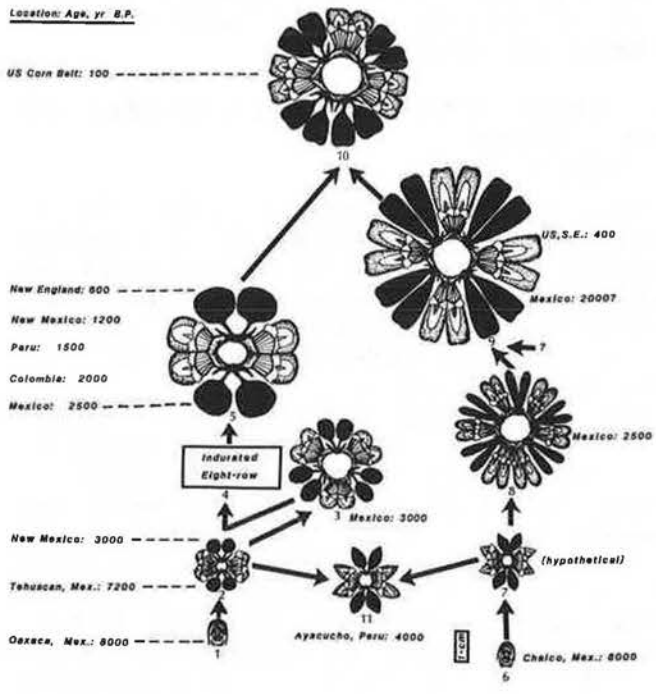


Fig. 1. On the lower left, there is kernel exposure from Guerrero (Balsas) teosinte by rachilla elongation with teosinte's cupule still unchanged.

Fig. 2. With the addition of two more traits, a pairing of female spikelets and a switch to multiranking, the first cob of Tehuacan corn was evolved.

Fig. 3. There was an increase in cob diameter together with an increase in the level of ranking represented here by a 12-rowed ear of Chapalote.

Fig. 4. When the larger supply system of the Chapalote ear is combined with the less demanding low-level ranking, the surplus photosynthate may be either deposited in the form of cob induration or kernel enlargement.

Fig. 5. Large kerneled eight-rowed corn arose independently in widespread areas from the Northern Flints of New England (A.D. 1300) to the giant Cuzco corn of Peru (A.D. 1500).

Fig. 6. Meanwhile a parallel sequence starting with the domestication of Chalco (Central Plateau) teosinte was based on a different system of kernel exposure by cupule reduction.

Fig. 7. A hypothetical race of eight-rowed corn, almost without cupules and with short rachillae, that is similar to the Peruvian race, Confite Morocho, known from archaeological remains about 4000 B.P. (before present) at Ayacucho (see Fig. 11).

Fig. 8. Palomero Toluqueno is an ancient indigenous race from the Mexico City area that is considered here to have evolved soon after domestication of Chalco Teosinte. It has reduced cupules, short rachillas and elongate kernels.

Fig. 9. Pepitilla is clearly related to Palomero Toluqueno. It is ancestral in varying degrees to the Southern Dents, especially to Shoe-Peg with its narrow deep kernels and to Gourd-Seed with its broad, deep kernels. These races all have reduced cupules and short rachillae.

Fig. 10. The Corn-Belt Dent is known to be a hybrid between the Northern Flint (5) and Southern Dent (9) pathways.

Nothing in the evidence from isozyme variation or DNA sequencing presented so far rules out my hypothesis of a biphyletic origin of maize by two systems for kernel exposure. The patterns of isozyme variation between Andean maize and the Mexican races of Pepetilla and Palomero Toluqueno as well as Chalco teosinte should be compared.

On the vital function of the ligule in maize

--Walton C. Galinat

Maize, like most grasses, is notoriously susceptible to stalk rots and carries young axillary buds which can not develop under a leaf sheath carrying a pool of water together with various debris such as drowned insects. The answer for the grasses was to evolve a collar or ligule that fits like a gasket against the stalk at the junction of the blade and leaf sheath. This excludes water from draining down the mid-rib of the leaf blade into the pocket of space between the leaf sheath and the internodal groove caused by divergence of the axillary bud. In doing this, the ligule also excludes conditions for possible stalk rot infection and allows the axillary bud to develop and respire.

In the related families of sedges and rushes that grow in a wetland habitat, there are no ligules, the stalks are rot resistant and the leaf sheaths collect water and drowned insects. Like a pitcher plant, the drowned insects apparently provide the main source of nitrogen for these plants that is necessary for survival in the nitrogen-poor wetland habitat.

WEST LAFAYETTE, INDIANA
Purdue University

The rescue of late field pollinations

--J. Bennetzen and P. San Miguel

Due to the severe drought conditions and a low soil moisture content at the time of our latest planting this summer, many of the seed in our nursery did not germinate until the middle of July. This necessitated some very late pollination dates, from late August through mid-September. In particular, potential mutants from one acre of a screen for a Mutator-associated inactivation of *Rf*-c* were not pollinated until September 15. Since early October frosts are common in the Lafayette area, we were not certain whether any of these ears would survive. Rather than panic (or, more correctly, in addition to attaining a reasonable level of

panic), we decided to take advantage of this situation to test various approaches for the rescue of late field pollinations.

We had constructed an *Rf*-c/Rf*-c* cms-C Mutator stock (in a mixed B73/B77/B78/Q66/Q67 background) and crossed this as a female to male Mo17 (*rf*-c/rf*-c* cms-N). Over 200 ears of this material were generated, and each ear was individually shucked and shelled. In the summer of 1988, about one-third of this material was machine planted, on an ear-by-ear basis. As the plants matured, we walked down each row and detasseled all male fertile plants with a machete. Apparent male sterile plants were observed at a frequency of about one in 300. Most or all of these apparent *Rf*-c* mutants were probably escapes due to physiological events causing poor male fertility. These male steriles were all hand pollinated by Mo17 on September 15. The rest of September and the first two weeks of October were unusually cool, with daytime highs in the 51 F-90 F range (averaging 71.2 F) and lows in the 21 F-69 F range (averaging 45.9 F). Normal high and low temperature averages in our area for this time of year are 74.2 F and 46.6 F, respectively. In all cases, temperatures were recorded at the National Weather Service Station at the Purdue Agronomy Farm, 1.2 miles from our nursery. We also received above average rainfall during this period. Subfreezing temperatures of 31 F, 29 F, and 28 F were recorded on October 6, 7, and 12, respectively. Our first hard frost was predicted for the early morning hours of October 13, when the temperature dropped to 21 F. The fertilized materials in the *Rf*-c* mutant screen were subdivided into five categories. One group of plants was left in the field to check for resistance to this temperature; the plants in a second group were each covered on the afternoon of October 12 with thirty- gallon black trash bags; ears from plants in the third group were harvested and shucked on October 12, while (following the suggestion of Dr. Janice Clark) ears from a fourth group were harvested but not shucked; and, in the fifth group, whole plants were uprooted. In the latter three groups, all ears or plants were left in open pollination bags in a well ventilated environment at approximately 70 F. On October 13, the trash bags were removed from the covered plants after the air temperature increased to 30 F. Between October 13 and October 24, conditions in the field remained unusually cool and damp, although the temperature did not drop below 32 F. All ears were harvested, shucked, and dried on October 24. Forty seed off each ear were soaked overnight in tap water and germinated on wet towels in a humidified incubator. As shown in Table 1, major differences were observed in the germination competence of seed from each experimental group.

Table 1.

Harvest date	Plant or ear harvested	Bagged in field	Shuck date	Germ. Eff. Range	Germ. Eff. Avg.
10/12	Ear	No	10/12	5-13%	7.8%
10/12	Ear	No	10/25	46-98%	82%
10/12	Plant	No	10/25	63-98%	85%
10/25	Ear	Yes	10/25	0-23%	6.3%
10/25	Ear	No	10/25	0-29%	19%

The most effective approach for rescuing these late pollinations was removal of the whole ear from the field and allowing the unshucked ear to further mature in a warm environment. Harvesting the entire plant carrying the fertilized ear, and allowing a similar maturation indoors, was not significantly superior to harvesting only the ear. Removing the husk from a harvested, immature ear was a major negative factor, decreasing seed viability from 82% to 8%. Leaving these young ears in the field was also less successful, whether or not the plants were covered during the hard frost. Many of these ears had no germinating seed, while a few had reasonable percentages of survivors.

On ears that gave low germination frequencies of 5% to 30% (both those samples that were left in the field during and after the hard frost and those immature ears that were both harvested and shucked on October 12), the seed in the middle of the ear was most likely to germinate (Table 2). It is not known whether this reflects a faster maturation rate, a greater resistance to freezing and/or desiccation, and/or a more efficient post- harvest translocation of nutrients to these mid-ear kernels.

Table 2.

Kernel position in row	Germination from ears left in field until 10/25	Germination from ears harvested and shucked on 10/12
1 (base of ear)	2/28*	1/12**
2	0/28	2/12
3	1/28	0/12
4	0/28	4/12
5	0/28	2/12
6	2/28	3/12
7	2/28	4/12
8	2/28	4/12
9	1/28	3/12
10	0/28	2/12
11	0/28	1/12
12	0/28	0/12
13	0/28	1/12
14	0/28	1/12
15	1/28	2/12
16	0/28	0/12
17	0/28	1/12
18 (toward tip of ear)	1/28	0/12

* = 28 rows analyzed from 7 ears.

** = 12 rows analyzed from 3 ears.

In an adjacent field, plants in slow-maturing opaque backgrounds were crossed between late August and early September. Crosses performed less than four weeks prior to harvesting demonstrated little or no decrease in germination efficiency if stored as unshucked ears at room temperature for an additional 23 days (Table 3).

Table 3.

Harvest date	Shuck date	Cross date	# of ears	Germination Eff. Range	Germination Eff. Avg.	Kernels scored
10/2	10/25	8/18	20	70-100%	92%	10
10/2	10/25	8/19	5	60-100%	86%	10
10/2	10/25	8/24	5	0-90%	64%	10
10/2	10/25	8/25	14	20-100%	77%	10
10/2	10/25	8/26	25	0-100%	77%	10
10/2	10/25	8/28	8	80-100%	95%	10
10/2	10/25	8/31	11	10-100%	76%	10
10/2	10/25	9/7	5	50-100%	66%	10
10/24	10/24	9/7	7	0-40%	20%	20

Taken together, these results indicate that seed which has matured less than four weeks post-pollination can be rescued by harvesting the unshucked ear and allowing it to mature indoors. We did not determine to what degree the 21 F hard frost of October 13 decreased germination efficiency compared to any possible negative effects of the

prolonged retention of the ears in a cool, damp field environment. All things being equal, it might be appropriate to rescue vital immature ears from the field even in the absence of an imminent freeze if the ensuing field conditions are likely to be continuously cool and damp until the next hard frost.

A note on RFLP probes: their dissemination and utilization

--J. L. Bennetzen

In our laboratory, we have made extensive use of the maize restriction fragment length polymorphism (RFLP) probes isolated and mapped by the Burr laboratory at Brookhaven, the Helentjaris laboratory at Native Plants (Inc.), and the Hoisington laboratory at the University of Missouri, Columbia. We have obtained most of these cloned probes directly from Dr. Hoisington, who has generously volunteered to operate a clearinghouse for these materials. Due to the numerous and continuing requests for these probes from a variety of sources and the large size of the RFLP library so far assembled, Dr. Hoisington's laboratory can take quite a while to send out the material and must forward it with only a minimum amount of descriptive information. This has led to a number of problems. For instance, most of the RFLP probes hybridize to two or more fragments on gel blot hybridization analysis, regardless of the restriction enzymes employed. This is presumably due, in part, to the predicted polyploid origin of the maize genome and, in part, to the routine presence of multicopy gene families in eukaryotes. In general, only one of these two or more bands is associated with a polymorphism in the mapping inbreds employed by Dr. Hoisington. This RFLP is then mapped and the clone designated as a marker for a specific chromosomal segment. However, anyone receiving this probe and employing it in a linkage analysis may actually be following a polymorphism for one of the unmapped bands.

Dr. Hoisington has all of the information in his notebooks regarding the number of bands any probe hybridizes to, their approximate size with specific restriction enzymes, and the identity of the polymorphic band mapped. Anyone wishing to make use of this information is allowed free access to all these notebooks upon visiting Dr. Hoisington's lab, but the material is too voluminous to send out along with each of several hundred RFLP probes. Moreover, Dr. Hoisington has not had the personnel available even to fully assemble this information in an easily digested form.

I would like to propose that a handling fee be required to obtain RFLP probes along with all of the pertinent information allowing the material's optimal utilization. This information would include probe source, probe size, probe cloning enzymes, and a digitized reproduction of the hybridization patterns observed for the probe with the inbreds and restriction enzymes employed by Dr. Hoisington in his mapping studies. Dr. Hoisington has both the data and the technology to provide this information but lacks the delegated personnel. A handling fee of $20 per clone (prepaid) would allow the hiring of a full-time technical assistant dedicated exclusively to the assembly and provision of RFLP clones and accompanying information. Compared to the current process, delivery of materials would be accelerated and their utility greatly enhanced. As a secondary but perhaps significant outcome, a handling fee would discourage requests by "collectors" with little or no immediate need for the probes. Finally, this fee would relieve Dr. Hoisington's lab of the burden of materials expense, postage costs, and personnel commitment that they have, to date, generously provided to the maize genetics community. The $20 handling charge would, of course, be waived in case of need. This fee level is half the amount charged by the American Type Culture Collection (ATCC) for microbial stocks.

For the long term, it would be most desirable to obtain commitment for the support of a maize RFLP supply house from federal or charitable sources. However, since such support is neither clearly available nor on the near horizon, I feel that charging an RFLP probe handling fee would be a valuable interim (preferably) or final solution to the current problems associated with the broad dissemination and efficient utilization of maize RFLP probes.

YANGZHOU, PEOPLES REPUBLIC OF CHINA
Jiangsu Agricultural College

Character and inheritance of a new Y-type cytoplasmic male-sterile line

--Zin Tai-chen, Dan De-xiang, Xu Ming-liang and Liu Da-wen

The breeding process. The maintainer lines were selected as males to cross with restorer lines, which may bear sterile cytoplasms, and backcrossed to the same lines repeatedly. New sterile lines may be obtained in this way. The concrete methods are as follows: In 1978 some restorer lines such as Wa 8 were selected as females to cross with inbred lines including B37. No sterile offspring have been detected in the F1 progeny. But in following F2 populations, 6 sterile individuals came to light in 1 cross of Wai-si 137xB37. In 1982 we duplicated the same experiment, and other restorer lines involving A170 were selected as females to cross with maintainer lines such as L107 to obtain 19 combinations. Male-sterile individuals came out in 3 F2 populations and appear to have normal male spikes, no anthers exserted, and fewer and anomalous pollen grains in anthers. The sterile lines bred later showed stable sterility and could readily be applied in hybrid production. The variable fertility restoration responses have been shown to correspond to the different sources of male-sterile cytoplasms, which in turn were derived from the respective restorer lines. Therefore, the former (derived from the cross of Wa 8 x Wei-si 137) and the latter (derived from the cross of A170 x L107) were named YI and YII type, respectively.

Research on their resistant reactions against HmT (*Helminthosporium maydis* race T), fertility restoration patterns, hereditary traits, light microscopy, electron microscopic ultrastructure and biochemical character have been conducted rather systematically for several years. According to the results, it has been fundamentally considered that the Y-type sterile lines are new in maize and differ from the C, T and S groups.

The explanation may be illustrated as follows: The induced assay was done for the resistance of Y type male-sterile lines against *H. maydis* (*Helminthosporium maydis*)

and *H. turcicum* (*Helminthosporium turcicum*) in 1982 and 1984. The results indicated that YI and YII type male-sterile lines showed some resistance to *H. maydis* and *H. turcicum*.

Since 1981, 62 combinations have been obtained by crossing 46 inbred lines with the T group male sterile line, 26 combinations by crossing 20 inbred lines with the S group sterile line, 172 combinations by crossing 82 inbred lines with the C group sterile line, 372 combinations by crossing 126 inbred lines with YI type sterile line and 632 combinations by crossing 191 inbred lines with the YII type sterile line. According to Duvick's standard for classification, the male fertility of the F1 generation was investigated with the finding that the relationship between restoration and maintenance for the Y type sterile line was different from that of the other 3 groups of sterile lines.

There was a distinct difference in the pollen abortion between C group and Y type sterile lines; the pollen abortion in the C group sterile line occurred at the early mononucleate stage after the division of the tetrad, while in the Y type sterile line it occurred at the late microspore stage.

The analysis of esterase isozyme was done by disc polyacrylamide gel electrophoresis for materials from C group, T group, S group and Y type sterile lines, for which C group and Y type sterile lines belong to a genetic background with the same genotype but different cytoplasms. The results revealed that these 4 groups (types) of sterile lines and their maintainer lines had different zymograms of esterase isozyme with their own specific character; they also differed in the number and order of the bands and the stained degree for each band varied with different groups (types) of sterile lines.

Four maintainer lines which bear a pair of restorer genes for the Y type sterile cytoplasm were selected as recurrent parents to cross with Wu 15, and then backcrossed repeatedly to transform Y type restorer lines with the recurrent parental backgrounds. The major gene's restoring capacity was assayed for each transforming restorer line at every backcross pedigree. The simple linear regression equation with replication was postulated to describe the correlation between the changes of nuclear ratios of a given recurrent parent and the corresponding changes of the major gene's restoring capacity. According to this equation, the expectation of the major gene's restoring capacity in various recurrent parental nuclear backgrounds or their differences may be possible when the nonrecurrent parent nuclei were completely replaced by recurrent ones. The results are as follows: The more the backcrosses, the higher the restoring capacity for those transforming restorer lines, assigned RYms330 and RYms632. In reverse, the restoring capacity decreased as the backcross pedigrees went up for RYms Santuan. No significant difference was detected for RYms02. The expected major gene's restoring capacity showed significant differences among various transformed restorer lines when Wu 15's nucleus was absolutely substituted by the recurrent parental nucleus. These differences of the restoring capacity between nonrecurrent and recurrent parent or among various recurrent parents may be attributed to the variable number or functions of the minor or modifying genes in different nuclear backgrounds, which influence the major gene's fertility expression quantitatively.

Finally, we believe that further testing is needed before it can be assigned to a group. Y type sterile lines have shown some favorable characters, such as the stable inheritance of pollen abortion, ready application in hybrid production, possessing many restorer lines in multitudinous inbred lines, and strong resistance against HmT and so on. They are regarded as a valuable germplasm source and have important applied value in seed production.

III. ZEALAND 1989

This is a summary of selected genetic research information (e.g., new factors; mapping; cloning) reported in this News Letter and in recent literature ("r" refers to numbered references in the Recent Maize Publications section). The breadth of coverage here has been reduced in favor of a new feature: This year the Symbol Index refers by number to all current published research involving genetic materials; comments on this new aid would be welcome.
BS = Base Sequence; BSH = Broad Sense Heritability; gca, sca = general and specific combining ability; QTL = Quantitative Trait Loci; RM = Restriction Map
* in symbols identifies loci needing allelism tests, documentation, or standardization of the symbol.

CHROMOSOME 1
BNL5.62 - *BNL8.05*(unc. by TB-1Sb) - *NPI411* - *BNL10.38* - *BNL1.326* - *BNL12.06* - *P1* - *BNL7.21* - *BNL1.556* - *BNL5.59* - *Mdh4* - *BNL17.06* - *BNL8.10* - *BNL15.18* - *BNL17.04* - *Adh1* - *BNL7.25* - *Phi1* - *BNL8.29* - *Acp4* - *BNL6.32* --Burr & r56
duplicate *NPI* loci, chroms. 1&2: *82&32, 109&273, 243&242, 243&244, 246&340.* 1&3: *109&358.* 1&4: *Adh1&Adh2, 96&95.* 1&5: *226&75, Phy1&Phy2, 357&282.* 1&6: *205&63, 246&245, 205&102.* 1&7: *96&316, 109&335, Sod4&Sod2.* 1&8: *205&301.* 1&9: *93&209, 99&97, 225&98* --Helentjaris & r245
1L distal: *Dia2* - 12.6 - *Acp4* --Wendel & r682
Cat2 cDNA: BS --Redinbaugh & r521
UMC107 - 6.9 - *Kn1* - 8 - *BNL7.25, UMC37* - 35 - *Kn1, BNL8.10* - 23 - *Kn1, Kn1* - 26 - *BNL8.29* --Mathern & 63:2
Kn1-2F11 clone --Veit & 63:2
wlu5 (*wl*-266A*) on 1L, not allelic to *lw1* or *w18*; - 26 - *bz2*. *w18* (*w*-495A*, allelic to *w*-571C*) on 1L, not allelic to *lw1*. *Blh1* (Bleached-1593) - 22 - T1-9c *wx1*. *Ws4* (*pg*-1589*) - 14 - T1-9(4995) *wx1*; *Ws4* - 30 - T1-9c --Neuffer 63:62
mono-1 characteristics --Weber 63:100
Glb1 (replaces *Prot1*): cDNA, genomic clones --Belanger & 63:115
w-8345* unc. by TB-1Sb, allelic *w*-4889* & *w*-013-3* & *w*-8254*. *w*-8054* unc. by TB-1La, not allelic *w*-4791* or *w*-oro6577*. *w*-018-3* not unc. by TB-1La or TB-1Sb, not allelic *w*-4889*. *w*-4791* unc. by TB-1Lb(?). *w*-oro6577* unc. by TB-1La. *v*-j(str)5688* not allelic *v*-055-4* or *v*-8983* or *f1*. *v22* (*v*-8983*) unc. by TB-1La, allelic *v*-055-4*. *v*-8943* unc. by TB-1Sb. *rd1* - 10 - *f1, rd1* - 9 - *gs1, rd1* - 9 - *bm2* (F2 repulsion). *rs2* - 35 - *ms17, rs2* - 0 - *as1* (F2 repulsion). *id1* - 4 - *br1, id1* - 0 - *an1, id1* - 3 - *gs1, id1* - 11 - *bm2* (F2 repulsion). *yelgr*-4484* in chrom. 1 (E. G. Anderson) --Derived from Stock Center notes of 1972.

CHROMOSOME 2
NPI239 - *BNL8.45* - *NPI421A*(unc. by TB-3La-2S) - *B1* - *NPI269A* - *BNL12.36* - *BNL8.04* - *BNL10.42* - *NPI271* - *BNL12.09* - *NPI456*(unc. by TB-1Sb-2L) - *Dia1* - *BNL6.20* - *NPI298* - *BNL17.03-C2*[*C2(BNL17.03)*] --Burr & r56
duplicate *NPI* loci, chroms. 2&1: *32&82, 273&109, 242&243, 244&243, 340&246.* 2&2: *242&244.* 2&3: *273&358.* 2&4: *11&317, 294&359, Ssu2&Ssu1.* 2&5: *329&115.* 2&6: *294&361, 340&245.* 2&7: *4&5, 46&35, 49&44, 118&113, 123&47, 210&59, 221&216, 273&335, 337&45, 352&353, 367&30, 405&277, Alr1&Alr2.* 2&10: *254&350, 320&321, 365&22* --Helentjaris & r245
Tripsacum segment (*Ws3 Lg1 Gl2*) homologies --Maguire r373
Rf4 and other restorer factors for cms-C --Vidakovic r658
Dia1 - 22.2 - *B1* --Wendel & r682
rDNA5S of maize lines & varieties, teosintes, *Tripsacum*s: RM --Zimmer & r726
B1 genomic sequence, RM --Chandler & 63:77
mono-2 characteristics --Weber 63:100
TB-2Sa uncovers *B1* --Echt & 63:114
restorers of cms-S (*Rf3*): recombination with T2-9d *wx1* varies from 8.3% to independence, within and between sources --Laughnan & 63:120,121
w3 & *wt** & *v4* unc. by TB-1Sb-2L*A*464. *w*-4670* unc. by TB-3La-2S6270, not by TB-1Sb-2L*A*464, not allelic *w3*. *v*-5537* not allelic *v4*; *wx* T2-9b - 33 - *v*, lg1* - 49 - *v*, gl2* - 38 - *v*, gs2* - 50 - *v**. *ws3* allelic *v*-7752* & *v*-8945* & *v*-8949* & *v*-8991*. *ba2* - 45 - *gs2*. --Derived from Stock Center notes of 1972.

CHROMOSOME 3
E8 - *BNL8.15* - *BNL8.35* - *Tpi4* - *BNL6.06* - *Pgd2* - *BNL5.37* - *BNL8.01* - *BNL10.24A* - *BNL15.20* - *BNL6.16* - *BNL3.18* - *BNL1.297* - *Mdh3* - *BNLA1*[*A1(BNLA1)*] - *NPI425* --Burr & r56
wx1 T3-9c associated with high-pH tolerance --Champoux & r75
duplicate *NPI* loci, chroms. 3&1: *358&109.* 3&2: *358&273.* 3&5: *Tpi4&Tpi5, 249&341.* 3&6: *Me1&Me2.* 3&7: *358&335.* 3&8: *52&328, 70&69, 108&204, 201&3, 202&114, 218&218, 338&339, 364&276, 425&426, Tpi4&Tpi3.* 3&9: *89&14* --Helentjaris & r245
Cin4 elements: in *A1*, *Cin4-1*; BS, ORFs --Schwarz-Sommer & r570
brn1 - 7 - *cr1* --Stinard 63:9
T1-3(5597) - 18 - *a1*; T2-3d - 18 - *a1* --Chang & 63:21
Wi2 (Wilted-1540) - 11 - T3-9c *wx1* --Neuffer 63:63
mono-3 & TB-3La hypoploid characteristics --Weber 63:100
Cg1 - 21 - *Wrk1* - 2 - *Lg3* --Poethig 63:101
w-8905* prob. not unc. by TB-3La, not allelic *cl1*. *w*-022-15* prob. not unc. by TB-3La, not allelic *cl1* or *w*-8336*. *w*-8336* not unc. by TB-3La. *v*-8623* (may be lethal) not unc. by TB-3Sb, not allelic *v*-8959* or *v*-8995*. *v*-8995* not unc. by TB-3La. *v*-8959* unc. by TB-3La, allelic *v*-9003* & *v*-8609* & *v*-8630*. *hook-br* unc. by TB-3Sb. *yg*-W23* unc. by TB-3La. *yel*-5787* unc. by TB-3La, allelic *yel*-8630*. *Lg3* - 2 - *ys3*. --Derived from Stock Center notes of 1972.

CHROMOSOME 4
AGR115r - *BNLAdh2*[*Adh2(BNLAdh2)*] - *BNL5.46* - *Aco1* - *BNL15.27* - *BNL15.45* - *BNL7.65*(unc. by TB-9Sb-4L6504) - *BNL10.05* - *BNL17.05-rbcS*[*rbcS(BNL17.05-rbcS)*] - *BNLC2*[*C2(BNLC2)*] - *BNL5.67* - *BNL8.23* - *BNL15.07* - *NPI451* --Burr & r56
wx1 T4-9g associated with high-pH tolerance --Champoux & r75
duplicate *NPI* loci, chroms. 4&1: *Adh2&Adh1, 95&96, 84&36.* 4&2: *317&11, 359&294, Ssu1&Ssu2.* 4&5: *203&116, 292&363.* 4&6: *6&7, 359&361.* 4&7: *95&316.* 4&9: *208&25.* 4&10: *208&366* --Helentjaris & r245
Aco1 - 6.2 - *su1* --Wendel & r682
T1-4(8602) - 3.5 - *c2*; T4-6(033-16) - 20 - *c2* --Chang & 63:21
Ph1 - 11 - *Ri1* - 31 - *su1* - 4 - *gl3* by allometric analysis --Miranda & 63:49
mono-4 & TB-4L hypoploid characteristics --Weber 63:100

gl16 allelic *gl4*. *o1* not unc. by TB-4Sa, *o1* - 30 - *Ts5*, *o1*- 0 - *gl3*. *nec*-016-15* allelic *nec*-rd*, not unc. by TB-4Sa. *v23* (*v*-8914*) unc. by TB-4Sa, *v23* - 47 - *Ts5*, *v23* - 6 - *su1*, *v23* - 24 - *zb6*, *v23* - 43 - *gl3*. *ra3* - 46 - *gl3*, *ra3* - 41 - *su1*, *ra3* - 48 - *zb6*. --Derived from Stock Center notes of 1972.

CHROMOSOME 5
BNL8.33 - *NPI409* - *BNL6.25* - *Pgm2* - *BNL7.56* - *BNL5.02* - *BNL6.10* - *BNL6.22* - *Mdh5* - *BNL1.30* - *BNL10.06* - *BNL7.43* - *BNL7.71* - *BNL4.36* - *Amp3* - *BNL5.71* - *BNL10.12L* - *BNL5.40* - *Got2* - *BNL5.24*(unc. by TB-5La) --Burr & r56
duplicate *NPI* loci, chroms. 5&1: *75&226*, *Phy2&Phy1*, *282&357*. 5&2: *115&329*. 5&3: *Tpi5&Tpi4*, *341&249*. 5&4: *116&203*, *363&292*. 5&6: *53&302*. 5&8: *Tpi3&Tpi5* --Helentjaris & r245
Cat1 cDNA: RM, BS --Redinbaugh & r521
T2-5(032-9) - <15 - *a2*; T4-5e - <15 - *a2* --Chang & 63:21
hcf108 unc. by TB-5Sb --Cook & 63:65
mono-5 characteristics --Weber 63:100
NPI288: BS --Helentjaris & 63:109
sh4 - 1.3 - *pr1* --Echt 63:113
v-6373* not allelic *v12* or *v2* or *v*-8982*. *v*-8982* allelic *v3*. *w*-021-7* not unc. by TB-5La. *nec*-5-9-5614* allelic *yelgrnec*-6853* & *nec*-7476*; not allelic *nec*-8624* (may not be in chr. 5) or *nec*-8376* or *yelnec*-7281* or *yg*-8951*. *yelnec*-8549* allelic *yelnec*-7281* & *yelnec*-5931*, not allelic *nec*-8376*. *br3* - 25 - T5-9d *wx1*. *zb3* - 44 - *a2*, *zb3* - 41 - *bm1*, *zb3* - 33 - *pr1*, *zb3* - 0 - *v2*. *gl8* - 50 - *a2*, *gl8* - 43 - *bm1*, *gl8* - 15 - *pr1*, *gl8* - 46 - *v2*. --Derived from Stock Center notes of 1972.

CHROMOSOME 6
BNL6.29 - NOR - *NPI235* - *Pgd1* - *Enp1* - *Pl1* - *NPI223* - *Hex2* - *BNL3.03* - *BNL15.37* - *BNL5.47* - *Idh2* - *Mdh2* --Burr & r56
duplicate *NPI* loci, chroms. 6&1: *63&205*, *245&246*, *102&205*. 6&2: *361&294*, *245&340*. 6&3: *Me2&Me1*. 6&4: *7&6*, *361&359*. 6&5: *302&53*. 6&6: *63&102*. 6&8: *2&1*, *9&206*, *63&301*, *101&103*, *102&301*, *Pdk1&Pdk2*. 6&9: *418&416* --Helentjaris & r245
NOR: rDNA17S, rDNA5.8S, rDNA26S: RM --Phillips & r492
6S: *Adk1* - 8.1 - *rgd1* --Wendel & r682
rDNA18S, rDNA5.8S, rDNA26S of maize lines & varieties, teosintes, *Tripsacums*: RM --Zimmer & r726
sr4 (*stp*-65A*) on 6L --Neuffer 63:62
mono-6 characteristics --Weber 63:100
w14 (*w*-8657*) allelic *w*-025-12* & *w*-035-2* & *w*-5946* & *w*-8050* & *w*-6853* & *w*-1-74302*, not allelic *w15* or *w*-8963* or *w*-8954* or *w*-8624*. *w1* allelic *w*-7366*, not to *w*-8624* or *w*-8954* or *w*-8963*. *w15* (*w*-8896*) not allelic *w*-8626* or *w*-8363* or *w*-8954*. *l10* not allelic *l12* or *yel*-4-6-4447* or *yel*-8631* or *yel*-7285* or *yel*-039-13*. *yel*-8631* not allelic *yel*-7285* or *yel*-039-13* or *yel*-4-6-4447*. *yel*-039-13* not allelic *yel*-7285* or *yel*-4-6-4447*. *pg11 pg12* allelic *pg*-6656* & *pg*-48-040-8* & *yg*-6853*. --Derived from Stock Center notes of 1972.

CHROMOSOME 7
NPI400 - *BNLO2*[*O2*(*BNLO2*)] - *BNL15.40*(unc. by TB-7Lb) - *BNLZpB36*[*ZpB36*(*BNLZpB36*)] - *BNL15.21* - *BNL4.24* - *BNL13.24* - *NPI283* - *BNL8.32* - *BNL8.21* - *BNL8.37* - *BNL14.07* - *BNL7.61* - *BNL8.39* - *NPI113* - *BNL16.06* - *BNL8.44* --Burr & r56
hcf104, *Mu*-elicited; characterization --Cook & r95
duplicate *NPI* loci, chroms. 7&1: *316&96*, *335&109*, *Sod2&Sod4*. 7&2: *5&4*, *35&46*, *44&49*, *113&118*, *47&123*, *59&210*, *216&221*, *335&273*, *45&337*, *353&352*, *30&367*, *277&405*, *Alr2&Alr1*. 7&3: *335&358*. 7&4: *316&95* --Helentjaris & r245
o2-m5 clone: RM --Motto & r424
hcf103 (allelic to *hcf114*), *hcf104*, *hcf111*, & *gl*-1258* (allelic to *gl*-1253-6*) unc. by TB-7Lb --Cook & 63:65
mono-7 and TB-7Sb hypoploid characteristics --Weber 63:100
gl1 - 11 - *Tp1* - 3 - *sl1* --Poethig 63:101
v-8647* not unc. by TB-7Lb, not allelic *v5*. *yel*-7748* unc. by *TB-7Lb*. *str*-2-7C44* allelic *ij1*. *ts-br** - 0 - *bd1* (may be allele of *bd1*). *yel*-7748* not close enough to *ra1*, *gl2*, *ij1*, or *bd1* to affect F2 ratios. --Derived from Stock Center notes of 1972.

CHROMOSOME 8
NPI220 - *BNL13.05* - *BNL9.11* - *Mdh1* - *BNL10.39* - *BNL9.44L* - *BNL1.45* - *BNL9.08* - *BNL7.08* - *BNLAct1*[*Act1*(*BNLAct1*)] - *BNL2.369* - *BNL8.26* - *BNL12.30* - *BNL17.01* - *Idh1* - *BNL10.24B* - *NPI268* - *BNL10.12U* - *NPI414* --Burr & r56
wx1 T8-9d associated with high-pH tolerance --Champoux & r75
pro1 (*o6*): b-32 protein, cDNA & genomic clones, BS --DiFonzo & r138; Hartings & 63:29
duplicate *NPI* loci, chroms. 8&1: *301&205*. 8&3: *328&52*, *69&70*, *204&108*, *3&201*, *114&202*, *218&219*, *339&338*, *276&364*, *426&425*, *Tpi3&Tpi4*. 8&5: *Tpi3&Tpi5*. 8&6: *1&2*, *206&9*, *301&63*, *103&101*, *301&102*, *Pdk2&Pdk1* --Helentjaris & r245
Bif1 - 20 - *Sdw1* --England & Neuffer 63:61, 63:63
hcf102 unc. by TB-8Lc --Cook & 63:65
mono-8 characteristics --Weber 63:100
gl-g* unc. by TB-8La, allelic *gl*-5249*. *w*-017-14* not unc. by TB-8La, allelic *w*-8925* & *w*-034-16* & *w*-053-4* & *w*-8635* & *w*-8963*, not close enough to *j1* or *ms8* to affect F2 ratios. *v*-6661* allelic *v16*. *yel*-024-5* unc. by TB-8La, affects ratios of *j1* & *ms8* in F2 repulsion. *nec1* (*nec*-6697*) not unc. by TB-8La, allelic *sienna*-7748*, *nec*-025-4*, affects ratios of *j1* and *ms8* in F2 repulsion. --Derived from Stock Center notes of 1972.

CHROMOSOME 9
NPI253 - *BNLC1*[*C1*(*BNLC1*)] - *BNLSh1*[*Sh1*(*BNLSh1*)] - *BNLBz1*[*Bz1*(*BNLBz1*)] - *BNL3.06* - *BNLWx1*[*Wx1*(*BNLWx1*)] - *BNL5.10* - *Acp1* - *BNL7.13* - *BNL5.04* - *BNL8.17* - *BNLSs2*[*Ss2*(*BNLSs2*)](unc. by TB-9Lc) - *BNL5.09* - *BNL14.26* - *BNL14.28* --Burr & r56
hcf113, *Mu*-elicited; characterization --Cook & r95
sucrose synthase-2 (*Sus2*): cDNA,RM --Gupta & r229
sh1 upstream region: DNaseI hypersensitive sites; micrococcal nuclease & S1 nuclease sensitive sites --Frommer & r187, Werr & r683
duplicate *NPI* loci, chroms. 9&1: *209&93*, *97&99*, *98&225*, *403&404*, *427&428*. 9&3: *14&89*. 9&4: *25&208*. 9&6: *416&418*. 9&9: *Css1&Sh1*. 9&10: *25&366* --Helentjaris & r245
Bz1-McC, *Bz1-W22*, *bz1-R*: BS --Ralston & r515
Bz1-wm, *bz1-m2* RM; *Ds1 Ac* inserts BS --Schiefelbein & r562
Wx1-Hy, *Wx1-W23* upstream: BS, RM --Spell & r614
T6-9(5454) - 39 - *wx1* --Chang & 63:21
hcf113 unc. by TB-9Sb --Cook & 63:65

mono-9 & TB-9Lc hypoploid characteristics --Weber 63:100
w11 not unc. by TB-9Sb or TB-9Lc, allelic *w*-8951*, not allelic *w*-9000* or *w*-8889* or *w*-034-15* or *w*-8950*, *w11* - 6 - *wx1*. *w*-8950* not unc. by TB-9Sb, not allelic *w*-034-15* or *w*-9000* or *w*-8889*. *w*-8889* distal to TB-9Lc, not allelic *w*-034-15* or *w*-9000*. *w*-034-5* not unc. by TB-9Sb or TB-9Lc, not allelic *w*-8950*. *l6* unc. by TB-9Sb, not allelic *yg2*. *l7* unc. by TB-9Sb, not allelic *yg2*. *yel*-034-16* unc. by TB-9Lc, not allelic *l7*. *yg*-5588* unc. by TB-9Sb. *pg11 pg12* allelic *pg*-8925* & *yg*-8563* & *yg*-8622* & *yg*-8322* & *yg*-4484* & *yg*-8962*. --Derived from Stock Center notes of 1972.

CHROMOSOME 10
BNL10.17 - *BNL3.04* - *NPI285* - *Glu1* - *NPI264* - *NPI269B* - *BNL10.13* - *BNL17.07*(unc. by TB-10L19) - *BNL17.02* - *BNL7.49* - *NPI306* --Burr & r56
wx1 T9-10b associated with high-pH tolerance --Champoux & r75
r1-nj:1, RM --Dellaporta & r127
Bz1-McC, *Bz1-McC2*, *Bz1-W22* and upstream: BS --Furtek & r191
duplicate *NPI* loci, chroms. 10&2: *350&254*, *321&320*, *22&365*. 10&4: *366&208*. 10&9: *366&25* --Helentjaris & r245
rust (*Puccinia sorghi*) R in IL677a single-gene recessive, *rp*-677a* --Kim & r306
bz1-m4D6856 and *Ds* insert, RM, BS --Klein & r311
TB-10La substitutions, heterosis for yield; tassel-branch number & kernel weight affected --Lamkey & r337
Glu1 - <1 - *Sad1* --Wendel & r682
mono-10 characteristics --Weber 63:100
w-9000* not unc. by TB-10La, not allelic *w2*. *v*-8574* unc. by TB-10La, not allelic *v18*. *yel*-8793* not unc. by TB-10La, allelic *yel*-8962* & *yel*-5344* & *yel*-8454*. *yel*-8721* not unc. by TB-10La, not allelic *yel*-8454* or *yel*-8962*. *yg*-8574* unc. by TB-10La, not allelic *oy1*. --Derived from Stock Center notes of 1972.

UNPLACED
hcf103 hcf105 hcf108 hcf109 hcf110 hcf111 hcf112 hcf113 Mu-elicited; characterizations --Cook & r95
Cat3 cDNA: RM, BS --Redinbaugh & r521
betaine deficiency, recessive --Rhodes & r531
Sod3 cDNA: RM, BS --White & r690
Dee-m857345*, *Dee*-m857513*: defective endosperm mutables --Pan & 63:18
Wrp1: dominant dwarf, wrinkled-corrugated leaves --Bockholt & 63:56
id-A972*: indeterminate growth habit; *idd*-2286*: indeterminate dwarf. *gl22* (*gl*-478C*) duplicate with *gl21*. *Fbr1* (Few-branched-1602). --Neuffer & 63:62
lbl1: leaf bladeless --Miles 63:66

B CHROMOSOME
Mu-hybridizing sequences --Patrosso & r473
region 4 (centromere plus short arm) nondisjunctional control --Carlson 63:81

TRANSPOSABLE ELEMENTS (see also specific loci affected)
Mu1.4-B37, *Mu4*, *Mu5*, *MRS-A* (internal) RM, BS, methylation vs. active *Mu* --Chandler & r77 r78
Uq distribution in populations and inbreds --Cormack & r98
Ac transcript, cDNA, 4 introns --Finnegan & r177
Ds1-related sequences *Ds101*, *Ds103*, *Ds105*, *Ds123*, *Ds130*, *Ds132*, *bz1-wm*, *wx1-m1*: BS --Gerlach & r203
Ds1 in *Bz1-wm*, BS; vs. *Adh1-Fm335* & *wx1-m1* --Schiefelbein & r562
Cin4 elements *Cin4-1*, *-15*, *-23*, *-151*, *-162*, *-198*, *-232* --Schwarz-Sommer & r570
MRS-A (*Mu*-related sequence): RM, BS --Talbert & r630
Ac termini 3' & 5': BS --Zhou & 63:5

NUCLEAR cDNAs, GENOMIC CLONES, AND PROBES
autonomously replicating sequences (ARS) via yeast: two families, repetitive, dispersed; RM --Berlani & r34, r35
glyceraldehyde-3-phosphate (GAPDHase) dehydrogenases, chloroplast vs. cytosolic polypeptides, cDNAs, BS --Brinkmann & r48; Quigley & r512
zein19kDa, zein21kDa upstream promoters --Brown & r53
nitrate reductase (NRase, NADH:NRase) cDNA, BS --Gowri & r217
aldolase, BS --Dennis & r131
Arabidopsis telomere clone pAtT4, cross-hybridization --Richards & r532
glutelin-2 28kDa cDNA, RM --Gallardo & r197
phosphoproteins of embryogenesis, 23-25kDa, ABA-induced: cDNA --Goday & r211
glutathione S-transferase I, III (GSH-S-tr) cDNAs, BS --Grove & r225
Helminthosporium maydis R selection, haploid embryonic clones --Guo & r228
ear rot (*Fusarium*) R, selection advance --Jovicevic & r280, Sultan & r626
chemical mutagens, agronomic variability --Khristova r304
zein10kDa cDNA, BS --Kirihara & r310
nitrite reductase (NiRase) cDNA, BS --Lahners & r335
Ant1, adenine nucleotide translocator, cDNA, BS (Baker & Leaver, Nucl. Ac. Res. 13:5857, 1985) --Lonsdale r358
zein19kDa cDNA, upstream RM, BS --Maier & r377; Roussell & r543
pyruvate,orthophosphate dikinase (PPDKase) cDNA, BS --Matsuoka & r390
glutamine synthetase (GSase; gln-synthase) (*E. coli gln* rescue) cDNA, BS --Snustad & r609
proline-rich cell wall glycoprotein family cDNA, BS --Stiefel & r621
phospholipid transfer protein (PLTP) cDNA, BS --Tchang & r633
lipid-body protein L3 cDNA --Vance & r650
zein19kDa constructs, BS --Wallace & r672
phosphoenolpyruvate carboxylase (PEPcase) cDNA, BS --Yanagisawa & r714
anaerobic protein (ANP31) 31kDa, cDNA --Kammerer & 63:38
zein-associated protein (ZAP27kDa), cDNA: RM --Chen & 63:102
ubiquitin cDNA: BS --Chen & 63:102

185bp knob sequence hybridization vs. maturity --Phillips & 63:103

CHLOROPLAST
ctDNA-23S, -4.5S, -5S, -R: BS --Fejes & r173
ctDNA-tRNAproUGG, -tRNAtrpCCA: BS --Lukens & r367
ctDNA-rpL36 (-secX), -rpL16, -rpS8, -rpL14, -rpS11, -rpL36: BS --Markmann-Mulisch & r381,r382
ctDNA-rpl23: BS --McLaughlin & r398
ctDNA-tRNAcysGCA: BS --Meinke & r401
ctDNA-rpoA: BS --Ruf & r545
ctDNA-ndhD, -ndhE, -psaC: RM, BS --Schantz & r559
ctDNA genotypes in maizes and teosintes --Doebley & 63:108

MITOCHONDRIA
cms-S reversions in Wf9; RM alterations in S1- and S2-homologous regions and coxI --Escote-Carlson & r165
mtDNA-B37: RM --Fauron & r168
mtDNA-ct23S, -ct4.5S, -ct5S, -ctR: BS --Fejes & r173
mtDNA-S1, integrated: BS --Garcia & r199
mtDNA-S2ORF1: BS similar to T7 phage & yeast RNA polymerases --Kuzmin & r332; Oeser r451
mtDNA-coxIII: BS --McCarty & r393
mtDNA-atp9, mtDNA-coxIII;BS --Mulligan & r430
mtDNA-1.4kb: BS --Smith & r602
mtDNA-tRNAcysGCA, -tRNAserGCU, -tRNAUAA: RM, BS --Wintz & r707

RESISTANCE/TOLERANCE/QUANTITATIVE INHERITANCE ANALYSIS/GERMPLASM
seedling vigor vs. yield diallel --Ajala & r2
multi-ear vs. one-ear --Angelov r9
drying injury diallel --Bdliya & r26
smut (*Ustilago zeae*) R diallel --Balashova & r17
prolificacy vs. yield, root/stalk strength --Brotslaw & r52
sib-mated lines from S2 vs. original S2 --Carlone & r64
high-pH R (in Purple 3036-1) associated with chromosomes 2, 3, 4, 6, 8, 10 --Champoux & r75
pith cell death: gca>sca, BSH 86% --Colbert & r93
European corn borer, southern armyworm R vs. complex carbohydrates, lignin & silica --Coors r96
recurrent selection in half-sib vs. selfed families, compared per se and in testcrosses; BSH --Coors r97
leaf rust (*Puccinia sorghi* Schw.) R in PI sources --Davis & r117
leaf blight (*Drechslera maydis*) and maize-borer (*Chilo partellus*) R: selection in populations --Dey & r134
blackbird R --Dolbeer & r145
cool-emergence rate --Eagles r153
bentazon R dominant --Fleming & r178
K uptake, K utilization; inbred differences --Furlani & r190
cold tolerance selection; isozyme alleles --Gardner & r200
cold tolerance & maturity, allozyme frequencies --Guse & r230
Fusarium R, inbreds --Hart & r237
valine R (ValR) selected in culture, dominant --Hibberd r248
stalk quality; stalk rot R [*Stenocarpella maydis* (Berk.) Sutton = *Diplodia maydis* (Berk.) Sacc., *Colletotrichum graminicola* (Ces.) G. W. Wils.] in tropical germplasm adapted derivatives, inbreds and hybrids; topcross performance, stalk quality, stalk rot --Holley & r250, r251
gray leaf spot (*Cercospora zeae-maydis* Tehon & Daniels) R, gca>sca, additive --Huff & r258
cell death in leaf midribs (senescence); BSH --Kang & r290
Stewart's wilt (*Erwinia stewartii* E. F. Smith) R, diallel, gca>sca, BSH --Kang & r292
nitrate uptake, nitrate reductase, N partitioning diallel --Katsantonis & r293
iron uptake vs. yield, gca --Kovacevik r322
TB-10La substitutions, heterosis for yield; tassel-branch number, kernel weight --Lamkey & r337
testcrosses of F2 vs. BC and predicted selection response --Melchinger & r402
lysine-threonine R (Ltr*) selected in culture; dominant --Miao & r410
NO_3 differences in stalks of inbreds --Mollaretti & r416
flint-dent diallel gca, sca --Moreno-Gonzalez r421
stalk rot [*Gibberella zeae* (Schw.) Petch] R selection --Mostafa & r422
populations, corn belt & exotic, heterotic patterns --Mungoma & r432
European corn borer (*Ostrinia nubilalis* Hbn.) 2d brood R in populations --Ordas & r458
anther culture response, selection increase --Petolino & r490
stability statistics & repeatability, genotype x environment comparisons --Pham & r491
photosynthetic rate mutations --Pok & r503
recurrent selection (for yield) methods compared, intra- vs. interpopulation; 10 populations --Rodriguez & r535
nematode R (Pratylenchus zeae, P. brachyurus): BSH; two dominant additive genes --Sawazaki & r558
N uptake & utilization among hybrids --Schepers & r560
aflatoxin (*Aspergillus flavus* Link ex Fr.) R in inbreds --Scott & r573
Exserohilum turcicum lesion expansion, diallel; gca>sca --Sigulas & r590
N uptake and utilization, differences in lines --Silva r591, r592, r593
diversity in inbreds & hybrids, isozymes and protein polymorphisms --Smith r604, Smith & r605
maize weevil (*Sitophilus zeamais* Motschulsky) R & pericarp surface --Tipping & r636
yield vs. maturity, ear weight, ear length, plant height, kernels/row, 100 kernel weight; diallel --Tyagi & r645
flood tolerance, inbreds & hybrids --VanToai & r651
ear length, diallel --Vedeneev r655
kernels per ear, diallel --Vedeneev r656

head smut (ear & tassel smut) [*Sphacelotheca reiliana* (Kuhn) Clint.] R, diallel; gca; R additive & partially dominant --Whythe & r691
earworm (*Heliothis zea* Boddie) R in PI sources --Wilson r703
nematode [*Meloidogyne incognita* Kofoid & White, *M. arenaria* (Neal) Chitwood] R in inbreds, diallel --Williams & r700, Windham & r705
fall armyworm (*Spodoptera frugiperda* J. E. Smith) R in fresh foliage vs. lab bioassay --Wiseman & r708
herbicide tolerance, inbreds --Wych & r711
root lodging R, discriminant function analysis for pulling R, fresh wt., & gravity center --Yamada & r712
salt tolerance: selection in embryogenic cultures --Lupotto & 63:31
L-glufosinate (BASTA) tolerance: selection in embryogenic cultures --Lupotto & 63:31
maize dwarf mosaic virus (MDMV) R --Roane & 63:40,41,42
Angoumois moth (*Sitophilus oryzae*, *S. zeamais*) and corn weevil (*Sitotroga cerealella* Oliver) R vs. multiple aleurone --Miranda & 63:47
oil variants --Keith & 63:73
cold tolerance, S2 lines and testcrosses --Quang & 63:94
earworm R vs. silk pH & nonbrowning silks --Byrne & 63:94
chlorsulfuron R: selection in gametophyte --Sari Gorla & 63:98
sethoxydim & haloxyfop R: selection in culture --Parker & 63:104
QTLs for days to silk (chrom. 6L, 7S), yield (1L, 2L, 3L, 4L, 5S, 9L), percent harvest moisture (1cent., 4S, 4L, 5S, 6L) with RFLPs --Beckman & 63:113

--Assembled unrestricted by Prof. Ligate

IV. MAIZE GENETICS COOPERATION STOCK CENTER

During calendar 1988, 3349 seed samples were supplied in response to 215 requests (letters or phone calls). The corresponding figures for 1987 were 2270 seed samples and 184 requests. Last year was a record year both for the volume of seed samples provided and the number of requests and other inquiries. It is not yet clear whether these increased numbers represent a transition to a higher continuing level of stock distribution.

During the past summer, several hundred families were devoted to seed increase of A-A reciprocal translocations. Because most of these stocks have good agronomic quality, they resulted in generally satisfactory increases. In addition, there were extensive plantings of genetic stocks involving chromosomes 1, 2, 3 and 4 that will require repeat plantings since their reduced vigor was insufficient to cope with severe drouth conditions in spite of repeated irrigation.

In observation plantings, good pedigree information was obtained especially from testcrosses of inversions and reciprocal translocations, from allelism tests and from seedling tests in greenhouse sandbenches.

Following Dr. Fletcher's departure from the Stock Center program at the beginning of last summer, we have been operating without backup professional support. However, we expect to employ professionally and technically qualified personnel as replacement by spring. We hope this will permit us to give renewed attention to re-evaluating the individual components in the total stock collection with a view to improving the selection and quality of the inventory. In addition, we plan to seek seed increases of the numerous stocks of symbolized new genes that have been assembled in the past several years in order that these valuable items may be added to future stock listings.

Each year several dozen samples representing uncharacterized, untested, unidentified newly-acquired traits are received for potential inclusion in our stock collection. As a result, we now have numerous different phenotypic categories, each including several items, that are awaiting further evaluation and testing. We will be happy to provide initial small seed source samples to those of you who have a special interest in studying specific types of mutants and are willing to conduct the allelism testing or chromosome location required to identify new loci.

E. B. Patterson
S-116 Turner Hall
Agronomy Department
University of Illinois
1102 S. Goodwin Avenue
Urbana, IL 61801
Phone (217)-333-9641
-6631 (Lab)

CATALOG OF STOCKS

(Entries marked ** designate stocks that may be available in homozygous state.)

CHROMOSOME 1

101A *sr zb4 P-WW* **
101B *sr P-WR* **
101C *sr P-WW* **
101D *sr P-RR* **
102A *sr P-WR an gs bm2* **
102B *sr P-WR an bm2*
102C *sr P-RW ad bm2* **
103C *sr P-WR bm2* **
103D *vp5*
103G *sr P-RR bm2* **
104B *zb4 ts2 P-WW bm2*
105C *zb4 P-WW br* **
105E *ms17*
106A *zb4 P-WW bm2*
106B *ts2 P-RR*
106D *ts2 P-WW br bm2*
107A *P-CR* **
107B *P-RR* **
107C *P-RW* **
107D *P-CW* **
107E *P-MO* **
107F *P-VV* **
108C *P-RR br f an gs bm2*
109A *P-RR an ad bm2*
109B *P-RR an gs bm2* **
109D *P-RR ad bm2* **
109E *P-WR br f* **
110A *P-WR an Kn bm2*
110B *P-WR an Kn*
110C *P-WR an ad1 bm2*
110D *P-WR an bm2*
110E *P-WR ad bm2*
110F *P-WR br Vg*
110G *P-WR br f gs bm2*
110H *P-WR br f bm2* **
111A *P-WW rs2*
111B *P-WW rs2 br*
111C *P-WW as br f bm2*
111D *P-WW hm br f*
112B *P-WW br f bm2* **
112E *as*
112H *P-WW br* **
113A *as br2*
113B *rd*
113C *br f* **
113E *br f Kn*
114A *br f Kn Ts6*
114B *br f Kn bm2*
114D *Vg*
115A *Vg an bm2*
115B *Vg br2 bm2*
115D *bz2-m, m; A A2 C Pr* **
116A *bz2-m, M; A A2 C R Pr*
116C *an bm2*
116D *an-bz2-6923 (Df)*
116E *an br*
117A *br2* **
117B *br2 bm2* **
117D *tb-8963* **
117E *Kn*
118A *Kn Ts6*
118B *Kn bm2*
118D *Kn Ts6 bm2*
119C *gs* **
119D *gs bm2* **
119F *bm2* **
120B *nec2*
121A *ms14*
121B *mi*
121C *D8*
121D *lls*
121E tiny
122A TB-1La
122B TB-1Sb
125A *Les2*

CHROMOSOME 2

201B *ws3 lg gl2 B sk*
201E *ws3 lg gl2 B ts*
201F *ws3 lg gl2 b* **
203B *al* **
203D *al lg*
204F *al lg gl2 B sk*
205B *lg* **
205C *lg gl2* **
206A *lg gl2 B* **
206B *lg gl2 B gs2* **
208B *lg gl2 B sk*
208C *lg gl2 B sk v4*
208D *lg gl2 B v4*
208E *lg gl2 b* **
208F *lg gl2 b gs2* **
208G *lg gl2 b gs2 Ch* **
208H *gl2* **
209E *lg gl2 b sk*
209F *lg gl2 b sk fl*
209G *lg gl2 sk fl v4*
210A *lg gl2 b sk v4*
211A *lg gl2 b fl* **
212A *lg gl2 b wt v4* **
212B *lg gl2 b fl v4* **
212C *lg gl2 b fl v4 Ch* **
212D *lg gl2 b v4*
212E *lg gl2 b v4 Ch* **
213B *lg gl2 wt* **
213C *lg gl2 w3*
213D *lg gl2 w3 Ch*
213E *lg gl2 b Ch* **
214C *d5*
214D *B gl11*
214E *B ts*
215A *gl14*
215B *gl11* **
215C *ut*

215E *fl* **
216B *fl v4 Ht*
216D *fl w3*
216E *fl v4 w3*
216F *fl w3 Ch*
216G *fl v4 w3 Ch*
217A *ts*
217B *v4* **
217D *v4 Ht Ch*
217E *w3 Ht Ch*
218A *w3*
218C *w3 Ch*
218D *Ht* (source A and B) **
218E *ba2*
219A *B-Peru ("R2"); r A A2 C*
219B *b ("r2"); r-g A A2 C* **
219C *Ch*
220A *Les*
220B 2 2T T2 (T=*Tripsacum*)
221A *gs2* **
222A TB-1Sb-2L 4464
222B TB-3La-2S 6270
223A Primary Trisomic 2
224A *w*-4670*
224B *v*-5537* **
224F *w*-062-3*
224G *yel *-8630*
224H *whp*

CHROMOSOME 3

301A *cr* **
301B *cr d* **
301C *cr d Lg3*
302A *d=d-6016* (rosette)
302B *d rt*
302E *d* (tall) **
303A *d rt Lg3*
303B *d Rf lg2* **
303F *g2=v19=g5=pg14* **
304B *d ys3 Rg*
305A *d Lg3*
305D *d Rg*
306A *d Rg ts4 lg2*
306D *d Rg ts4*
307A *d pm* **
308B *d ts4* **
308C *d lg2 a-m; A2 C R Dt*
308D *d a-m; A2 C R Dt* **
308G *d ts4 a-m; A2 C R Dt*
309A *ra2 Rg ts4 lg2*
309B *ra2 ys3 Lg3 Rg*
309D *ra2 Rg lg2*
309E *ra2 pm lg2* **
309F *ra2 Lg3 ys3*
310A *ra2 ts4* **
310C *ra2 lg2* **
310D *Cg*
311A *cl*
311B *cl ; Clm2*
311C *cl; Clm3* **
311D *cl-p; Clm4* **
311E *rt* **
311F *ys3* **
311G *ys3 Lg3*
312C *ys3 ts4 lg2*
312D *Lg3* **
316A *ts4* **
316B *ts4 na*
317A *ts4 na pm*
317C *ts4 lg2 a-m A2 C R Dt*
318A *ig* **
318B *ba*
318C *w*-7748=y10*
319A *lg2 A-b et; A2 C R Dt* **
319C *lg2 a-m et; A2 C R dt* **
319D *lg2 a-m et; A2 C R Dt* **
319F *lg2 a-st et; A2 C R Dt* **
320A *lg2* **
320C *na lg2*
320D *A sh2; A2 C R B Pl dt* **
321A *A-d31; A2 C R* **
322E *a-m; A2 C R B Pl dt* **
322F *a-m; A2 C R b pl dt* **
323A *a-m; A2 C R Dt* **
323B *a-m; A2 C R B Pl Dt* **
323C *a-m sh2; A2 C R B Pl dt*
323D *a-m sh2; A2 C R B Pl Dt*
323E *a-m et; A2 C R Dt* **
324A *a-st; A2 C R Dt* **
324B *a-st sh2; A2 C R Dt* **
324E *a-st et; A2 C R Dt* **
324G *a-st; A2 C R dt* **
325A *a-p et; A2 C R dt* **
325B *a-p et; A2 C R B Pl Dt* **
325C *a-x1*
325D *a-x3*
325G *a3* **
326B *up*
326C *Rp3* **
326D *te* **
327A TB-3La
327B TB-3Sb
328A Primary Trisomic 3
330A *h* **
331E TB-3Lf
331F TB-3Lg
331K TB-3Ll

CHROMOSOME 4

401D *Ga-S* **
401E *Ga-S; y*
402B *st Ts5*
402D *Ts5*
403A *Ts5 fl2*
403B *Ts5 su*
403C *Ts5 la su gl3*
405B *la*
406C *fl2* **
406D *fl2 su* **
407D *su* **
407E *su-am* **
407F *su-am du*
408B *su bm3* **
408C *su zb6* **
409A *su zb6 Tu* **
410D *su zb6 gl3* **
411A *su gl4 j2*
412E *su j2 gl3* **
414C *gl4 o* **
412B *su j2*
412C *su gl3* **
413A *su o* **
413B *su gl4* **
414A *bt2* **
408E *bm3* **
415A *j2*
415B *j2 c2; A A2 C R* **
415C *j2 C2; A A2 C R*
416A *Tu*
416B *Tu-l* 1st
416C *Tu-l* 2nd
416D *Tu-d*
416E *Tu-md*
416F *Tu gl3*
417A *j2 gl3*
417B *v8* **
417C *gl3*
417D *gl3 o*
418A *gl3 dp* **
418B *c2; A A2 C R* **
418C *C2; A A2 C R* **
418E *dp* **
418F *o* **
418G *v17* **
419A *v23*
419B *su gl3 ra3*
419F *Dt6 gl3; a1* **
420B TB-9Sb-4L6504
420I TB-9Sb-4L6222
421A TB-4Sa
421C TB-7Lb-4L4698
422A Primary Trisomic 4
423A TB-4Lb
423B TB-4Lc
423C TB-4Ld
423D TB-4Le
423E TB-4Lf

CHROMOSOME 5

501A *am a2; A A2 C R*
501B *lu* **
501C *lu sh4*
501D *ms13*
501K *gl17 v3*
502A *gl17 a2 bt v2; A C R*
502B *A2 vp7=ps pr; A C R*
502C *A2 bm bt pr ys; A C R*
502D *A2 bm pr; A C R*
503A *A2 bm pr ys; A C R* **
503D *A2 bt v3 pr; A C R*
504A *A2 bt pr; A C R*
506A *A2 v3 pr; A C R* **
506B *A2 pr; A C R* **
506C *A2 pr v2; A C R*
506D *A2 pr na2; A C R*
505B *A2 pr ys; A C R* **
506F *A2 pr v12; A C R* **
506L *A2 pr br3; A C R*
507A *a2; A C R* **
508A *a2 bm bt pr; A C R* **
508B *a2 bm bt pr ys; A C R*
510A *a2 bm pr v2; A C R* **
510B *A2 bm pr eg; A C R* **
511A *a2 bt v3 pr; A C R*
511B *a2 bt v3 Pr; A C R*
511C *a2 bt pr; A C R* **
512A *a2 bt v2; A C R*
512B *a2 v3 pr; A C R*
513A *a2 pr; A C R* **
513B *a2 pr; A C R B Pl*
513C *a2 pr v2; A C R*
515A *vp2*
515C *vp7=ps*
516A *bm yg; Ch*
516B *bt* **
516G *bm pr yg; A C R*
517A *v3* **
517B *ae* **
516D *td ae*
518B *gl8* **
518C *na2*
518D *lw2*
518F *sh4 v2*
519A *ys* **
519B *eg* **
519C *v2*
519D *yg* **
519E *pr yg; A C R* **
520B *v12* **
520C *br3* **
521A *nec3*
522A TB-5La
522B TB-5Lb
522C TB-5Sc
523A Primary Trisomic 5

CHROMOSOME 6

601A *rgd po y*
601B *rgd po Y*
601C *rgd y*
601D *rgd Y*
601E *po = ms6*
601F *po y pl*
601G *po y Pl*
602A *po y wi*
602C *y = pb = w*-n* **
602D *y rhm* **
602E *po y wi pl*
603E *y pb4*
604A *y pb4 pl* **
604B *y pb4 Pl*
604H *y ms*
605A *y wi Pl*
605C *y pg11 ; Wx pg12*
605D *y pg11 wi ; wx pg12*
605E *Y wi Pl* **
605F *Y wi pl* **
606A *Y pg11; Wx pg12*
606B *y pg11 ; wx pg12* **
606C *Y pg11 ; wx pg12*
606D *y pg11 su2 ; wx pg12*
606E *y pl*
606F *y Pl*
607A *y Pl Bh ; c sh wx A A2 R* **
607B *y pl Bh ; c sh wx A A2 R* **
607C *y su2* **
608A *y l10*
608B *Y l12*
609A *Y pb4* **
609B *Y wi pl* **
609C *Y wi Pl* **
609D *Y su2* **
610A *wi*
610B *Pl Dt2 ; a-m A2 C R* **
610C *pl sm ; P-RR* **
611A *Pl sm ; P-RR* **
611B *Pl sm py ; P-RR*
611D *Pt*
611F *Pl sm Pt;P-RR*
612B *ms6*
612C *l*-4923*
612D *oro*
613A 2NOR ; *a2 bm pr v2*
614A TB-6Lb
614B TB-6Sa
614C TB-6Lc
615A Primary Trisomic 6

CHROMOSOME 7

701A *Hs o2 v5 ra gl*
701B *In-D*
701C *In-D gl*
701D *o2* **
702A *o2 v5* **
702B *o2 v5 ra gl*
702C *o2 v5 ra gl sl*
702D *o2 v5 ra gl Tp*
702E *o2 v5 ra gl ij*
703A *o2 v5 gl* **
703D *o2 ra gl*
704A *o2 ra gl ij*
704B *o2 ra gl sl*
705A *o2 gl*
705B *o2 gl sl* **
705C *o2 ij*
705D *o2 bd*
706A *o2 sl*
706B *o2 ij bd*
707A *y8 v5 gl*
707B *in ; A2 pr A C R*
707C *in gl ; A2 pr A C R* **
707D *v5* **
707E *up9*
707F *y8 gl* **
708A *ra*
709A *gl* **
710A *gl Tp*
710B *gl mn2* **
710E *gl o5 = pg**
710H *ms7 gl Tp*
711A *Tp*
711B *ij* **
711C *gl sl va*
712A *ms7*
712B *ms7 Tp*
713A *Bn* **
713B *bd*
714A *Pn*
714B *o5* **
714C *o5 mn2 gl*
714D *va*
715A *Dt3; a-m A2 C R*
716A *v*-8647*
716B *yel*-7748*
717A TB-7Lb
718A Primary Trisomic 7

CHROMOSOME 8

801A *gl18* **
801B *v16* **
801D *v16 ms8 j*
801F *v16 j gl18* **
801G *v16 gl18*
802A *v16 ms8 j gl18*
803A *ms8*
803B *nec*
803C *ms8 j gl18*
804A *v21* **
805B *nec v21*
805C *gl18 v21*
805D *fl3 j ms8*
806A TB-8La
806B TB-8Lb
807A Primary Trisomic 8
809A TB-8Lc

CHROMOSOME 9

901B *yg2 C sh bz; A A2 R*
901C *yg2 C sh bz wx; A A2 R* **
901D *yg2 C-I sh bz wx; A A2 R*
901E *yg2 C bz wx; A A2 R* **
902A *yg2 c sh bz wx; A A2 R* **
902B *yg2 c sh wx; A A2 R* **
902C *yg2 c sh wx gl15; A A2 R* **
902D *yg2 c sh wx gl15 K-S9; A A2 R*
902E *yg2 c bz wx; A A2 R*
924A *wd-Ring C-I; A A2 R*
903A *C sh bz; A A2 R* **
903B *C sh bz wx; A A2 R* **
903D *C-I sh bz wx; A A2 R* **
904A *C sh bz wx gl15 bm4; A A2 R*
904B *C sh; A A2 R* **
904C *C sh wx; A A2 R*
904D *C wx ar; A A2 R* **
904E *C sh bz wx bm4; A A2 R* **
904F *C sh bz gl15 bm4; A A2 R* **
905A *C sh wx K-L9; A A2 R* **
905B *C sh ms2; A A2 R*
905C *C bz Wx; A A2 R* **
905E *C sh wx v; A A2 R* **
906A *C Ds wx; A A2 R Pr y* **
906B *C Ds wx; A A2 R pr Y* **
906C *C-I Ds Wx; A A2 R* **
906D *C-I; A A2 R* **
907A *C wx; A A2 R* **
907B *C wx; A A2 R B Pl* **
907C *C wx; A A2 R b Pl* **
907D *C wx; A A2 R B pl* **
907E *C-I wx; A A2 R y* **
907F *C-I wx; A A2 R y B pl* **
908A *C wx ar da; A A2 R* **
908B *C wx v; A A2 R* **
908C *C wx v; A A2 R Pl* **
908D *C wx gl15; A A2 R* **
908E *C wx gl15; A A2 R pr*
908F *C wx da;A R*
909A *C wx Bf; A A2 R* **
909B *c bz wx; A A2 R* **
909C *c sh bz wx; A A2 R y* **
909D *c sh wx; A A2 R* **
909E *c sh wx v ; A A2 R* **
909F *c sh wx gl15; A A2 R* **
909G *c sh wx ms2; A A2 R*
910D *c; A A2 R* **
911A *c wx; A A2 R y* **
911B *c wx v; A A2 R* **
911C *c wx gl15; A A2 R* **
911D *c wx Bf; A A2 R* **
912A *sh* **
912B *sh wx v* **
912E *lo2*
913A *sh wx* **
913B *sh wx v gl15; A A2 C R* **
914A *wx d3* **
915A *wx* (Other alleles from O. Nelson avail.) **
915B *wx-a* **
915D *wx pg12 bm4; y pg11*
916A *wx v* **
916C *wx bk2* **
917A *wx Bf* **
917C *v* **
917D *ms2*
917E *gl15* **
917F *d3*
918A *gl15 Bf* **
918B *gl15 bm4* **
918D *Wc* **
918F *Wx Bf* **
919A *bm4* **
919B *Bf bm4* **
919C *l6*
919D *l7*
920A *yel*-034-16*
920B *w*-4889*
920C *w*-8889*
920E *w*-8950*
920F *w*-9000*
920G Tp9 N9 N3 Df3
921A TB-9La
921B TB-9Sb
921C TB-9Lc
922A Primary Trisomic 9

CHROMOSOME 10

X01A *oy*
X01B *oy R; A A2 C* **
X01C *oy bf2* **
X01E *oy bf2 R; A A2 C* **
X02A *oy ms11*
X02D *oy du R; A A2 C* **
X02E *oy du r; A A2 C*
X02G *oy zn*
X02H *oy ms10*
X02I *oy bf2 ms10*
X03B *Og* **
X03C *Og B Pl* **
X04A *Og du R; A C R* **

X04B *ms11*
X04D *bf2* **
X04E *Og; C-I B Pl*
X05A *bf2 zn* **
X05B *bf2 li g r; A A2 C*
X05C *bf2 g R sr2; A A2 C* **
X05E *bf2 sr2*
X05F *bf2 ms10*
X06A *bf2 r sr; A A2 C* **
X06C *nl g R; A A2 C* **
X07B *nl g R sr2; A A2 C*
X07C *y9*
X07E *nl g r sr2; A C* **
X07D *nl* **
X09B *li g R; A A2 C* **
X09F *ms10*
X10A *du* **
X10D *du g r; A C R* **
X10F *zn* **
X10G *du v18* **
X11A *zn g* **
X11B *zn g R sr2; A A2 C*
X11D *Tp2 g r; A A2 C* **
X11E *g R sr2; A A2 C* **
X11F *g r; A A2 C* **
X12A *g r sr2* **
X12E *g R; A A2 C* **
X12F *g r-ch* K10; *A A2 C* **
X13A *g R-g* K10; *A A2 C*
X13B *g R-g sr2; A A2 C* **
X13D *g r-r sr2; A A2 C* **
X13F *g r-;r A C wx* **
X14A *isr r-r; A A2 C* **
X15A *isr r-r sr2; A A2 C* **
X15C *R-g; A A2 C* **
X16A *r-g sr2; A A2 C* **
X16B *r* K10; *A A2 C*
X16C *R-ch; A A2 C B Pl* **
X17A *r-g; A A2 C* **
X15D *r-ch Pl; A A2 C* **
X17B *r-r; A A2 C* **
X17C *R-mb; A A2 C* **
X17D *R-nj; A A2 C* **
X17E *R-r; A A2 C* **
X17F *R-nj* purple embryo Chase **
X18A *R-lsk; A A2 C* **
X18B *R-sk-nc-2; A A2 C* **
X18C *R-st; A A2 C* **
X18D *R-sk; A A2 C* **
X18E *R-st Mst* **
X18G *R-scm2; bz2 A A2* **
X25A *R-scm2; a-st A2 C C2* **
X25B *R-scm2; c2 A A2 C* **
X25C *R-scm122; pr A A2 C C2* **
X25D *R-scm2; a2 A C C2* **
X25E *R-scm2; c A A2 C2* **
X19A *Lc* **
X19B *u2*
X19C *w2 l*
X19D *o7* **
X20B *l* **
X20C *v18* **
X20F *yel*-8721*
X21A TB-10La
X22A TB-10Sc
X21B TB-10L19
X23A Primary Trisomic 10

UNPLACED GENES

U235A *dv*
U235B *dy*
U335A *el*
U435A *l4*
U635A *Rs*
U533A *vl3*
U935A *ws ws2*
U933A *o9*
U933B *o10*
U933C *o11*
U933D *o13*

MULTIPLE GENE STOCKS

M141B *A A2 C C2 R-g Pr B pl*
M141C *A A2 C C2 R-g b Pl*
MX17A *A A2 C C2 r-g Pr b pl*
M241B *A A2 C C2 r-g Pr B pl*
M342A *A A2 c C2 R-g Pr B pl*
M341B *A A2 C C2 R-r Pr B pl*
M341C *A A2 C C2 R-r Pr b Pl*
M441B *A A2 C C2 R-r Pr B pl wx*
M441C *A A2 C C2 R Pr*
M641A *A A2 C C2 R Pr wx*
MX41A *A A2 C C2 R pr y wx gl*
M941A *A A2 c C2 R Pr y wx*
M741A *A A2 C C2 r Pr Y wx*
MX41B *su pr y gl wx ; A A2 C C2 R*
M841A *A su pr ; A2 C C2 R*
MX41C *bz2 a c2 a2 pr Y/y c bz wx r*
M841B *a su A2 C C2 R*
MX40A *bm2 lg a su pr y gl j wx g*
M841C colored scutellum
MX41D *a su pr y gl wx A2 C C2 R*
MX40B *ts2 ; sk*
MX40C *lg gl2 wt ; a-m A2 C C2 R Dt*
M741B *A A2 C C2 R-nj* ; purple embryo S. Chase
M741C Stock 6 : Hi-haploid *R-r B Pl*

POPCORNS

P142A Amber Pearl
P142B Argentine
P142C Black Beauty
P242a Hulless
P242B Ladyfinger
P242C Ohio Yellow
P342A Red
P342B Strawberry
P342C Supergold
P342D South American
P442A Tom Thumb
P442B White Rice

EXOTICS AND VARIETIES

E542A Black Mexican Sweet Corn (with B-chromosomes)
E542B Black Mexican Sweet Corn (without B-chromosomes)
E642A Knobless Tama Flint
E642C Knobless Wilbur's Flint
E442A Gaspe Flint
E642B Gourdseed
E742A Maiz Chapalote
E942B Missouri Cob Corn
E742B Papago Flour Corn
E842A Tama Flint
E942A Winnebago Flint
E842B Zapalote Chico

TETRAPLOID STOCKS

N103A *P-RR*
N102C *a A2 C R Dt*
N104B *pr ; A A2 C R*
N105A *y*
N106C *wx*
N107A *g A A2 C R*
N107C Synthetic B
N107B W23 conversion
N104C *su wx*
N106D *sh Wx ; Y*
N106E *sh wx; y*

CYTOPLASMIC TRAITS

C738A NCS2
C738B NCS3

CYTOPLASMIC STERILES AND RESTORERS

C836A WF9-(T)	*rf rf2*
C836B WF9	*rf rf2*
C736A R213	*Rf rf2*
C736B Ky21	*Rf Rf2*

WAXY RECIPROCAL TRANSLOCATIONS

WX01A *wx* T1-9c (1S.48; 9L.22) * Sx
WX02A *wx* T1-9(4995) (1L.19; 9S.20) * Sx
WX03A *wx* T1-9(8389) (1L.74; 9L.13) * Sx
WX04A *wx* T2-9c (2S.49; 9S.33) * Sx
WX05A *wx* T2-9b (2S.18; 9L.22) * Sx
WX06A *wx* T2-9d (2L.83; 9L.27)
WX07A *wx* T3-9(8447) (3S.44; 9L.14)
WX08A *wx* T3-9c (3L.09; 9L.12) * Sx
WX10A *wx* T4-9e (4S.53; 9L.26) * Sx
WX11A *wx* T4-9g (4S.27; 9L.27) * Sx
WX12A *wx* T4-9(5657) (4L.33; 9S.25) * Sx
WX13A *wx* T4-9b (4L.90; 9L.29) * Sx
WX14A *wx* T5-9c (5S.07; 9L.10) * Sx
WX15A *wx* T5-9(4817) (5L.06; 9S.07)
WX16A *wx* T5-9d (5L.14; 9L.10)
WX17A *wx* T5-9a (5L.69; 9S.17) * Sx
WX18A *wx* T6-9(4778) (6S.80; 9L.30)
WX20A *wx* y T6-9b (6L.10; 9S.37) * Sx
WX21A *wx* T6-9(4505) (6L.13; 9ctr.)
WX22A *wx* T7-9(4363) (7ctr.; 9ctr.) * Sx
WX23A *wx* T7-9a (7L.63; 9S.07) * Sx
WX24A *wx* T8-9d (8L.09; 9L.16) * Sx
WX25A *wx* T8-9(6673) (8L.35; 9S.31) * Sx
WX26A *wx* T9-10(8630) (9S.28; 10L.37)
WX27A *wx* T9-10b (9S.13; 10S.40) * Sx
WX28A *wx* T5-9(8386) (5L.87; 9S.13)

NON-WAXY RECIPROCAL TRANSLOCATIONS

WX30A *Wx* T1-9c (1S.48; 9L.22) * Sx
WX30B *Wx* T1-9(4995) (1L.19; 9S.20) * Sx
WX30C *Wx* T1-9(8389) (1L.74; 9L.13) * Sx
WX31A *Wx* T2-9c (2L.49; 9S.33) *
WX31B *Wx* T2-9b (2S.18; 9L.22) * Sx
WX32A *Wx* T3-9(8447) (3S.44; 9L.14) * Sx
WX32B *Wx* T3-9(8562) (3L.65; 9L.22) * Sx
WX32C *Wx* T3-9c (3L.09; 9L.12) * Sx
WX33A *Wx* T4-9e (4S.53; 9L.26) * Sx
WX33B *Wx* T4-9(5657) (4L.33; 9S.25) * Sx
WX33C *Wx* T4-9g (4S.27; 9L.27)
WX34A *Wx* T5-9c (5S.07; 9L.10) * Sx
WX34B *Wx* T5-9(4817) (5L.06; 9S.07) M14 only
WX34C *Wx* T4-9b (4L.90; 9L.29)
WX35A *Wx* T5-9(8386) (5L.87; 9S.13) * Sx
WX35B *Wx* T5-9a (5L.69; 9S.17)
WX35C *Wx* T5-9d (5L.14; 9L.10)
WX36A *Wx* T6-9(4778) (6S.80; 9L.30) * Sx
WX37A *Wx* T6-9(8768) (6L.89; 9S.61) * Sx
WX37B *Wx* T7-9(4363) (7ctr.; 9ctr.) *
WX37C *Wx* T6-9(4505) (6L.13; 9ctr.)
WX38A *Wx* T7-9a (7L.63; 9S.07) * Sx
WX38B *Wx* T8-9d (8L.09; 9L.16) * Sx
WX38C *Wx* T8-9(6673) (8L.35; 9S.31) * Sx

WX39A *Wx* T9-10(8630) (9S.28; 10L37) M14 only
WX39B *Wx* T9-10b (9S.13; 10 S.40) * Sx

* = Homozygotes available in both M14 & W23 backgrounds

Sx = Single cross of homozygotes between M14 & W23 versions available

INVERSIONS

I143A Inv1a (1S.86-L.50)
I143B Inv1c (1S.35-L.01)
I143C Inv1d (1L.55-L.92)
I143D Inv1 (5131-10) (1L.46-L.82)
I444A Inv2a (2S.70-L.80)
I243A Inv2 (8865) (2S.06-L.05)
I243B Inv2 (5392-4) (2L.13-L.51)
I343A Inv3a (3L.38-L.95)
I343B Inv3L (3L.19-L.72)
I343C Inv3 (3716) (3L.09-L.81)
I443A Inv4b (4L.40-L.96)
I443B Inv4c (4S.86-L.62)
I543A Inv4e (4L.16-L.81)
I743A Inv5 (8623) (5S.67-L.69)
I743B Inv6 (8452) (6S.77-L.33)
I843A Inv6 (8604) (6S.85-L.32)
I743C Inv6 (3712) (6S.76-L.63)
I943A Inv7 (5803) (7L.17-L.61)
I943B Inv7 (8540) (7L.12-L.92)
I943C Inv7 (3717) (7S.32-L.30)
IX43A Inv8a (8S.38-S.15)
I344A Inv9a (9S.70-L.90)
IX43B Inv9b (9S.05-L.87)
IX43C Inv9c (9S.10-L.67)

Ed. Note

Cooperators (that means you) need the Stock Center.
The Stock Center needs Cooperators (this means you) to:

(1) Send stocks of new factors you have reported in this News Letter or in publications, and stocks of new combinations, to the collection. A list of mutants not represented in the collection is given in MNL 61:115.

(2) Inform the Stock Center on your experience with materials received from the collection.

(3) Acknowledge the source of the stocks for research when you publish, and advice or help you have received in development of your research project.

CHROMOSOME 1L

Mapping of EMS-induced mutants provided by M.G. Neuffer: *white*-495A* and *white*-571C* are albino lethals that have been shown to be allelic by complementation tests (MNL 61:114, 1987), but not allelic to *lw1* or to *wl*-266A*. This locus, designated *w18* (MNL63), is linked to *wx* T1-9(8389) [1L.74] but not to *wx* T1-9(035-10) [1L.89] (MNL 62:124, 1988). Data given below show that this locus is also linked to *bz2*. Since the *gs* and *bm2* classes are greater than expected, it is likely that this locus falls somewhere between them. F2 data, (*bz2 gs bm2* x *w18-495A*) selfed (Total = 505, of which 377 are not *w*):

++	+*w*	*bz2* +	*bz2 w*	Chi-Square = 20	
260	114	117	14	p<.0000	r= .32±.04

Class	Number	Class	Number
+++	185	*bz2* ++	18
+*gs*+	11	*bz2 gs* +	35
++*bm2*	33	*bz2* + *bm2*	6
+*gs bm2*	31	*bz2 gs bm2*	58

wlu5 (white-luteus-266A)* is an albino lethal not allelic to *wh*-495A* nor to *lw1*. Data from 1973 F2 progeny show it to be loosely linked to *bz2* (Chi-Square = 34.9; p .0000; r = .40±.02). No distortions of the expected ratios were seen in crosses with *bm2* in repulsion, so it is likely that the locus is near *br2* and *hm1* in the proximal region of 1L.

zb7 (was *zb*-101*) is a non-lethal mutant that has a clear "zebra-stripe" phenotype in seedlings. It is linked to *wx* T1-9(8389) and is very close to *gs1* on 1L (MNL 62:124, 1988). Examination of 1884 F2 progeny of a cross between *bz2 gs bm2* and *zb7* has failed to identify a single *gs zb7* plant. The one case of this reported last year was incorrect. Most likely *gs1* and *zb7* are very closely linked or the double homozygote is too weak to survive in field conditions. A *bz2 zb7 bm2* homozygous stock has been developed and will be given to the Stock Center.

Waxy translocation stocks: *wx* T1-9(4995) [1L.19; 9S.20]: There may be a problem with the Coop version of this stock. Plants descended from Coop 82-078-05 selfed have shown no linkage between *wx* and T, unlike the 2.58% recombination reported in MNL 39:106-109, 1965.

wx T1-9(5622) [1L.10; 9L.12]: We have developed this waxy translocation as an additional marker for the proximal part of 1L. The *wx* - T crossover percentage has not yet been determined.

Isozyme-morphological-RFLP marker mapping: DNA from plants involved in two mapping projects (MNL 61:86, 1987 and 62:102, 1988) was isolated so that RFLP markers could be mapped in relation to isozyme and morphological markers on 1L. Data were analyzed using the program LINKAGE-1 (copyright K.A. Suiter, J.F. Wendel, and J.S. Case). The results are given below. Numbers between loci refer to map distance in centiMorgans. Data from 236 testcross progeny, region *br1 - Pgm1:*

br1- 3.0 -*Amp1*- 12.7 -UMC37- 1.3 -*Mdh4*- 1.7 -*bz2*- 9.3 -BNL8.10- 13.1 -*Pgm1*

Data from 200 testcross progeny, region *gs1 - Acp4:*

gs1- 2.5 -UMC72B- 11.5 -*Phi1*- 7.0 -BNL8.29- 3.5 -*bm2*- 4.5 -UMC84- 10.5 -BNL6.32- 0.5 -*Acp4*

Mathern and Hake report in this volume the relation of *Adh1* and *Kn1* to each other and to several RFLP markers.

Paul H. Sisco

CHROMOSOME 3S

Two new mutations were placed on 3S this year. P. Stinard mapped the *brn1* locus 6.7m.u. distal to *cr*, and *Wrk1* was placed between *Lg3* and *cl* by S. Poethig. In addition, preliminary data indicate that *h* is located between *Lg3* and *Cg1*, about 12cM from *Cg1*. This would place it in the vicinity of *ra2*. It should also be noted that *Tp3* is closely linked to *Cg1*, and may be an allele of this locus.

g2----------*E8*---*brn1*----*cr1*------*Cg1*------*d1*---*ra2*-------*cl1*--*Wrk1*--*Lg3*--*Rg1*
0 14 19 26 35 44 49 60 62 65 67

S. Poethig

CHROMOSOME 4L

At this writing no new genes have been mapped. Progress has been made in placing a male sterile mutant from Neuffer as well as an F3 generation with *dp1 gl3* x Mo17 recombinants to use in zein gene mapping and for Mo17 "delay factor" placement. The TB method is being used too.

Dale M. Steffensen

CHROMOSOME 5S

Published and unpublished data are partway collected and a complete summary is planned.

Mary Polacco

CHROMOSOME 6S

Ed Weck has volunteered to be Coordinator for this arm.

CHROMOSOME 10S

Plantings of mapping experiments were detrimentally affected by drought, and we have to repeat these experiments this year.

Marc C. Albertsen

WORKING LINKAGE MAPS

On the following pages are the current working maps for each chromosome. As you can see, the maps are becoming quite complex with the addition of RFLPs. The traditional linkage map based on conventional factors and isozymes is presented in the center. Each linkage map represents the order and recombinational distances, in centimorgans (1% recombination = 1 cM), for those genes for which sufficient information is available to make a reasonable judgment of their location. Each chromosome is arranged beginning with the most distal gene in the short arm. Locations of the centromeres are indicated according to the best available data from cytogenetic studies. The physical map of each chromosome, immediately to the left of each linkage map, is drawn with the length of each arm in proportion to the ratio of the length of that arm to the length on chromosome 1. Locations of the B-A translocations, which generate hemizygous segments, are shown as TB-...; placement on the physical map is in accordance with observed breakpoints; placement on the linkage map is in relation to genes uncovered or not uncovered. The vertical line associated with simple B-A translocations represents the segment within which the breakpoint is located (genes distal to the line on that arm should be uncovered). In the case of compound translocations, the associated vertical line on the linkage map for the first arm involved (e.g., 1L of TB-1La-5S8041) defines the segment within which the second breakpoint is located (genes distal to the line are not uncovered). On the map of the second arm involved (5S, in the example), genes distal to the associated line are uncovered (as they are with simple B-A translocations). TB's shown spanning one or more genes may or may not uncover the indicated gene or genes. Immediately to the right of the linkage map are those genes that have some information leading to a "rough" placement on the map, either near a gene already on the map or to a region of the map. Further to the right are those genes which have been only placed to chromosome (represented by the vertical line with arrows at both ends) or to one arm (represented by a vertical line running from near the centromere to the end of the arm).

To left of each chromosome's physical map are the current versions of the public restriction fragment length polymorphism (RFLP) maps developed by Ben Burr at the Brookhaven National Labs (on the left) and my lab at the University of Missouri (on the right). The Burr map was developed using a set of recombinant inbreds while our map was developed using an F2 population. Both maps contain a number of loci in common as well as a number of conventional loci. When possible, the most distal common markers on the two RFLP maps were set equal by lengthening one of the maps. The two maps were then positioned relative to the conventional map by equating a common, well-mapped locus (or loci). It should be noted that the actual distances between loci in each map are not drawn to the same scale; however, it was felt that by equating common loci in each map, some idea as to relative position could be gained. On the right of the Hoisington map are preliminary localizations of conventional markers based on work at Missouri and that performed by other researchers. I have tried to indicate the relative placement of the gene or genes along the RFLP map. The horizontal ticks indicate the RFLP loci used in mapping the gene or genes.

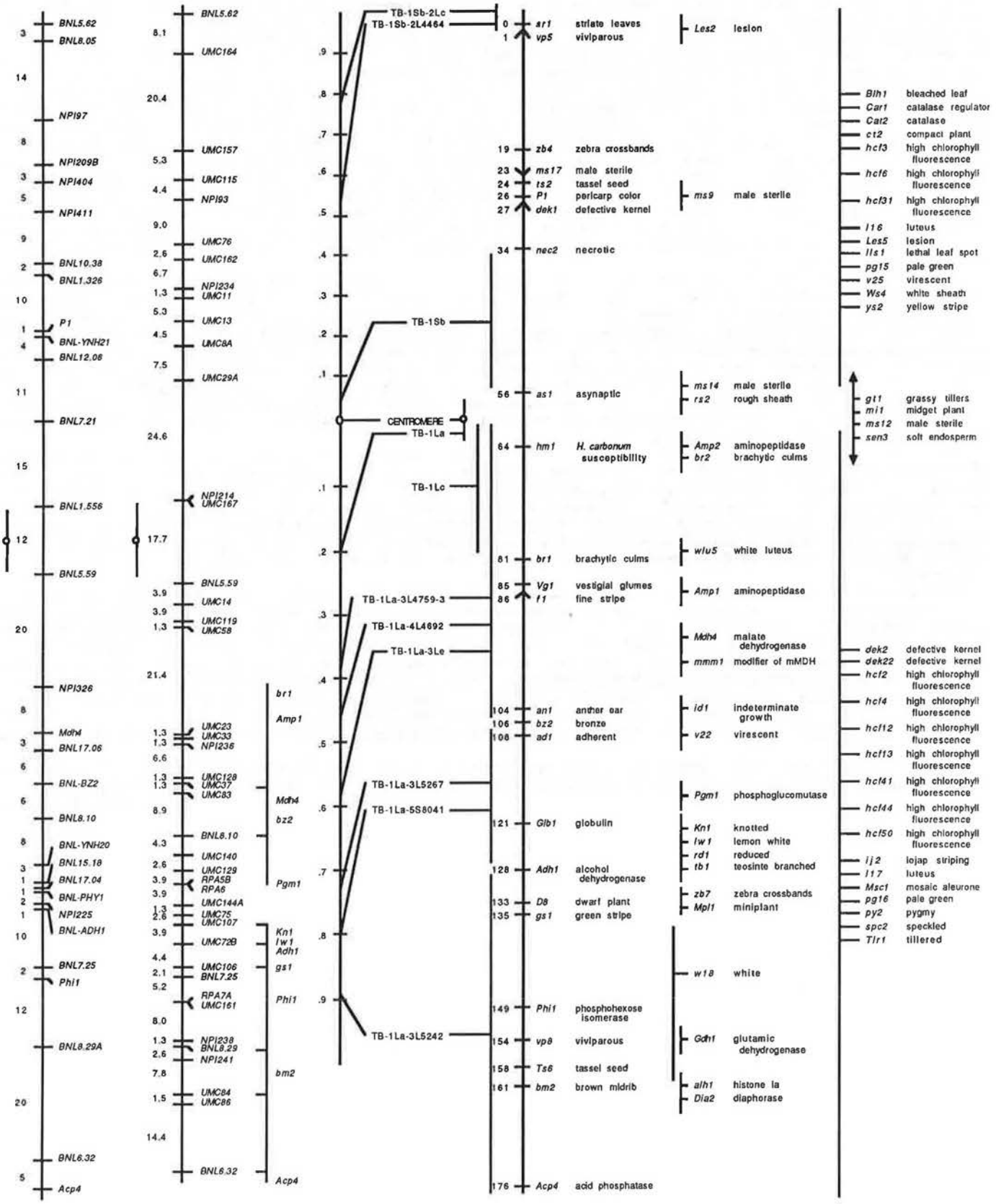
The mapping proposal which resulted from the efforts of several members of the maize community was partially funded by NSF. The major efforts will be focused at Missouri with sufficient funding to permit the highest priority mapping, to provide some common equipment among the proposed collaborators, and to provide for the production of maps and data summaries in the Newsletter. Our focus will be to extend the current set of public RFLP markers, to correlate these to physical locations in the genome through the use of A-A and B-A translocations, and to interval map as many conventional loci as possible (the initial focus will be on the loci on the main linkage map). It is important to stress that this effort in no way decreases the need for others to map (either traditionally or with RFLPs). What this project will do is provide a means by which these data can be assembled and distributed to all interested researchers.

Finally, the importance of placing loci defined by probes of known function cannot be overstressed. In a number of cases these provide very accurate ties to the conventional map and, in the very least, provide functional significance to a particular region of the genome which will be important as further additional studies (particularly in the area of quantitative genetics) progresses. Therefore, I would like to make a plea that if you have a clone for a known function and know, or at least believe that it hybridizes to a maize genomic sequence, please attempt to map the locus (or loci). This can be accomplished in a couple of ways (and I recommend doing both). First the set of recombinant inbreds should be probed and the data sent to Ben Burr for analysis. Secondly, it would be appreciated if the probe could be sent to Missouri for mapping in F2 and testcross populations. We would also be able to use the probe in our correlation to physical and conventional markers. Included at the end of this Newsletter is a sample form indicating the desired information for each clone you provide. If you have any questions regarding mapping of RFLP loci (both old and new), please give me a call.

As usual, any comments and/or changes to the maps are greatly appreciated. Also, the entire genelist and working maps are reproduced in the latest version of Corn and Corn Improvement and will be published in the next version of Genetic Maps.

Dave Hoisington

CHROMOSOME 1



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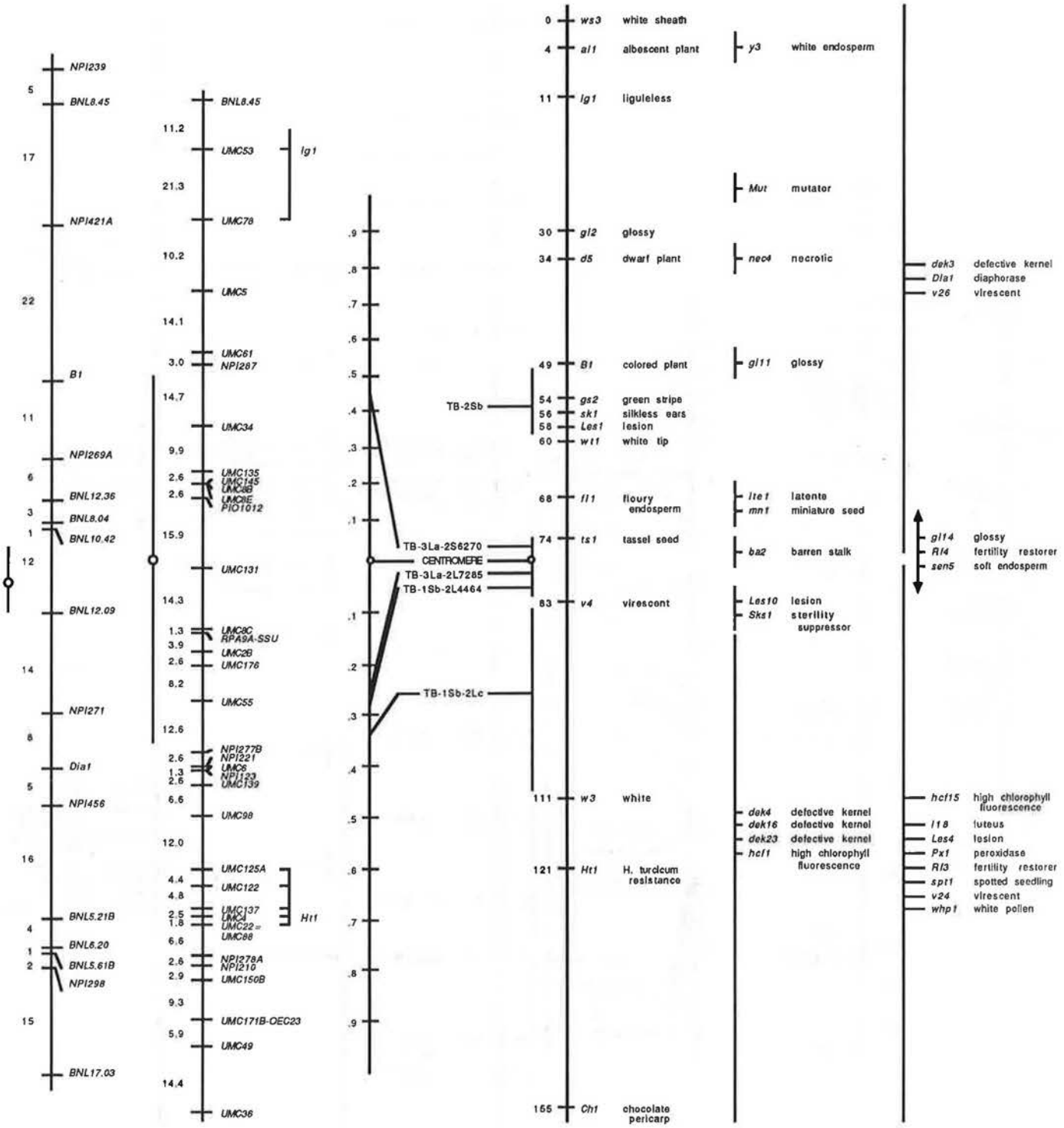

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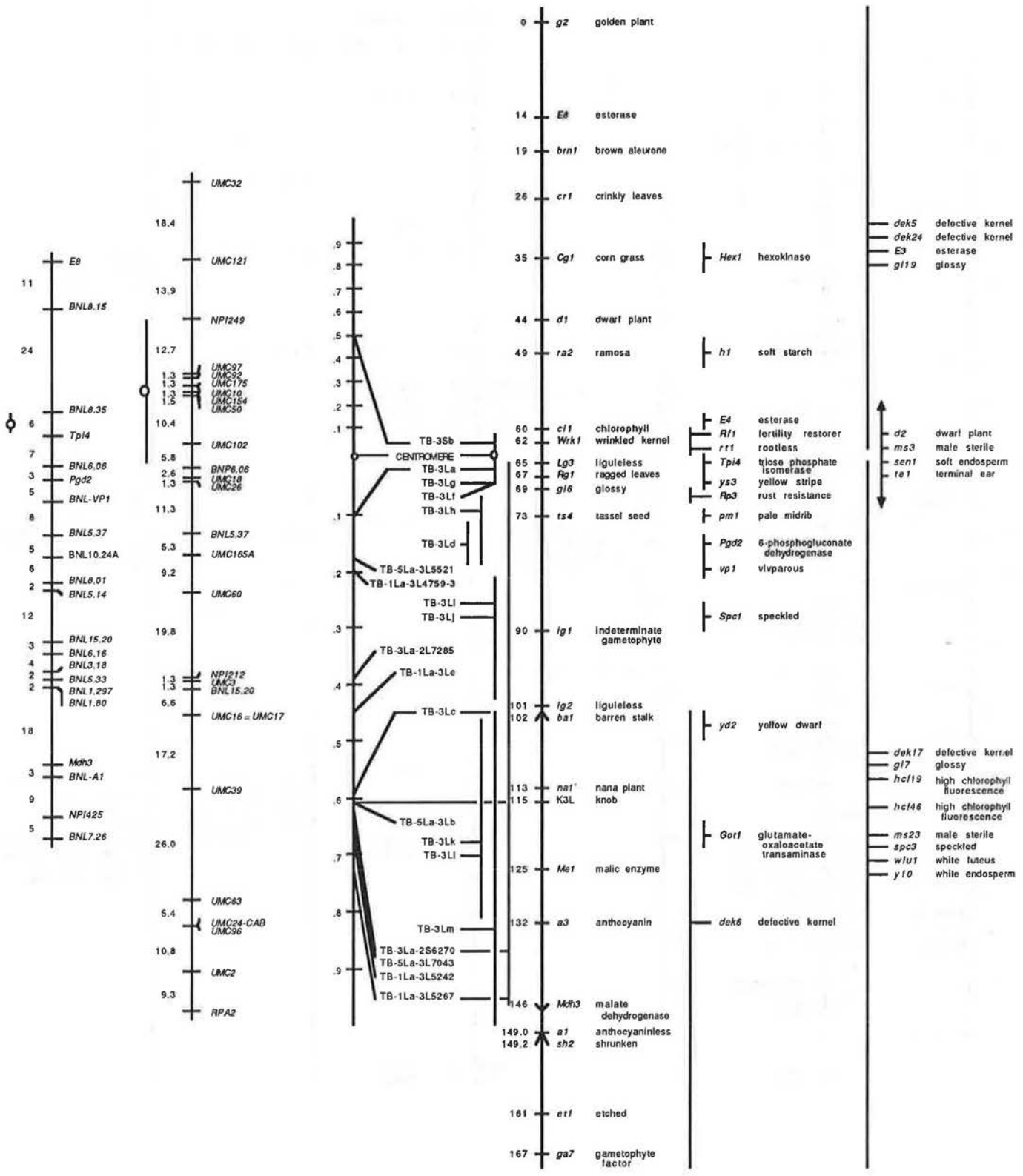

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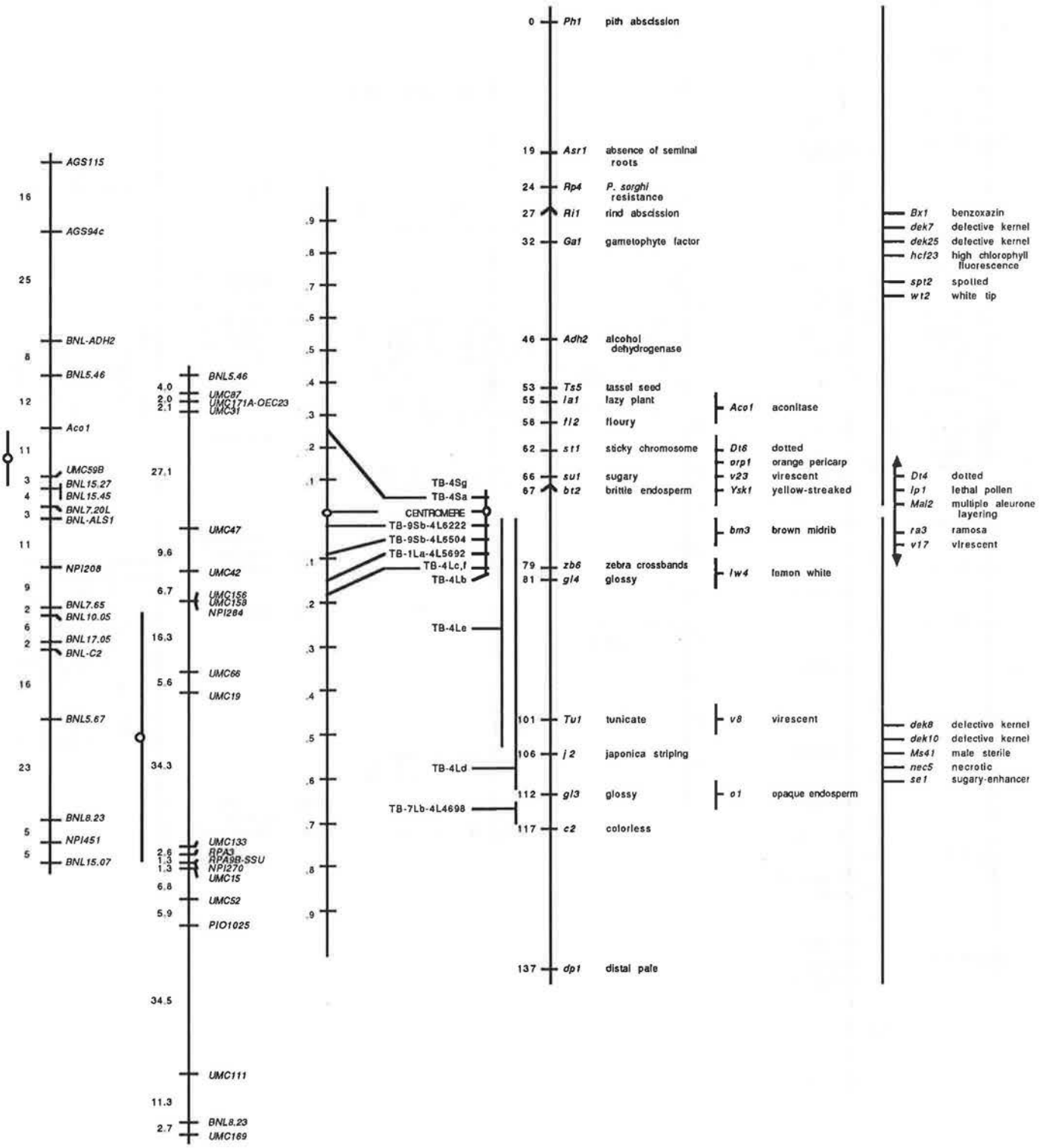

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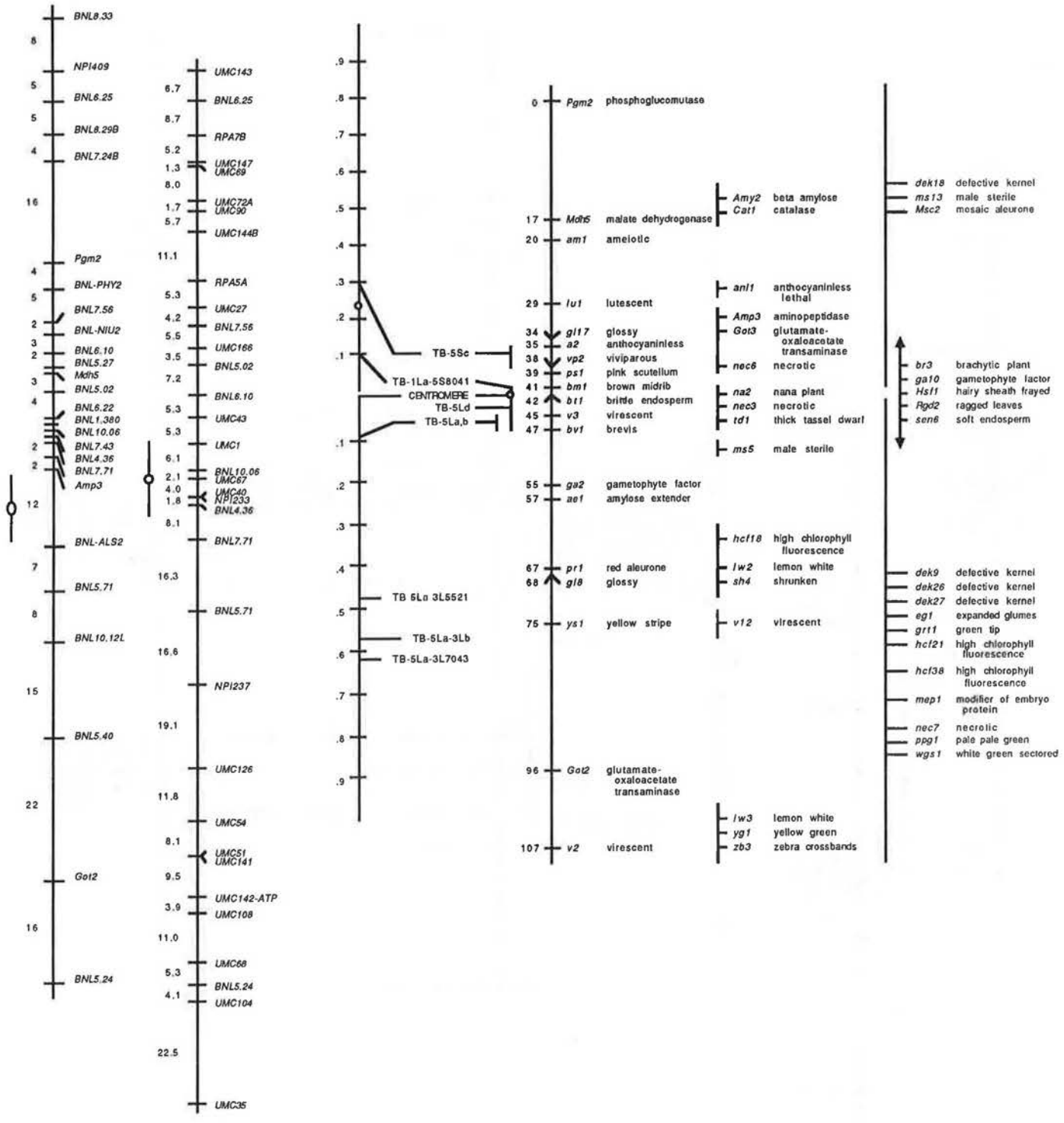

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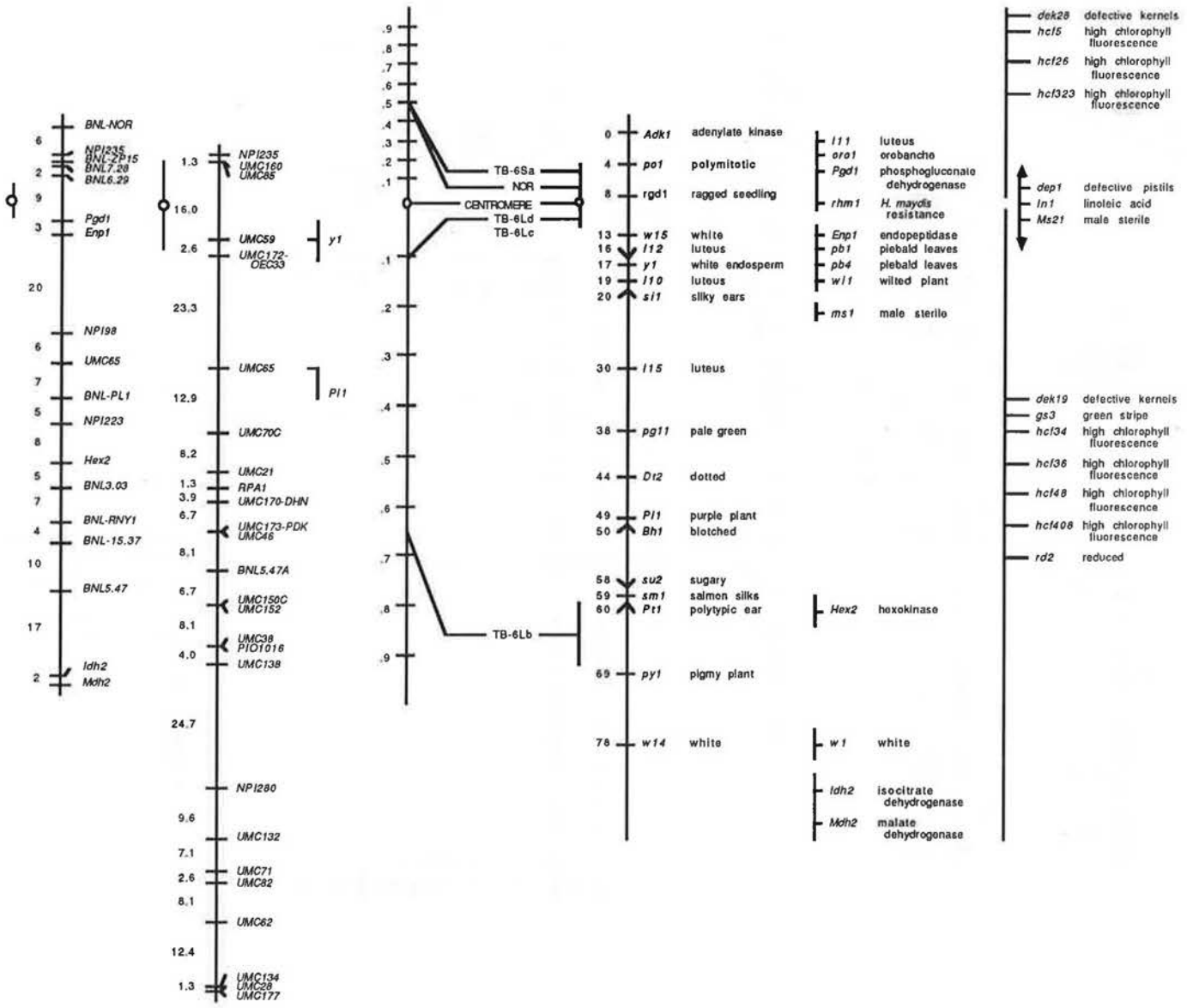

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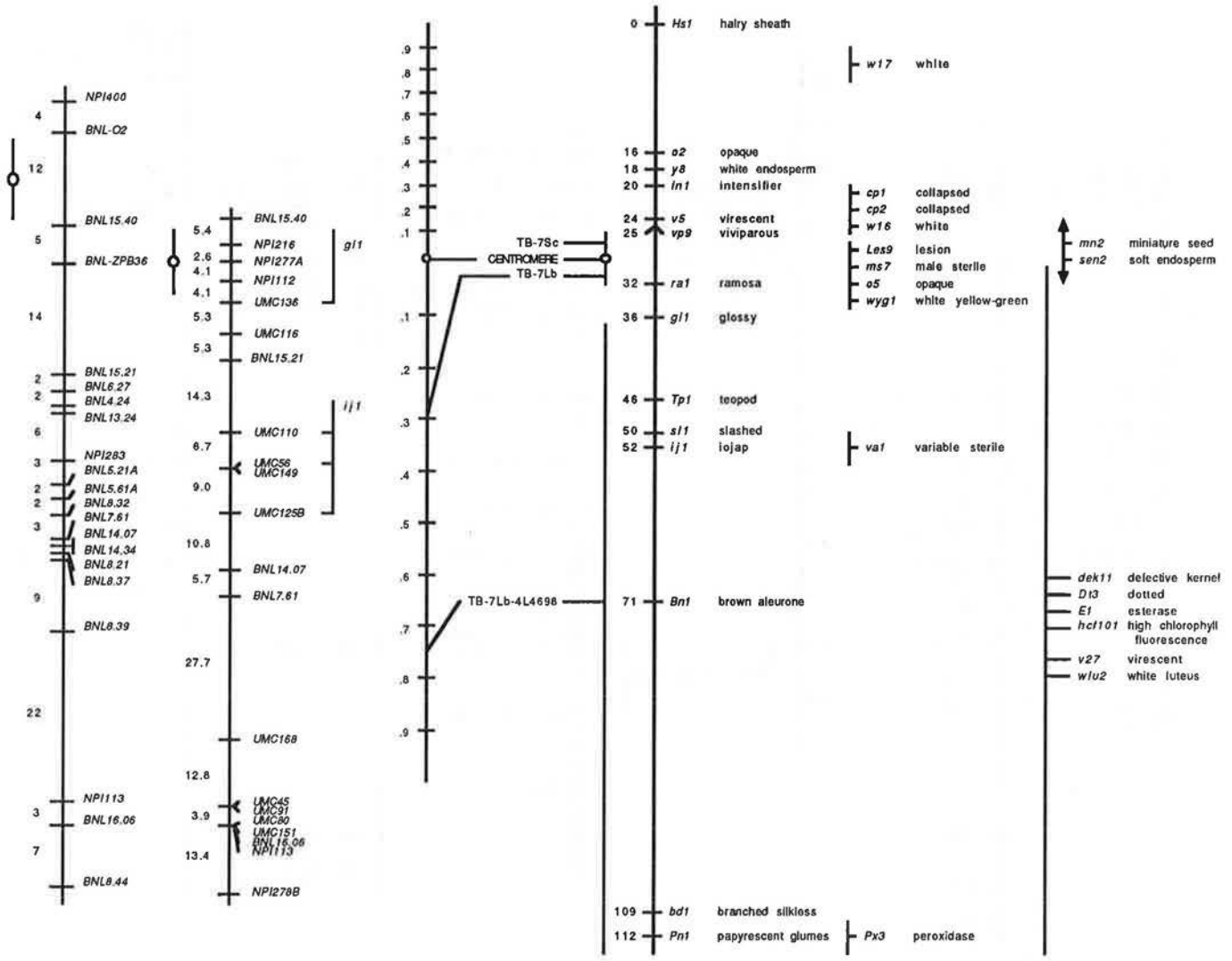

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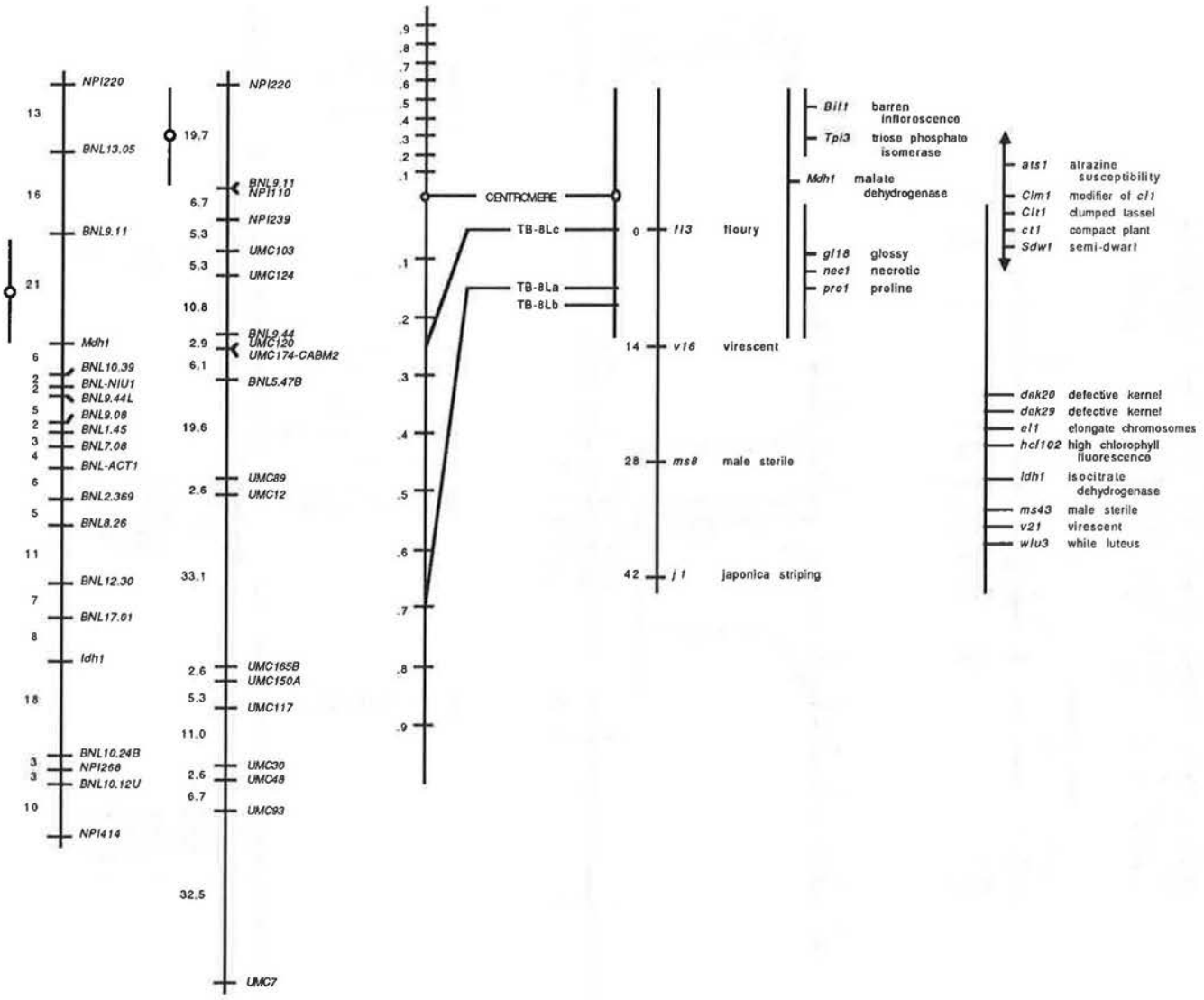

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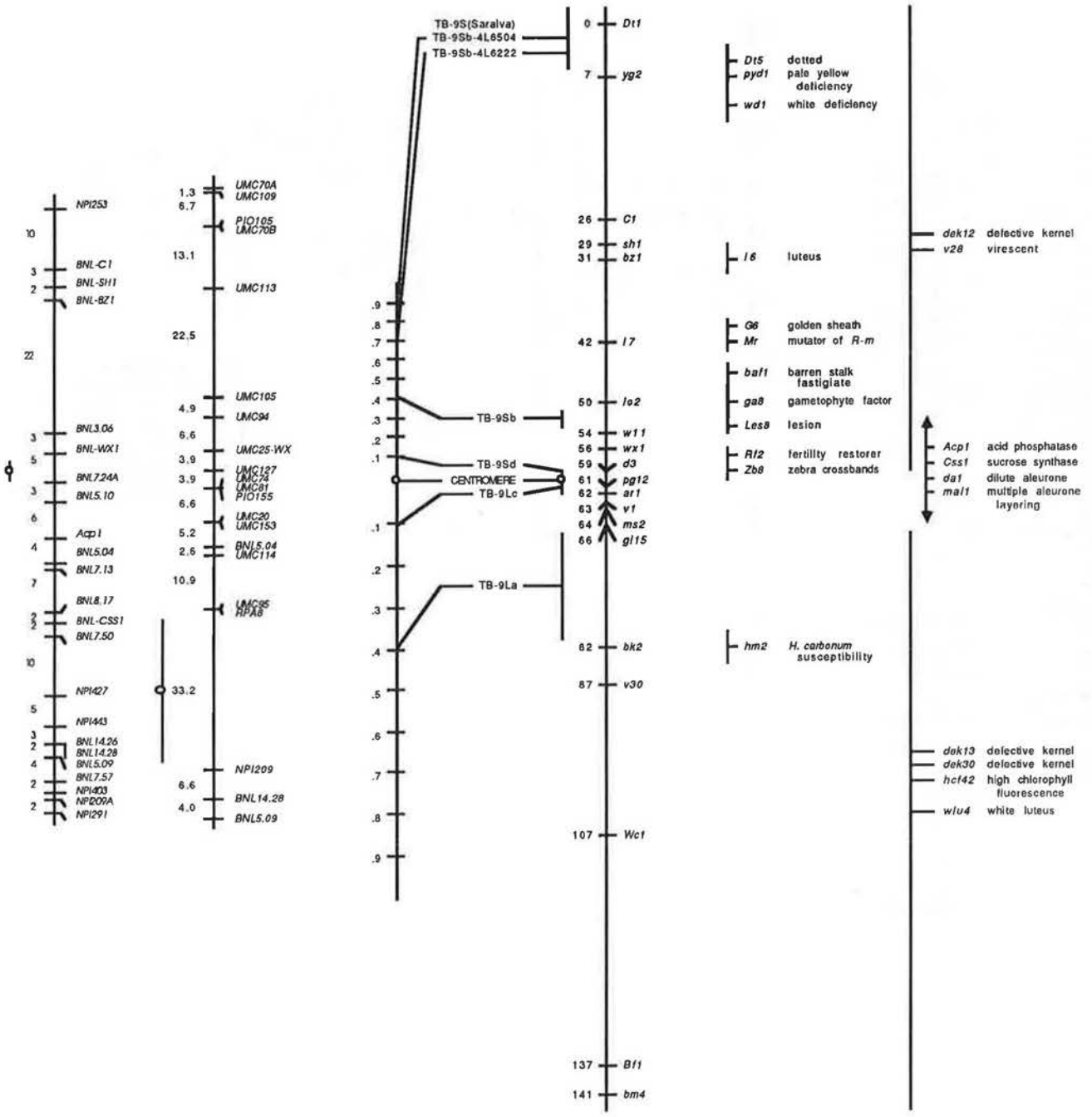

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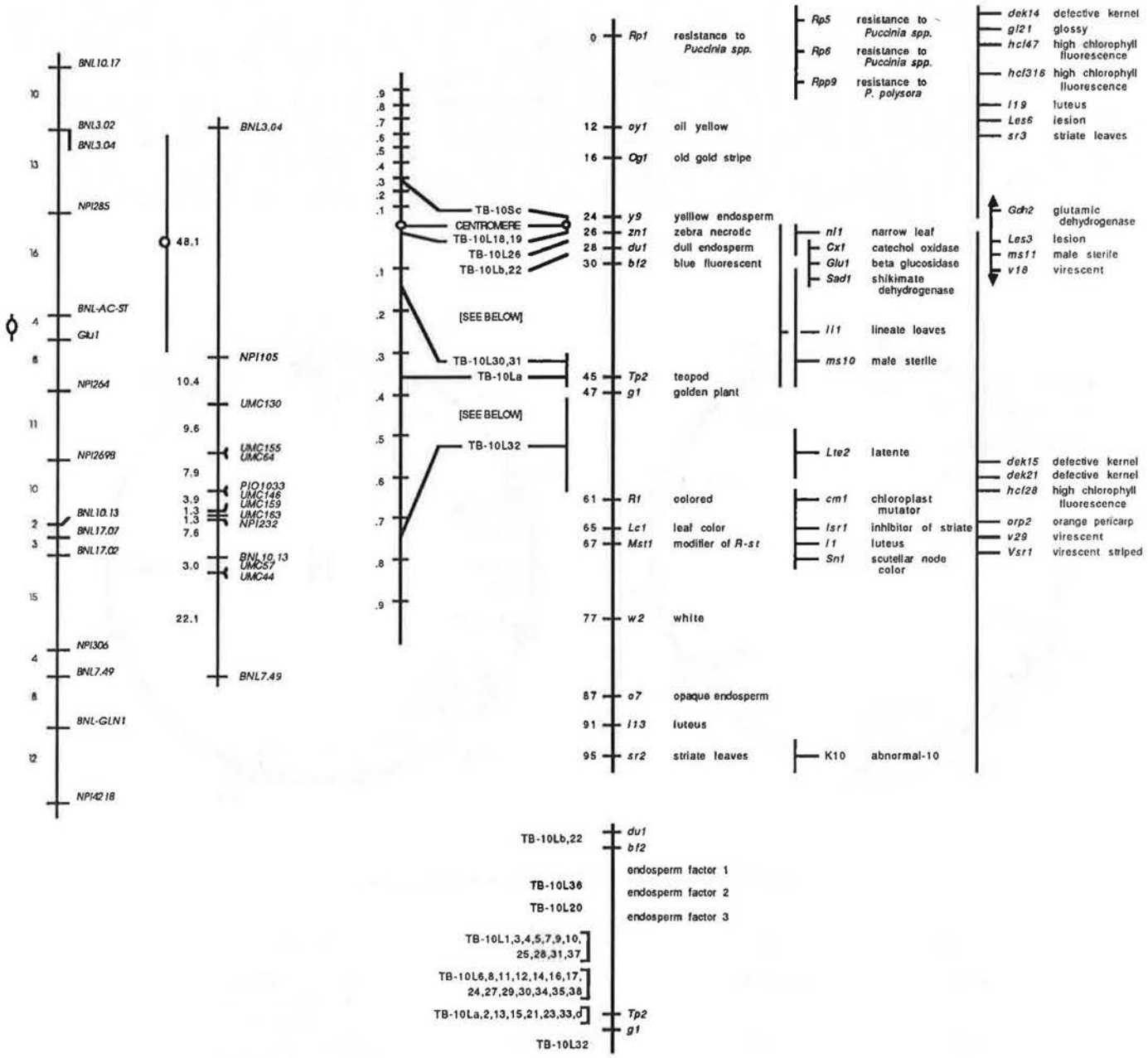


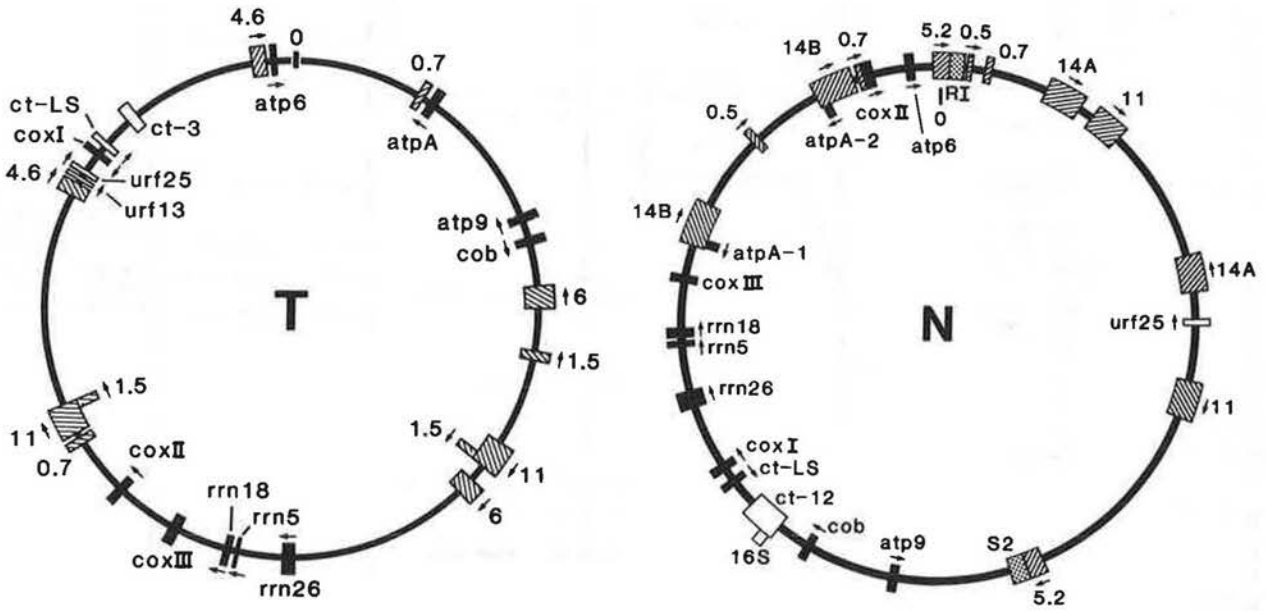
Physical map of the Zea mays mitochondrial genome from the male fertile cytoplasm and the cytoplasmic male sterile cms-T (genotype B37)

--C. Fauron, HHMI, 743 Wintrobe Building, University of Utah, Salt Lake City, UT 84132

This abstract presents the latest and complete restriction enzyme mapping data for the mitochondrial genome of N and cms-T (genotype B37). The N maize mtDNA has been mapped by Lonsdale et al. (1984 - genotype Wf9) and Fauron and Havlik (1988 - genotype B37). The cms-T mtDNA has been mapped by Fauron et al. (1989 genotype B37). The entire sequence complexity can be represented on a 570 kb and 540 kb circular molecule for N and cms-T respectively (see Figure 1). The circular maps are also shown linearized (Figure 2) at the *Sma*I site internal to the 5.2 kb repeat in N (position 3048, Houchins et al. 1986).

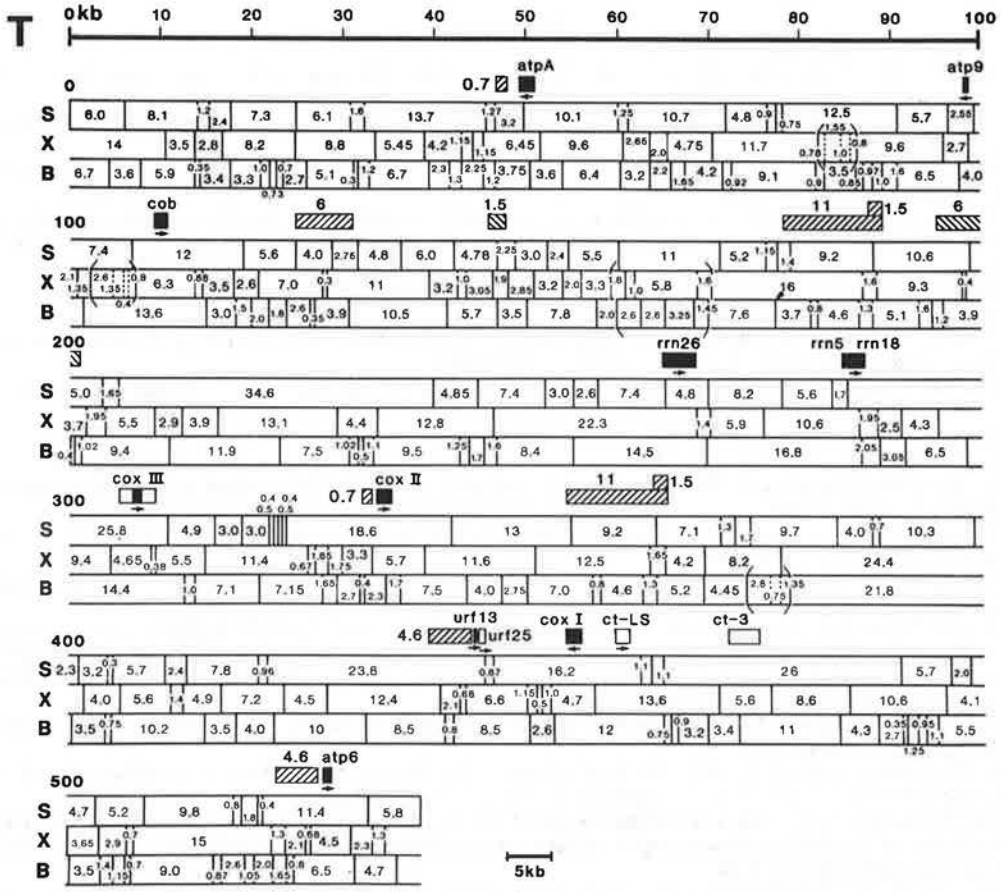
Each line represents a 100kb stretch of DNA. The scale is shown on the top of the figure. The relative orders of some contiguous *Bam*HI or *Xho*I fragments have not been determined and are placed in parentheses. The fragment size is in kb. The repeats are represented by hatched boxes with their size in kb. Inverted repeats have hatching of different orientation. The rRNA and protein coding genes are represented by black boxes. The arrow indicates their orientation 5' - 3'. The *coxIII* gene couldn't be precisely mapped, so it is placed within a larger box. For the mapping of the tRNA genes, see the data from D. L. Lonsdale in MNL 61 (p. 148). The data about the location of the cms-T and N mitochondrial genes are compiled in the table. A detailed comparison between the N and cms-T genome has been published (Fauron and Havlik, 1989). See also abstract in this issue.

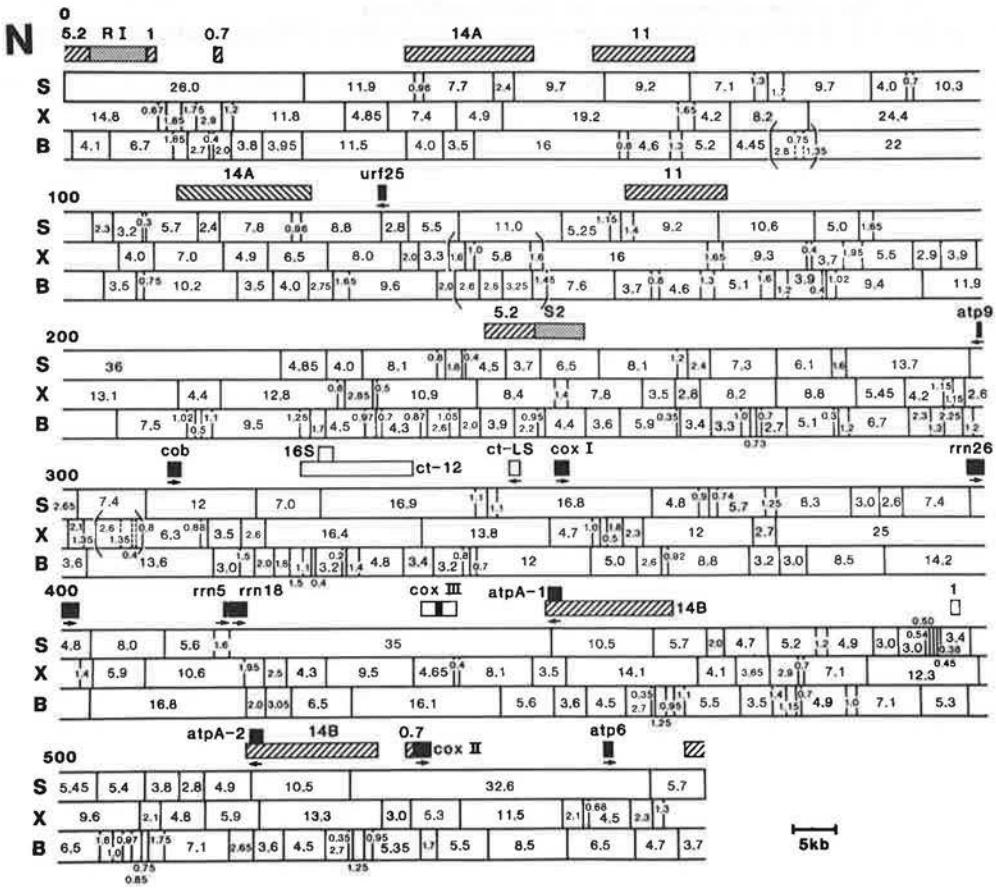
More information about those two maps is available upon request.



Location of the cms-T and N Mitochondrial Genes.

genes	Map Position (5' -- 3') cms-T	N	Source
atpA	50.8 - 49.3	454.2 - 452.7	Isaac et al 1985
		521.8 - 520.3	Braun et Levings 1985
			Dawson et al 1986
atp9	98 - 97.8	299.6 - 299.4	Dewey et al 1985
			Mulligan et al 1988
cob	109.4 - 110.6	311.6 - 312.8	Dawson et al 1984
			Dawson et al 1986
rrn26	263 - 266.5	398.2 - 401.7	Dale et al 1984
			Dawson et al 1986
rrn5	288.9 - 289	417.7 - 417.8	Chao et al 1983
			Dawson et al 1986
rrn18	289.1 - 291.1	417.9 - 419.9	Chao et al 1984
			Dawson et al 1986
coxIII	(305.6 - 309.6)	(439 - 443)	McCarty et al 1986
coxII	334 - 335.5	538 - 539.5	Fox et al 1981
			Dawson et al 1986
urf-13	424.6 - 424.9	---	Dewey et al 1986
urf-25	425 - 425.7	135.2 - 134.5	Dewey et al 1986
coxI	456.4 - 454.8	353 - 355	Isaac et al 1985
			Dawson et al 1986
atp6	528 - 528.9	558.4 - 559.3	Dewey et al 1985


T
0kb 10 20 30 40 50 60 70 80 90 100
atpA
atp9
cob
rrn26
rrn5 rrn18
cox III
cox II
urf13
urf25
cox I
ct-LS
ct-3
atp6
S
X
B
5kb


N
5.2 RI 1
0.7
14A
11
urf25
5.2 S2
atp9
cob
16S
ct-12
ct-LS
cox I
rrn26
rrn5 rrn18
cox III
atpA-1
14B
atpA-2
atp6
cox II
S
X
B
5kb

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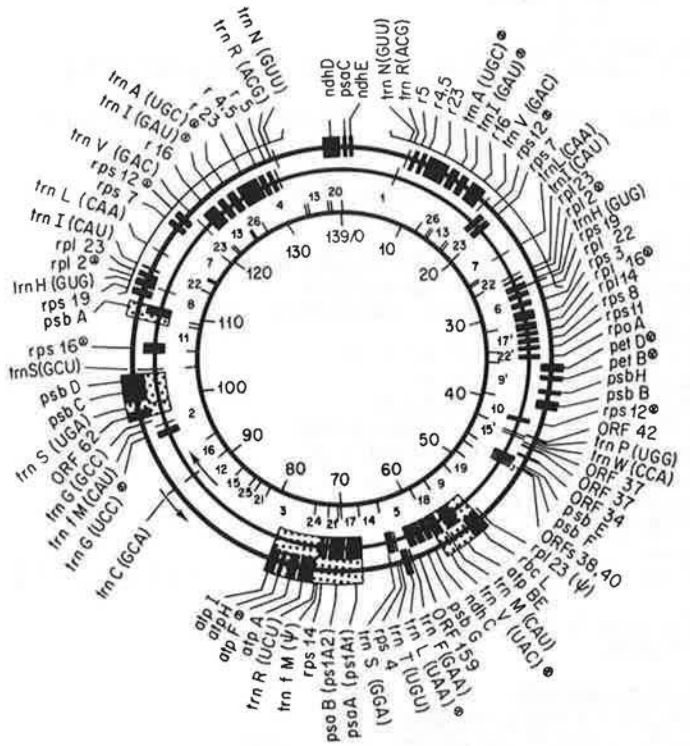
GENETIC MAP OF THE ZEA MAYS PLASTID CHROMOSOME

Steven Rodermel and Lawrence Bogorad, Department of Cellular and Developmental Biology, Harvard University, Cambridge, Massachusetts, USA 02138

The nucleotide sequences of 14 maize chloroplast genes have been published since last year's News Letter. The locations of these genes are shown on the circular map of the chromosome (below), and their products are briefly described in the following table. See last year's News Letter (MNL 62: 148) for descriptions of maize plastid chromosome organization and plastid gene nomenclature, and for descriptions of the other genes on the circular map.



RECENTLY REPORTED ZEA MAYS PLASTID GENES AND GENE PRODUCTS

Gene Product	Gene	Reference
Transfer RNAs:		
Cysteine	trnC (GCA)	Meinke et al., 1988
Proline	trnP (UGG)	Lukens and Bogorad, 1988
Serine	trnS (GCU)	Russell et al., 1987
Tryptophan	trnW (CCA)	Lukens and Bogorad, 1988
Photosystem I Components:		
Apoprotein of iron-sulfur centers A and B	psaC	Schantz and Bogorad, 1988
70S Ribosomal Proteins:		
S7	rps7	Giese et al., 1987
S8	rps8	Markmann-Mulisch and Subramanian, 1988
S11	rps11	Ruf and Kossel, 1988
S12	rps12	Giese et al., 1987
L14	rpl14	Markmann-Mulisch and Subramanian, 1988
L23 (pseudogene)	rpl23(U)	Bowman et al., 1988
Putative NADH Dehyrogenase Components:		
ND4	ndhD	Schantz and Bogorad, 1988
ND4L	ndhE	Schantz and Bogorad, 1988
Other stromal proteins:		
Alpha subunit of DNA-dependent RNA polymerase	rpoA	Ruf and Kossel, 1988

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Abbott, Albert G., 338 Long Hall, Clemson University, Clemson, SC, 29634-1903
Agnell, Dana A., 621-E Nazareth St., Raleigh, NC, 27606
Agrawal, B. D., G. B. Pant Univ Agr Tech, Pantnagar 263145, India
Agrema, Hesham A., 42 Mumphis Street, Camp Caesar, Alexandria 21321 5970525, Egypt
Ainsworth, Thomas C., Ainsworth Seed Company, Mason City, IL, 62664, 217-482-3281
Albertsen, M. C., Pioneer Hi-Bred Internatl, P. O. Box 38, Johnston, IA, 50131, 515-270-3648
Alexander, Larry, Sigco Research, Box 289, Breckenridge, MN, 56520, 218-643-2155
Alfenito, Mark R., 16 Divinity Ave., Harvard Biol. Labs., Cambridge, MA, 02138
Alleman, M., Department of Genetics, University of Wisconsin, Madison,WI, 53706, 608-262-3286
Allen, Jim, Laboratory of Genetics, University of Wisconsin, Madison, WI, 53706, 608-262-3286
Altschuler, Marsha I., Dept. of Biology, Williams College, Williamstown, MA, 01267, 413-597-2497
Alvarez, Angel, Departamento de Genetica, Estacion Exp de Aula Dei, Apartado 202, 50080 Zaragoza, Spain
Alvey, David, FFR Cooperative, 4112 East State Road 225, W. Lafayette,IN, 47906, 317-567-2115
Amano, Etsuo, Inst of Radiation Breeding, PO Box 3, Ohmiya-machi, Naka-gun, Ibaraki-Ken 319-22, Japan
Andrew, R. H., Department of Agronomy, University of Wisconsin, Madison, WI, 53706, 608-262-2587
Antonelli, N. Michele, Dept. of Genetics, Iowa State University, Ames,IA, 50011
Armstrong, Charles L., Monsanto, GG4H, 700 Chesterfield Village Pkwy, St. Louis, MO, 63198, 314-537-7229
Arnold, Jerry M., Asgrow Seed Co., 9866 Highway 66, Newburgh, IN, 47630
Aromose, O. S. A., Biological Sciences Dept, University of Benin, BeninCity, Nigeria
Arthur, Gary D., Holden's Foundation Seeds Inc, RR 1, Box 149, Franklin, IN, 46131, 317-535-8357
Ashman, R. B., Dept of Botany, Purdue University, W. Lafayette, IN, 47907, 317-494-4634
Atherly, Alan G., 8 Curtiss Hall, Genetics Dept, Iowa State Univ, Ames,IA, 50011
Athma, Prasanna, Cold Spring Harbor Lab, P.O. Box 100, Cold Spring Harbor, NY, 11724
Auger, Donald L., Biology Department, Box 8238, University Station, University of North Dakota, Grand Forks, ND, 58202, 701-777-3674
Ayala-Osuna, Juan, Depto Agrar Vet Fac Cienc, Universidade Estad Paulista, Rodovia Carlos Tonnani, Km 5, 14870 Jaboticabal SP, Brazil
Aycock, Harold S., 5776 Vesper Dr, South Beloit, IL, 61080
Ayers, J. E., Dept Plant Pathology, 211 Buckhout Lab, Pennsylvania State Univ, University Park, PA, 16802, 814-865-7776
Badenhorst, Coerie, Saffola, Box 36, Boskop 2528, South Africa
Baier, John W., 1255 Fifield Hall, University of Florida, Gainesville,FL, 32611
Bailey, Bryan A., 304 Curtis Hall, Agronomy Dept., University of Missouri, Columbia, MO, 65211, 314-882-3410
Bailey-Serres, Julia, Genetics Dept, Mulford Hall, Univ Calif Berkeley,Berkeley, CA, 94720, 415-642-7948
Baker, Barbara, USDA, Plant Gene Expression Center, 800 Buchanan Street, Albany, CA, 94710, 415-559-5912
Baker, Raymond, Pioneer Hybrid Seed Co, Johnston, IA, 50131, 515-278-2108
Banks, Jody, Carnegie Inst of Wash, 115 W. University Parkway, Baltimore, MD, 21210, 301-467-1414
Banuett, F., Dept Biochem Biophys, Univ of California, San Francisco, CA, 94143, 415-666-4985
Bar-Tsur, Avri, Dept Maize Breed/Genet, Newe Yaar Exper Sta 31999, PO Haifa, Israel, 04-933-186
Barry, Edward G., Dept of Biology, CB# 3280, Univ of North Carolina, Chapel Hill, NC, 27599, 919-962-6933
Bass, Hank W., Botany Dept, Box 7612, North Carolina State Univ, Raleigh, NC, 27695-7612, 919-737-3570
Baszczynski, C. L., Allelix Inc, Agric Division, 6850 Goreway Drive, Mississauga, Ont L4V 1P1, Canada, 416-677-0831
Bateman, Brenda S., Dept of Genetics, North Carolina State Univ, Box 7614, Raleigh, NC, 27695-7614
Bates, Lynn S., Alteca Ltd., 731 McCall Road, Manhattan, KS, 66502, 913-537-9773
Baxter, Donald Ray, Dept Biol Sci, Stanford University, Stanford, CA, 94305, 415-723-3902
Bayliss, Michael W., Imperial Chem Ind PLC, Internatl Seeds Business, Jealott's Hill Research Stn, Bracknell Berks RG12 6EY, U K, 0344-424701
Beach, Larry R., Pioneer Hi-Bred Internatl, Department of Biotechnology, Box 38, Johnston, IA, 50131, 515-270-3633
Bear, Robert P., Noble Bear, Inc, PO Box 950, Decatur, IL, 62525, 217-422-5621
Beavis, W. D., Pioneer Hi-Bred Int., Box 85, Johnston, IA, 50131
Beckett, Jack, Curtis Hall, University of Missouri, Columbia, MO, 65211, 314-882-8214
Becraft, Philip, Genetics Department, Univ of California-Berkely, Berkeley, CA, 94702, 415-642-7948
Bedinger, Patricia, Dept of Biol, 010A Coker Hall, U of North Carolina,Chapel Hill, NC, 27599-3280, 919-962-6943
Behr, Fritz, 4614 Ames Street, Madison, WI, 53711
Belanger, Faith, 1102 S Goodwin Street, Urbana, IL, 61801
Bendbow, Evelyn, Curtis Hall, Univ of Missouri, Columbia, MO, 65211
Benner, Michael, Waksman Inst Microbiol, Rutgers Univ, P.O. Box 759, Piscataway, NJ, 08854, 201-932-3801
Bennett, M. D., Plant Breeding Institute, Maris Lane, Trumpington, Cambridge CB2 2LQ, U K, 0223 840411
Bennetzen, Jeff, Dept of Biological Sciences, Purdue University, W. Lafayette, IN, 47907, 317-494-4763
Benton, W. D., Genbank c/o Intelligenetics, 700 E. El Camino Real, Mountain View, CA, 94040, 415-962-7360
Benzion, G., 303 W. Lanvale St., Baltimore, MD, 21217, 301-529-3386
Bergquist, Richard R., Plant Pathologist, 401 East Sixth Street, El Paso, IL, 61738, 309-527-6000
Bergstrom, Gary C., 316 Plant Sci Bldg, Cornell University, Ithaca, NY,14853, 607-255-7849
Bertaux, Philippe, Lifaco Seed Corp, PO Box 278, Kirkland, IL, 60146, 815-522-3246
Berthaud, Julien, Ressources Genetiques, et Amelior des Plantes Tropic,ORSTOM, BP 5045, 34032 Montpellier Cedex, France
Berville, A., INRA, Station d'Amelior des Plantes, Lab de Genet et Mutag, BV 1540, 21034 Dijon Cedex, France
Bianchi, Angelo, Ist Sper Cerealicoltura, Via Cassia, 176, 00191 Rome,Italy, 06-3285705
Birchler, J., Room 290, The Biological Labs, Harvard University, 16 Divinity Avenue, Cambridge, MA, 02138, 617-495-4272
Bird, Robert McK., Inst Study Plants, Food & Man , P.O. Box 5142, Raleigh, NC, 27650, 919-787-8452
Bjarnason, Magni, CIMMYT, Apdo. Postal 6-641, 06600 Mexico, D.F., Mexico
Blakey, Cynthia Ann, Curtis Hall, University of Missouri, Columbia, MO,65211, 314-882-2033
Bocanski, Jan, Faculty of Agriculture, University of Novi Sad, 21000 Novi Sad, Yugoslavia
Bogorad, L., Biol Labs, Harvard Univ, 16 Divinity Ave, Cambridge, MA, 02138, 617-495-4292
Bohning, Kermit, Cargill Research, 311 River Ave North, P.O. Box 307, Belmond, IA, 50421
Bokde, S., UNDP-FAO, C. P. 1335, Luanda, Angola, Africa

Bolen, P. L., NRRL-USDA, 1815 N. Univ St, Peoria, IL, 61614, 309-685-4011 EXT 272
Bommineni, V. R., Department of Plant Sciences, University of Western Ontario, London, Ontario N6A 5B7, Canada, 519-679-2111
Boothe, Joe G., Plant Biotechnology Institute, Saskatoon, S7N 0W9, Canada
Borner, T., Sekt Biol, Humboldt Univ, Invalidenstr 43 DDR-1040, Berlin,E Germany
Boston, Rebecca S., Box 7612, Dept Botany, North Carolina State Univ, Raleigh, NC, 27695-7612, 919-737-2727
Bouchard, Robert A., Dept of Biol, The College of Wooster, Wooster, OH,44691, 216-263-2026
Bouck, Dan, Agway Inc Crop Serv, Syracuse, NY, 13221
Boyer, C., Dept Hort Penn State Univ, 103 Tyson Building, University Park, PA, 16802, 814-863-3371
Brady, Kevin P., Department of Biology, Indiana University, Bloomington, IN, 47405, 812-335-4234
Brakke, M., Dept of Plant Path, Univ of Nebraska-Lincoln, Lincoln, NE,68583, 402-472-3166
Brar, G. S., Agracetus Corp, 8520 University Green, Middleton, WI, 53562, 608-836-7300
Brawn, Robert I., 15 Douglas Drive, Ayr, Ontario NOB 1E0, Canada, 519-632-7954
Brettell, R., CSIRO Div Plant Industry, P.O. Box 1600, Canberra City ACT 2601, Australia, 062-46-5581
Brewbaker, James, Horticulture, Univ of Hawaii, 3190 Maile Way, Honolulu, HI, 96822, 808-948-7985
Briggs, Robert W., Funk Seeds International, 1300 W Washington Street,P.O. Box 2911, Bloomington, IL, 61702-2911, 309-829-9461
Briggs, Steven, Cold Spring Harbor Lab, P.O. Box 100, Cold Spring Harbor, NY, 11724, (516) 367-8468
Britt, Anne Bagg, Dept. Biol. Sciences, Stanford Univ, Stanford, CA, 94305
Brown, D., Dept of Biology, Bishops University, Lennoxville Quebec, Canada, 819-569-9551 EXT 289
Brown, R. P., Ohio Foundation Seeds, Croton, OH, 43013, 614-893-2501
Brown, Steven, Northrup King Co., Stanton, MN, 55081, (507)663-7633
Brown, William L., Pioneer Hibred Internatl, 6800 Pioneer Parkway, Johnston, IA, 50131, 515-270-3155
Browne, C., Curtis Hall, University of Missouri, Columbia, MO, 65211, 314-882-2674
Buckner, Brent, Dept Genetics, Iowa State Univ, Ames, IA, 50011, 515-294-4618
Buescher, Pat, Custom Farm Seed, Box 160, Momence, IL, 60954, 815-472-2433
Bullock, W. Paul, 424 Oliver Avenue, Ames, IA, 50010
Burgess, Diane, DNA Plant Technology, 6701 San Pablo Avenue, Oakland, CA, 94608, 415-547-2395
Burmood, T., Jacques Seed Company, 720 St. Croix Street, Prescott, WI,54021, 715-262-3223
Burnham, C. R., Agronomy & Plant Genetics, University of Minnesota, St.Paul, MN, 55108, 612-644-7797
Burr, Benjamin, Biology Dept, Brookhaven National Lab, Upton, NY, 11973, 516-282-3396
Bussard, James B., Curtis Hall, University of Missouri, Columbia, MO, 65211, 314-882-3410
Butler, Edward, Dept Agron, Curtis Hall, University of Missouri, Columbia, MO, 65211
Butnaru, Gallia, Institutul Agronomic Timisoara, Disciplina de Genetic,C. Post 136; O.P. 1, Timisoara 1900, Romania
Byrne, P. F., CIMMYT, Lisboa 27, Apdo. Postal 6-641, Col. Juarez, Deleg. Cuauhtemoc, 06600 Mexico, D.F., Mexico
Callaway, M. Brett, 604 Bradfield Hall, Dept of Plant Breeding, CornellUniv, Ithaca, NY , 14853
Callis, Judy, Horticulture Dept, Univ Wisconsin, Madison, WI, 53706
Camara-Hernandez, J., Altolaguirre 1295, Buenos Aires 1427, Argentina,51-6464
Cambolive, M., GIE Pioneer-France, Epuiseau, 4I290 Oucques, France
Cande, Zac, Dept of Botany, Univ of California, Berkley, CA, 94720, (415) 642-1669
Capelaro, Ademir, CIBA GEIGY QUIMICA S/A, R. Eduardo Marquez, 56, CaixaPostal, 585, Uberlandia-MG 38.400, Brazil, (034) 236-6520
Carlson, G., North Central Region, USDA-ARS MWA, 1815 N. University, Peoria, IL, 61604
Carlson, John E., Biotechnology Lab, 6174 Univ Boulevard, Univ of British Columbia, Vancouver, BC V6T1W5, Canada, 604-228-4733
Carlson, Lawrence A., 7 N. Winthrop Street, St. Paul, MN, 55119, 612-738-8812
Carlson, Peter S., Crop Genetics International, 7170 Standard Dr., Hanover, MD, 21076
Carlson, Wayne, Botany Department, University of Iowa, Iowa City, IA, 52240, 319-335-1316
Caruso, John L., Dept Biol Sci, University of Cincinnati, Cincinnati, OH, 45221
Chandlee, Joel M., Dept Plant Science, Univ Rhode Island, Kingston, RI,02881, 401-792-2529
Chandler, Vicki, Department of Biology, Institute of Molecular Biology,University of Oregon, Eugene, OR, 97403, 503-686-5136
Chang, Ming-Tang, Garst Seed Company, Highway 210, P.O. Box 500, Slater, IA, 50244, 515-685-3574
Chang, Ruying, Agronomy Dept, Iowa State Univ, Ames, IA, 50011, 515-294-7883
Chang, S. C., Known-You Seed Co. Ltd., 55 Min-Yi Rd., Yen-Pu Hsiang, Pingtung Hsieng, 904, Taiwan
Chase, Christine D., Plant Pathol Dept, Univ of Florida, Gainesville, FL, 32611, 904-392-3631
Chase, Sherret S., Chase Road, P.O. Box 193, Shokan, NY, 12481, 914-657-2392
Chaudhary, V. K., Dept Genetics, Rajendra Agr Univ, Bihar, Pusa (Samastipur)-848125, India
Chen, Che-Hong, Section Plant Biol, Plant Sci Bldg, Cornell Univ, Ithaca, NY, 14853, 607-255-8423
Chen, Keqin, Dept Genet & Cell Biol, University of Minnesota, St. Paul,MN, 55108, 612-624-3015
Choe, Bong-Ho, Agronomy Dept, College of Agriculture, Chungnam NationalUniversity, Dae-Jon 300-31, Korea
Chojecki, Jan, Imperial Chem Industries PLC, Jealott's Hill Research Sta, Bracknell Berks RG12 6EY, U K
Chomet, Paul, 345 Mulford Hall, Genetics Dept, Univ California, Berkeley, CA, 94720
Chou, Tau-San, Geo. J. Ball, Inc., P.O. Box 335, West Chicago, IL, 60185, 312-231-3600
Chourey, Prem, USDA ARS, Plant Pathology Department, University of Florida, Gainesville, FL, 32611, 904-392-7237
Christensen, Dean W., Funk Seeds Internatl, P.O. Box 477, Algona, IA, 50511, 515-295-9223
Chughtai, Sajjad R., Maize Program, NARC, P.O. NIH, Islamabad, Pakistan, 82005226
Clark, Jan, Purdue University, Dept of Biological Sciences, W. Lafayette, IN, 47906, 317-494-4919
Clark, Raymond L., USDA Plant Introduction Sta, Iowa State University,Ames, IA, 50010, 515-294-3255
Coe, E. H., Curtis Hall, University of Missouri, Columbia, MO, 65211, 314-882-2768
Colasanti, Joseph J., Cold Spring Harbor Lab, P.O. Box 100, Cold SpringHarbor, NY, 11724, 516-367-8469
Colbert, Terry, Funk Seeds International, P.O. Box 921, Union City, TN,38261, 901-885-7538
Colless, J. M., Agric Research Sta, Grafton, NSW, 2460 Australia, 066-447210
Cone, Karen C., Biological Sciences, University of Missouri, Columbia,MO, 65211, 314-882-2118
Conrad, Peter L, Dept of Biol Sciences, State Univ of New York, Plattsburgh, NY, 12901, 518-564-5271
Cook, Bill, 308 Tucker, University of Missouri, Columbia, MO, 65211, 314-882-3481
Coors, James G., Department of Agronomy, University of Wisconsin, 1575Linden Drive, Madison, WI, 53706, 608-262-7959
Cornu, A., Sta d'Amelior des Plantes, B V 1540, 21034 Dijon, France, 80-237221
Costa-Rodrigues, L., Estacao Agron. Nacional, 2780 Oeiras, Portugal
Cowen, Neil, United AgriSeeds, P.O. Box 4011, Champaign, IL, 61820, 217-373-5300

Crane, Paul L., Dept of Agronomy, Purdue University, W. Lafayette, IN,47907, 317-494-8088
Crawford, C. G., Northern Regional Res Ctr, USDA-ARS, 1815 N. University Street, Peoria, IL, 61604, FTS 360-4011
Creech, Roy G., Agronomy, PO Box 5248, Mississippi State University, Mississippi State, MS, 39762, 601-325-2459 EXT 2311
Cress, Dean, Agricultural Biotechnology, Rohm and Haas Company, 727 Norristown Road, Spring House, PA, 19477
Cresse, April, Dept Biol Sci, Purdue University, W. Lafayette, IN, 47907
Crosbie, T. M., Garst Seed Co., Highway 210, P.O. Box 500, Slater, IA,50244, 515-685-3574
Cross, Harold, Dept Crop & Weed Sci, North Dakota State Univ, Fargo, ND, 58102, 701-237-8138
Crowe, Todd G., Department of Plant Sci, Biol & Geol Bldg, Rm 231, London, Ontario N6A 5B7, Canada
Cuany, Robin L., Department of Agronomy, Colorado State University, Fort Collins, CO, 80523, 303-491-6832
Cullis, Christopher A., Department of Biology, Case Western Reserve, Cleveland, OH, 44106, 216-368-5362
Cummings, D. P., Dekalb-Pfizer Genetics, Box 367, Windfall, IN, 46076,317-945-7121
Curtis, Christine A., Curtis Hall, University of Missouri, Columbia, MO, 65211
Cutter, Gary L., Cargill, Inc, 324 Troy Street, Covington, OH, 45318, 513-473-5265
D'Halluin, Kathleen, Plant Genetics Systems, Lab Gent, Jozef Plateaustraat 22-B 9000, Gent, Belgium, (32) (97) 24255
Da Silva, W. J., Genet Evol, Univ Campinas, Cidad Univ Barao Geraldo, Campinas SP, Brazil
Dales, David, Biol and Geol Bldg, Rm 231, London, Ontario, Canada N6A 5B7, 679-2111 ext. 6468
Dallmier, Donald J., Sturdy Grow Hybrids Inc, Box 94, Arcola, IL, 61910, 217-268-3838
Dankov, Toma, Ivan Assen Str.-93, Sofia 1124, Bulgaria, 43-82-73
Darrah, L., 110 Curtis Hall, University of Missouri, Columbia, MO, 65211, 314-882-7707
Dash, Sudhansu, G-412 Agronomy, Ames, IA, 50011, 515-294-7883
Davis, David W., Horticultural Science Dept, 286 Alderman Hall-Univ Minn, 1970 Folwell Avenue, St. Paul, MN, 55108
Dawe, R. Kelly, Dept Genetics, University of California, Berkeley, CA,94720, 415-642-8058
Day, Janet, Dept Plant Biol, 289 Morrill, University of Illinois, 505 S. Goodwin, Urbana, IL, 61801, 217-333-2919
Day, Peter R., Center for Ag Molec Biology, Cook College, Rutgers Univ,Martin Hall, Rm 101A, P.O. 231, New Brunswick, NJ, 08903-0231
De Jong, A. W., Zelder B. V., Postbus 26, 6590 AA GENNEP, Nederland
De Leon, Carlos, CIMMYT, P.O. Box 9-188, Bangkok 10900, Thailand
De Leon, Diego Gonzalez, 302 Curtis Hall, University of Missouri, Columbia, MO, 65211
Dellaporta, Steve, Department of Biology, P.O. Box 6666, Yale University, New Haven, CT, 06511
Demopulos, J. T., U. of Alabama-Birmingham, UAB/Immunogenetics Program,Birmingham, AL, 35294, 205-934-2516
Dempsey, Ellen, Biology Dept, Indiana University, Bloomington, IN, 47401, 812-335-4061
Dennis, Elizabeth, CSIRO, Division of Plant Industry, P.O. Box 1600, Canberra, ACT 2601, Australia
Deutsch, James A., CIMMYT, Lisboa 27, Apdo. Post. 6-641 , 06600 MexicoD. F., Mexico
Dhillon, B. S., Inst Pl Breed & Pop Genetics, University of Hohenheim,PO Box 700 562-304, D-7000 Stuttgart-70 , Germany Federal Republic
Di Fonzo, Natale, Ist Sper Cereal, Via Stezzano 24, P.O. 164, 24100 Bergamo, Italy, 035-248823
Dilworth, Machi, USDA ARS OGPS, Room 112, W. Auditors Bldg., 15th & Independence Ave., SW, Washington, DC, 20251
Dodd, J. L., Professional Seed Research, 411 Dugan Rd, Sugar Grove, IL,60554
Doebley, John F., Botany Department, University of Minnesota, St. Paul,MN, 55108, 612-625-3702
Dolfini, Silvana Faccio, Dipartimento di Genetica, University of Milano, Via Celoria, 26, 20133 Milano, Italy
Dollinger, E. J., Ohio Agric Res & Devel Center, Wooster, OH, 44691, 216-263-3893
Dombrink-Kurtzman, Mary Ann, USDA/ARS, 1815 N. University St., Peoria ,IL, 61604
Dooner, Hugo K., Advanced Genetic Sciences Inc, 6701 San Pablo Avenue,Oakland, CA, 94608, 415-547-2395
Doring, H.-P., Inst Genetik, Univ Koln, D-5000 Koln 41, Weyertal 121, WGermany, 0221-4702465
Dotson, Stanton, Dept of Agronomy & Pl Genetics, Borlaug Hall, 1991 Buford Circle, St. Paul, MN, 55108
Dowe, Mike E. Jr., Dept Biochemistry, Spaulding Life Sci Bldg, Univ NewHampshire, Durham, NH, 03824
Dowling, Edward J., C/O Jacques Seed Co, P.O. Box 370, Lincoln, IL, 62656, 217-735-2770
Doyle, G. G., Curtis Hall, University of Missouri, Columbia, MO, 65211,314-882-8215
Dudley, John W., 1102 S Goodwin Ave, University of Illinois, Urbana, IL, 61801, 217-333-9640
Duncan, Dave, Dept Agron, S-332 Turner Hall, University of Illinois, 1102 S. Goodwin, Urbana, IL, 61801, 217-333-9465
Duvick, Donald N., Pioneer Hi-Bred Internat, 400 Locust Street, 700 Capitol Square, Des Moines, IA, 50309, 515-245-3591
Duvick, Jonathan P., Pioneer Hi-Bred Internatl, Dept of Biotechnology Res, 7300 N.W. 62nd Ave, Box 38, Johnston, IA, 50131, 515-270-3176
Dwyer, L., Land Resource Res Ctr, Agriculture Canada, Bldg 74 CEF, Ottawa, Ontario K1A 0C6, Canada
Earle, Elizabeth D., Dept Plant Breed & Biom, Cornell University, 516 Bradfield, Ithaca, NY, 14853-1902, 607-255-3102
Eberhart, Steve, USDA ARS Natl Seed Storage Lab, Colorado State Univ, Fort Collins, CO, 80523, 303-484-0402
Echt, Craig, Plant Pathology Department, University of Arizona, Tucson,AZ, 85721, 602-621-3381
Edwards, Marlin, Green Giant, 1201 N. 4th Street, Le Sueur, MN, 56058,612-665-3515
Eichholtz, David A., Monsanto, AA3E, 700 Chesterfield Village Pkwy, St.Louis, MO, 63198, 314-537-6227
Elliott, Beth, Cold Spring Harbor Lab, P. O. Box 100 , Cold Spring Harbor, NY, 11724, 516-367-8330
Endo, Toru, National Inst of Genetics, Misima 411, Japan, 0559-75-0771EXT 319
England, Dan, Curtis Hall, Univ of Missouri, Columbia, MO, 65211, 314-882-7818
Esen, Asim, Dept Biology, Va Polytech Inst & State Univ, Blacksburg, VA, 24061, 703-961-5894
Eubanks, Mary W., Dept of Crop Science, N Carolina State U., Box 7620,Raleigh, NC, 27695-7620, 919-737-2647
Everett, Herbert L., Cornell University, 520 Bradfield Hall, Ithaca, NY, 14853, 607-255-1667
Evola, S. V., CIBA-GEIGY Corporation, P.O. Box 12257, Research TrianglePark, NC, 27709-2257, 919-549-8164
Fahey, Jed, Crop Genetics International, 7170 Standard Drive, Hanover,MD, 21076, 301-379-0030
Farish, Guy, Department of Biology, University of North Dakota, Box 8238 University Station, Grand Forks, ND, 58202, 701-777-3676
Fauron, Christiane, University of Utah, 743 Wintrobe Building, Salt Lake City, UT, 84132, 801-581-5192
Fedoroff, Nina, Carnegie Inst Wash, 115 West Univ Parkway, Baltimore, MD, 21210, 301-467-1414
Feiler, Heidi, 16562 Drexel Avenue, South Holland, IL, 60473, 312-333-0937
Feix, G., Institut Fur Biologie III, Universitat Freiburg, Freiburg, WGermany, 0761-2032724
Feldman, Lewis J., Department of Botany, University of California, Berkeley, CA, 94720
Ferguson, V., Custom Farm Seed, 2773 N Main, Decatur, IL, 62526, 217-875-2826
Ferl, R. J., Dept Vegetable Crops, Univ Florida, Gainesville, FL, 32611, 904-392-2370

Filion, W. G., Erindale Campus Biology, University of Toronto, 3359 Mississauga Road, Mississauga, Ontario, Canada L5L1C6, 416-828-5326
Fincher, Robert R., Pioneer Hi-Bred Internatl, Dept of Biotechnology Research, P.O. Box 38, Johnston, IA, 50131, 515-270-3650
Fletcher, G., Agronomy Dept, S-122 Turner, University of Illinois, 1102S. Goodwin, Urbana, IL, 61801, 217-333-9644
Flores, Catalina, Ceres Int. A.P. 484, Los Mochis, Sinaloa, Mexico 81200, Mexico, 681-2-1502
Foley, Robert, RPA-C Associates, 505 W. Saginaw, P.O. Box 54, Breckenridge, MI, 48615
Foley, Terry, Holden's Foundation Seeds Inc, P.O. Box 839, Williamsburg, IA, 52361, 319-668-1100
Fong, Franklin, Dept Soil & Crop Sci, Texas A&M University, College Station, TX, 77843-2474
Fowler, John, Department of Genetics, 345 Mulford Hall, University of California, Berkeley, CA, 94720, 415-642-8058
Fowler, Robert G., Dept Biological Sciences, San Jose State University,San Jose, CA, 95192, 408-277-2355
Fowler, Tom, Ohio State Biotechnology Ctr, 244 Rightmire Hall, 1060 Carmack Road, Columbus, OH, 43210, 614-292-3349
Fraley, Robb, Monsanto, BB3B, 700 Chesterfield Village Pkwy, St. Louis,MO, 63198, 314-537-6204
Francis, T. R., Northrup King Co., R. R. 1, Arva, N0M 1C0, Canada, 519-659-5130
Freeling, Michael, Dept of Genetics, Univ of California, Berkeley, CA,94720, 415-642-7085
Frei, Mark, Northrup King Seed, Via Comparina 2, Madignano 26020, Italy, 065-381797
Friedrich, James W., Maize Genetic Resources, Inc, 421 Angier Highway,Benson, NC, 27504, 919-934-9908
Frisch, David, Department of Agronomy, University of Minnesota, St. Paul, MN, 55108, 612-625-5215
Fromm, Michael, USDA ARS PGEC, 800 Buchanan Street, Albany, CA, 94710,415-559-5908
Gabay-Laughnan, S., Plant Biology/289 Morrill Hall, University of Illinois, 505 S. Goodwin Avenue, Urbana, IL, 61801, 217-333-2919
Gain, Jeffrey E., National Corn Growers Assoc., 1000 Executive Parkway,Suite 105, St. Louis, MO, 63141
Galinat, Walton C., Sub Exp Sta, Univ Mass, 240 Beaver Street, Waltham,MA, 02254, 617-969-6523
Gallais, A., G.I.S. du Moulon, Ferme du Moulon, 91190 Gif sur Yvette, France
Garcia, Ramon, EniChem Americas Inc., 2000 Princeton Park Corp Ctr, Monmouth Jct., NJ, 08852
Garcia-Olmedo, F., Dept Biochemistry, E T S Ingenieros Agronomos, 28040-Madrid, Spain, 1-24444807
Gardiner, Jack, Curtis Hall - Agronomy, University of Missouri, Columbia, MO, 65211
Gardner, C. A. C., Pioneer Hybrid Internatl, Highway 65 South, PO Box 218, Carrollton, MO, 64633, 816-542-3780
Gardner, C. O., Department of Agronomy, University of Nebraska, Lincoln, NE, 68583-0915
Garwood, D. L., Garwood Seed Company, RR 1, Box 20, Stonington, IL, 62567, 217-325-3715
Gavazzi, Giuseppe, Universita de Milano, Dept of Genetics & Microbiol,Via Celoria 26, 20133 Milano, Italy, 02-230823
Gay, Philippe, CIBA-GEIGY Ltd, Agricultural Division, CH-4002 Basel, Switzerland, 061-37-44-57
Geadelmann, J. L., Holden's Foundation Seeds, Inc, Rt. 1, Box 112, Stanton, MN, 55018, 507-263-3476
Gebauer, Juan E., Corn Breeding, Casilla 190, Buin, Chile
Geiger, Hartwig H., Univ Hohenheim, Inst Pflanzenzucht, Postfach 700562(350), D-7000 Stuttgart 70, W Germany
Gengenbach, B.G., Agron & Plant Genetics, Univ of Minnesota, St Paul, MN, 55108, 612-625-6282
Georgiev, Trifon, Technical University, "A. Kanchev", Rousse 7004, Bulgaria, 082-23469
Gerdes, James, 405C Eagle Hts, Madison, WI, 53705, 608-233-6730
Gierl, Alfons, Max-Planck Inst., Egelspfad, D-5000 Koln-30 Vogelsang, WGermany, 49-221-5062-242
Gillies, Christopher, Botany Bldg A12, Univ of Sydney, Sydney NSW 2006,Australia, 02-692-2688
Glover, David V., Dept of Agronomy, Purdue University, W. Lafayette, IN, 47907, 317-494-8067
Godshalk, E. Brent, Dekalb-Pfizer Genetics, 3100 Sycamore Road, Dekalb,IA, 60115, 815-758-9372
Goldman, Stephen, Dept of Biology, University of Toledo, Toledo, OH, 43606, 419-537-4197
Golubovskaya, I. N., Dept of Genetics, N I Vavilov , All-Union Res InstPlant Ind, Hertzen Street, 42, 190 000 Leningrad, USSR
Goodman, Major M., Department of Crop Sciences, North Carolina State Univ, P.O. Box 7620, Raleigh, NC, 27695, 919-737-2704
Gordon, P. N., Department of Biology, 918 Kline Biology Tower, Yale Univ, P.O. Box 6666, New Haven, CT, 06511-8112
Gould, Alan R., United Agriseeds, Inc., P. O. Box 4011, Champaign, IL,61820, 217-373-5300
Gracen, V. E., Cargill Hi-Bred Seed, Box 9300, Minneapolis, MN, 55440,612-475-7244
Grant, David, Pioneer Hi-Bred Internatl, Box 38, 7300 N.W. 62nd Avenue,Johnston, IA, 50131, 515-270-3503
Green, M. M., Dept of Genetics, University of California, Davis, CA, 95616, 916-752-1189 EXT 6295
Greenblatt, I. M., U-131, Molecular & Cell Bio, Univ of Connecticut, 354 Mansfield Rd, Storrs, CT, 06268, 203-486-2915
Grier, Stephen L., Sunseeds, 1120-220th St West, Farmington, MN, 55024,612-463-4646
Grobman, Alexander, Grobman Genotecnica SA, Apartado 5161, Lima 1, Peru, 234464
Gross, D. F., Dekalb-Pfizer Genetics, Corn Research Center, 3100 Sycamore Road, Dekalb, IL, 60115, 815-756-7333
Grula, John, Phytogen, 101 Waverly Drive, Pasadena, CA, 91105, 818-792-1802
Grumbles, Robert M., Dept Molec Biophys & Biochem, 260 Whitney Avenue 414 KBT, P.O. Box 6666, New Haven, CT, 06511, 203-432-2606
Guffy, Richard D, Dept of Genetics, North Carolina State Univ, Box 7614, Raleigh, NC, 27695-7614, 919-737-2280
Gupta, Manju, 2050 Concourse Drive, San Jose, Ca, 95131-1818, (408) 433-9800
Haag, Wayne L., c/o CIAT, 1380 NW 78th Ave, Miami, FL, 33126
Hack, Ethan, Botany Department, Bessey Hall, Iowa State University, Ames, IA, 50011
Hagemann, Kim M., G412 Agronomy, Ames, IA, 50011, (515) 294-7883
Hagemann, R., Dept Genetics, Martin Luther University, Domplatz 1, 4020Halle/S., E Germany
Hake, S., USDA-ARS-PGEC, 800 Buchanan Street, Albany, CA, 94710, 415-559-5907
Hallauer, Arnel R., Agronomy Building, Iowa State University, Ames, IA,50010, 515-294-3052
Hallberg, Boone, Cent de Grad, Inst Tec Oaxaca, Calzs . Technologico Y,Gral. Wilfrido Massieu S/N., Oaxaca, Oax. C.P. 68000, Mexico, 6-44-13
Hamilton, R. H., Buckhout Laboratory, Pennsylvania State Univ, University Park, PA, 16802, 814-865-8800
Hamilton, R. I., Agriculture Canada, Plant Research Centre, Forage Bldg12, Ottawa, Ontario K1A 0C6, Canada
Han, Changdeok, Curtis Hall, University of Missouri, Columbia, MO, 65211
Hannah, L. C., Veg Crops Dept, Univ of Florida, IFAS, Gainesville, FL,32611, 904-392-7919
Hansel, W. C., Hansel Cons & Mgmt, Box 283, Carrollton, MO, 64633, 816-542-1616
Hansen, Dale J., NPI, 417 Wakara Way, Salt Lake City , UT, 84108
Hansen, Leon A. , Rogers Brothers Seed Co., P.O. Box 4727, Boise, ID, 83711-0727, 208-322-7272
Harberd, Nicholas, AFRC Inst Plant Sci Res, Cambridge Lab, Maris Lane,Trumpington, Cambridge CB2 2JB, UK, 011-44-233-840932
Hardeman, Kris, Department of Biology, Institute of Molecular Biology,University of Oregon, Eugene, OR, 97403
Harn, Chee, Dept Botany & Plant Path, Oregon State University, Corvallis, OR, 97331, 503-754-3451
Harper, David C., Holden's Foundation Seeds Inc., P.O. Box 839, Williamsburg, IA, 52361, 319-668-1100

Harris, John W., Dept of Biology, Tennessee Tech Univ, Cookeville, TN,38505, 615-528-3143
Harris, Linda, Institute of Molecular Biol, Univ of Oregon, Eugene, OR,97403
Harris-Cramer, Jane, Agrigenetics, 5649 East Buckeye Road, Madison, WI,53716, 608-221-5000 ext 353
Hartley, Tony, Sungene Technologies Corp., 2050 Concourse Drive, San Jose, CA, 95131-1818
Hartman, Carl J., 155 South St. Rd. 2, Valparaiso, IN, 46383
Hauptli, Holly, Calgene Inc, 1920 Fifth Street, Davis, CA, 95616, 916-753-6313
Hawk, James A., Dept Plant Science, University of Delaware, Newark, DE,19711, 302-738-2531
Headrick, John, Plant Pathology Department, Univ Ill, N-519 Turner Hall, 1102 S. Goodwin Avenue, Urbana, IL, 61801, 217-333-7514
Helentjaris, Tim, NPI, 417 Wakara Way, Salt Lake City, UT, 84108, 801-582-0144
Helm, James, Agronomy Department, North Dakota State University, Fargo,ND, 58105
Henderson, Clarion B., Illinois Foundation Seeds, PO Box 722, Champaign, IL, 61820, 217-485-6260
Hendrickx, Margaretha, Dept Biol Sci, Purdue University, W. Lafayette,IN, 47907
Herrmann, R., Botany Department, Ludwig-Maximilans Univ Munich, Manzenger Str 67, 8 Munchen 19, W. Germany
Hile, Glenn C., Northrup King Co, RD1, Box 480, Mannheim, PA, 17545
Ho, David, Department of Biology, Washington University, St. Louis, MO,63130, 314-889-4632
Hodges, Tom, Botany & Plant Pathology, Purdue University, W. Lafayette,IN, 47907
Hoegemeyer, Thomas C., Hoegemeyer Hybrids Inc, Rt 2, Hooper, NE, 68031,402-654-3399
Hoisington, David, Curtis Hall, Univ of Missouri, Columbia, MO, 65211,314-882-2033
Holden, A., Corn States Hybrid Serv, 2505 McKinley Ave., Des Moines, IA, 50321, 515-285-3091
Hole, David , Soil & Crop Sci. Dept., Plant Sci. Bldg., Texas A&M University, College Station, TX, 77843, 409-845-1126
Holley, Randall N., NC State University, 1236 WMS Dept. Crop Sciences,Carry, NC, 27695-7620
Hooker, A. L., Plant Molecular Biology Ctr, Northern Illinois Univ, DeKalb, IL, 60115, 815-753-7841
Huang, Yi Xiang, Crop Breed & Cult Res Inst, Sichuan Academy Agr Sci, Chengdu, Sichuan Province, China
Hubbard, Jon, Asgrow Seed Company, PO Box L, San Juan Bautista, CA, 95131
Huff, Crystal A., Northrup King Company, Rd #1 Box 480, Manheim, PA, 17545, 717-898-1630
Hulbert, Scott, Purdue University, Dept of Biol Sciences, W. Lafayette,IN, 47907, 317-494-4919
Hunsperger, John P., Dept Genet & Cell Biol, University of Minnesota, St. Paul, MN, 55108, 612-624-3015
Hunt, Marjorie, Div Biol Sci, Tucker Hall, Univ Missouri, Columbia, MO,65211
Hunter, R. B., Funk Seeds International , Box 18300, Greensboro, NC, 27417
Iltis, Hugh H., Dept of Botany, Univ of Wisconsin, Madison, WI, 53706,608-256-7247
Ingram, David S., Botany Department, University of Cambridge, Downing Street, Cambridge CB2 3EA, U K, 0223-61414
Inoue, Yasuaki, National Grassland Res Inst, 768 Nishinasuno, Tochigi 329-27, Japan
Irish, Erin, Department of Biology, Yale University, New Haven, CT, 06511
Isaac, Peter, The Nickerson Seed Co Ltd, Cambridge Science Park, MiltonRoad, Cambridge CB4 4WE, UK, 0223-862933
Isenhour, David J., Department of Entomology, Univ Ga, Coastal Plain Exp Sta, P.O. Box 748, Tifton, GA, 31793-0748, 912-386-3374
Ishige, Teruo, Department of Cell Biology, Natl Inst Agrobiol Resources, Kannondai Tsukuba, Ibaraki 305, Japan
Jacobs, M., Plant Genetics Univ Brussels, 65 Paardenstraat, B-1640st Genesius-Rode, Belgium, 2-358-65-22
Jaesan, Kim, Dept. of Agron. & Plant Genet., St. Paul, MN, 55108
Jakob, Karl M., Department of Plant Genetics, Weizmann Inst Sci, Rehovot, Israel, 054-83301
James, Martha G., Genetics Department, 8 Curtiss Hall, Iowa State University, Ames, IA, 50010, 515-292-3984
Jardine, Douglas J., Throckmorton Hall, Kansas State University, Manhattan, KS, 66506, 913-532-5810
Jauhar, Prem P., USDA ARS, Forage & Range Res Lab, Utah State Univ, Logan, UT, 84322-6300, 801-750-3074
Jellen, Eric H., Univ of Minnesota, 411 Borlaug Hall, St. Paul, MN, 55108
Jewell, David C., CIMMYT - Maize Program, Lisboa 27, Apdo Postal 6-641,Mexico D.F. 06600, Mexico, 525-761-3311 EXT 1110
Jimenez, J., Biology Dept, Purdue Univ, Fort Wayne, IN, 46805, 219-482-5253
Johal, G., Cold Spring Harbor Lab., P. O. Box 100, Cold Spring Harbor,NY, NY, 11724
Johns, Mitrick A., Dept Biological Sciences, Northern Illinois University, DeKalb, IL, 60115, 815-753-7836
Johnson, E. C., Escagen Corp., 830 Bransten Road, San Carlos, CA, 94070, 415-595-5335
Johnson, Elizabeth, Box 371, Kaunakakai, HI, 96748, 808-553-5468
Johnson, M. W., Dept of Agronomy, Pennsylvania State Univ, University Park, PA, 16802, 814-865-0324
Johnson, Scott, McCurdy Seed Co, East Main Street, Fremont, IA, 52561,515-933-4291
Johri, M. M., Molecular Biol Unit, Tata Inst Fundamental Res, Bombay 400 005, India, 219111/ext 338
Jondle, Doug, Department of Agronomy, University of Wisconsin, 1575 Linden Drive, Madison, WI, 53706
Jones, J. K., Department of Agricultural Bot, University of Reading, Whiteknights, Reading RG6 2AS, UK, 0734-875123 EXT 7905
Jones, John Edward, 4949 Battery Lane, Apt #311, Bethesda, MD, 20814
Jones, R. K., Dept Agron & Plant Genetics, Univ of Minnesota, St. Paul,MN, 55108
Jordan, Berry, Dept Foods & Nutrition, Dawson Hall, Univ Georgia, Athens, GA, 30602
Josephson, L. M., 350 Plant Sci Bldg, Univ of Tennessee, Knoxville, TN,37916, 615-974-7391
Julstrom, P., Dekalb-Pfizer Genetics, 3100 Sycamore Road, Dekalb, IL, 60115
Jupe, Eldon R., Department of Biochemistry, 322 Choppin Hall, LouisianaState University, Baton Rouge, LA, 70803, 504-388-1554
Juvik, J. A., Dept of Horticulture, University of Illinois, 1103 West Dorner Drive, Urbana, IL, 61801, 217-333-1966
Kahler, A. L., Biogenetic Services, Inc, 2308 6th Street E., P.O. Box 710, Brookings, SD, 57006, 605-697-8500
Kamimura, Shoji, Kunisada Plant Breed Station, 1211 Ichiba, Akabori-mura, Sawa-gun, Gunma-ken 379-22, Japan
Kang, M. S., Department of Agronomy, Louisiana State University, BatonRouge, LA, 70803, 504-388-2110
Kannenberg, L. W., Dept of Crop Science, University of Guelph, Guelph,Ontario N1G 2W1, Canada, 519-824-4120 EXT 2506
Karpoff, A. J., Dept of Biology, Univ of Louisville, Louisville, KY, 40292, 502-588-6771
Kasha, K. J., Dept of Crop Science, Univ of Guelph, Guelph, Ontario N1G2W1, Canada, 519-824-4120 EXT 2507
Kato Y., T. A., Centro de Genetica, Colegio de Postgraduados, Chapingo56230, Mexico, (5)5-85-07-28
Katsuta, Masumi, 210 Curtis Hall, Dept of Agronomy, 210 Curtis Hall, Columbia, MO, 625211, 882-2674
Keim, Kent R., Sunseeds Genetics, Inc., 1200 Anderson Corner Road, Parma, ID, 83660, 208-674-2208
Kelley, Phil, University of Nebraska, School of Biology, Lincoln, NE, 68588, 402-472-1683
Kermicle, Jerry, Dept of Genetics, University of Wisconsin, Madison, WI, 53706, 608-262-1253
Kerr, E. A., Stokes Seeds Limited, 39 James Street, P.O. Box 10, St. Catharines, Ontario L2R 6R6, Canada, 416-688-4300
Keyes, Carol A. Lemke, 12773 Castlebar Dr, St. Louis, MO, 63146
Khavkin, Emil E., All-Union Res Institute of, Agricultural Biotechnology , 12 Pskovskaya str., bld. 4, I27253 Moscow, USSR
Kim, Soon Kwon, Maize Research Program, Internatl Inst Tropical Agr, PMB 5320, Ibadan, Nigeria

Kind, K., Curtis Hall, Univ of Missouri, Columbia, MO, 65211, 314-882-2674
Kindiger, Bryan, Wilson Hybrids, Inc, P.O. Box 391, Harlan, IA, 51537,712-755-3841
Kinugawa, Kenjiro, 4-23-3, Takakuradai, Sakai, Osaka 590-01, Japan, 0722-91-5497
Kirihara, Julie, Molecular Genetics Inc., 10320 Bren Road East, Minnetonka, MN, 55343, 612-935-7335
Kiss, Charles, Northrup King Semences, 18 Avenue Gollieni, 49130 Les Ponts de Ce, France, (41) 663831
Kleese, Roger, Molecular Genetics, 10320 Bren Road East, Minnetonka, MN, 55343, 612-935-7335
Klein, Anita S., Department of Biochemistry, Spaulding Life Science Bldg, University of New Hampshire, Durham, NH, 03824, 603-862-2455
Koeppler, Shawn, Dept. Agron. & Plant Genet., 1991 Buford Circle , St.Paul , MN., 55108
Kohashi, Josue, Dept of Botany, Colegio de Postgrad, Chapingo, Edo de Mex, Mexico
Koinuma, Keiichi, Natl. Grassl. Res. Inst., 768 Nishinasuno, Tochigi, 329-27, Japan
Konstantinov, Kosana, Maize Research Institute, P.O. Box 89, 11081 Zemun, Yugoslavia
Konstantinov, Yu., Siberian Inst Plant Phys Bioch, USSR Academy of Sciences, P.O. Box 1243, Irkutsk, USSR
Kowles, R. V., Biology Department, St Marys College, Winona, MN, 55987,507-452-4430 EXT 1554
Kriz, A. L., Dept Agronomy, Turner Hall, Univ Illinois, Urbana, IL, 61801
Kuehnle, Adelheid, Dept. Horticulture, 3190 Maile Way, Honolulu, HI, 96822
Laible, Charles A., Research Department, Funk Seeds International, Bloomington, IL, 61701, 309-829-9461
Lambert, A., Semences Nickerson, 49160 Longue, Jumelles, France
Lamkey, Kendall, Agronomy Building, Iowa State University, Ames, IA, 50010, 515-294-7826
Lampoh, E., Crops Res Inst, PO Box 20, KPEVE, Volta Reg, Ghana, 2 ext 5
Lander, Eric S., Whitehead Institute, 9 Cambridge Center, Cambridge, MA, 02142, 617-258-5192
Lane, Barbara, Genetics Department, University of California, Berkeley,CA, 94720, 415-642-8058
Langdale, Jane, Biology Department, P.O. Box 6666, Yale University, NewHaven, CT, 06511, 203-432-3862
Langston-Unkefer, Pat J., INC-4, Mail Stop C345, Los Alamos National Lab, Los Alamos, NM, 87545, 505-665-2556
Larkin, John C., Dept Biochemistry, Univ Minnesota, 1479 Gortner Ave, St Paul, MN, 55108
Larkins, B., Dept Plant Sciences, University of Arizona, Tucson, AZ, 85721
Larson, Russell L., Curtis Hall, University of Missouri, Columbia, MO,65211, 314-882-3410
Laughnan, John R., Plant Biology/289 Morrill Hall, U of Illinois/505 SGoodwin Av, Urbana, IL, 61801, 217-333-2919
Laurie, David, AFRC Inst Plant Science Res, Maris Lane, Trumpington, Cambridge CB2 2JB, UK, 0223-840932
Lavapaurya, Tavat, Dept Horticulture, Kasetsart University, Bangkok 10900, Thailand
Law, Colin N., AFRC Inst Plant Sci Res, Cambridge Laboratory, Maris Lane, Trumpington, Cambridge CB2 2JB, UK
Leaver, C. J., Dept Botany, Kings Bldgs, U. of Edinburgh/Mayfield Road,Edinburgh EH9 3JH, Scotland, 031-667-1081 EXT 3304
Lee, Chong, Oil Palm Res Station, PO Box 207, Banting, Selangor, Malaysia
Lee, Elizabeth, 1571 Agronomy, Iowa State University, Ames, IA, 50011
Lee, Michael, Department of Agronomy, Iowa State University, Ames, IA,50011, 515-294-3052
Lee, Sharon K., Department of Botany, University of Rhode Island, Kingston, RI, 02881, 401-792-2625
Leja, Carol, Dept Plant Biol, 289 Morrill, University of Illinois, 505S. Goodwin, Urbana, IL, 61801, 217-333-2919
Leland, Tim, Funk Seeds International, 1300 West Washington, Bloomington, IL, 61701, 309-829-9461
Leroy, Philippe, Biosem, 24 Avenue de Landais, 63170 Aubiere, France, (33) 73-26-87-34
Leto, K. J., Agric Prod Dept, DuPont Co, Stine Laboratory S215, ElktonRoad, PO Box 30, Newark, DE, 19714, 302-366-5170
Levings, C. S., Department of Genetics, North Carolina State Univ, Box7614, Raleigh, NC, 27695-7614, 919-737-7115
Levites, E. V., Inst Cytol Genetics, Novosibirsk, USSR
Li, Ching Hsiung, Chinese Acad Agric Sci, Department of Crop Science, Beijing, China
Li, Jigeng, Institute of Genetics, Academia Sinica, Beijing, China
Lim, Yong Pyo, Dept Biol, Yale Univ, P.O. Box 6666, New Haven, CT, 06511
Lin, Bor-yaw, Genetics Institute, 87 Cambridge Park Drive, Cambridge, MA, 02140, 617-876-1170
Lindsey, M. F., Dekalb-Pfizer Genetics, Box 408, Dayton, IA, 50530, 515-547-2550
Liu, Changnong, Dept of Genetics & Cell Biol, 250 Bioscience Center, 1445 Gortner Ave, St. Paul, MN, 55708
Loeffel, F. A., NAPB Res. Center, Rural Route #2, Brookston, IN, 47923,317-563-3111
Lonsdale, D. M., Plant Breeding Institute, Maris Lane, Trumpington, Cambridge CB2 2LQ, UK
Lorenzoni, C., Istituto di Genetica Vegetale, Universita Cattolica, 29100 Piacenza, Italy
Lorz, Horst, Max-Planck-Institut Fur, Zuchtungsforschung, D5000 Koln 30, W Germany, 221-5062-316
Lowe, Brenda, PGEC - USDA, 800 Buchanan St, Albany, CA, 94710
Lower, W., 235 Environ Trace Ctr, Univ of Missouri, Columbia, MO, 65211, 314-445-8595
Ludwig, Steven, Department of Botany, University of Georgia, Athens, GA, 30602
Luehrsen, Ken, Dept of Biological Sciences, Stanford Univ, Stanford, CA, 94305, 415-723-2609
Lundquist, Ron, Molecular Genetics Inc, 10320 Bren Road East, Minnetonka, MN, 55343
Ma, Hongchang, Dept of Agronomy, Kansas State University, Manhattan, KS, 66502
Ma, Yu, Agrigenetics, 5649 East Buckeye Road, Madison, WI, 53716, 608-221-5000
Mackey, C. J., DeKalb-Pfizer Genetics, Pfizer Central Research, EasternPoint Road, Groton, CT, 06340
MacRae, Amy F., Dept of Botany and Plant Sci, University of California,Riverside, CA, 92521, (714) 787-3543
Magoja, J. L., Univer Nac de Lomas de Zamora, CC9S, 1832-Lomas de Zamora, Argentina, 243-0233
Maguire, Marjorie, Zoology Department, University of Texas, Austin, TX,78712, 512-471-7451
Maheshwari, J. K., National Botanical Res Inst, Lucknow-226001, India
Malone, Carl, 205 McKinley Avenue, Milford, IL, 60953, 815-889-4079
Mans, Rusty J., Dept of Biochem/J245 JHMHC, Univ of Florida, Gainesville, FL, 32610, 904-392-2332
Manzocchi, Lucia A., Consiglio Nazionale Ricerche, Istituto BiosintesiVegetali, Via Bassini 15, 20133 Milano, Italy
Marchand, J. L., F Chemin de l'IRAT, Ligne Paradis, 97410 St. Pierre, Reunion
Mareck, Jim, I.I.T.A., PMB 5320, Oyo Road, Ibadan, Nigeria
Marquez-Sanchez, F., Campo Agricola Experimental, Valle de Mexico, Coordinacion de Maiz, Zoua Sur, Chapingo, Mex. 56230, Mexico, 585-0728
Marshall, Lori, 411 Borlaug Hall, 1991 Upper Buford Circle, Univ of Minnesota, St. Paul, MN, 55108, 612-625-8107
Martienssen, R. A., Cold Spring Harbor Labs, P.O. Box 100, Cold SpringHarbor, NY, 11724, 516-367-8467
Martin, Michael J., Garst Seed Co., P.O. Box 500, Slater, IA, 50244
Martin Sanchez, J. A., Esc T Sup Ingenieros Agron, Carretera de HuescaKm 3, Lerida 25006, Spain, 973-233841
Marton, Csaba Lajos, 2462 Martonvasar, P.O.B. 19, Hungary, 26-45774

Mascarenhas, Joseph P., Department of Biol Sci, State University of NewYork, Albany, NY, 12222, 518-457-3300
Mascia, P., Cargill Hi-Bred Seed Division, RR 3, Box 751, Aurora, IL, 60506, 312-892-4331
Mashnenkov, A. S., Krasnodar Res Inst Agric, Krasnodar, USSR
Masson, Patrick, Carnegie Inst Wash, 115 West Univ Parkway, Baltimore,MD, 21210, 301-467-1414
Mather, Diane E., Plant Sci Dept - Macdonald Col, McGill Univ - 21111 Lakeshore, Ste-Anne-de-Bellevue, Quebec H9X 1C0, Canada, (514) 398-7855
Mathews, Paul, Dept Biol Sci, Davis Hall, Lehman College, City Univ NewYork, Bronx, NY, 10468
Mathur, D. S., Div. of Genetics, Indian Agr Res Inst, New Delhi, India
Mazoti, Luis B., C. Croce 145, 1832 Lomas de Zamora, Argentina
McBride, A. C., Phyto Dynamics, Inc., 624 South 775 E, Lafayette, IN, 47905, 317-296-3497
McCarty, Donald R., Vegetable Crops Department, 1255 Fifield Hall, University of Florida, Gainesville, FL, 32611, 904-392-9087
McClain, Eugene F., Dept Agronomy & Soils, Clemson University, Clemson,SC, 29634-0359, 803-656-3102
McClintock, Barbara, Cold Spring Harbor Laboratory, Cold Spring Harbor,NY, 11724, 516-367-8385
McConnell, D. J., Genetics, Trinity College, University of Dublin, Dublin 2, Ireland
McCormick, Sheila, USDA-ARS-PGEC, 800 Buchanan Street, Albany, CA, 94710, 415-559-5906
McCoy, T., Department of Plant Sciences, University of Arizona, Tucson,AZ, 85721, 602-621-1014
McCurdy, Leroy, P.O. Box 77, McCurdy Seed Co, Fremont, IA, 52561, 515-933-4291
McDaniel, R. G., Dept of Plant Sciences, University of Arizona, Tucson,AZ, 85721, 602-261-3357
McFerson, John, Asgrow Seed Co., 1060 E. Highway 14, RR #1, Janesville, WI, 53546, (608)755-1777
McMullen, Michael, USDA ARS, Department of Agronomy, OARDC, Wooster, OH, 44691, 216-263-3894
McWhirter, K. S., 127 Victoria Road, West Pennant Hills, N.S.W. 2120, Australia, 02-484-7417
Melia-Hancock, Susan, Curtis Hall - Agronomy, University of Missouri, Columbia, MO, 65211
Mello-Sampayo, Tristao, Centro di Biologia, Inst Gulbenkian de Ciancia,Oeiras, Portugal
Messing, Jo, Rutgers, The State Univ, Waksman Institute, P.O. Box 759,Piscataway, NJ, 08855, 201-932-4256
Meyer, Annette, 101 Vegetable Crops Building, 1103 W. Dorner Dr., University of Illinois, Urbana, IL, 61801
Michel, Kenneth, Department of Biology, Slippery Rock University, Slippery Rock, PA, 16057, 412-794-7326
Micu, V. E., Moldav Inst Maize & Sorghum, Kriulianskii Raion Krcv-Nova,278335 Moldavian SSR, USSR
Miernyk, Jan, Northern Regional Res Lab, USDA-ARS, 1815 N. University Street, Peoria, IL, 61604, FTS 360-4011
Mies, David, Northrup King, Box Z, St. Joseph, IL, 61873
Mihm, John A., CIMMYT, Apdo Postal 6-641, Deleg Cuauhtemoc, 06600 Mexico, DF, Mexico, 905-595-4-2100
Miksche, J. P., USDA NPS, Bldg 005, Rm 239, BARC-W, Beltsville, MD, 20705
Mikula, Bernard C., Defiance College, Defiance, OH, 43512, 419-784-4010EXT 426
Miles, Donald, Tucker Hall, University of Missouri, Columbia, MO, 65211, 314-882-7933
Miranda, L. T. De, Instituto Agronomico, Caixa Postal, 28, 13.100 Campinas SP, Brazil, 0192-420235
Mitchell II, Roger E., Dept Genet & Cell Biol, University of Minnesota,St. Paul, MN, 55108
Mock, James J., Northrup King Company, 7500 Olson Memorial Highway, P.O. Box 959, Minneapolis, MN, 55440
Modena, Stephen, Dept. of Crop Science, North Carolina State Univ., Box7620, Raleigh, NC, 27695, 919-737-2246
Mogford, D. J., Asgrow S. Africa, P.O. Box 912-653, 0127 Silverton, S Africa
Molina, Maria del Carmen, Inst Fitotec Santa Catalina, Llavallol, Argentina
Moll, Robert H., Department of Genetics, North Carolina State Univ, Box7614, Raleigh, NC, 27695-7614, 919-737-2289
Molnar, S. J., Agriculture Canada, Plant Biotech, Plant Res Ctr, Ottawa, Ontario K1A 0C6, Canada, 613-995-3700 EXT 7560
Morris, Rosalind, Department of Agronomy, University of Nebraska, Lincoln, NE, 68583-0915, 402-472-1534
Morse, Richard, Cargill Hybrid Seed, 3864 Preemption Rd, Geneva, NY, 14456, 315-789-8626
Mottinger, John, Department of Botany, University of Rhode Island, Kingston, RI, 02881, 401-792-2625
Mourad, George, 5, Mostafa M. Ismail, Mazarita, Alexandria, Egypt, 011-203-4823745
Mousel, Alan, Funk Seeds International, PO Box 417, Bluffton, IN, 47614
Muenchrath, Deborah A., Dept Agron & Plant Genet, Univ Minnesota, 1991Buford Circle, St. Paul, MN, 55108, 612-625-1208
Mukai, Yasushi, 1-11-10, Tachibana-Higashi, 880 Miyazaki, Japan, 0985-27-1838
Mukherjee, B. K., Cummings Lab, Indian Agr Res Inst, New Delhi 110012,India, 586198
Mulcahy, David, Botany Department, Univ of Massachusetts, Amherst, MA,01003, 413-545-2238
Murphy, C. F., USDA NPS, Bldg 005, Rm. 239, BARC-W, Beltsville, MD, 20705, 301-344-1560
Murphy, T. C., The Upjohn Company, 9612-50-1, Kalamazoo, MI, 49001, 616-384-2651
Murray, Michael G., Agrigenetics, 5649 E Buckeye Rd, Madison, WI, 53716, 608-221-5000
Muszynski, Michael G., Department of Genetics, 8 Curtiss Hall, Iowa State University, Ames, IA, 50010, 515-292-3984
Myers, Jr, Oval, Dept of Plant & Soil Science, Southern Illinois Univ,Carbondale, IL, 62901, 618-453-2496
Nagle, B. J., Dekalb-Pfizer Genetics, Box 33, Mason City, IL, 62664, 217-482-3213
Nasser, DeLill S., Eukaryotic Genetics Program, Division of Cell Biology, National Science Foundation, Washington, DC, 20550, 202-357-0112
Nebiolo, Christine M., Dept of Biology, Allegheny College, Carnegie Hall, Meadville, PA, 16335, 814-724-5360
Nel, P. M., Dept of Genetics, Univ of The Witwatersrand, Johannesburg 2001, S Africa, 011-716-2134
Nelson, Timothy, Department of Biology, PO Box 6666, Yale University, New Haven, CT, 06511-8112, 203-432-3860
Nelson, Jr., O. E., Department of Genetics, University of Wisconsin, Madison, WI, 53706, 608-262-3344
Nemeth, J., Gabonater Kutatointezet, Szeged, Also Kikoto sor 9, Hungary, 62-17-025
Nesticky, Milan, Maize Research Institute, 917 52 Trnava, Czechoslovakia
Neuffer, M. G., Curtis Hall, University of Missouri, Columbia, MO, 65211, 314-882-7735
Newhouse, Keith, American Cyanamid Co, P.O. Box 600, Princeton, NJ, 08540, 609-799-0400
Newton, K., Dept Biol Sci, University of Missouri, Columbia, MO, 65211,314-882-4049
Nguyen, Henry T., Dept of Agronomy, Box 4169, Texas Tech University, Lubbock, TX, 79409-4169, 806-742-2837
Nichols, Jeff, Institute of Molecular Biol, University of Oregon, Eugene, OR, 97403
Nickerson, N. H., Department of Biology, Tufts University, Medford, MA,02155, 617-381-3544
Nider, Fabio, Dekalb Argentina Saciai, C.C. 25, 2741 Salto, Buenos Aires, Argentina, 0474-22350
Noble, Jr., Stephen W., Dept of Corn Breeding, Pioneer Hi-Bred InternatInc, P.O. Box 409, Johnston, IA, 50131-0409, 515-270-3318
Notani, N. K., Bhabha Atomic Res Centre, Biology Division, Mod Lab, Trombay, Bombay 400085, India, 5510323
Nubel, Douglas, Pfister Hybrid Corn Co, El Paso, IL, 61738, 309-527-6008
Nykaza, Scott, Callahan Seeds, 1122 East 169th Street, Westfield, IN, 46074, 317-896-5551
O'Brien, David, Dept of Botany, University of Rhode Island, Kingston, RI, 02881

Oaks, Ann, Dept. of Botany, University of Guelph, Guelph, Ont. N1G 2W1,Canada
Oberthur Caldwell, Elizabeth E., G412, Agronomy Building, Agronomy Department, Iowa State University, Ames, IA, 50010, 515-294-7883
Ogle, Charles W., P.O. Box 484, Sugar Grove, IL, 60554, 312-466-4742
Ohta, Y., Inst Agr & Forestry, Univ of Tsukuba, Tsukuba City, Ibaraki-Ken 305, Japan, 0298-53-6691
Oishi, Karen, Molec & Cell Biol Dept, University of Arizona, Tucson, AZ, 85721, 602-621-3381
Okagaki, Ron, Lab of Genetics, 445 Henry Mall, Univ of Wisconsin, Madison, WI, 53706, 608-262-3287
Okuno, K., Field Crops Div., Hokuriku National Agr Exp Sta, 1-2-1 Inada, Joetsu, Niigata 943-01, Japan
Ondiviela, Jose, Agrar Investigacion S.A., Monasterio de Cogullada, 50014 Zaragoza, Spain
Osler, R. D., CIMMYT, Apartado Postal 6-641, 06600 Mexico D.F., Mexico,(905) 5-85-43-55
Osterman, John, School of Life Sciences, University of Nebraska-Lincoln, Lincoln, NE, 68588, 402-472-5129
Ottaviano, E., Dpt. of Genetics and Microbiol, Via Celoria 26, 20133 Milano, Italy, 02-230823
Paez, Alix V., PO Box 316, Pioneer Hybrid Intern Inc, 6800 Pioneer Pkwy, Johnston, IA, 50131, 515-270-3145
Palmer, Susan E., Department of Pediatrics, University of Iowa Hospital, Iowa City, IA, 52242
Pan, David, Laboratory of Genetics, B14 Genetics Building, University of Wisconson, Madison, WI, 53706, 608-262-3287
Pan, Yong-Bao, 714 Pammel Court, Ames, IA, 50010, 515-296-7946
Pardee, William D., 252 Emerson Hall, Cornell University, Ithaca, NY, 14853
Pareddy, Dayakar R., United AgriSeeds, Inc., P.O. Box 4011, Champaign,IL, 61820
Park, Keun Yong, Crop Experiment Station, Admin of Rural Development, Suwon 170, Korea, 82-0331-6-2166
Paterniani, Ernesto, Univ Sao Paulo Esc Sup Agr, Luiz de Queiroz, Piracicaba, Brazil
Patterson, Earl, Agron Dept, S-116 Turner Hall, University of Illinois,1102 S. Goodwin Avenue, Urbana, IL, 61801, 217-333-9641
Patterson, Garth, Department of Biology, Institute of Molecular Biology, University of Oregon, Eugene, OR, 97403
Paul, Anna-Lisa, Dept Veg Crops, c/o Rob Ferl, Univ Florida, Gainesville, FL, 32611, 904-392-2370
Pauly, Michael H., Rogers Brothers Seed Co., P.O. Box 1069, Nampa, ID,83653, 208-466-0319
Pawar, S. E., Biol/Agric Div, Bhabha Atomic Research Center, Trombay Bombay 40085, India, 5514910 ext 2340
Payne, Peter I., Plant Breeding International, Cambridge, Maris Lane, Trumpington, Cambridge CB2 2LQ, U K
Peacock, W. J., Division of Plant Industry, CSIRO, Canberra ACT 2601, Australia, 062-465250
Pepe, John, Cargill Seed Research, P.O. Box 774, Grinnell, IA, 50112
Peschke, Virginia M., Dept Botany, Campus Box 1137, Washington Univ, St. Louis, MO, 63130
Peters, D. W., Asgrow Seed Company, Box 50, Monmouth, IL, 61462
Peterson, Peter A., Dept of Agronomy, Iowa State University, Ames, IA,50011, 515-294-9652
Peterson, Thomas A., Cold Spg. Harbor Lab, P.O. Box 100, Cold Spg. Harbor, NY, 11724, 516-367-8825
Petzold, Ruth, Dept of Genetics, North Carolina State Univ, Box 7614, Raleigh, NC, 27695-7614
Phillips, Ronald, Agronomy & Pl Genetics, University of Minnesota, St.Paul, MN, 55108, 612-625-1213
Phinney, Bernard O., Dept of Biology, 405 Hilgard Ave., UCLA, Los Angeles, CA, 90024, 213-825-3177
Pienaar, R. de V., Dept Genet & Plant Breeding, University of Stellenbosch, 7600 Stellenbosch, S. Africa
Pinter, Lajos, Crop Science Dept, Univ Keszthely, P.O. Box 71, H-8361 Keszthely, Hungary
Podolskaya, Anna P., N. I. Vavilov All Union Inst, of Plant Industry,44 Herzen Street, 190000, Leningrad, USSR
Poethig, R. Scott, Biology Department, Leidy Labs, Univ Penn, 415 University Ave, Philadelphia, PA, 19104-6018, 215-898-8915
Pogna, N. E., Ist Sper Cerealicoltura, Via Mulino, 3, 20079 S. Angelo Lodigiano, Milan, Italy
Polacco, Mary , Dept Biochemistry, 107 Schweitzer Hall, Univ of Missouri, Columbia, MO, 65211, 314-882-6484
Pollacsek, M., Station D Amelior Des Pl-INRA, 63039 Clermont Ferrand, France, 73-62-43-01
Pollak, Linda, Dept of Agronomy, Iowa State Univ, Ames, IA, 50011, 515-294-3052
Pollmer, W. G., Universitat Hohenheim, Postf 700562-304, Garbenstrasse9, 7000 Stuttgart 70, W Germany
Polson, David E, Canners Seed Corporation, PO Box 18, Lewisville, ID, 83431
Poneleit, Charles G., Agronomy, University of Kentucky, Lexington, KY,40546-0091, 606-257-4934
Pratt, Richard C., Agronomy Department, OSU/OARDC, 1680 Madison Avenue,Wooster, OH, 44691, 216-263-3972
Price, Carl, Waksman Inst, Piscataway, NJ, 08855-0759, 201-932-2920
Pring, D. R., Dept of Plant Pathology, University of Florida, Gainesville, FL, 32611, 904-392-3638
Procunier, James D., Zoology Department, Univ of Western Ontario, London, Ontario N6A 5B7, Canada, 519-679-2111x6783
Pryor, A. J., Div Plant Ind CSIRO, PO Box 1600, Canberra Act 2601, Australia, 062-46-5896
Qin, Tai-chen, Department of Agronomy, Jiangsu Agriculture College, Jiangsu, Yangchou, China
Quail, Peter H., USDA-ARS-PGEC, 800 Buchanan Street, Albany, CA, 94710,415-559-5900
Raboy, Victor, USDA/ARS, Plant & Soil Science Dept, MT State University, Bozeman, MT, 59717, 406-994-5054
Rabson, Robert, Biological Energy Sciences, Office of Basic Energy Sci,U.S. Dept of Energy, ER-17 GTN, Washington, DC, 20545, 301-353-2873
Racchi, M. L., Univ di Milano, Dept Genetics & Microbiology, Via Celoria 26, 20133 Milan, Italy
Radicella, Pablo, Institute of Molecular Biol, University of Oregon, Eugene, OR, 97403
Ragsdale, D., National Corn Growers Assoc., 1000 Executive Parkway, Suite 105, St. Louis, MO, 63141
Rakha, Farouk A., Dep Genetics Fac Agric, Alexandria University, Alexandria, Egypt, 966386
Ralston, Ed, Advanced Genetic Sciences, 6701 San Pablo, Oakland, CA, 94608, 415-547-2395
Ramsey, Ann, RR2, Box 15, Rochester, IL, 62563, 217-498-9374
Rao, P. N., Dept of Botany, Andhra University, Visakhapatnam 530003, India, 64871 ext 390
Rashid, Abdul, Agronomy Dept, Iowa State University, Ames, IA, 50011
Reddy, A. R., School of Life Sci, Univ of Hyderabad, Hyderabad-500001,India, 253901, extn:226
Reddy, G. M., Department of Genetics, Osmania University, Hyderabad-500007, India
Reddy, G. Madhavi, Curtis Hall, University of Missouri, Columbia, MO, 65211
Redei, G. P., Curtis Hall, University of Missouri, Columbia, MO, 65211,314-882-6434
Rees, Hubert, Agricultural Botany Dept, Univ College of Wales, Aberystwyth, Wales, U K, 0790-3111
Reid, W. R., Asgrow Seed Company, P.O. Box 460, Parkersburg, IA, 50665
Reksodihardjo, S., Pioneer Agricultura Ltda, Caixa Postal 131, 86100 Londrina PR, Brazil, 0432 271512
Rhoades, M. M., Biology Dept, Indiana University, Bloomington, IN, 47401, 812-335-9319
Rhodes, C. A., Sandoz Crop Protection Corp., 975 California Ave., PaloAlto, CA, 94304, 415-354-3527
Rice, Thomas B., Dekalb-Pfizer Genetics, 3100 Sycamore Road, Dekalb, IL, 60115
Ries, Matthew N., Cargill Hybrid Seeds, Box 405, Seward, NE, 68434, 402-643-2549
Rifaat, Mahmoud M., c/o Dr. Asim Esen, Dept of Biology, Derring Hall, VPI & SU, Blacksburg, VA, 24061

Rinehart, Karl, Box 187, Marshalltown, IA, 50158, 515-752-2170
Rivin, Carol, Dept. of Botany, Oregon State University, Corvallis, OR,97331, 503-754-4433
Robbins, Jr., W. A., P.O. Box 158, Ag. Alumni Seed Imp. Assn., Romney,IN, 47981
Roberts, Jean L., Lilly Research Labs, Greenfield, IN, 46140, 317-467-4788
Robertson, Donald S., Department of Genetics, Iowa State University, Ames, IA, 50010, 515-294-3908
Robison, Glenn, Dekalb-Pfizer Genetics, Sycamore Road, Dekalb, IL, 60115, 815-758-9531
Rodermel, S., The Biological Laboratories, Harvard University, 16 Divinity Avenue, Cambridge, MA, 02138, 617-495-4260
Rodrigues, Delmo Diogo, Sem Cargill Ltda, Sitio Sao Jao Barao Geraldo,Caixa Postal, 6553, Campinas SP 13.100, Brazil, 0192-39-13-81
Roebbelen, G., Inst f. Pflanzenbau, Von Sieboldstr. 8, D-3400 Goettingen, W Germany
Romero-Severson, Jeanne, Agrigenetics, 5649 E. Buckeye Road, Madison, WI, 53716, 608-221-5000
Romig, R. W., Northrup King Co., P.O. Box 959, Minneapolis, MN, 55440
Roseman, Robin R., Department of Botany, University of Iowa, Iowa City,IA, 52240, 319-335-1331
Rosielle, A., Asgrow Seed Co, 634 E. Lincoln Way, Ames, IA, 50010, 515-232-7170
Rossman, E. C., Dept of Crop & Soil Sci, Michigan State University, East Lansing, MI, 48823, 517-355-2289
Roura, Lluis Bosch, Departament d'Agronomia, Escola Superior d'Agricultura, Urgell 187, 08036 Barcelona, Spain, 93-2304207
Roussell, Deborah L., Div Biol Sci, Tucker Hall, Univ of Missouri, Columbia, MO, 65211, 314-882-8033
Rubenstein, Irwin, 220 Bioscience Center, Dept Plant Biology, University of Minnesota, St Paul, MN, 55108, 612-624-2716
Russell, W. A., Dept of Agronomy, Iowa State University, Ames, IA, 50010
Russell, W. Ken, Garst Seed Co, PO Box 500, Slater, IA, 50244
Ryan, G. S., E.E.A. INTA, 5988 Manfredi, (Cordoba), Argentina
Sachs, M. M., Department of Biology, Campus Box 1137, Washington University, St. Louis, MO, 63130, 314-889-4631
Saedler, H., Max-Planck Inst Zuchtungsf, Egelspfad, D 5000 Koln 30 Vogelsang, W Germany, 221-5062200
Sager, Ruth, DANA-FARBER, Cancer Institute, Boston, MA, 02115, 617-732-3380
Saghai, Mohammad, Dept. of Agronomy, VPI & SU, Blacksburg, VA, 24061
Saha, B. C., Tirhut College of Agriculture, P.O. Dholi, Dist. Muzaffarpur, Bihar-813210, India
Salamini, Francesco, Max Planck Inst Zuchtungsf, Abt Pflanzenz Ertragsphysiol, D-5000 Koln 30, W Germany
Santos, M. A., Dep Biol Molec Agro, Centro Invest Desarrollo, Gorge Girona 18-24, 08034 Barcelona, Spain
Saraiva, L. S., Genetica Dept Biol Geral, Univ Fed Vicosa, 36570 MinasGerais, Brazil
Sarkar, K. R., Division of Genetics, Indian Agric Res Inst, New Delhi 110012, India
Saura, Fulgencio, Div Agron Basica, Dpto Tecnologia , Univ Nacional Lujan, CC 221-6700 Lujan(Bs As), Argentina
Sauvaire, D., CIRAD - IRAT, BP 5035, 34032 Montpellier Cedex, France
Savidan, Yves H., Dept Plant Sci, Utah State Univ, Logan, UT, 84322-4820, 801-752-1629
Sayed, Sayed Galal, PO Box 953, Cairo, Egypt, 3491619
Schaeffer, Jurgen R., Dept of Plant + Soil Sci, Montana State University, Bozeman, MT, 59715, 406-994-5066
Schiefelbein, John W., Michigan State University, 210 Plant Biology Bldg, East Lansing, MI, 48824, 517-355-5197
Schillinger, John, Asgrow Seed Company, 9672-190-16, 7000 Portage Rd.,Kalamazoo, MI, 49001
Schmidt, Daria, 1523 Agronomy, Iowa State University, Ames, IA, 50011
Schmidt, Robert J., Univ of California-San Diego, Dept of Biology, C-016, San Diego, CA, 92093, 619-534-1636
Schnable, Patrick, Dept of Genetics, Curtiss Hall , Iowa State Univ, Ames, IA, 50011
Schneerman, Martha C, Dept Biol Sciences, Illinois State Univ, Normal,IL, 61761
Schon, Astrid, Dept Mol Biophys Biochem, 260 Whitney Ave, P.O. Box 6666, New Haven, CT, 06511
Schulman, Alan H., Helsinki University, Recombinant DNA Lab, Karvaamokuja 3, 00380 Helsinki, Finland, 011-358-0-402-7286
Schwartz, Drew, Biology Dept, Indiana University, Bloomington, IN, 47401, 812-335-6060
Schwer, Joseph F., Agrigenetics Corporation, Centre Plaza North, 35575Curtis Blvd, Suite 300, Eastlake, OH, 44094, 216-942-2210
Scott, Gene E., Agronomy Dept, Box 5248, Mississippi State University,Mississippi State, MS, 39762, 601-325-2311
Scowcroft, W., Biotechnica Canada Inc., 170,6815 8 St. N.E., Calgary, Alberta T2E 7H7, Canada
Setter, Tim L., Department of Agronomy, Cornell University, Ithaca, NY,14853
Sevilla P., Ricardo, Programa de Maiz, Univ Nacional Agraria, Aptdo 456, La Molina, Lima, Peru
Shadley, Jeff and Gwen Krill-, 5200 Blackstone Apt. 1110, Chicago, IL,60615
Shafer, John, 3031 N Roselawn Drive, Logansport, IN, 46947, 219-753-5547
Shands, Henry, Natl Program Staff, USDA-ARS, Beltsville, MD, 20705, 301-344-3311
Sharma, N. D., Division of Biochemistry, Indian Agric Res Inst, New Delhi 110012, India
Shattuck-Eidens, Donna M., NPI, 417 Wakara Way, Salt Lake City, UT, 84108
Shaver, D. L., Plant Br Dept, Cornnuts, 24 Winham St, Salinas, CA, 93901, 408-424-1023
Shcherbak, V. S., Krasnodar Res Inst Agric, Krasnodar, USSR
Sheen, Jen, Molecular Biology Dept, Wellman 8, MGH, Boston, MA, 02114,617-726-5916
Shepherd, Nancy, DuPont E402/4243, Wilmington, DE, 19898, 302-695-1519
Sheridan, W. F., Biology Department, Univ of North Dakota, PO Box 8238Univ Station, Grand Forks, ND, 58202, 701-777-4479 or 777-4206
Shilo, Judith, Dept of Biological Chemistry, The Hebrew University, Jerusalem 91904, Israel 91904
Short, Kent E., CIMMYT, Mid Altitude Maize Res Stn, P.O. Box MP 163, MtPleasant, Harare , Zimbabwe, 35534
Siddiqui, Khushnood A., Plant Genetics Division, A.E.A.R.C., Tandojam Sind, Pakistan
Simcox, K., Dept Biological Sciences, Purdue University, West Lafayette, IN, 47907
Simonds, S. Caprice, 1253 Fifield Hall, University of Florida, Gainesville, FL, 32611, 904-392-9087
Sims, Lynne E., Pioneer Hi-Bred Internatl, Dept Biotechnology, P.O. Box38, Johnston, IA, 50131
Singh, N. N., Cereal Research Lab, Indian Agricultural Res Inst, New Delhi 110012, India
Sisco, P. H., USDA-ARS, Dept Crop Science, North Carolina State Univ, P.O. Box 7620, Raleigh, NC, 27695-7620, 919-737-2704
Smail, Virgil W., BioTECHNICA Agriculture, Inc., 7300 W. 110th St. Suite 540, Overland Park, KS, 66210, (913) 661-0611
Smith, Alan G., Dept Hort & Landscape Arch, 356 Alderman Hall, Univ. ofMN, 1970 Folwell Av., St Paul, MN, 55108
Smith, James D., PO Box 2132, Department Soil & Crop Sci, Texas A & M University, College Station, TX, 77843, 409-845-8276
Smith, Margaret E., Cornell Univ, 252 Emerson Hall, Dept of Plant Breeding, Ithaca, NY, 14853, 607-255-1654
Smith, Oscar S., Pioneer Hi-Bred Internatl, Plant Breeding Division, P.O. Box 85, Johnston, IA, 50131, 515-270-3300
Smith, Steve, Pioneer Hi-Bred Internatl, Plant Breeding Division, 7300N.W. 62nd Avenue, Johnston, IA, 50131, 515-270-3353
Snape, John W., Inst Plant Sci Res, Cambridge Laboratory, Maris Lane, Trumpington, Cambridge CB2 2LQ, UK, 0223-840411
Soll, Dieter, Dept Molec Biophys & Biochem, 260 Whitney Avenue, Box 6666, Yale University, New Haven, CT, 06511
Sollinger, John, Dept. of Botany, Oregon State University , Corvallis,OR, 97331

Somers, David A., Dept Agron & Plant Genet, University of Minnesota, St. Paul, MN, 55108, 612-625-5769
Sorenson, John C., Experimental Ag Sci, The Upjohn Co, Kalamazoo, MI, 49001, 616-385-4582
Sorrentino (Brown), Jennifer, Cold Spring Harbor Lab, P.O. Box 100, Cold Spring Harbor, NY, 11724, 516-367-9808
Sotomayor-Rios, A., Tropical Agric Res Stn, P.O. Box 70, Mayaguez, PR,00709
Sperling, David W., Robson Seed Farms Corp, PO Box 270, Hall, NY, 14463, 716-526-5879
Sprague, G. F., Dept Agron, S-120 Turner Hall, University of Illinois,1102 S. Goodwin Avenue, Urbana, IL, 61801, 217-333-4254
Springer, Patricia, Dept Biol Sci, Purdue University, W. Lafayette, IN,47907, (317) 494-4919
Springer, Warren, Northrup King Company, Stanton, MN, 55081, 507-645-5621
Sriwatanapongse, Sutat, CIMMYT, P.O. Box 39 Emek, Ankara, Turkey
Stack, Stephen, Department of Botany, Colorado State University, Fort Collins, CO, 80523
Stadler, Joan, 8 Curtiss Hall, Genetics Dept, Iowa State University, Ames, IA, 50011, 515-294-0337
Starlinger, P., Inst Genetik, Univ Koln, D-5000 Koln 41, Weyertal 121,WGermany, 221-470-2465
Staub, Rick W., Dept of Biology, Carleton College, Northfield, MN, 55057, 507-663-4385
Steffensen, D. M., 506 Morrill Hall, Cell Biol, Univ of Illinois, Urbana, IL, 61801, 217-333-3087
Stern, Herbert, Dept of Biology, Univ of Calif-San Diego, La Jolla, CA,92037, 619-534-3746
Stiles, J. I., Dept. of Pl & Molec Physiology, Univ. Hawaii at Manoa, Honolulu, HI, 96822, 808-948-7354
Stinard, Philip, Dept Genetics, Iowa State Univ, Ames, IA, 50011
Stout, John T., Upjohn Company, 9602-18-2, Kalamazoo, MI, 49001, 616-385-4583
Strissel, Jerry, Wilson Hybrids, Inc, P.O. Box 391, Harlan, IA, 51537,712-755-3841
Strommer, Judith, Dept of Genetics, Univ of Georgia, Athens, GA, 30602,404-542-7980
Stroup, D., 4740 Conn. Ave. NW #613, Washington, DC, 20008
Stuber, Charles W., Dept of Genetics, North Carolina State Univ, Box 7614, Raleigh, NC, 27695-7614, 919-737-2289 FTS672-4564
Styles, E. D., Biology, Univ of Victoria, Victoria, British Columbia V8W 2Y2, Canada, 604-721-7101
Suga, Tetsuya, Japan Tobacco Inc, Plant Brdg Genetics Res Lab, 700 Higashibara Toyoda-cho, Iwata-gun Shizuoka-ken438, Japan
Sullivan, Sue, Edward Funk and Sons, P.O. Box 67, Kentland, IN, 47951
Sullivan, Tom, Laboratory of Genetics, 445 Henry Mall, University of Wisconsin, Madison, WI, 53706, 608-262-3287
Sundaresan, V., Cold Spring Harbor Lab, P.O. Box 100, Cold Spring Harbor, NY, 11724
Sundberg, Marshall D., Department of Botany, 508 Life Science Bldg, Louisiana State University, Baton Rouge, LA, 70803-1705, 504-388-8563
Sung, Tong Ming, Dept of Agronomy, Beijing Agric Univ, 912 Research Building, Beijing 100094, China, 28-5831
Suprasanna, P., Department of Genetics, Osmania University, Hyderabad-500 007, A.P., India
Sussex, I. M., Dept Biol, PO Box 6666, Yale University, New Haven, CT,06511, 203-432-3858
Swaminathan, M. S., B-4/142, Safdarjang Enclave, New Delhi 110029, India, 67-9069
Tadic, Bojana, Maize Research Institute, P.O. Box 89, 11081 Zemun, Yugoslavia, 011-217434
Talbert, Luther, Institute of Molecular Biology, University of Oregon,Eugene, OR, 97402, 503-686-5136
Tang, Ching-Yan, Maize Program, CIMMYT, Lisboa 27, Apdo Postal 6-641, Col. Juarez , 06600 Mexico D.F., Mexico
Taylor, Brian H., Dept of Biology, Texas A&M Univ, College Station, TX,77843-3258, 409-845-7754
Taylor, Loverine P., 35 Naranja Way, Portola Valley, CA, 94025
Taylor, W. C., CSIRO, Division of Plant Industry, G.P.O. Box 1600, Canberra ACT 2601, Australia, (62)46-5223
Teixeira, Margarida de A., Inst Nac de Investigacao Agrar, Quinta dos Peoes, Gualtar, 4700 Braga, Portugal, 24511
Thomann, Estela, Dept. of Botany, Oregon State University, Corvallis, OR, 97331
Thompson, Jack C., FFR Coop, P.O. Box 10, Providence Forge, VA, 23140,804-966-2888
Thompson, Steven A., United Agriseeds, P.O. Box 4011, Champaign, IL, 61820, 217-373-5300
Thornbury, David W., S-305 Ag Science Center N, Dept Plant Path, University of Kentucky, Lexington, KY, 40546, 606-257-2681
Thorstenson, Yvonne, Dept of Genetics, Univ of California, 345 MulfordHall, Berkeley, CA, 94720, 415-642-8058
Tierney, Mary L., Biotechnology Center-OSU, Rightmire Hall, 1060 Carmack Road, Columbus, OH, 43210-1002, 614-422-3348
Timothy, David H., Dept of Crop Science, North Carolina State Univ, Box7620, Raleigh, NC, 27695-7620, 919-834-5161
Ting, Chu-Po, Shandong Agricultural College, Taian, Shandong, China
Ting, Yu-Chen, Biology, Boston College, Chestnut Hill 67, Boston, MA, 02167, 617-552-3551
Tonelli, Chiara, Dept of Genetics & Microbiol, Univ Milano, Via Celoria26, 20133 Milano, Italy, 02-230823
Tracy, W. F., Department of Agronomy, University of Wisconsin, Madison,WI, 53706, 608-262-1390
Treat, Calvin, Department of Agronomy, University of Wisconsin, 1575 Linden Drive, Madison, WI, 53706
Troyer, A. F., Dekalb-Pfizer Genetics, 3100 Sycamore Road, Dekalb, IL,60115, 815-756-3671
Tsai, C. Y., Dept Bot Plant Pathol, Purdue University, W. Lafayette, IN, 47907, 317-494-4640
Tsuchiya, T., Dept of Agronomy, Colorado State University, Fort Collins, CO, 80523, 303-491-6648 EXT 6237
Tuttle, Annmarie, Ciba-Geigy Corporation, P.O. Box 12257, Research Triangle Park, NC, 27709-2257
Ulrich, V., Div Plant & Soil Sci, West Virginia Univ, Morgantown, WV, 26506, 304-293-6258
Underwood, Douglas, Dept. of Botany, Oregon State University, Corvallis, OR, 97331
Vallejos, Ruben H., CEFOBI, Suipacha 531, 2000 Rosario, Argentina
Van Schaik, N., Dept Genetics/U Witwatersrand, PO Wits 2050, S Africa,(011) 716-2125
Vanderslice, Olin L., Agway Inc, 2 Parkwood Circle, Cortland, NY, 13045
Vantoai, Tara T., USDA Agronomy, 590 Woody Hayes Dr, Columbus, OH, 43210, 614-292-9806
Varagona, Rita, Department of Botany, University of Georgia, Athens, GA, 30605, 404-542-3732
Vasal, S. K., Londres 40 1 Piso, Apartado Postal 6-641, Mexico 6, D.F.,Mexico, 5-85-43-55 EXT 115
Vasil, Indra K., Dept Vegetable Crops, 511 Bartram Hall West, Gainesville, FL, 32611, 904-392-1193
Veit, Bruce, USDA ARS PGEC, 800 Buchanan Street, Albany, CA, 94710, 415-559-5907
Veitenheimer, Erich E., Dekalb-Pfizer Genetics Inc, 603 W. Main St., Palmyra, PA, 17078
Veretnik, Stella, Dept Genet & Cell Biol, University of Minnesota, St.Paul, MN, 55108
Verma, S. C., Botany Department, Panjab University, Chandigarh-160014,India
Vierling, Richard, Plant and Soil Science, Box 4169, Lubbock, TX, 79409-4169
Violic, Alejandro D., CIMMYT, Cristobal Colon 3773 Dep. 23, Santiago (Las Condes), Chile
Viotti, Angelo, Ist Biosintesi Vegetali, Via Bassini 15, 20133 Milano,Italy
Vodkin, Lila Ott, Dept Agron, S-110 Turner Hall, Univ Illinois, 1102 S.Goodwin, Urbana, IL, 61801, 217-244-6147
Vogel, Julie, Dept Genetics, Univ of California, Berkeley, CA, 94720, 415-642-8058
Volk, B., Agronomy Dept, 135 Mumford, Univ of Missouri, Columbia, MO, 65211, 314-882-2801
Von Bulow, J. F. W., Univ Brasilia, Cx Postal 15-2958, 70.910 BrasiliaDF, Brazil, 274-0022 r.2429

Von Wettstein, D., Carlsberg Lab, Gamle Carlsbergvej 10 , DK-2500 Copenhagen Valby, Denmark, 01-221022
Voris, Merle, Box 457, Windfall, IN, 46076, 317-945-7777
Vulchinkov, Stefan, Maize Research Institute, Kneja, Bulgaria
Waines, J. Giles, Dept of Botany/Plant Sciences, University of California, Riverside, CA, 92521, 714-787-3706
Walbot, V., Dept Biol Sci, Stanford Univ, Stanford, CA, 94305, 415-723-2227
Walden, D. B., Dept of Plant Sciences, Univ of Western Ontario, LondonN6A 5B7, Canada, 519-679-6757
Walker, John, Biol Sci, 110 Tucker Hall, Univ of Missouri, Columbia, MO, 65211, 314-882-3583
Walton, Jonathan, DOE Plant Research Lab, Michigan State University, East Lansing, MI, 48824, 517-353-4885
Wan, Yuechun, Dept of Agronomy, Turner Hall, 1102 S. Goodwin Ave, Urbana, IL, 61801
Wang, Andrew S., Sungene Technologies Corp , 2050 Concourse Drive, SanJose, CA, 95131, 408-433-9800
Wang, Yunxia, Department of Horticulture, Purdue University, W. Lafayette, IN, 47905, (317) 494-7111
Warnock, S. J., Campbell Institute for, Research & Technology, Route 1,Box 1314, Davis, CA, 95616-9610, 916-753-2116
Wassom, Clyde E., Agronomy Dept, Kansas State Univ, Manhattan, KS, 66506, 913-532-7253
Weaver, Sam H., The Quaker Oats Company, P.O. Box 9001, Chicago, IL, 60604-9001
Weber, David, Dept of Biological Sci, Illinois State Univ, Normal, IL,61761, 309-438-2685
Weck, Edward A., Northrup King Co., Route 19, Stanton, MN, 55081
Wendel, J. F., Department of Botany, Bessey Hall, Iowa State University, Ames, IA, 50011, 515-294-7172
Wessells, Catherine P. B., 28015 Stonehenge Lane, Eugene, OR, 97402
Wessler, Sue, Dept of Botany, Univ of Georgia, Athens, GA, 30602, 404-542-3732
West, D. P., Research Dept, Jacques Seed Company, Prescott, WI, 54021,715-262-3162
West, Dennis R., Dept Plant and Soil Sci, Univ of Tennessee, Knoxville,TN, 37901-1071, 615-974-8826
Whalen, R. H., Dept of Biology, South Dakota State Univ, Brookings, SD,57007, 605-688-4553
Whitwood, W., Musser Seed Co., P.O. Box 787, Caldwell, ID, 83605, 208-459-8243
Widholm, J. M., Dept of Agronomy, U Illinois/1102 S. Goodwin, Urbana, IL, 61801, 217-333-1279
Widstrom, Neil W., Coastal Plain Exp Sta, PO Box 748, Tifton, GA, 31793, 912-382-6904
Wienand, Udo, Max Planck Institute, Egelspfad, D-5000 Koln-Vogelsang 30, W Germany
Wilkes, H. Garrison, Biology-College II, Univ of Mass/Boston, Boston, MA, 02125-3393, 617-929-8448
Williams, Robert E., PO Box 294, Pittsfield, IL, 62363, 217-285-2530
Williams, Terrill E., Pioneer Hi-Bred Internatl, P.O. Box 128, New Holland, PA, 17557
Williams, W. P., Crop Sci Res Lab, PO Box 5248, Miss. State, MS, 39762,601-325-2311
Willman, Mark R., Beatrice/Hunt-Wesson Inc, 463 U.S. Hwy. 30 East, Valparaiso, IN, 46383
Wilson, Curtis M., USDA ARS NRRC, 1815 North University Street, Peoria,IL, 61604, 309-685-4011
Wise, Roger, Max-Planck-Institut Zuchtung., D-5000 Koln 30, West Germany, 49-221-5062-440
Wong, James R., Sogetal, Inc., 3876 Bay Center Place, Hayward, CA, 94545, 415-785-1881
Woodham, Carl, Dept Biology, Illinois State Univ, Normal, IL, 61761
Woodman, James C., Molec Genetics Inc, 10320 Bren Road East, Minnetonka, MN, 55343, 612-935-7335
Woodruff, Dorde, 6366 Cobblerock Lane, Salt Lake City, UT, 84121-2304,801-277-5526
Wozniak, Chris A., USDA ARS, B-006, Rm 118, BARC-West, Beltsville, MD,20705
Wright, Allen, Dept Agron, Curtis Hall, Univ Missouri, Columbia, MO, 65211
Wurtzel, Eleanore, Dept Biol Sci, Davis Hall, Lehman College, City UnivNew York, Bronx, NY, 10468, 212-960-8483
Xie, You-Ju, College of Biology, Beijing Agricultural Univ, Beijing, China
Xu, Wenwei, Waters Hall, University of Missouri, Columbia, MO, 65211
Yamada, Minoru, Dept Plant Breed., Nat Grassl, Research Inst, Sembonmatsu, Nishi-nasuno, Nas, Tochigi, 329-27, Japan, 0287-36-0111 ext.285
Yano, Masahiro, Hokuriku Natl Agr Exper Sta, 1-2-1, Inada, Joetsu, Niigata 943-01, Japan
Yates, Don, Downing Foundation Seeds, Inc, 2380 Harrison Road, New Madison, OH, 45346, 513-997-3813
Yatou, Osamu, Institute of Radiation Breed, P.O. Box 3, Ohmiya-machi, Naka-gun, Ibaraki-ken, 319-22, Japan
Youngquist, Wayne C., Agronomy Dept, Univ of Nebraska, Lincoln, NE, 68583, 402-472-1531
Zabala, Gracia, Plant Biol/289 Morrill Hall, Univ Illinois, 505 S. Goodwin Avenue, Urbana, IL, 61801, 217-333-2919
Zabulionis, Ray, Department of Plant Sci, B & G Building, University ofWestern Ontario, London, Ontario N6A 5B7, Canada
Zarowitz, Michael, Escagenetics, 830 Bransten Road, San Carlos, CA, 94070, 415-595-5335
Zeng, M. Q., 100012 Institute of Genetics, Academia Sinica, Beijing, China, 44,6551-556
Zhao, Ji-ping, Department of Agronomy, Shandong Agric University, Taian, Shandong Province, China
Zhao, Zuo-Yu, Cold Spring Harbor Laboratory, PO Box 100, Delbruck, ColdSpring Harbor, NY, 11724, 516-367-8826
Zidwick, Mary Jo, Cargill Research Department, Box 9300, Minneapolis, MN, 55440
Zimmer, Elizabeth, Department of Biochemistry, 322 Choppin Hall, Louisiana State University, Baton Rouge, LA, 70803, 504-388-1556
Zuber, M. S., Curtis Hall, University of Missouri, Columbia, MO, 65211,314-882-7707

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VIII. SYMBOL INDEX

("r" refers to numbered references in the Recent Maize Publications section)

IX. AUTHOR AND NAME INDEX

("r" refers to numbered references in the Recent Maize Publications section)
(* identifies articles authored in this Newsletter)

CLONE INFORMATION SHEET
(PLEASE SUPPLY FOR EACH CLONE)

CLONE DESIGNATION: _____ ISOLATING LAB/PERSON: _____

IS THIS A KNOWN SEQUENCE CLONE (circle one)? YES NO

IF YES, FOR WHAT PRODUCT or LOCUS: _____

CLONE TYPE (genomic, cDNA, etc.): _____ SIZE: _____

ISOLATED FROM WHAT ORGANISM: _____

RESTRICTION MAP/SEQUENCE INFORMATION (supply reference, if possible):

SOUTHERN BLOT INFORMATION

LINE ANALYZED	ENZYME(S) TRIED	# BANDS SEEN	APPROX. MW

AMOUNT OF PURIFIED PLASMID SUPPLIED (10-100 ug): _____

VECTOR: _____ ENZYME(S) TO CUT OUT INSERT: _____

HOST OF SUPPLIED STAB CULTURE: _____ MEDIA: _____

CAN WE FREELY DISTRIBUTE THIS CLONE (circle one)? YES NO

CONTACT PERSON REGARDING CLONE:

NAME: _____

ADDRESS: _____

_____

_____

_____

PHONE: _____

COMPUTER ADDRESS: _____

SEND CLONES AND INFORMATION TO:

DR. DAVID HOISINGTON
302 CURTIS HALL
UNIVERSITY OF MISSOURI
COLUMBIA, MISSOURI 65211

PHONE: 314/882-2033

This is an informal news letter by which working research information on the genetics and cytogenetics of maize is shared. The information and data are shared by Cooperators with the understanding that they will not be used in publications without their specific consent.

Notes for the 1990 Maize Genetics Cooperation News Letter need to be in my hands by January 1. Be concise, not formal, but include specific data, observations and methods. A double-spaced, letter-quality copy of your text is needed. Please follow the simple style (title; authors; minimal citations) used in this issue. Whenever possible send an electronic version on 3-1/2 or 5-1/4 floppy disk, identifying the operating system (e.g., MS-DOS) and the word processor (e.g., Microsoft Word). Figures, charts and tables should be **compact** and camera-ready.

Subscription information is provided on the form included in this issue.

Author and Name Indexes	
Nos. 3 through 43	Appendix to No. 44, 1970
Nos. 44 through 50	No. 50
Nos. 51 to date	Annual in each issue
Symbol Indexes	
Nos. 12 through 35	Appendix to No. 36, 1962
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Genetic Stocks	In this issue
Translocations	No. 55
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Cytogenetic Working Maps	No. 52; Hoisington 59:159 & 60:149
Gene List	No. 62; Coe et al., 1989
Working Linkage Maps	In this issue

Cooperators (that means you) need the Stock Center.
The Stock Center needs Cooperators (this means you) to:

(1) Send stocks of new factors you have reported in this News Letter or in publications, and stocks of new combinations, to the collection. A list of mutants not represented in the collection is given in MNL 61:115.

(2) Inform the Stock Center on your experience with materials received from the collection.

(3) Acknowledge the source of the stocks for research when you publish, and advice or help you have received in development of your research project.

Cooperators Clone Home! Each functionally defined clone enhances the map, and mapping information enhances further exploration of the function. Your clone is wanted; please see page 141.