genotype and two Rg y si/Rg y Si genotypes clearly establish that y is between rg and si. Experiments with po and Pl are in progress to determine orientation.

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2. The location of y on chromosome 6.

Linkage tests of $y\text{-}su_2/Y\text{-}Su_2$ in homozygous translocation T6-10b (6L.17,10L.14) showed 65 $Y\ Su_2$:110 $Y\ su_2$:118 $y\ Su_2$:59 $y\ su_2$ which gives 35.2% recombination and indicates the translocation point to be to the left of y with y on the long arm. Data presented by Patterson (1958 Newsletter p. 64) showed recombination between y and R to be 18.8% in the homozygous translocation placing the break on 6 to the right of y. These two sets of data are compatible only if the break on 6 is in the short arm as Burnham (Genetics, 1950) indicated. If the break on 6 is in fact in the short arm, the possibility of y also being on the short arm is not ruled out.

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1. Intracistron recombination at the Wx/wx locus.

The dependence of the type of starch (amylopectin vs. amylopectin + amylose) produced in a microspore on the genotype at the Wx/wx locus of the microspore itself and not the parental plant allows a test in maize for the occurrence of intracistron recombination. The barrier to the investigation of such a phenomenon in higher organisms is our inability to handle populations of sufficient size to detect the infrequent recombinants if such exist. In this system, however, the requisite numbers are easily available since a maize plant produces millions of microspores and since slides containing 50,000 or more microspores can be prepared and scored in twenty to twenty-five minutes.

If two independently occurring waxy mutants at the Wx/wx locus represent changes at different mutational sites within the cistron and if recombination between such sites is a reality, it should be detectable in preparations from the pollen produced by the F_1 between the mutant stocks. One of the products of recombination would be a reconstituted functional locus; in this case some amylose would be formed, and a microspore carrying such a locus would stain black with a KI, I_2 stain in contrast to the brownish color typical of waxy microspores. Where in a cross between 2 waxy mutants the frequency of such black (normal)

microspores exceeds the mean of the frequencies of black microspores in the pollen produced by the parents, it could be an indication of such recombination. Further, the frequencies of normal microspores in the various all-combination crosses between a series of waxy mutants should be a function of the distances between the sites concerned and allow a preliminary mapping of the locus.

With such a project in mind, a number of independently occurring waxy mutants were collected. These are listed in Table 1. All possible crosses were made between the different mutants. Tassel sections were collected in 70% alcohol just prior to pollen shedding from a number of the F_1 plants of each cross. Subsequently slides were prepared according to a standard technique to be described later. The total population of microspores for each slide was estimated by the sum of six grid counts over the surface of the slide x a constant. Normal microspores were located and marked by a drop of Kodak Opaque.

Table 1

Mutant	Source
C	Maize Coop; Chr. 9 tester, Rec'd. 1951.
90	Brunson; Mutation in Inbred 90.
H21	Brunson; Mutation in Inbred H21.
B	Bear; Mutation in Breeding Material.
a	Kramer; The wx[a] isolated in Argentina.

Table 2 gives the results for the parental stocks and the crosses both for plants grown in the greenhouse in the winter of 1957-1958 and for plants grown in the field in the summer of 1958. The results are not strictly comparable since the greenhouse data consist of the results of repeated analyses of not more than 8 plants from the same cross between two mutants. The field data include a greater but variable number of plants from all crosses between two mutant stocks. Reference to Table 2 shows that for the parental stocks there are low but measurable frequencies of normal microspores. The figure for any one mutant stock presumably includes the products of back-mutation at the waxy locus, suppressor mutation, and contamination of tassel samples by the lodging of wind-blown normal pollen. Crosses between different mutant stocks may give frequencies ranging from those no higher than the parental stocks to those which are many-fold higher.

Table 2. Estimates of Incidences of Normal Microspores

Stock	'57-'58 Greenhouse Est.No.Microspores x 10^3	'57-'58 Greenhouse $\bar{x}$ No.Normal x 10^{-5} ± sx	1958 Field Est.No.Microspores x 10^3	1958 Field $\bar{x}$ No.Normal x 10^{-5} ± sx
a	61	0	---	---
B	503	1.6 ± 0.6	472	3.8 ± 1.2
C	554	1.1 ± 0.4	444	0.7 ± 0.4
H21	509	2.0 ± 0.4	559	2.7 ± 0.8
90	615	1.6 ± 0.5	353	0.3 ± 0.4
H21 x B/	576	35.6 ± 4.2	987	28.1 ± 2.2
H21 x 90	522	33.1 ± 3.7	1168	31.8 ± 2.7
C x H21/	1174	54.3 ± 3.9	1218	46.0 ± 2.7
90 x B	537	1.9 ± 1.2	717	1.4 ± 0.6
90 x C	1165	75.3 ± 4.0	596	88.0 ± 5.7
C x B/	550	23.6 ± 2.0	1077	29.5 ± 2.9
Rec. C x C*	---	---	252	1.3 ± 0.9
C x a	289	4.5	---	---
a x H21	385	29.4	---	---
a x 90	287	2.4	---	---
a x B	354	0.3	---	---

* Recovered C = $\{[(C \times \text{Inbred Tr}) \mathcal{F} \times \text{Tr}] \mathcal{Q} \times \text{Tr}\} \mathcal{Q}$ with sh wx segregates being selected after each selfing.
/ 1958 field data includes also the reciprocal cross.

Where a number of crosses between two mutant stocks were made, the progeny from each cross was sampled. In all cases there was good agreement between the different progenies within a cross. In some cases, reciprocal crosses were available. Data again showed good agreement. The results for two sets of progenies are reported in Table 3.

Table 3. Normal Microspores in Individual Progenies

Cross	No. Plants	Est. No. Microspores x 10^3	Est. No. Normal x 10^{-5}
(C x H21)-1	6	252	50.7
(C x H21)-2	4	224	40.0
(C x H21)-3	3	248	40.7
(C x H21)-4	5	273	46.2
H21 x C	3	221	49.3
Total	21	1216	46.0
(H21 x B)-1	4	272	23.3
(H21 x B)-2	5	357	31.9
B x H21	6	358	28.3
Total	15	987	28.1

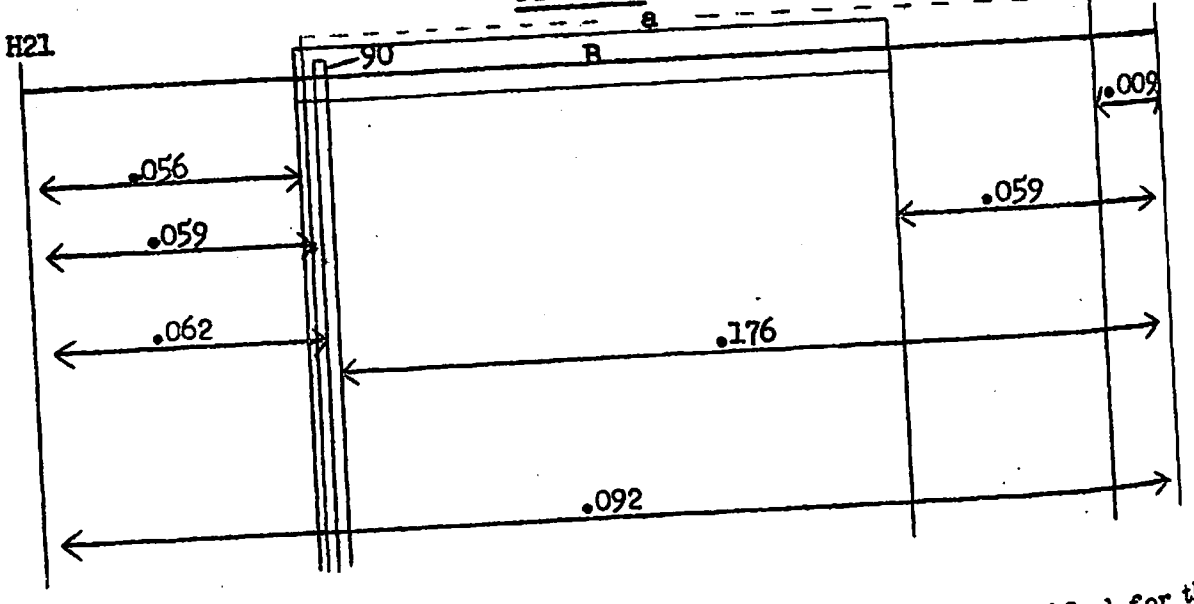
For most crosses which had a high incidence of normal microspores, a number of individual anther preparations were scored. In all cases the distribution of numbers of normal microspores per anther showed good agreement with a Poisson distribution. Thus the bulk preparations for any cross give a good estimate of the probability that any microspore will be normal in phenotype.

Results from the 1958-59 greenhouse planting of the BC progeny (90 x C)x C show 5 plants with no normal microspores and 4 plants with a mean of 63×10^{-5} normal microspores per plant.

Considering the above data, it seems justifiable to conclude; (1) that each of the alleles is different from any other allele, (2) that there is a characteristic frequency of normal microspores for each cross between two mutants, and this frequency is obtained every time the cross is made, (3) heterosis, per se, cannot account for the normal microspores as witness the frequencies in 90 x B and Rec. C x C, and (4) the normal microspores are the result of recombination within the locus.

If this last hypothesis is correct, it should be possible to use the data from the crosses to establish a linear order for the mutants within the locus. This can be done since only one arrangement appears to satisfy the data. This arrangement is shown in Figure 1. Note, however, that the criterion of additivity is not satisfied. This may be a consequence of the heterogeneous backgrounds in which the mutations occurred. Unquestionably, for a study of genetic fine structure, it would be desirable to induce a series of mutants in the same stock where one could be sure that the critical locus prior to mutation was the same in every case. This is being done presently.

FIGURE 1*



* The frequencies of normal microspores observed have been doubled for the purpose of map construction. The figures given are percentages.

The fact that several mutants (B and a) appear to have some size may also be due to the heterogeneous backgrounds of the mutants. One cannot discount, however, the possibility that it is actual. If the genesis of a mutant were functional locus + gene controlling element within the locus, then the mutant would appear as a block in studies of this type. Several waxy mutants which are known to have had such an origin have been included in our crosses, and data should soon be available on this point.

Functional complementation if it occurs should be revealed in the endosperm of the seed resulting from the cross between two mutants. Analyses are not complete as yet, but interactions of a magnitude which would suggest that the two mutants crossed are located in different cistrons have not been observed. Still measurable interactions are present. The percentage of amylose in all crosses involving wx^a, for example, is substantially greater (100%) than the percentage of amylose in either parent. More data on amylose percentage in various crosses are being obtained.

The details of the technique used may be pertinent. The tassel samples are collected as mentioned in 70% alcohol. A "curing" period of several weeks is desirable since newly collected microspores do not stain as readily with a standard strength stain as do those which have been collected longer. The standard stain formulation is 25 ml. of water, 250 mg. of potassium iodide, and 45 mg. of iodine. The stain is mixed approximately 20 hours before use and placed on a shaker over night. One hour before use, 1 drop of "Tween 80" is added and then 0.5 grams of Baker's gelatin. The mixture is heated for 5 minutes on a warm hot plate.

In preparing the slides, 24 anthers are selected--the 3 anthers from the more mature floret of 8 glumes which are just beginning to open. These are placed in the small stainless steel cup of a Virtis Microhomogenizer together with 0.8 ml. of the stain. The mixture is homogenized for 2 minutes after which it is strained through cheesecloth onto the surface of a lantern slide. The microspores are dispersed as evenly as possible and covered with a 50 x 75 mm cover slip. After the mixture has set, the edges of the cover slip are coated with colorless nail polish. Such preparations will keep for several days without desiccation and can be scored at any time in that period.

O. E. Nelson, Jr.

2. Gene controlling elements of the a_1^{pm} system.

Notes dealing with a mutable a_1^p allele have appeared in the News-Letter for the past several years. By and large they have been concerned with the analysis of A_1 locus components through the use of patterns of mutation presented by this mutable locus. This letter, on the other hand, will deal with the gene controlling elements involved.