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A ZYGOTIC LETHAL IN CHROMOSOME 1 OF MAIZE AND ITS LINKAGE WITH NEIGHBORING GENES¹

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DISTORTED F₂ RATIOS OF CHARACTERS LINKED WITH A ZYGOTIC LETHAL

ABOLIVIAN maize with mosaic red pericarp and cob, here designated by the symbol *M-M*,² crossed with a local inbred strain of maize having white pericarp and cob, *W-W*, produced in F₁ 21 *M-M* and 28 *W-W* plants, not far from the 1:1 relation expected on the assumption that the *M-M* parent was heterozygous for pericarp and cob color, *M-M/W-W*. In F₂ and segregating F₃ cultures, however, there were 130 *M-M* and 64 *W-W* plants obviously a 2:1 instead of the 3:1 relation expected. Later cultures increased these records to 747 *M-M* and 382 *W-W* plants, a deviation of only 6 ± 10.5 from a 2:1 ratio. Of 18 *M-M* plants tested, all were heterozygous for pericarp color, though one-third were expected to be homozygous. The odds against the chance occurrence of this deviation of 6 ± 1.3 are over 500:1. This suggested that the 2:1 ratio might be brought about by the elimination of homozygous *M-M* plants.

To test this possibility, *M-M* plants were crossed with an inbred *W-R* strain. The F₁ cultures consisted of 195 *M-R* and 183 *W-R* plants (really *M-M/W-R* and *W-W/W-R*). In F₂ and later segregating cultures from *M-R* parents, there were 1238 plants with mosaic and 617 with white pericarp, again a 2:1 relation. The 617 were *W-R* as expected. Of the 1238 plants with mosaic pericarp, 1217 were *M-R* and 21 *M-M*, that is, 1217 were heterozygous *M-M/W-R* and only 21 homozygous *M-M/M-M*. The fortunate circumstance that, in crosses involving *M-M* with *W-R* all heterozygous plants are *M-R* and homozygous ones *M-M*, makes it possible to determine hetero- and homozygosity by mere inspection of the ears, thus avoiding the necessity of growing innumerable progenies. The results

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² The gene *P* for pericarp color has numerous alleles (ANDERSON 1924; EMERSON, BEADLE, and FRASER 1935). Thus, the symbols *P^{rr}*, *P^{wr}*, *P^{vv}*, etc., indicate red pericarp and red cob, white (colorless) pericarp and white cob, variegated pericarp and variegated cob, respectively. For convenience in this paper, the locus of these genes is indicated by *P* without superscripts and the several alleles by the superscripts alone, the first letter for pericarp and the second for cob color as follows: *R-R* red pericarp and cob, *M-M* mosaic pericarp and cob, *V-V* variegated pericarp and cob, *W-W* white pericarp and cob, *W-R* white pericarp and red cob. The symbols *M-R* or *V-R* are used to indicate mosaic or variegated pericarp associated with red cob because of the dominance of mosaic or variegated over white pericarp and of self red over variegated or mosaic cob in the heterozygotes *M-M/W-R* or *V-V/W-R*.

recorded here indicate clearly that the 2:1 relation of mosaic to white is due to the almost complete elimination of the expected 25 percent of homozygous *M-M* plants. This result is ascribed to a recessive zygotic lethal, *zl*, closely linked with *P*. This hypothesis is supported by the observation that the ears of selfed plants heterozygous for *zl* show partial sterility. Theoretically there should be 25 percent of missing kernels, but it is usually impossible to find that many vacancies. Reciprocal crosses of heterozygous *zl* plants with homozygous normals give a full set of kernels, thus showing that no gametic lethal is involved.

The inbred *W-R* strain used in the crosses noted above was segregating for a then unknown recessive male-sterile gene, now known as *ms*₁₇. Of *F*₂ and later cultures in which *ms*³ was involved there were 673 normal and 341 *ms* plants, clearly a 2:1 relation. Moreover, of the 673 normal plants, 641 were *M-R*, six *M-M* and 26 *W-R*, while of the *ms* plants 329 were *W-R* and 12 *M-R*. Evidently, *ms* is closely linked with *P* and with *zl*.

It has long been known that tassel-seed 2, *ts*₂, is closely linked with *P*. It was assumed, therefore, that it must be linked with *ms* and with *zl*. Mosaic-pericarp plants of the cultures recorded above were, therefore, crossed with *W-R* plants carrying *ts*.³ Parental *M-R* plants gave in *F*₂ and later generations 376 normal and 176 *ts* plants. Of the normals, six were *M-M*, 357 *M-R*, and 13 *W-R*; while, of the *ts* plants 10 were *M-R* and 166 *W-R*. Here *zl*, again, as expected, eliminated most of the homozygous *M-M* plants resulting thereby not only in a 2:1 ratio of colored to white pericarp but also in a 2:1 ratio of normal to *ts* plants.

The data presented above show that *zl* is linked with the dominant mosaic red pericarp and the normal allele of *zl* with the recessive white pericarp. The convenient allelic series of pericarp-color genes makes it easily possible to arrange a cross in which *zl* is linked with a recessive pericarp color and its dominant normal allele with a dominant color, and this without having to use a previous crossover. Self-red pericarp and cob, *R-R*, is dominant to *M-M* just as *M-M* is to *W-W*. Maize with *M-M zl* was crossed with *R-R ts* and resulted in normal *R-R F*₁ plants. There resulted in *F*₂ 123 normal and 49 *ts* plants. All the *ts* and all but one of the normal plants were *R-R*; the one exception was *M-M*. The ratio of *R-R* to *M-M* was 171:1. Later records increased these numbers to 705:7. Some of the *F*₁ *R-R* plants involved here were also crossed with normal *W-W* plants and produced in the test-cross generation 93 *R-R* and 95 *M-M* plants (*R-R/W-W* and *M-M/W-W*, respectively). Obviously *R-R/M-M F*₁ can give *M-M* plants in *F*₂ only when *zl* is lost from at least one *M-M* gamete (*M-M zl/M-M* +).

³ Since *ms*₁₇ and *ts*₂ are the only male-sterile and tassel-seed genes referred to in this paper, they are here designated by the symbols *ms* and *ts* without subscripts.

INTENSITY OF LINKAGE BETWEEN *zl*, *P*, *ms*₁₇, AND *ts*₂

It is evident from the foregoing that in chromosome 1 of maize there is a group of four closely linked genes, *P*, *ms*, *ts*, and *zl*. No unusual difficulty is experienced in determining the percent of recombination between *P* and *ts* or *P* and *ms*. The following summary (table 1) is taken from EMERSON, BEADLE, and FRASER 1935:

TABLE 1

X Y	LINKAGE PHASE	X Y	X y	x Y	x y	TOTAL	RECOMBINATIONS	
							NUM-BER	PER-CENT
<i>Ms</i> ₁₇ <i>P</i>	<i>C B</i>	1307	36	45	1318	2706	81	3.0
<i>P Ts</i> ₂	<i>C B</i>	1558	19	21	1510	3108	40	
	<i>R B</i>	0	94	92	2	188	2	
						3296	42	

These results indicate that *ts* is nearer *P* than *ms* is and that the two are either approximately 1.7 or approximately 4.3 units apart. It is impossible, however, to measure this distance by ordinary genetic tests, without excessive effort. Male-sterile 17 almost never produces viable pollen and most lines of tassel-seed 2 are almost entirely pistillate flowered. It is, therefore, in most instances, impossible to identify the double recessive. The occurrence of a double recessive, however, doubtless by crossing over, has been demonstrated. Crosses of male-sterile with tassel-seed (one or other parent heterozygous normal) gave normals in *F*₁ and, in *F*₂ and later segregating generations, normal, male-sterile, and tassel-seed plants in the following numbers, respectively: 428, 196, 161, approximately a 2:1:1 relation. One of these male-steriles with variegated pericarp and red cob, *V-V ms/W-R ms*, was crossed by a normal *W-R*, resulting in normal *F*₁'s *V-V ms/W-R+* and *W-R ms/W-R+*. One of these *V-R* normals on being selfed gave in *F*₂ 15 *V-R* normal, four *W-R* normal, six *V-V ts*, and no *ms*. Fortunately, this same *V-R* normal parent had been crossed on both *W-R ts* and *W-R ms*. The first of these test crosses resulted in nine *W-R* normal and 16 *V-R ts* and the second in 22 *W-R* normal and 12 *V-R ms*. Obviously, the original male-sterile plant that had been crossed by a *W-R* normal carried *ts* as well as *ms*. The *F*₁ was *V-V ms ts/W-R++* and the *F*₂ *ts* plants were really double recessives, *ms ts*. This situation could have resulted only by a crossover between *ms* and *ts*.

Since homozygous *zl* plants never appear, backcross data cannot be obtained and linkage intensity for *P* and *zl* must, therefore, be calculated from *F*₂ progenies. As shown earlier in this account, plants heterozygous

for $M-M$, $W-R$ and zl ($M-M\ zl/W-R+$) give in F_2 a 2:1 ratio of mosaic to white pericarp, the former associated almost always with red cob as in the F_1 parent. The few mosaic-cob plants observed must have resulted from crossovers whereby zl was lost from one $M-M$ chromosome, and were, therefore, $M-M+ / M-M\ zl$. From the percent of $M-M$ plants the percent of crossing over can be calculated. In F_2 from $M-M\ zl/W-R+$, the percent of $M-M$ plants (homozygous dominants) in the whole population should vary from 0 to 25 as the percent of crossing over varies from 0 to 50. Similarly, in F_2 from $M-M\ zl/R-R+$, the percent of $M-M$ plants (homozygous recessive) should vary from 0 to 25 with percent of crossing over varying from 0 to 50. From an observed percent of $M-M$ plants in F_2 , the percent of crossing over can be calculated by interpolation in a table prepared for the purpose. The available F_2 data for the two classes of heterozygotes, including records published earlier (EMERSON, BEADLE, FRASER 1935) are given in table 2.

TABLE 2

F ₁ GENOTYPE	TYPES OF F ₂ PLANTS				TOTAL	PERCENT <i>M-M</i>	PERCENT CROSSING OVER
	<i>R-R</i>	<i>M-M</i>	<i>M-R</i>	<i>W-R</i>			
<i>M-M\ zl/R-R+</i>	705	7	—	—	712	0.983	1.487
<i>M-M\ zl/W-R+</i>	—	28	1861	1012	2891	0.969	1.466
Total		35			3603	0.971	1.469

As shown in this table, the percent of crossing over between P and zl is 1.5. Since the crossover percent for $P\ ts$ is 1.3 and for $P\ ms$ 3.0, zl must be about 0.2 or 2.8 from ts and 1.5 or 4.5 from ms , depending on whether zl is on the same side of P as ts or ms or on the opposite side. The nature of the characters involved, zygotic lethal, tassel-seed, male-sterile, make it impossible to use the ordinary three-point back-cross test as a means of determining the exact loci of this group of genes, but fortunately another method can be employed.

LINEAR ORDER OF THE GENES: P , ms_{17} , AND zl

Since, to hand pollinate all plants involved in this study would require an inordinate amount of work and since crossovers are infrequent with genes so closely linked, it has usually happened that crossover plants had been open-pollinated before they were detected. But the close linkage renders crossover chromosomes relatively stable so that the new association of genes can be studied even though the plant first exhibiting it had been open pollinated. To avoid undue confusion, cultures produced by the selfed heterozygotes $M-M\ zl/W-R\ ms$ and $M-M\ zl/W-R\ ts$ were grown on the two sides of an isolated block. Since ms and ts plants produce little

or no viable pollen, the pollen produced in such isolated fields consists of a mixture of about 50 percent $M-M\ zl$, 25 percent $W-R\ ms$, and 25 percent $W-R\ ts$. This mixture is, of course, modified slightly by the pollen of the few crossover plants present in the block and by new crossover gametes of non-crossover plants. On one occasion, the open-pollinated block adjoined a field consisting largely of normal $W-R$ and $W-W$ plants.

In the populations making up these partly isolated blocks, three types of crossover plants can be detected by mere observation without resort to progeny tests. In cultures grown from selfed $M-M\ zl/W-R\ ms$, these three types are as follows:

I—Homozygous $M-M$ plants, usually $M-M\ +/M-M\ zl$.

II—Male sterile $M-R$ plants, usually $M-M\ ms/W-R\ ms$.

III—Normal $W-R$ plants, usually $W-R\ +/W-R\ ms$.

These three crossover types will be considered one by one in an attempt to discover the linear order of the genes P , ms , and zl . Later similar types from cultures grown from selfed $M-M\ zl/W-R\ ts$ will be considered.

There are only three possible linear orders of the genes P , ms , and zl as shown below for the heterozygote $M-M\ zl/W-R\ ms$:

<i>Alpha</i>	<i>Beta</i>	<i>Gamma</i>
$\begin{array}{ccc} + & M-M & zl \\ ms & W-R & + \end{array}$	$\begin{array}{ccc} M-M & zl & + \\ W-R & + & ms \end{array}$	$\begin{array}{ccc} M-M & + & zl \\ W-R & ms & + \end{array}$

Type I crossovers, homozygous $M-M$ plants, can arise only by replacement of zl by its dominant allele in at least one $M-M$ chromosome. This might be accomplished by a crossover in region 2 of *alpha*, region 1 of *beta*, or in either region 1 or 2 of *gamma*. Since no one of these genes can be more than five units from any other one, it is fair to assume that double crossing over never or very rarely ever occurs. Without double crossing over, when zl is lost from the $M-M$ chromosome, that chromosome could *never* acquire ms simultaneously if the order is that shown in *alpha*, must *always* acquire it if the order is that of *beta*, and might or might not acquire it if the order is that of *gamma*. The problem, therefore, is to determine whether on losing zl the $M-M$ chromosome *never*, *always*, or *sometimes* picks up ms .

Type II crossovers, male-sterile mosaic-pericarp plants, might arise by a crossover in region 1 of *alpha* order, in either region 1 or 2 of *beta* order, or in region 1 of *gamma* order. Without double crossing over, when the $M-M$ chromosome acquires ms it can *never* lose zl , if the order is that of *alpha*, may or may not lose it if the order is that of *beta* and must *always* lose it if the order is that of *gamma*. The problem is then to determine whether the $M-M$ chromosome on acquiring ms *never*, *sometimes*, or *always* loses zl .

Type III crossovers, normal *W-R* plants, might arise from a crossover in region 1 of *alpha*, in either region 1 or 2 of *beta*, or in region 1 of *gamma*. Without double crossing over, when *ms* is lost from the *W-R* chromosome, that chromosome could *never* pick up *zl* if the order of genes is that of *alpha*, might or might not if the order is *beta*, and must *always* do so if the order is *gamma*. What is to be determined, therefore, is whether the *W-R* chromosome, on losing *ms*, acquires *zl* *never*, *sometimes*, or *always*.

These several alternatives are summarized for ready reference in the following tabular statement, table 3:

TABLE 3

CROSSOVER TYPE	CHROMOSOME EXCHANGE	GENE ORDER		
		<i>ms P zl</i> <i>alpha</i>	<i>P zl ms</i> <i>beta</i>	<i>P ms zl</i> <i>gamma</i>
I	<i>M-M</i> loses <i>zl</i> and acquires <i>ms</i>	never	always	sometimes
II	<i>M-M</i> acquires <i>ms</i> and loses <i>zl</i>	never	sometimes	always
III	<i>W-R</i> loses <i>ms</i> and acquires <i>zl</i>	never	sometimes	always

Evidence from crossover type I

In cultures coming from selfed heterozygotes *M-M zl/W-R ms*, 28 homozygous *M-M* plants were found and studied more or less adequately. All these plants had been grown in isolated or semi-isolated blocks from selfed seed and the seeds produced by them had been open-pollinated, the available pollen having been about 50 percent *M-M zl*, 25-50 percent *W-R ms*, and 0 to 25 percent *W-R ts*. Small F_1 cultures were grown from each of the 28 open-fertilized ears. In all there were 443 F_1 plants and all of them were normal, not a single one male-sterile. Since 25 to 50 percent of the pollen produced in the isolated block the preceding year was *ms*, it is highly probable that some *ms* plants would have appeared in F_1 if the *M-M* chromosome had gained *ms* simultaneously with the loss of *zl*.

A few plants of most F_1 cultures were selfed and F_2 progenies grown. Thirty selfed F_1 's were homozygous *M-M*, presumably *M-M +/M-M zl*, and 16 were heterozygous *M-R*, presumably *M-M +/W-R ms*.

The F_2 progenies of the 30 *M-M* plants totaled 1139 individuals, of which 1100 were normal and 39 *ms*. For some of the 39 plants recorded as *ms* identification was uncertain because of injury to the tassels, but in most instances identification was probably correct. It is significant that all of the 39 *ms* plants occurred in six of the 30 F_2 cultures, the other 24 cultures having consisted of normal plants only. Moreover, 36 of the 39 *ms* plants occurred in four of the six cultures, the other two cultures having had only three *ms* plants. The distribution of normal and *ms* plants in the two classes of cultures is shown in table 4:

TABLE 4

NUMBER OF F ₂ CULTURES	TYPES OF F ₁ PLANTS		TOTAL	PERCENT MALE-STERILE
	NORMAL	MALE-STERILE		
Four	208	36	244	14.7
Two	91	3	94	3.2

The genotype of the F₁ plants which gave rise to the two F₂ cultures showing 3.2 percent of *ms* plants was probably $+M-M+/ms\ M-M\ zl$. The 3.2 percent of *ms* plants corresponds to a crossover percentage between *ms* and *zl* of 4.8, which is not far from the maximum of 4.5 calculated earlier from the linkage of *P ms* and *P zl*. But the 14.7 percent of *ms* plants in the other four cultures could hardly have come from an F₁ of that genotype, for 14.7 percent recessive corresponds to a crossover percentage of slightly more than 25. If the four F₁'s were $+M-M+/ms\ M-M+$, 25 percent instead of about 15 percent of the F₂ population should have been *ms*. That the parents of all these F₁ plants were $+M-M+/+M-M\ zl$ is suggested by the fact that sibs of four of these F₁'s threw no *ms* plants in F₂. It seems probable that *ms* might have come from crossover pollen in the open-pollinated isolation block while the loss of *zl* had certainly occurred in the selfed plants one generation earlier.

The F₂ progenies of the 16 heterozygous *M-R* selfed F₁'s grown from open-pollinated crossover *M-M* plants exhibited two strongly contrasting types of segregation, as shown in table 5:

TABLE 5

NUMBER OF F ₂ CULTURES	TYPES OF F ₂ PLANTS					TOTAL
	<i>M-M+</i>	<i>M-R+</i>	<i>M-R ms</i>	<i>W-R+</i>	<i>W-R ms</i>	
Nine	84	129	11	11	71	306
Seven	1	138	17	9	81	246

Records of the seven F₂ cultures are approximately those expected in F₂ from selfed F₁'s of the genotype $+M-M\ zl/ms\ W-R+$. If the original crossover *M-M* plants were $M-M+/M-M\ zl$ and if 25 to 50 percent of the pollen of the field was *W-R ms*, as postulated, about one-fourth to one-half of the F₁ *M-R* plants should have been $+M-M\ zl/ms\ W-R+$. That is, the crossover chromosome was not involved and these seven cultures are irrelevant to this discussion. The other nine F₂ cultures with 84 *M-M*, 140 *M-R*, and 82 *W-R* plants, the latter mostly *ms*, are about what should be expected from selfed F₁'s of the constitution $+M-M+/ms\ W-R+$. The 11 *M-R ms* and 11 *W-R+* plants are to be regarded merely as the result of a relatively high crossover frequency in the F₁ plants. These nine cultures afford no evidence that the *M-M* chromosome acquired *ms* when it lost *zl*.

As a general statement, therefore, it is fair to conclude that, when the *M-M* chromosome loses *zl* by crossing over, it does not simultaneously pick up *ms* or that it very rarely does so. It follows from this that linear order *alpha*, *ms P zl*, is the most likely one but that order *gamma*, *M-M ms zl*, is perhaps a possibility. The most significant conclusion, however, is that order *beta*, *M-M zl ms*, is impossible because *ms* certainly is not *always* acquired with the loss of *zl*.

Evidence from crossover type II

In addition to homozygous *M-M* plants arising because of the loss of *zl* from an *M-M* chromosome, considered in the preceding discussion, there are found *M-R ms* plants (*M-M ms/W-R ms*) coming from the addition, by crossing over, of *ms* to the *M-M* chromosome. In the same open-pollinated blocks in which type I crossovers were found, 56 type II crossovers were identified. *F*₁ cultures from seed of all these crossover plants were grown and a few ears of each selfed or crossed with appropriate tester stocks. The *F*₁ cultures had a total of 754 plants, 445 of which were normal and 309 *ms*. This is in strong contrast with *F*₁ of type I crossovers in which no *ms* plants appeared.

The *F*₁ *ms* plants were mostly *M-R*, presumably *M-M ms/W-R ms*. The problem is to determine whether the *M-M ms* chromosome carries *zl* also. Several of these *ms* plants were pollinated by normal *M-R* plants of a tester stock, the genotype of which was known to be *+M-M zl/ms W-R+*. If the *F*₁ *ms* plants so crossed carried *zl* in the *M-M* chromosome, *zl M-M ms*, no *M-M* plants should appear in the progeny except from new crossovers by which *zl* might be lost. Two sharply separable types of progenies were obtained, 30 of one type and two of the other, as shown in table 6:

TABLE 6

NUMBER OF CULTURES	TYPES OF TEST-CROSS PLANTS						TOTAL
	<i>M-M+</i>	<i>M-R+</i>	<i>M-M ms</i>	<i>M-R ms</i>	<i>W-R+</i>	<i>W-R ms</i>	
Thirty	23	481	0	454	33	465	1456
Two	24	24	9	44	0	33	134

Of the 1456 plants of the 30 cultures, 958 had mosaic to 498 white pericarp, and 537 were normal to 919 male-sterile, not far from 2:1 and 1:2 ratios, respectively. This behavior, together with the occurrence of relatively few *M-M* plants, indicates clearly that the 30 *F*₁ plants from which these test-cross progenies came had both *zl* and *ms* in their *M-M* chromosomes. It follows, therefore, that the *M-M* chromosome did not, in these instances, lose *zl* when it acquired *ms*.

The other two cultures, however, gave strikingly different results. Of the 134 plants, 101 had mosaic and 33 white pericarp, a 3:1 ratio. And of the mosaic-pericarp plants, 33 were *M-M* and 68 *M-R*. Obviously the *M-M* chromosomes of these two F_1 's carried *ms* but not *zl*. This situation might have occurred by the loss of *zl* simultaneously with the acquisition of *ms*, but this is not the only possibility. The acquisition of *ms* might have occurred in the selfed parents of some of the isolation-block plants and some of the gametes of the resulting plants in the isolation-block might have lost *zl*. In either case the *ms* parents of these two test-cross cultures must have been *ms M-M* +/*ms W-R* +.

So far only the *ms* plants appearing in the progenies of type II crossovers found in isolation blocks have been considered. If, in general, *zl* is retained by the *M-M* chromosome when it acquires *ms*, the normal plants grown from open-fertilized seed of type II crossovers should be of no significance in this discussion. If these plants are *ms M-M zl/ms W-R* + and are pollinated with a mixture of +*M-M zl* and *ms W-R* + pollen, the only normal plants of the next generation should be *ms W-R* +/+*M-M zl*. In short, the crossover plant contributes to these normal plants of the next generation only the non-crossover chromosome *ms W-R* +.

The F_1 progenies of open-pollinated type II crossovers contained normal *M-M* and *M-R* plants whose selfed progenies gave results of significance for the problem here under consideration. Of 26 F_1 normal *M-M* and *M-R* plants, in eight instances the crossover parent had obviously been pollinated in part with *W-R ts*₂ pollen, in seven with *W-R* pollen, and in 11 with *W-W* pollen. The resulting F_2 cultures of the three groups are given separately in table 7:

TABLE 7

NUMBER OF F_2 CULTURES	TYPES OF F_2 PLANTS								TOTAL
	<i>M-M</i>	<i>M-M</i>	<i>M-R</i>	<i>M-R</i>	<i>M-R</i>	<i>W-R</i>	<i>W R</i>	<i>W-W</i>	
	+	<i>ms</i>	+	<i>ms</i>	<i>ts</i>	+	<i>ts</i>	+	
Eight	0	1	138	34	2	18	80	—	273
Seven	0	1	226	14	—	123	—	—	364
Eleven	477	21	—	—	—	—	—	230	736

In the first group of eight and the second one of seven cultures from selfed *M-R* normals, only one *M-M* plant occurred in each; the respective numbers of plants with mosaic and with white pericarp were 175 to 98 and 241 to 123; and the respective numbers of normal and *ms* plants were 238 to 35 and 349 to 15. Evidently *zl* was here associated with *M-M* and *ms*, thus preventing, except by new crossovers, the appearance of homozygous *M-M* and *ms* plants and thereby resulting in 2:1 ratios of colored to white

pericarp. Such behavior is that expected from selfed plants of the respective genotypes $ms\ M-M\ zl/ts\ W-R+$ and $ms\ M-M\ zl/+W-R+$. In the third group of 11 cultures from selfed $M-M$ normals, there were 498 plants with colored to 238 with white pericarp, a 2:1 ratio; and there were 715 normal to 21 ms plants. Obviously the 11 selfed $M-M$ plants had the genotype $ms\ M-M\ zl/+W-W+$.

In 56 of the 58 tests of type II crossovers, it is certain that the $M-M$ chromosome did not lose zl when, by crossing over, it gained ms . In only two of the 58 tests, might zl have been lost at the same time that ms was acquired by this chromosome. And, even here, the loss of zl might have occurred one generation later than the acquisition of ms . These results indicate strongly that linear order $alpha$, $ms\ P\ zl$, is the correct one but do not disprove the possibility that the order might be that of $beta$, $P\ zl\ ms$. The most significant conclusion derived from these records is that order $gamma$, $P\ ms\ zl$ is impossible, because certainly zl is not *always* lost when ms is gained by the $M-M$ chromosome.

It will be recalled that the records from 37 F_2 cultures grown in the study of crossover type I, homozygous $M-M$, indicated order $alpha$ as the most probable but did not exclude order $gamma$, while the records of the 58 cultures used in a study of crossover type II, $M-M\ ms$, indicated order $alpha$ as most probable without excluding the possibility of order $beta$. But the crucial conclusions are that the type I crossovers studied could not possibly have come from order $beta$ and that the type II crossovers studied would have been impossible with order $gamma$. The only possible order, therefore, is $alpha$, that is, with P located between ms and zl .

Evidence from crossover type III

Even though the evidence already presented from studies of crossover types I and II is conclusive, it is perhaps worth while to present the few data available from studies of type III crossovers, normal $W-R$ plants. The problem here is to determine whether the $W-R$ chromosome acquires zl on losing ms by crossing over. In the isolation blocks, $+M-M\ zl$ and $ms\ W-R+$, pollen was abundant. If the $W-R$ chromosome, on losing ms were to gain zl , gametes with the crossover chromosome $W-R\ zl$ could produce no seed when fertilized with an $M-M\ zl$ gamete. Yet, the progenies of open-pollinated $W-R$ crossover plants consisted largely of normal $M-R$ and $W-R$ plants. Many of the $M-R$ plants were, naturally, the result of fertilization of a non-crossover $W-R\ ms$ egg by an $M-M\ zl$ sperm and are of no importance in this study. Five selfed $M-R$ plants, however, threw normal $M-R$ and $W-R$ plants in F_2 , in all, 211 of the former and 108 of the latter. Certainly in these five instances zl was not acquired by the $W-R$ chromosome when it lost ms . The 2:1 ratio and the absence of $M-M$

plants is, of course, brought about by the presence of *zl* associated with *M-M*. Six *W-R* plants in progenies of open-pollinated *W-R* normals were, evidently, the result of pollination with *W-W* pollen. Their F_2 progenies included 472 *W-R* and 145 *W-W* plants, all normals. This is approximately a 3:1 ratio, as is expected in F_2 from selfed plants of the constitution $+W-R+/+W-W+$. In these instances also the *W-R* chromosome did not gain *zl* on losing *ms*. This is the result expected with the *alpha* order of genes.

But one *M-R* and one *W-R* plant gave different F_2 results. The former produced 129 *M-R* to 16 *W-R* plants, as might have been expected if it was *W-R zl/M-M+*. The latter produced 117 *W-R* to 56 *W-W* plants, a 2:1 ratio, expected if it was *W-R zl/W-W+*. Such results can not be interpreted on the basis of a single crossover with the *alpha* order of genes, and *beta* and *gamma* orders have been shown to be impossible by studies of crossover types II and I, respectively. It is not necessary, however, to postulate double crossing over in this short region of chromosome 1. The F_1 plant, which gave in F_2 129 *M-R* to 16 *W-R* plants and which was presumably *W-R zl/M-M+*, had two *W-R* sibs in which the *W-R* chromosome did not carry *zl*. In this case and also in case of the F_1 *W-R* plant which gave 117 *W-R* to 56 *W-W* plants in F_2 , the crossovers by which the *W-R* chromosome lost *ms* certainly occurred in the selfed parents of the isolation-block cultures in which the crossovers were first detected. If these two isolation-block plants had been $+W-R+/ms W-R+$ the $+W-R+$ chromosome could not have picked up *zl* by crossing over because its homolog, the commonly present *ms W-R+* chromosome, did not carry *zl*. If, however, these two plants were $+W-R+/ms W-R zl$, the first *W-R* chromosome of at least one gamete might have gained *zl* from the second *W-R* chromosome. But the genotype here postulated presupposes its formation by the union of two crossover gametes, $+W-R+$ and *ms W-R zl*.

Moreover, the seed from which one of the F_1 plants grew must have been formed from fertilization with a crossover pollen grain, $+ M-M +$. Whether this concatenation of unusual events is less or more likely than double crossing over in a region of about five crossover units or than the occurrence of a new *zl* mutation, the problem does not affect the well grounded conclusion that the postulated *alpha* order of genes, *ms P zl*, is the actual one.

LINEAR ORDER OF THE GENES *P*, *ts*₂, AND *zl*

In an attempt to determine the linear order of the genes *P*, *ts*, and *zl*, the same method was used as in similar studies involving *ms*, already reported. Progenies of selfed plants of the genotype $+ M-M zl/ts W-R +$ were grown at the side of a field made up largely of $+ W-R +$ and $+ W-W$

+ plants, or in an isolated block containing on one side progenies of selfed plants of the constitution + *M-M zl/ms W-R* +. The plants were open-pollinated and, in the isolation-block, the available pollen consisted of approximately 50 percent *zl M-M* +, from 50 to 25 percent + *W-R ts*, and from 0 to 25 percent + *W-R ms*. Crossover types I and II and possible gene orders, *alpha*, *beta*, *gamma*, are the same as given in the preceding account involving *ms*, except that *ts* is substituted for *ms* in each case.

Evidence from crossover type I

Thirteen F_1 cultures were grown from open-pollinated plants of crossover type I, homozygous *M-M*. Of these F_1 plants, 286 were normal and none *ts*. This indicates that, when *zl* was lost from the *M-M* chromosome, *ts* was not gained. Thirty-five of these normals, 15 *M-M* and 20 *M-R*, were selfed and F_2 progenies grown from them. Records of like cultures are grouped together in table 8.

TABLE 8

NUMBER OF CULTURES	TYPES OF F_2 PLANTS									TOTAL
	<i>M-M</i> +	<i>M-M</i> <i>ts</i>	<i>M-R</i> +	<i>M-R</i> <i>ts</i>	<i>M R</i> <i>ms</i>	<i>W-R</i> +	<i>W-R</i> <i>ts</i>	<i>W-R</i> <i>ms</i>	<i>W-W</i> +	
Nine	5	—	154	5	—	7	94	—	—	265
Eight	56	1	88	—	—	6	49	—	—	200
Three	21	—	35	—	1	5	—	14	—	76
Ten	227	—	—	—	—	—	—	—	—	227
Five	115	—	—	—	—	—	—	—	28	143

The F_1 parents of the first group of nine cultures were, doubtless, *zl M-M* +/+ *W-R ts*. Evidently, the non-crossover chromosome, *zl M-M*, was involved here, and the records, therefore, are of no use in this discussion. The F_1 parents of the other four groups of eight, three, ten, and five cultures, apparently had, respectively, the genotypes + *M-M* +/*ts W-R* +, + *M-M* +/*ms W-R* +, + *M-M* +/+ *M-M zl* or + *M-M* +/+ *M-M* +, and + *M-M* +/+ *W-W* +. It is obvious that in none of the original crossovers concerned in these tests did the *M-M* chromosome, on losing *zl*, gain *ts*. This indicates the *alpha* order of genes or the *gamma* order with all the crossovers occurring in region 2. The most significant conclusion is that the *beta* order is impossible.

Evidence from crossover type II

Only nine open-pollinated crossover plants of type II, *M-R ts*, were studied. Their F_1 progenies consisted of 69 normal and 89 *ts* plants. Thirteen normal plants, one *M-M* and 12 *M-R* were selfed and seven *ts* plants, all *M-R*, were crossed by + *M-M zl/ts W-R* +. The records of the F_2 and test-cross progenies are presented in three groups in table 9:

TABLE 9

NUMBER OF CULTURES	TYPES OF F ₂ AND TEST-CROSS PLANTS					TOTAL
	<i>M-M</i> +	<i>M-R</i> +	<i>W-R</i> +	<i>M-R ts</i>	<i>W-R ts</i>	
Twelve	1	130	4	4	70	209
One	3 ²	—	—	—	—	3 ²
Seven	2	77	2	71	76	221

The first group of 12 cultures, evidently, was derived from F₁ plants of the genotype + *M-M zl/ts W-R* +. The non-crossover *W-R ts*, not the crossover *M-M ts* chromosome, was involved. These records, therefore, are of no use in this study. The one F₂ culture might have come from an F₁ of the constitutions *ts M-M zl/+ M-M* +, and it could not have come from *ts M-M* +/+ *M-M zl*. The seven cultures from *M-R ts* F₁ plants crossed by + *M-M zl/ts W-R* + could have come only from *ts* plants of the genotype *ts M-M zl/ts W-R* +. All the evidence, therefore, indicates that *zl* is not lost when *ts* is acquired by a crossover in the *M-M* chromosome. This indicates that the *alpha* gene order is the correct one or that, if the *beta* order, all the crossovers occurred in region 2. The most significant conclusion is that the *gamma* order is impossible.

Since the evidence from crossover type I proves the *beta* order impossible and from type II shows the *gamma* order to be impossible, the actual order is the *alpha* one with *P* between *zl* and *ts*. This is the same order found for *ms*, *P*, and *zl*. The genes *ts* and *ms* must, therefore, be on the same side of *P*. The order for *ts* and *ms* can be inferred from the crossover percentages of 1.3 for *P ts* and 3.0 for *P ms*. Moreover, the one double recessive *ms ts* plant noted earlier could have arisen by a single crossover in the genotype from which it came only if the order of genes is *ms*, *ts*, *P*. The order of the four genes, therefore, is believed to be *ms ts P zl* or *zl P ts ms*.

POSSIBLE DOUBLE CROSSING OVER IN THE REGION *ms* TO *zl*

Here and there throughout the preceding account, crossover plants have been reported the genotypes of which might have arisen from a single crossover if the order of genes had been that of the postulated *beta* or *gamma* and could not have come from a single crossover with the *alpha* order which has been demonstrated to be the actual one. Any of these exceptional crossovers could be accounted for by double crossing over with the *alpha* order of genes, but, as has been shown, that assumption is not the only possible one. It has been pointed out that two separate crossovers, one in the open-pollination-block, where the crossover plants were discovered, and one in the preceding generation, furnished an adequate interpretation. In one instance, it was necessary even to assume the union of

two crossover gametes in one generation plus the functioning of a third crossover gamete in the next generation. Unlikely as this peculiar chain of events may be, it is perhaps quite as likely as the occurrence of a double crossover in a region of approximately 4.5 crossover units. One dislikes to postulate the occurrence of new mutations, though the possibility is not excluded.

One exceptional crossover plant, not mentioned previously, can not be dismissed so easily. The case, therefore, deserves somewhat detailed treatment. The exceptional behavior occurred in the tester stock involving the progeny of selfed $+ M-M\ zl/ms\ W-R +$. A culture of this stock consisted of 55 $M-R +$, one $M-R\ ms$, and 26 $W-R\ ms$ plants. Individual Number 10 of this culture, an $M-R$ normal, was selfed and the resulting progeny contained 25 $M-R +$, two $M-M +$, and 14 $W-R\ ms$ plants. All this is, of course, typical behavior for this stock. Pollen of this same Number 10 plant was used to pollinate a normal $R-R$ plant and 40 normal $R-R\ F_1$ plants resulted. They were presumed to be $+ M-M\ zl/+ R-R +$, and $ms\ W-R +/+ R-R +$. Four selfed $R-R$ plants of this F_1 culture gave F_2 progenies such as expected on this assumption. Two of the four F_1 plants were, obviously, $ms\ W-R +/+ R-R +$, and need be given no further consideration. Two other F_1 plants were, doubtless, $+ M-M\ zl/+ R-R +$ as expected. Their F_2 progenies from selfing consisted of 181 $R-R$ normals; and their F_1 progenies, when crossed on a normal $W-W$ stock, had 93 $R-R$ and 95 $M-M$ plants.

One normal $R-R$ of the original F_1 culture of 40, however, on being selfed produced an F_2 progeny of 184 $R-R +$, four $R-R\ ms$, one $M-M +$, and 58 $M-M\ ms$. The genotype of this F_1 must have been $ms\ M-M +/+ R-R +$. One gamete of individual Number 10 of the tester stock crossed on normal $R-R$ must, therefore, have been $ms\ M-M +$. In short, when zl was lost (type I crossover) ms was gained, or, when ms was acquired (type II crossover) zl was not retained. There is no possibility that the genotype here concerned could have arisen by separate crossovers in plants of different generations, except the chance, unfortunately not always excluded, that a stray grain of pollen, instead of a pollen grain from individual Number 10 of the tester stock, functioned in the production of the one normal $R-R$ plant which was responsible for this involved discussion. The almost insurmountable difficulty with the suggestion of the accidental functioning of a stray grain of pollen in the otherwise controlled cross-pollination is that, so far as known, no stock of maize in which ms was associated with $M-M$ without zl was in existence at the time. In fact the first known occurrence of this association of genes was in the progeny of the selfed plant here under discussion. This leaves the alternatives of postulating (1) a mutation of normal to ms in the $M-M$ chromosome coincidentally with the

TABLE 10

F ₁ GENOTYPE	PARENTAL COMBI- NATIONS	RECOMBINATIONS			TOTAL
		REGION 1	REGION 2	REGIONS 1 AND 2	
$\frac{P \quad + \quad br}{+ \quad T \text{ I-5b} \quad +}$	242	71 15.8%	108 24.1%	28 6.2%	449*
$\frac{sr \quad P \quad +}{+ \quad + \quad T \text{ I-5b}}$	178	89 23.7%	88 23.5%	20 5.3%	375*
$\frac{ms_{17} \quad P \quad +}{+ \quad + \quad T \text{ I-5b}}$	51	5 5.7%	28 32.2%	3 3.4%	87
$\frac{ts_2 \quad P \quad +}{+ \quad + \quad T \text{ I-5b}}$	169	3 1.4%	39 13.7%	0	211
$\frac{P \quad + \quad br}{+ \quad T \text{ I-5c} \quad +}$	195	60 18.1%	58 17.5%	19 5.7%	332*
$\frac{sr \quad P \quad +}{+ \quad + \quad T \text{ I-5c}}$	80 50 — 130	21 17 — 38 16.7%	39 14 — 53 23.2%	6 1 — 7 3.1%	146* 82 — 228
$\frac{T \text{ I-3a} \quad + \quad +}{+ \quad br \quad bm_2}$	48	30 22.1%	37 27.2%	21 15.4%	136*
$\frac{P \quad + \quad br}{+ \quad T \text{ I-3a} \quad +}$	104	20 10.3%	56 28.7%	15 7.7%	195*
$\frac{sr \quad P \quad +}{+ \quad + \quad T \text{ I-3a}}$	43	21 27.6%	11 14.5%	1 1.3%	76*
$\frac{ms_{17} \quad P \quad +}{+ \quad + \quad T \text{ I-3a}}$	87	2 2.0%	12 11.9%	0	101
$\frac{ts_2 \quad P \quad +}{+ \quad + \quad T \text{ I-3a}}$	246	4 1.3%	65 20.6%	0	315
$\frac{sr \quad + \quad P}{+ \quad ms_{17} \quad +}$	40	9 18.0%	1 2.0%	0	50
$\frac{+ \quad ms_{17} \quad P}{T \text{ I-2c} \quad + \quad +}$	309	60 15.7%	8 2.0%	4 1.0%	381
$\frac{+ \quad ts_2 \quad +}{T \text{ I-2c} \quad + \quad P}$	1175	249 17.4%	11 0.8%	1 0.1%	1436

* Unpublished data from E. G. ANDERSON.

crossover by which it lost *zl*, or a mutation of *zl* to its normal allele when *ms* was added by crossing over, (2) a somatic crossover in addition to a meiotic one, or (3) double crossing over in a region of less than five cross-over units. If a double crossover did occur in this instance, it happened in a region shorter than that commonly given as the minimum for *Drosophila*.

ORIENTATION OF THE *ms*₁₇ *ts*₂ *P* *zl* COMPLEX IN CHROMOSOME I

It has been shown that the order of genes here under discussion is *ms ts P zl* or *zl P ts ms*. Orientation of this group with respect to other genes of the short arm of chromosome 1 of maize had to await the discovery of suitable markers near enough to *P* to be usable. Such markers are now available in certain reciprocal translocations involving the short arm of chromosome 1 (ANDERSON 1935). These translocations, T 1-2c, 1-3a, 1-5b, 1-5c, were furnished by DR. E. G. ANDERSON of California Institute of Technology, who has also given permission to use unpublished data included with other three-point back-cross records in table 10.

The data presented in the above table show that the breaks in T 1-5b, 1-5c, and 1-3a are to the right of *P*, between *P* and *br*. The data involving these three translocations show further that *sr*, *ms*₁₇, and *ts*₂ are to the left of *P*. Finally, they show that T 1-2c is to the left of *ms*₁₇ and *ts*₂. This is in accord with back-cross data showing that T 1-2c is very near *sr*, as follows:

$$\begin{array}{cccccc} + & \text{T 1-2c} & + & \text{T} & ++ & sr & \text{T} & sr & + \\ \hline sr & + & 151 & 1 & 1 & 1 & 144 & = 297. \end{array}$$

Crossover percent *sr*—T 1-2c = c.7.

The available data place this group of genes of the short arm of chromosome 1 of maize in the following order:

$$sr \leftarrow ms_{17} \text{ 1.7 } ts_2 \text{ 1.3 } P \text{ 1.5 } zl \rightarrow br.$$

The gene *zb*₄, reported by H. K. HAYES (unpublished) to be near *P*, has not been tested with *ms*₁₇ or *ts*₂.

SUMMARY

A Bolivian maize with mosaic red pericarp and cob was found to have a recessive zygotic lethal, *zl*, closely linked with the pericarp-color gene, *P* and the near-by genes male-sterile 17, *ms*₁₇, and tassel-seed 2, *ts*₂. The effect of *zl* is to prevent homozygosis of genes with which it is closely linked, and thus to change a 3:1 to a 2:1 F₂ ratio when linked with a dominant gene or to prevent the occurrence of the one class when linked with a recessive gene.

The nature of the characters studied made impossible the use of ordinary methods of determining the linear order of the genes concerned. The method employed, based on the assumption of very rare or no double crossing over in so short a region, may be illustrated as follows: The three possible orders of the genes *P*, *ms*₁₇, and *zl* and the associations of the three genes in the heterozygotes studied are:

<i>Alpha</i> order	<i>Beta</i> order	<i>Gamma</i> order
$\frac{+ \quad P \quad zl}{ms_{17} \quad + \quad +}$	$\frac{P \quad zl \quad +}{+ \quad + \quad ms_{17}}$	$\frac{P \quad + \quad zl}{+ \quad ms_{17} \quad +}$

When, by crossing over, *P* loses *zl* it could never acquire *ms*₁₇ if the gene order is *alpha* and must always acquire it if the order is *beta*; and when *P* gains *ms*₁₇ it could never lose *zl* in the *alpha* order and must always lose it in the *gamma* order. Many tests of easily detectable crossover types demonstrated that *P* on losing *zl* does not gain *ms*₁₇ and on gaining *ms*₁₇ does not lose *zl*, thus eliminating the possibility of the *beta* and *gamma* orders. The postulated *alpha* order, *ms*₁₇ *P* *zl*, is, therefore, the actual one. Similar tests involving *ts*₂ proved the order to be *ts*₂ *P* *zl*.

Tests with appropriate markers, mostly reciprocal translocations with chromosome 1 breaks near *P*, have shown that *zl* is to the right of *P* with *ms*₁₇ and *ts*₂ to its left. The genetic map for this short region of the short arm of chromosome 1 is:

$$sr \leftarrow ms_{17} \text{ 1.7 } ts_2 \text{ 1.3 } P \text{ 1.5 } zl \rightarrow br.$$

The occurrence of several crossover plants, which could not have resulted from a single crossover with the order of genes as given above, were readily accounted for by two separate crossovers in succeeding generations. The one exception to this was a plant the change in whose genotype could have occurred only (1) by a single crossover between two genes accompanied by mutation of a third gene, (2) by a single crossover preceded by at least one somatic crossover, or (3) by a double crossover in a genetic distance of approximately 4.5 crossover units.

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