(5) Observations not expressed in the table show that the double heterozygotes have an intermediate phenotype with fewer and less striking large lesions and fewer small lesions, although both are clearly expressed.

(6) A comparison of large lesion plants from family 37 with the same type in family 39 (Table 1) shows no appreciable difference at the lowest temperature (72^{o}) but a marked increase in the expression in family 39 at 84^{o}. Table 2 shows this to be mostly due to the expression in the double mutant plants.

(7) The same comparison for the small lesion mutant (Les2) shows a striking reduction in the lesions on plants from the Les x Les2 cross (family 39) as compared to the backcross (family 38) at low temperatures while at the highest temperature there is no appreciable difference.

(8) Changing the temperature from 72^{o} to 84^{o} for the Les x Les2 material produces a striking and opposite reversal of expression for both lesion types; the effect is more abrupt and complete than in either family carrying the single mutants.

From these observations it can be concluded that Les is expressed best at low temperatures (around 72^{o}) and not at high temperatures, while Les2 is expressed best at temperatures around 84^{o} and reduced at lower temperatures. Changes in temperature may abruptly alter the expression of either mutant according to its prescribed response. Double heterozygotes have an intermediate expression with both mutants expressed at lower levels. In the double heterozygote the expression of Les2 is restricted or reduced at lower temperatures, while the expression of Les is enhanced in the higher temperature range. The Les x Les2 material also shows differences suggesting parental influence on the interaction of Les and Les2.

M.G. Neuffer

Comparative efficiency of seed and pollen treatments in mutation experiments — An important aspect of the design of mutation experiments is economy in terms of the plants to be handled in each generation. A common practice with autogamous plant species such as barley or Arabidopsis is to treat seed with a mutagenic agent, grow an M_1, self that to produce M_2 seed, grow the M_2 and look for mutant segregants. The size of the experiment is usually determined by a trade-off between the number of M_2 plants required to provide a statistically adequate sample and the number of plants that can be handled. With corn, which has separate male and female flowers (geitonogamy), the M_1 may be selfed or outcrossed to untreated material and the progeny in either case may be selfed to produce an M_3, which is examined for segregating mutants. The economics of the numbers grown in each generation and the samples taken is often not properly understood, but

it is directly dependent on the developmental pattern of the species being studied.

The proportion of mutant individuals appearing in the M_2 will depend on the number of primordial cells in the seed which contributes to the germ line. As shown in Table 1, the combined seed progeny of an M_1 plant may segregate 7:1, 15:1 or 31:1 with a germ cell number of two, four or eight, respectively, for

Table 1. The frequency of mutant individuals in the M_2 of an M_1 in which a mutant has been induced in one primordial cell for autogamous vs. geitonogamous plants with different primordial germ cell numbers.

	Germ cell number					
	1	2	4	6	8	10
M_2 ratio (autogamous)	3:1	7:1	15:1	23:1	31:1	39:1
M_2 ratio (geitonogamous)	3:1	15:1	63:1	143:1	255:1	399:1

autogamous plants and 15:1, 63:1 or 255:1 for the same germ cell numbers in geitonogamous plants. According to Anderson et al. (Genetics 34:639-646, 1949) and Steffensen (Amer. J. Bot. 55:354-369, 1968), the primordial germ cell number for the corn tassel is between four and eight, probably nearer eight. Since it is unlikely that a recessive mutant would appear in the M_2, the M_2 must be grown, selfed and tested. It has not been established whether the same primordial cells produce both male and female gametes, in which case the gametes would be mutually concordant, or whether different primordial cells are involved. Treatments applied and effective at the seed stage may affect any one of the primordial cells independently and produce a sector generating half normal and half mutant gametes. Assuming eight primordial cells and concordance, the population treated will be 16 genomes times the number of seeds treated. For those mutants having no selective effects 1/16 of the gametes from an M_1 plant undergoing a mutational event will carry the mutant. The M_1 plants may be either crossed to a standard strain or selfed, with the results indicated in Table 2 and discussed below.

If one treats 100 kernels, each carrying eight diploid primordial germ cells, the resulting M_1 plants will carry 1600 treated genomes. If the treatment produces mutants in 25% of the genomes (a frequency somewhat higher than is characteristically found for treatment at the seed stage, but used here in order to make equivalent comparisons with pollen treatment), then 400 mutants will have

Table 2. Comparison of the efficiency of different methods of treatment and handling for mutagenesis.

Treatment and handling	M_1 plants	Treated genomes	Mutants produced at 25% effectiveness	M_2 selfed	Mutants detected	Plants grown $M_1 + M_2$	Efficiency (Mutants per plant)
Seed: M_1 crossed; large M_2	100	1600*	400	4700*	380	4800	0.08
Seed: M_1 selfed; large M_2							
Concordance	100	1600	400	2300	380	2400	0.16
Non-concordance	100	3200*	800	4700	760	4800	0.16
Seed: M_1 selfed; minimal M_2							
Concordance	1000	16000	4000	1000	500	2000	0.25
Non-concordance	1000	32000*	8000	1000	500	2000	0.25
Pollen: M_1 selfed	2000	2000	500	--	500	2000	0.25

*assuming eight non-concordant primordial cells each for the tassel and ear
**required to detect 95% of mutants; 7200 would be required to detect 99%

been produced. Crossing the M_1 plants as female by a standard strain will produce 100 ears that will preserve all the mutants. The frequency with which the gametes and kernels will carry a particular mutant will be one in 16. A planting of these seeds with a subsequent self-fertilization will be required to express any recessive mutants. According to Hanson (Agron. J. 51:711-15, 1959), a sample of 47 individuals is required in order to have 95% certainty of obtaining one individual occurring at a frequency of one in 16. Therefore, to detect 95% (380) of the 400 mutants in the 100 ears will require planting 47 kernels from each ear, a total of 4700 plants to be selfed. Thus, an input of 4800 plants (100 M_1 plus 4700 M_2) will produce 380 mutants, an efficiency of 0.08 mutants per cultured plant.

Selfing the M_1 is more efficient than crossing by a standard strain. Assuming concordance, a double sample (from the ear and the tassel) is provided by each selfed M_1 ear, and only 23 M_2 kernels, or 2300 M_2 plants will give an efficiency of 0.16. With non-concordance, a self will provide one test each from two 8-celled primordial sets (ear and tassel) for a total of 3200 treated genomes and 800 mutants. These will require 4700 individuals to detect 760 mutants, resulting in an efficiency of 0.16, identical to the value for concordance. The tendency is to try to save all of the mutants by taking a very large sample from the M_1 ears, but this is counterproductive; for example, a sample of 52 kernels (5200 plants) would be required to save 99.9% of the mutants (efficiency 0.075). An additional problem arises from maximal sampling, namely the duplication and confounding of mutants. A single mutational event in a primordial cell will be duplicated many times through cell divisions before gamete formation, and many copies of the same mutant will be produced. For this reason, only one mutant of a particular type can be accepted in the progeny of each M_1 plant as a unique mutant. This is of considerable consequence, inasmuch as there are many loci whose recessive alleles have similar, confoundable phenotypes.

A more efficient sampling method from studies with Arabidopsis but generalized in application (G. P. Redei, Z. Pflanzenzuchtg. 73:87-96, 1974) is to treat large numbers of seeds and grow a large M_1 and then take a minimal sample from each M_1 for the M_2 to be selfed. For maize the minimal sample would consist of a single seed from each M_1 ear. Following previous assumptions, treatment of 1000 seeds will affect 16,000 genomes and produce 1000 plants that will carry 4000 mutants. Planting one seed from each M_1 will test 2000 gametes and save 500 of the mutants. In terms of total input, an investment of 2000 selfed plants (1000 M_1 and 1000 M_2) will yield 500 mutants for an efficiency of 0.25. Furthermore, since only one sample of two gametes is taken from each M_1 plant the

problem of duplicate copies is eliminated; if by chance (one in 256, again assuming concordance) both gametes contribute the same mutant, it will be homozygous immediately and will be recognizable as such.

An equally efficient approach with some additional advantages over that just presented is to treat pollen; an investment of less than 100 plants for treatment plus a test by selfing of 2000 M_1 plants will produce 500 mutants for an efficiency of 0.25 (slightly less because of the plants needed for the original crosses with treated pollen). Pollen treatment has several advantages: (1) Each mutant seen is an independent event, so that except for normal attrition all mutants produced are saved--this allows easy comparison of mutation rates and of the relative frequencies of different mutant types. (2) Variations among different lines or between sexes in primordial cell number are not a concern in the estimation and comparison of mutation rates. (3) Dominant mutants are easily recognized as such and are ready for immediate testing.

The economic advantage described here for minimum sampling in mutation experiments applies equally well to the development of elite lines for breeding programs.

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Genetic breakpoints of the B-A translocations of maize — The accompanying chromosome maps summarize the data I have collected on the genetic breakpoints of B-A translocations. The chromosome arm, cytological map position if known, and originator are given for each. The portion of each chromosome arm translocated to the centric segment of the B chromosome is shown as a broken line; therefore, all genes shown along the broken line were found to be "uncovered" by the translocation and all genes beside the solid portion were not uncovered.

I have tested all loci listed with the exception of hm (tested by Roman and Ullstrup, Agron. Jour. 43:450-454, 1951), gl4 (tested by Rakha and Robertson, Genetics 65:223-240, 1970) and, for TB-10(18) and TB-10(19), zn and du (tested by Lin, MGCNL 48:182-184). Loci that should be retested for confirmation of position with respect to the B-A translocation are marked by the symbol "#". Map positions of loci are usually given, if known, and other factors are inserted in what is believed to be the proper place or listed at the end of the arm.