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Instructions for use

Analysis of polymeric genes controlling a new form
of dotted leaves in maize

By

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(With 2 text-figures and 13 tables)

Introduction

Dotted leaves in maize are characterized by having many chlorophyll deficient spots on the leaf blade, and occur very rarely in the corn field. They have been reported to possess seven genic patterns in total: three of the patterns, *polkadot leaf* (*pk*), *yellow fleck* (*yf*) and *chloroblotch* (*cb*), by EYSTER (1924 and 1934); two others, *dotted leaf* (*dt*) and *blotched leaf* (*bl₁* and *bl₂*), by EMERSON (1923 and 1924); remaining one, *piebald* (pb_1, pb_2, pb_3 and pb_4), by DEMEREC (1926), and the last two, *maculate leaf* (*ma*) and another *blotched leaf* (bl_3, bl_4 and bl_5), by SUTÔ (1946 and 1948). Most of the genes have been found to be simple Mendelian recessives over the normal green and a few to be in recessive polymeric series, one set of which involves duplicate genes, pb_2 and pb_3 (DEMEREC 1926), and another set involves triplicate genes, bl_3, bl_4 and bl_5 (SUTÔ, 1948). And also there has been found a small amount of linkage data on five genes among them; namely three (*pk*, *yf* and bl_1) locating in chromosome 9 (EYSTER, 1934 and SUTÔ, 1948), one (*ma*) in chromosome 4 (SUTÔ and KATÔ, 1948), and the last one (bl_5) in chromosome 6 (SUTÔ, 1948), although no any information of the linkage with another genes has yet been announced.

The present blotch is very interesting in presenting an exceptional case of simple Mendelian characters. In a previous paper (SUTÔ, 1948), there is presented evidence to show that a set of independent triplicate genes are associated with the inheritance of blotched leaf. The present paper reports further studies on the same inheritance. It will be demonstrated that the present blotch must be determined by at least several members belonging to a set of polymeric genes, some of which are linked either with other genes or with each other.

The writer is greatly indebted to Professor H. MATSUURA, under

Material and method

In connection with a maize improvement program in our university, many self-pollinations were made for a variety of the northern type of yellow dent, widely growing in the southern part of Hokkaidô. Of self-pollinated ears from an inbred strain, S-199, in the third generation of selfing, only one ear was available as the material for the beginning of this investigation. Since a new type of chlorophyll characters was isolated from it in the summer of 1942, such a new chlorophyll character was called "*blotched leaf*", because it resembles in its general aspects that term as used by Emerson.

A systematic search for this pattern was made during the years 1943–1948 in the breeding field of Kônôkôsha situated in a suburb of Sapporo. Firstly, the mutant plants from S-199 were crossed with normal plants from various sources. Most of the crosses were made in the summers of 1943 and 1944, and a few in the summers of 1945 and 1946. The F_1 plants from all the crosses to a total number of twenty six were always normal, so that the mutant character should be recessive to the normal green. Next, segregations for the mutant phenotype were examined in the F_2 progenies from all of the crosses. It is very interesting to note that segregations into various ratios were found in only five crosses; two of them made in 1945 and the other three in 1946, and that in all of the others no any segregation at all was observed. Segregation-data from F_2 plants are listed in appendix Tables, from 9 to 12. As a further evidence for F_2 segregations those F_1 and F_2 normal plants were further selfed or back-crossed with the mutant parent during 1946 and 1947. Data from those back-crossing and F_3 populations are given in Tables 9, 10 and 13. Lastly, an effort was made to determine the linkage relation with several endosperm characters in the summers of 1947 and 1948. In only four crosses,

linkage tests have proved to be valuable in identifying gene-loci involved (Tables, 11-13).

For calculating the significance of deviation in segregated ratios and of its heterogeneity in interfamilies, between the expectation and observation, the χ^2 method reported by MATHER (1938) was used. Recombination values in the segregation were calculated by the formulae which are developed on the same principle as used by FISHER's method of maximum likelihood and those for the disturbed segregations were calculated by the product method (IMMER, 1930 and STEVENS, 1939).

Description of the character

A new type of chlorophyll deficiencies which is limited to small spots was referred as *"blotched leaf"* (SUTÔ 1948). Spots are formed indefinitely in the intervenial regions of leaf blade, and are brilliantly white or yellow in coloring (Figs. 1 and 2).

They are sometimes irregular in size, number and position. However, there may be as a rule a definite tendency of variation in number on the position of the leaves. The lower leaves of the plant are apparently normal green and the blotching is usually seen in about the 5th leaf of the plant. Those spots of the upper leaves are numerous and, in extreme cases, are arranged so closely as to form more or less irregular longitudinal bands in some of the interveinal regions (Fig. 2).

The character can be recognized in the matured stage, appearing at about the 5th or 6th leaf stage and showing better at the latter stages. The homozygous plant for blotch is normal in viability and good in classification. On account of the exquisite coloring of this pattern, the mature plant will be attractive to horticulturists for ornamental purposes.

The blotched leaf reported by EMERSON (1923) resembles this pattern, in number, size and distribution of the chlorophyll deficient spots, but the former is clearly distinguishable from the latter by having two distinct characteristics as follows:

1), In the character, there is a discolorment of spots according to the aging in appearance, while the color of the present blotch is always constant; white or yellow.

2), In the heritable manner, EMERSON's pattern is inherited as a simple Mendelian recessive character, while the present pattern is controlled by five or more genes behaving as a polymery series as described next.

Fig. 1. So-called *"blotched leaf"* plants associated with a type of *"wheat tassels"*.

Gene analysis

Up to the present time, twenty five different crosses have been made by the writer between the blotched plant and the normal inbred strains coming from different source respectively. All the F_1 plants from those crosses were normal in phenotype. The character should be inherited to be recessives. Progenies from F_1 plants which are heterozygous for the mutant should therefore segregate blotch in the F_2 generation. Contrary to the expectation, F_2 plants from all the crosses made during 1943 and 1944 were always green, giving a total

of about four thousand as follows (The numerals within parenthesis denote the observed number of F_2 plants):

1), Six crosses in 1943;
A-46 (375), A-84 (602), A-85 (860), A-87 (856), A-88 (418), and A-96 (277).

2), Ten crosses in 1944;
A-145 (74), A-154 (193), A-180 (30), A-182 (72), A-186 (60); A-227 (235), A-246 (262), A-261 (195), A-262 (310) and A-263 (89).

From this fact the writer became a consideration at that time; this may be a case of delayed inheritances conditioned by the predetermination of genes. In spite of such a conjecture, the hypothesis of polymeric inheritance has

Fig. 2. Partly dissected leaves from a blotched plant, characterized by the typical blotching at the upper position of culm (the left one) and by the normal type at the lower position (the right one).

been established, according to data from the seven crosses with different strains from above cited ones carried out in the next two years, 1945 and 1946.

Those seven crosses may be distinguished into three following types by the different ratios of the segregation from progenies in the F_2 generation;

1), In the first type including three crosses (A-392, A-408 and H-60), any segregation was not observed, similar to that in crosses in 1943 and 1944, so that F_2 plants were always normal in a total of about one thousand.

2), In the second type including one cross (A-390), a segregation into an approximate 63:1 ratio of green to blotch was observed.

3), In the third type including two remainders (H-26 and H-59), F_2 plants were of the segregation into ca. 15:1 ratio.

The first type including three crosses made in 1945 and all of other crosses made during 1943 and 1944 may probably be caused by quadruplicate or probably more genes in a polymeric series. Thus the F_2 plants should segregate blotch into a 256 or more: 1 ratio.
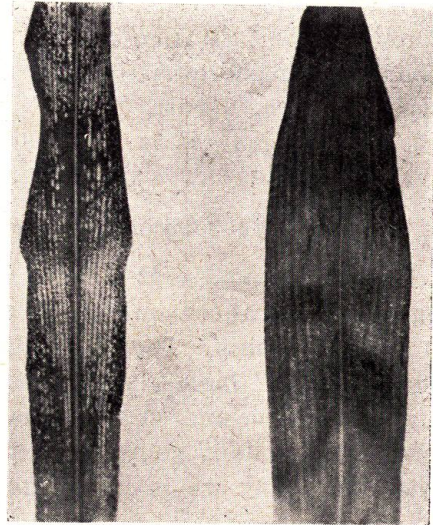
Owing to the very scant number, the chance that the recessive plants will be lost from observations in the segregation of such ratio should be considered to be most probable. Accordingly, it may be conclusive that, as a rule, at least five or more genes in order to secure the blotched leaf were involved in the normal plants from common strains investigated.

i). *Detection of triplicate genes,* bl_3, bl_4 *and* bl_5

Table 9 gives F_2 data from a cross, *A–390–1*. Twenty-one ears were segregated in approximately 63 : 1 ratio with a total of 1115 green to 19 blotched plants. The calculated numbers from expectancy are 1116.3 green and 17.7 blotch, giving a χ^2 of 0.00926 for the deviation. The fit is very close and so a conclusion may be justified that in this cross three independent genes were present, producing a blotched pattern when the plant are in homozygous recessive condition for all of them.

As further evidence on the presence of triplicate genes, eleven F_2 normal plants from *A–390–1* were chosen at random, and back-pollinated by the blotched parent. Normal plants from the F_2 generation should then be characterized by following four genotypes; (1) $[+/+, +/bl, +/bl]$ or $[+/+, +/+, +/bl]$ or $[+/+, +/+, +/+]$, (2) $[+/bl, +/bl, +/bl]$, (3) $[bl/bl, +/bl, +/bl]$ and (4) $[bl/bl, bl/bl, +/bl]$. Accordingly, blotched segregations in progenies from those back-crosses grouped into four different ratios, (1) all normal, (2) 7 : 1, (3) 3 : 1 and (4) 1 : 1, should be expected to occur in the frequency of 37 : 12 : 8 : 6 respectively. A summary of data from Table 9 is given in Table 1. F_2 normal parents were practically of four kinds in a total of 11 ears; namely six producing only green progeny, two falling in the 7 : 1 classes, two in the 3 : 1 class and last one in the 1 : 1 class. The fit is very close, χ^2 equalling

TABLE 1

Evidence for the existence of independently triplicate genes, bl_3, bl_4 and bl_5, from back-crossed data included in Table 9

Exp. ratio in each class ;	1 : 0	7 : 1	3 : 1	1 : 1
χ^2 for the exp. ratio ;	0	1.4563	0.7013	1.2500
Exp. freq. for the F_2 normal ;	37	12	8	6
Obs. freq. for the F_2 normal ;	6	2	2	1

χ^2 and P ; $\chi^2 = 0.2426$ $P = 0.95$–0.98 (0.9689)[1]

1) Number within parenthesis denotes a probability calculated by FISHER's direct method.

0.2426, and $P = 0.97$. It was therefore supposed that the F_1 plants had a genotype of tri-heterozygous forms $(bl/+, bl/+, bl/+)$ in regard to the blotched leaf.

The F_2 plants from two crosses, *H-62* and *H-59*, segregated in approximately 15 : 1 ratio, giving 671 green and 46 blotched plants with a χ^2 of 0.0149 in a deviation from expectancy. Table 10 gives this data. The segregation into a 15 : 1 ratio must be considered as a result of combination of triplicate genes, because the normal parent in these crosses was obtained from the F_2 normal plants in a cross *(A-390-1)* noted above. The genic constitution of the F_1 parent was therefore represented as *"bl/bl, +/bl, +/bl"*.

Furthermore, the thirteen F_2 normal ears from a cross *(H-59)* were selfed and then F_3 kernels were planted separately. The F_3 data is also arranged in Table 10. From the F_3 data it can be seen that three types of F_2 normal ears, (1) not segregating, (2) segregating into approximately 3 : 1 and (3) segregating into approximately 15 : 1, occurred in a ratio expected when duplicate genes acting independently were involved. Actually, the F_3 observed frequency from three types were as follows; (1) no blotch in a total of 290 plants, (2) 19 blotch in 336 and (3) 18 blotch in 90, respectively. This finding was entirely in accordance with the expectancy (Table 2). With respect to these three

TABLE 2

Evidence for the existence of independently duplicate genes,
bl_4 and bl_5, from F_3 data included in Table 10

Exp. ratio in each class;	1 : 0	15 : 1	3 : 1
χ^2 for the expected ratio;	0	0.1912	1.2000
Exp. freq. for the F_2 normal;	7	4	4
Obs. freq. for the F_2 normal;	6	4	3
χ^2 and P;		$\chi^2 = 0.0053$	$P = 0.99$.

phenotypes, thirteen ears segregated actually in the 6 : 4 : 3 ratio, being approximately in frequency of the theroetical 7 : 4 : 4 ratio. As shown by these F_3 behaviors, the approach to the theoretical proportion of the F_2 genotypes proved to be surprisingly close; the probability that the deviations were merely due to errors of ramdom sampling, being 99 percent. Thus it seems reasonable that the F_3 data give further reliable evidence for a conclusion from all the F_2 data.

In every case of the above three crosses, segregations showed a linkage relation between the yellow endosperm (Y_1) and blotched leaf. Accordingly, this indicates probably that one of the triplicate genes (bl_5) was closely linked with Y_1, although no crossover evidence was detected.

From a summary of data from the above three crosses, the conclusion was reached that the triplicate genes must be present in a homozygous recessive condition in order to yield the blotched phenotype, such being denoted by the gene symbols of bl_3, bl_4 and bl_5 respectively, one of which has its locus in chromosome 6. This finding has already been reported by the present author (1948) in a few words.

ii). *Detection of other two genes, bl_6 and bl_7*

F_2 data from a cross, *A-428*, is given in Table 12, interesting in presenting a linkage relation between two members of a set of triplicate genes for the blotched leaf. In this cross, the normal parent had three endosperm characters; *shrunken (sh)*, *waxy (wx)* and *white (y_1)*, while the blotched parent had three opposite characters of endosperm; *Sh*, *Wx* and *Y_1*. All of the plants of the F_1 generation were green, being the same as those of all other crosses. The F_2 kernels from four F_1 ears obtained by selfing were separated according to the endosperm characters. From the F_2 data, the segregation into almost exactly a 63 : 1 ratio in a total of 778 green and 16 blotched plants was as observed as if suggesting the existence of independently triplicate genes. Since about one half of the yellow kernels were planted in the field, in spite of all other kernels being planted, the observed data must be corrected according to the frequency of F_2 kernels in each class of the segregation. The important results from the corrected data (Table 12) will be summarized in following three points:

1). A segregation into about 32 : 1 ratio instead of the 63 : 1 was found in a total of 771 green and 23 blotched plants.

2). All the blotched plants segregated have grown up from yellow kernels, and blotched plant from white kernels did not occur.

3). A few of them, 2 out of 23, have arisen each from one with the *sh* and *sh-wx* in addition to *Y_1*.

These results will be discussed in detail in the next chapter. As for the first point, there is an objection to the idea of the segregation for triplicate genes. This must be considered together with F_2 data

from another cross, *A-391*, because the F_2 segregated ratios in two of those crosses are practically like each other (about 32 : 1).

Namely, seven F_1 ears from *A-391* were selfed and planted separately. F_2 data from each of seven ears are given in Table 11. The segregation was found to be different in appearance between progenies from each ear. But, the number of blotched plants observed was too small for the deviation to be calculated. Summing up the F_2 data, the segregation can be seen to occur in a ratio of approximately 32 : 1 (actually 23 blotch in a population of 870 plants), approaching to that in the segregation from *A-428*.

According to the χ^2 test (Table 3), the agreement in expectancy of a 63 : 1 rato is very poor in both crosses, *A-428* and *A-391*. There is

<div align="center">

TABLE 3

χ^2 test for the existence of triplicate genes (bl_4, bl_5 and bl_6), two of which are linked together, from the F_2 data in a population of 1664 plants detailed in Tables, 11 and 12

</div>

Factor	DF	For 631 : 1		For 32 : 1	
		χ^2	P	χ^2	P
Deviation	1	24.4200**	0.001	0.0003	0.99–0.98
Heterogeneity	1	0.1445	0.95–0.90	0.1524	0.95–0.90
Total	2	24.5645		0.1527	

** Highly significant.

only one or less chance in a thousand that the observed deviation is due to errors of random sampling. This probability is much too low to support the conclusion that in both crosses 63 : 1 is the expected ratio. That the blotch segregates into almost exactly a 32 : 1 ratio was also demonstrated by the χ^2 test in Table 3. The repeated occurrence of such a ratio suggests that it should hardly be attributed to deviations from other ratios, due entirely to errors of random sampling. The only simple ratio which is close enough to 32 : 1 to be considered is the 63 : 1 ratio. Thus, the 32 : 1 ratio should be a modified 63 : 1 ratio owing to the linkage relation between two members of triplicate genes. Since one of these linked genes is bl_5 as shown already in *A-390-1*, the other one was designated here to be bl_6. As a conclusion, it is very probable that the F_1 parent in both crosses had a genotype of bl_4/Bl_4, bl_5-bl_6/Bl_5-Bl_6.

Furthermore, the fifth gene, bl_7, was found in a back-cross, *A-390-2*.

The normal plant used was obtained from sister plants of the same strain as that used in *A–390-1*. The F_1 plants from a cross between the normal and blotched plants were further back-crossed with the blotched plants which were obtained from the F_2 progeny of the *A–390-1*. The backcrossed data from *A–390-2* are given in Table 13.

In spite of the anticipation that there must be the same triplicate segregation as occurred in the F_2 progeny from *A–390-1*, all the plants from five out of six ears (*A–390-2-1*) segregated in a ratio of approximately 15 : 1 with a total of 297 normal and 21 blotched plant. As shown in Table 4, the possibility for segregation into a 7 : 1 ratio is practically eliminated by the very poor agreement between observed

TABLE 4

χ^2 values computed on the basis of a 7 : 1 expectation in back-crossed data included in Tables 9 and 13

Factor	*A–390-1* and *A–390-2-2*			*A–390-2-1*		
	DF	χ^2	P	DF	χ^2	P
Deviation	1	1.6356	0.2	1	10.1018**	0.01–0.001
Heterogeneity	2	0.1596	0.95–0.90	4	0.4757	0.98–0.95
Total	3	1.7952		5	10.5775	

and calculated data. The chance that the observed deviation is due to errors of random sampling are about one in ten thousand equaling $\chi^2 = 10.1018$. On the other hand, a satisfactory agreement supports the possibility for segregation into approximately 15 : 1 ratio; exactly 14 : 1 (Table 5). This fact seems to indicate conclusively that in this backcross four independent genes should be involved to make it heterozygous, being designated as bl_3, bl_4, bl_5 and bl_7. Nevertheless, it can be seen in the progeny from only one, *Tx-18*, out of six back-crossed ears (*A–*

TABLE 5

χ^2 value computed on a expected ratio of 15 : 1 in the back-crossed data from *A–390-2-1* included in Table 13

Factor	DF	χ^2	P
Deviation	1	0.6162	0.5–0.3
Heterogeneity	4	3.7982	0.5–0.3
Total	5	4.4144	

390-2-2) that the segregation followed clearly a 7 : 1 ratio, although the numbers are very small ; actually 59 normal and 7 blotched plants. The F_1 parent should therefore be heterozygous for the triplicate genes, bl_3, bl_4 and bl_5, depending on the absence of both blotched genes, bl_6 and bl_7. This finding agrees entirely with that from *A-390-1*. It must be concluded that the F_1 parents in this cross, but one (*Tx-18*), had a genotype of [bl_3/Bl_3, bl_4/Bl_4, bl_5/Bl_5, bl_7/Bl_7].

Linkage analysis

That quadruplicate genes (bl_3, bl_4, bl_5 and bl_7) are independent in heritable behavior was practically proven by the close approximation to the theoretical segregation of green to blotched plants produced when the heterozygons plant from the various combinations of quadruplicate genes was either selfed or back-crossed. Under the present chapter, some amount of data has been accumulated to prove that three (bl_4 and bl_5–bl_6) of those polymeric genes are expected to belong in two separate linkage groups. Fortunately, those linked genes were involved in three crosses, *A-391*, *A-428* and *A-390-2*, pollinated in the summers of 1945 and 1946. In point of presenting both linkage relations, *A-428* gave the most interesting data amongst all of the crosses reported in the present paper.

i). Evidence for the location of bl_4 in chromosome 9

For the cross, *A-428*, two well-known genes (*sh* and *wx*) having their loci in the chromosome 9 were used to be the linkage marker. When F_1 plants from *A-428* were selfed, the number of blotched plants in the F_2 generation was 23 in a total of 794, only two of which have

TABLE 6

Evidence on a linkage relation existing between *sh–wx* and bl_4, estimated by the 2×2 contingency table through the data from *A-428*

	+	*bl*	Total
+	514	21	535
sh·wx	257	2	259
Total	771	23	794

$\chi^2 = 5.0328^*$ $P = 0.05-0.02$

grown from each of *sh·wx* and *wx* kernels.   Table 6 gives a summary
of the data in Table 12.   With respect to the genes, *bl*, *sh* and *wx*, these
data show strongly that there is a significant difference between ex-
pected and observed data when the calculation is made under the
assumption that two sets of the genes, *bl–sh* and *bl–wx*, are inherited
independently.   The χ^2 from equality between parental and recombi-
nating classes is 5.0328, which justifies a conclusion that one of the
bl–genes and *sh–wx* are linked together in the repulsion phase.   It was
demonstrated already that *A-428* is a tri-hybrid cross having the ge-
notype of "*bl_4/Bl_4, $bl_5–bl_6/Bl_5–Bl_6$*".   The conclusion that the *bl_4* gene must
therefore be located in chromosome 9 will also be drawn from the fact
that both the genes, *bl_5* and *bl_6*, are known to be in chromosome 6.

   If the above assumption is correct, then the observed classes are
a product of recombination of three linked genes, i. e. two coupling
genes for the endosperm character (*sh* and *wx*) and one of the *bl*–genes,
(*bl_4*).   If a calculation is made on that basis, then the percentage of
crossovers is estimated to be 24.65 $\pm$ 3.27 between *bl_4* and *sh*, and also
32.82 $\pm$ 3.13 between *bl_4* and *wx*, according to the product method.   There
is a possibility with respect to the order of those genes: that it may
be *$bl_4–sh–wx$*.   This possibility can be demonstrated by the two facts
that (1) the map distance between *sh* and *wx* is known to be about 20
units; actually the recombination value in this data being 19.14 $\pm$ 1.09
and (2) the recombination value between *bl_4* and *wx* is larger than that
between *bl_4* and *sh*.   According to a calculation from the *bl*–segregation,
the recombination value of *sh-wx* is 8.17, being far apart from the direct
value which is in accordance with the map distance.   Such a large
deviation between estimated and actual values will be considered to
be due to an error resulting from small number of samples, blotched
segregants numbering only two, out of 295, *sh* and *wx* plants in this
population.

ii).   *Evidence for the location of bl_5 and bl_6 in chromosome 6*

   The data concerning the linkage relation between *bl_5* and *bl_6* was
found in the two crosses, *A-391* and *A-428*, as summarized in Table 3.
It has already been suggested that the segregation into a 32 : 1 ratio
should be explained by the triplicate genes and that one (*bl_4*) of them
is linked with *sh–wx*, and remaining two (*bl_5* and *bl_6*) were linked to-
gether.   Thus, the 32 : 1 ratio should be a modified 63 : 1 ratio.   If this

suggestion is correct, then it is very clear that the ratio of segregation in coupling phase should be modified between the limits of 63 : 1 and 15 : 1 according to the closeness of linkage, while repulsion would tend to increase the ratio between those of 63 : 1 and 256 : 1. Owing to the fact that the observed ratio of segregation is an index of linkage intensity to estimate the recombination value between two of the triplicate genes, the formulae will be developed by the method of maximum likelihood (FISHER, 1936) as follows;

$$\text{Coupling}: \quad \frac{dm}{dp} = \frac{2(+)(1-p)}{15+2p-p^2} - \frac{2(bl)(1-p)}{1-2p+p^2} = 0 \quad \text{then,} \quad \frac{(1-p)^2}{16-(1-p)^2} = \frac{bl}{+}$$

$$\text{Repulsion}: \quad \frac{dm}{dp} = \frac{2(bl)p}{p^2} - \frac{2(+)p}{16-p^2} = 0 \quad \text{then,} \quad \frac{p^2}{16-p^2} = \frac{bl}{+}$$

where p is the recombination value and m represents the expected frequency in the phenotypic class. Therefore, the standard deviation ($\bar{s}p$) of p will be derived from the following equations[1]:

$$\text{Coupling}: \quad \bar{s}p = \sqrt{\frac{15+2p-p^2}{4n}}$$

$$\text{Repulsion}: \quad \bar{s}p = \sqrt{\frac{16-p^2}{4n}}$$

Substituting in the equation the data from both crosses (*A–391* and *A–428*), a recombination value (p) between bl_5 and bl_6 is estimated to be $30.37 \pm 1.26\%$ from a total of both the F_2 plants in *A–391* and *A–428*. Two crosses (*A–428* and *A–390–2*) were made between **different**

TABLE 7

Evidence on a linkage between Y_1 and bl_5, estimated by the 2×2 contingency table

a. Data of repulsion (*A–428*)				b. Data of coupling (*A–390–2–1*)			
	+	$\sim bl$	Total		+	bl	Total
Y_1	427	28	455	Y_1	198	3	201
+	156	(1)	156	+	99	18	117
Total	583	28	611	Total	297	21	318

$\chi^2 = 6.9715 - 8.7051^{**}$ $P = 0.01 - 0.001$ $\chi^2 = 20.9426^{**}$ $P < 0.001$

1) Since, in the equation of estimation, I_p is equated to

$$I_p = ip \times n = \frac{1}{V_p} = n \sum \left[\frac{1}{m} \left(\frac{dm}{dp} \right)^2 \right]$$

where V_p is the sample variance of p (from FISHER 1936).

plants with respect to the genic combination of the two alleles, $Y_1 y_1$ and $Bl_5 bl_5$. The data on the relation of those genes are arranged in Table 7. The difference from expectancy is very great when calculations are made on the assumption that they are inherited independently, χ^2 being 8.7051 for *A–428* and 20.9426 for *A–390–2*. The chances that this difference is due to random sampling are negligble, so that a conclusion is justified that a close linkage exists between $Y_1 \cdot y_1$ and $Bl_5 \cdot bl_5$.

As can be seen in Tables, 7, 12 and 13, there seems to be a difference in linkage intensity between the repulsion and coupling phases. The observed ratio from *A–428* and *A–390–2–2* is caused by repulsion with a complete linkage owing to non-occurrence of crossovers, actually the four phenotypic classes of *Bl–y*: *Bl–Y*: *bl–y*: *bl–Y* are present in the respective numbers; 427 : 156 : 0 : 28 in a F_2 population from *A–428*, and 33 : 26 : 0 : 7 in a back-crossed population from *A–390–2–2*. At the same time, the ratio observed in five ears from a backcross, *A–390–2–1*, is caused by coupling, disagreeing fairly with that of repulsion in linkage intensity, because there is some number of crossovers between y_1 and bl_5, giving actually a backcrossed population of 198 *Y–Bl* : 99 *y–Bl* : 3 *Y–bl* : 18 *y–bl*. The average recombination in this case was estimated to be $14.29 \pm 7.63\%$, according to the method of maximum likelihood.

It may be interesting to compare the data from repulsion with those obtained from the coupling phase. Why there should be any difference between them may not be evident. If such a difference does occur, then the possible explanation on an increasing value of recombination may be considered as an effect of either bl_7 or m as follows:

1. *Evidence as bl_7*. The blotched situation, in which segregation is modified by the linkage between bl_5 and bl_6, gives a tri-hybrid behavior in repulsion phase (*A–390–2–1* and *A–428*), and a quadri-hybrid behavior in coupling phase with respect to $yl–bl_5$. In spite of evidence of complete linkage from repulsion data, Y_1 and bl_5 can not be recognized to be a multiple allelic series, because there is a fact that in coupling phase a few crossovers were observed in the quadriplicate back-crossed population, being proved to be 14.29% and so they were able to be segregated.

2. *Evidence as m*. All the *bl*–plants originated directly from an inbred strain, *S–199*, occurred in both selfing and sib-mating progeny. As compared with this, when the *bl*–plant from the progeny of *A–390–2* were sib-crossed with each other, their progenies segregated into the 3 : 1 ratio giving actually a frequency of 60 blotched and 18 normal plants. These numbers are not large, but the fit is very close. (Such a ratio will be expected when one of *bl*–genes is heterozygous dominant).

Any interpretation of segregations for the blotched inheritance described to be in polymeric manner under all the foregoing crosses can not accout for this segregation. As an explanation of this fact, the use of a modifier for one of *bl*–genes may

be assumed to be most helpful. The modifier was designated here to be "m". It may be considered that the bl-gene is stable in the presence of dominant allele, M, and that a phenotypic change from recessive to dominant for a decided one of bl-genes (probably bl_7) occurs whenever the m gene is in the recessive homozygous condition. The $M \cdot m$ allele would usually involve to be homozygous dominant so that m might have arisen through a mutation in a bl-plant (bl-A-390-2). Further investigations on the $M \cdot m$ allele will give the evidence to draw the final conclusion.

The order of these genes may be given as either Y_1–bl_5–bl_6 or bl_5–Y_1–bl_6. The possibility of its being bl_5–bl_6–Y_1 should be eliminated by the fact that the recombination value between bl_5 and bl_6 is larger than that between bl_5 and Y_1. Further data will be necessary to ascertain which of such two possibilities is most likely.

Summary of segregation

A summary of all the data has been arranged in such a manner as to show that the segregation into seven different sets was realized in 62 ears resulting from 13 crosses (Table 8). Considering the population of 4895 plants as a whole, it was found that the total number of blotched plants does not differ significantly from the theoretical number which is determined by totalling the calculated numbers for each class. According to MATHER's method of the joint estimation, the χ^2 of deviation and of heterogeneity, which were merely due to errors of random sampling are equal to 0.0250 and 3.6230 respectively. The approach to the expected ratio of these segregations proves to be surprisingly close, so that it is farily indisputable to postulate the existence of hexaplicate genes as responsible for the present blotching. As far as the basis of view is concerned, it is reasonably certain that the following nine sets of the parent genotype may be expected in all the crosses investigated:

I. Normal parents:
    1), $bl_3.\ Bl_4.\ Y_1$–Bl_5–$bl_6.\ bl_7.\ M$ ·················· H–59
    2), $bl_3.\ Bl_4.\ yl_1$–Bl_5–$bl_6.\ bl_7.\ M$ ·················· H–62
    3), $Bl_3.\ Bl_4.\ Y_1$–Bl_5–$bl_6.\ bl_7.\ M$ ·················· A–390–1
    4), $Bl_3.\ Bl_4.\ y_1$–Bl_5–$bl_6.\ bl_7.\ M$ ·················· A–390–2–2
    5), $Bl_3.\ Bl_4.\ Y_1$–Bl_5–$bl_6.\ Bl_7.\ M$ ·················· A–390–2–1
    6), $bl_3.\ Bl_4.\ Y_1$–Bl_5–$Bl_6.\ bl_7.\ M$ ·················· A–391
    7), $bl_3.\ Bl_4$–sh–$wx.\ y_1$–Bl_5–$Bl_6.\ bl_7.\ M$ ·················· A–428

II. Blotched parents:
    8), $bl_3.\ bl_4.\ Y_1$–bl_5–$bl_6.\ bl_7.\ M$ ·················· S–199
    9), $bl_3.\ bl_4.\ Y_1/y_1$–bl_5–$bl_6.\ bl_7,\ M$ or M/m ······ A–390–2–bl

TABLE 8
Summary of data from all crossing progenies
segregated the blotched plants

Exp. ratio	Crossed pedigrees	Phase	No. of ears	No. of progenies	$I^{1)}$	$D^{2)}$	χ^2	
							D	$H^{3)}$
1 : 3	A–390–2–bl	F_2	2	78	312	— 6	0.1154	—
1 : 1	A–390–1	B	1	80	160	— 10	0.6250	—
3 : 1	H–59	F_3	3	90	360	+ 18	0.9000	1.4260
	A–390–1	B	2	154	616	— 18	0.5260	
	Total		2	5	244	976	0	1.4260	
7 : 1	A–390–1	B	2	259	2072	+ 51	1.2553	0.9136
	A–390–2–2	B	1	66	528	+ 10	0.1894	
	Total		2	3	325	2600	+ 61	1.4447	
15 : 1	H–59	F_2	8	634	10144	— 22	0.0477	0.3569
	H–62	F_2	2	83	1328	— 3	0.0068	
	H–59	F_3	4	335	5360	+ 31	0.1793	
	A–390–2–1	B	5	318	5088	— 18	0.0637	
	Total		4	19	1370	21920	— 6	0.3585	
32 : 1	A–391	F_2	7	870	28710	— 54	0.1015	0.1417
	A–428	F_2	4	794	26202	+ 35	0.0468	
	Total		2	11	1664	54912	— 19	0.1483	
63 : 1	A–390–1	F_2	21	1134	72576	— 82	0.0926	—
Total	(13)		13	62	4895	153456	— 62	0.0250 (3.6480)	3.6230

1) Amount of information.
2) Deviation from the expectancy.
3) Heterogeneity between hybrid families.

Perhaps this is the most interesting contribution in the present paper as evidence on the existence of a set of hexaplicate genes for the blotched leaf in maize. Of them, bl_4 was determined to locate in chromosome 9, and bl_5–bl_6 in chromosome 6. The remainning two (bl_3 and bl_7) were determined to each locate in other different chromosome than the above mentioned ones, but the linkage relations of them were impossible to detect within the scope of the present experimentation. Furthermore, different genetic factors in addition to them should be considered to be involved, because no segregation was usually observed in the progeny from a majority of the crosses between the blotched

and normal plants.

Discussion

According to EMERSON et al (1935), more than three hundred genes have been found in maize, of which not less than one hundred, about 30%, are known to be responsible for the chlorophyll character. If the same names were given to phynotypically similar characters according to EYSTER's classification (1934), all the chloroyhll characters can be placed in ten patterns which may be further grouped into two types from the standpoint of chlorosis. The first type is characterized by the amount of chlorosis, including six following patterns and seventy-one genes: (1) albino having 12 genes, (2) luteus 11 genes, (3) virescent 22 genes, (4) albescent 3 genes, (5) pale-green 10 genes and (6) yellow-green 13 genes; the second type is characterized by the distribution of chlorosis, including the four following patterns and about forty genes: (7) white leaf-base having 4 genes, (8) striped patterns 20 genes, (9) banded patterns 5 genes and (10) dotted patterns 12 genes.

The fundamental difference between these two types is that in the former the chlorophyll is either deficient or reduced in all the tissues, while in the latter chlorophyll deficiency is limited to only some tissues in a leaf. Accordingly, all the chlorophyll patterns belonging to the latter type may be considered as a mosaic consisting of contrasting characters, albino and green.

At any rate, all the genes reported as being responsible for chlorophyll defects have been found to be scattered in all of the chromosomes in maize. Most of them are inherited as a simple recessives respectively, and a few as duplicates.

The present blotch consists of contrasting characters, albino spots and green areas, segregating them on a leaf blade. It is therefore very reasonable to consider this blotch as a pattern of mosaic on the chlorophyll character. At least five genes for the blotch were found to be genetically distinct; they were designated as bl_3, bl_4, bl_5, bl_6 and bl_7. From the interaction and linkage relation of those genes, their normal alleles seem to act in a polymeric manner to cause the development of normal chlorophyll. Thus, segregated ratios into 15:1 and 63:1 or 32:1 green to blotch were observed when the blotch di- and tri-heterozygous plants respectively are selfed, and into 15:1, 7:1, 3:1 and 1:1 when quadri-, tri-, di- and mono-heterozygous plants re-

spectinely are back-crossed. In addition, it was suggested strongly
through the data from a majority of crosses carried out in the two
years, 1943 and 1944, that there are many of still other genes belonging
to a set of polymeric series as analysed in the present paper.

According to a linkage summary in maize reported by EMERSON et
al (1935), it is interesting to note that as to most of the genes in
polymeric series their loci have been known to be in the same chromo-
some, but the samples are very few, as follows: (1) au_1–au_2 in chromo-
some 9 (EYSTER 1933), (2) fr_1–fr_2 in 7 (JENKINS and POPE, 1935), (3) w_5–w_6
in 6 (DEMEREC, 1923) and (4) zl_1–zl_2 in 1 (EYSTER, 1934). Contrary to this
fact, four members of a set of hexaplicate genes, bl_3, bl_4, bl_5 or bl_6 and
bl_7, were found in the present investigation to be located in different
chromosomes, and only two members, bl_5 and bl_6, in the same chromo-
some. This finding is farily well in accordance with those on the
polymeric genes reported by DEMEREC (1923) and MANGELSDORF (1926
and 1930). DEMEREC reported that the segregation into a 63:1 ratio
in the inheritance of white seedling occurred in F_2 population from
crosses with unrelated stocks of maize. This ratio suggests the ex-
istence of independently triplicate genes, although this may be a 15:1
ratio in consequence of complete linkage between duplicate genes as
pointed out in his original paper. MANGELSDORF has reported at least
fifteen genes and nine distinct patterns from the analysis of the
inheritance of premature germination in maize. Three of nine
patterns are governed by three sets of independent polymeric genes
respectively, viz. duplicate (ge_6. ge_7), triplicate (ge_8. ge_9. ge_{10}) and quad-
ruplicate (ge_{11}. ge_{12}. ge_{13}. ge_{14}), and are inherited in a ratios of 15:1, 63:1
and 255:1 in the F_2 population. Practically his idea has been further
verified by the data from the F_3 progenies (MANGELSDRF, 1930).

Since the discovery of duplicate genes, which has been reported
independently by NILSON-EHLE (1909) and EAST (1910), many cases of
inheritance falling into the category of duplicate genes have been
recorded. There is an attractive speculation regarding the origin of
this hereditary mechanism, according to which polymeric genes have
arisen entirely through the chromosome duplication (SHULL 1926). From
the conception of secondary associations among non-homologous chromo-
somes in the complement with respect to the basic number of chromo-
somes, it has been demonstrated that the polymeric genes are indicative
of an ancestral chromosome in the course of evolution. It is, however,
difficult to conceive of such a view in maize owing to the following

facts:

1), There are sometimes duplicate genes included in the same chromosome. Different segments of such a chromosome should be of the same origin. Such idea is pertinent on the ground of the segments being homologous to each other.

2), As appears in such characters as blotch, white seedling and germinating seed in maize, some of the polymeric genes have been known to be scattered through various chromosomes in the complement, as well as some of the complementary genes as being scattered as reported by LINDSTROM (1923). In other words, there is an arithmetical limit in the number of duplications, which could have occurred to produce the ten pairs, while it is by no means certain that the limit in number of genes involved in a polymeric series has been found.

3), In the present case, it will be expected that at least four of chromosomes in the complement of maize should show considerable morphological resemblance. Nevertheless, it is a well-known fact that every chromosome in maize is morphologically identifiable, differing from each of the others in some essential feature.

It is being demonstrated that many genes belonging to a set of polymeric series are to be found in most, if not in all, of the chromosomes in the maize complement. Because, to those reported by DEMEREC (1923) and MANGELSDORF (1930) were added those of the present case. Accordingly, it is evident that genes affecting the same character may be recognized to be scattered through all of the chromosomes, although they are sometimes observed to locate in the same chromosome. For this reason, MANGELSDORF (1930) has been inclined believely to accept in the gradual transition of the duplicated segment of a chromosome. He came, therefore, to a conclusion on the origin of polymeric genes that every gene of a polymeric series in his germinating seeds is characterized respectively by a different linkage relation, and so he says:

> "It is possible that the hypothetical transition from tetrasomic to disomic inheritance, that is, from a tetraploid to a double diploid, has been acompanied by discernible morphological changes in chromosomes that were originally identical and homologous. The fact remains, however, that at present there is very little, if any, critical evidence that multiple factors arise through chromosome duplications. The hypothesis remains a plausible one. however, particularly in view of the fact that duplicate factors and polyploidy are both of frequent ocurrence in plants and of rare ocurrence in animal."

It may be highly probable that his speculation is supported very

well by the chromosome alteration hypothesis of LEWITSKY (1931) and
NAWASCHIN (1932), who postulated independently that a given chromo-
some can change in both directions, number and form, according to the
dislocation of the segment of a chromosome.   The formation of new
species by such an idea of the dislocation has been recently reestablished
experimentally by several workers; viz., in *Drosophila artificalis* (Koz-
HEVNIKOVA, 1936), a new type of *Dr. melanogaster* (DUBININ, 1936), *Crepis
nova I & II* (GERASSIMOVA, 1939) and others.   It is however impossible
to ascertain whether MANGELSDORF's hypothesis is correct or not, be-
cause there is as yet nothing of any experimental evidence in further
proof of the polymeric genes, chromosome duplications and chromosome
alterations as pointed out by him.

Two possibilities will be further considered with respect to the
polymeric mechanism of inheritance.   The first possibility will be sug-
gested by the excellent experiments reported by MANGELSDORF and
REEVES (1939) and MANGELSDORF (1947), in which four segments of a
given chromosome of *Tripsacum*, differentiating teosinte from maize,
could be transferred to the chromosomes of maize by making repeated
back-crossings with maize to the *Zea-Tripsacum* hybrid; two segments
to chromosome 4 in maize, one to chromosome 1 and the last one
probably to chromosome 5.   Each of these segments was demonstrated
to produce polymeric effects upon several of the dominant characters,
by which maize can be distinguished from teosinte.   KEPMTON (1924)
reported that the F_2 segregation from the crosses between maize and
teosinte represents a rate of 1 : 133, which is quite close to the ex-
pected ratio of 1 : 127 on the basis of quadruplicate gene segregation.
Such a dominant quadruplicate gene hypothesis was further proved by
the large amount of the crossed data supplied by MANGELSORF and REEVES
(1939) and MANGELSDORF (1947).   That is to say, they have been now
assuming that these four genes are in reality located in a small segment
of the chromosome which was received orginally by a probable trans-
location from *Tripsacum* to maize.   The polymeric inheritance of the
present blotch will be not impossible of interpreation by an assumption
that a sensitive segment of chromosome, which is responsible for the
blotched character in maize, was divided into several parts and each
of the parts was inserted into definite part of various chromosomes
by some form of chromosome alteration in the course of the evolution
of maize.

The second possibility will be supported by a physiological specula-

tion regarding the phenotypic appearance of mosaic character. It has been known to be a fact that the mosaic is formed by means of a given gene mutating to its opposite allele or eliminating itself in some parts of the somatic tissues of a single individual in the course of ontogeny. Accordingly, it may be inferred that the blotched pattern is not caused directly by the *bl* genes but by the formation of mosaic pattern, of which the albino areas are controlled by many *ll* genes behaving in a polymeric manner. It is also a well-known fact that the morphological character such as mosaic pattern has been proved to be an endo-product of chemical reactions occurring in the course of ontogeny. There are two types with respect to the reaction system; one consisting of only a single step and the other of many different steps. The former is usually controlled by a single gene, so that it is inheritable in a monogenic manner. While in the latter each of the genes is responsible for the respective step in a chain of chemically definable reactions, so that the number of genes may be indicative of the number of steps in a reaction system.

If the polymeric genes had arisen through chromosome duplication, they should be found all to be affecting one certain step in the reaction, and so account for different chromosomes which have originated from a single chromosome in the course of evolution. Only such an occurrence of polymeric genes will be considered equally well to be indicative of the primary basic number of chromosomes in the complement. However, it will be difficult to apply the present case to this assumption.

If it be acceptable that each part within a sensitive region of chromosome is responsible for its respective step of the reaction system, being necessary to the appearance of the blotched phenotype, then, the polymeric inheritance of present blotch will be expected in consequence of occurring alteration of chromosomes described in the first possibility in the course of evolution.

Summary

The present paper deals with a phase in the analysis of genes involved in the blotched plant. The principal findings brought out from the present data may be summarized as follows:

1), Blotched leaf is a new form of chlorophyll character isolated by inbreeding from a southern yellow dent variety in Hokkidô. The

pattern is characterized by many beautiful white or yellow spots, all of which are formed on the leaf blade.

2), The blotched phenotype must be controlled by many recessive *bl*-genes, at least more than five, belonging to a set of polymeric series. The analysis has advanced far enough to prove the existence of five *bl*-genes, bl_3, bl_4, bl_5, bl_6 and bl_7, and a probably recessive modifier, *m*, of the gene bl_7, but indications are very strong that in the blotch the existence of many more genes additional to them is necessary to express the blotched character.

3), Independent inter-relationships were found amongst the four *bl*-genes; bl_3, bl_4, bl_5 or bl_6 and bl_7. Accordingly, the two segregations into 15:1 and 63:1 green to blotched plants occurred in the F_2 population when di- and tri-heterozygous blotched plants respectively were selfed. The four segregated ratios, 1:1, 3:1, 7:1 and 15:1 green to blotched plants, were observed when mono-, di-, tri-, and quadri-heterozygous plants for *bl*-genes were back-crossed with the blotched parent.

4), Two members of a set of hexaplicate genes, bl_5 and bl_6, were linked together with about 30 percent of recombination in repulsion phase. Furthermore, one of them, bl_5, was very closely linked with a certain other genes, Y_1. This is apparently a case of complete linkage, although about 14 percent as crossovers were estimated to be in coupling phase when the bl_7 gene was involved in the parent plant.

The possible arrangement of the three above linked genes will be either Y_1–bl_5–bl_6 or bl_5–Y_1–bl_6 in chromosome 6.

5), The gene, bl_4, was located in chromosome 9, where it showed approximately 24% of recombination with *sh* and 33% with *wx*. The presumed order may be bl_4–*sh*–*wx*.

6), The remaining two genes, bl_3 and bl_7, were independent of Y_1 or *sh*-*wx*, and the linkage relation of them could not as yet be proven.

7), The mechanism for the appearance of blotched phenotype and the possible origin of polymeric genes through the chromosome alteration in the complement were discussed.

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Appendix-Tables, 9 to 13

TABLE 9

F_2 and its back-crossed progenies from *A-390-1*

Phase	Exp. ratio	Crossed pedigrees	No. of ears	Observed numbers			χ^2	
				Total	+	*bl*	$D^{1)}$	$H^{2)}$
F_2	63 : 1	*A-390-1*	21	1134	1115	19	0.0926	—
$B^{3)}$	1 : 0	*H-14*	1	27	27	0	0	
		H-18	1	81	81	0	0	
		H-20	1	82	82	0	0	
		H-21	1	86	86	0	0	0
		H-25	1	35	35	0	0	
		H-29	1	70	70	0	0	
		Total	6	381	381	0	0	
	7 : 1	*H-10*	1	180	161	19	0.6222	
		H-24	1	79	72	7	0.9566	0.1225
		Total	2	259	233	26	1.4563	
	3 : 1	*H-22*	1	76	52	24	1.7544	
		H-23	1	78	59	19	0.1068	1.1599
		Total	2	154	111	43	0.7013	
	1 : 1	*H-26*	1	80	35	45	1.2500	—

1) The χ^2 of deviation.
2) The χ^2 of heterogeneity.
3) Female (F$_2$ normal from *A-390-1*) ×Male (*bl*-parent).

TABLE 10

F_2 and F_3 progenies from the hybrids, *H-59* and *H-62*

Phase	Exp. ratio	Pedigrees	No. of plants			χ^2	
			Total	+	*bl*	*D*	*H*
F_2	15 : 1	*H-59*	634	593	41	*0.0149*	
		H-62	83	78	5	*0.0072*	*0.0072*
		Total	717	671	46	*0.0149*	
F_3	1 : 0	*H-59- 1*	47	47	0	*0*	
		H-59-22	78	78	0	*0*	
		H-59- 8	41	41	0	*0*	
		H-59-11	44	44	0	*0*	*0*
		H-59-40	40	40	0	*0*	
		H-59-41	40	40	0	*0*	
		Total	290	290	0	*0*	
	15 : 1	*H-59- 3*	120	113	7	*0.0355*	
		H-59- 4	83	79	4	*0.2655*	
		H-59- 7	105	99	6	*0.0514*	*0.2229*
		H-59-44	27	25	2	*0.0617*	
		Total	335	316	19	*0.1912*	
	3 : 1	*H-59- 6*	28	23	5	*0.7619*	
		H-59- 9	26	20	6	*0.0154*	
		H-59-12	36	29	7	*0.5926*	*0.1699*
		Total	90	72	18	*1.2000*	

TABLE 11

F₂ progenies from the hybrid, *A–391*

Crossed pedigrees	No. of plants		
	Total	+	*bl*
1	87	86	1
2	119	117	2
3	156	153	3
4	121	112	9
5	139	131	8
6	145	144	1
7	103	99	4
Total	870	842	28

TABLE 12.

F₂ progenies from four F₁ ears in the hybrid, *A–428*, from a cross between the normal plant with $y_1 \cdot sh-wx$ and the blotched plant with $Y_1 \cdot Sh-Wx$

Endosperm character	No. of obs. seeds	No. of plants observed			No. of plants corrected		
		Total	+	*bl*	Total	+	*bl*
Y_1	840	253	240	13	*405*	*384*	*21*
y_1	270	213	213	0	*130*	*130*	*0*
sh	134	85	85	0	*64*	*64*	*0*
wx	147	97	95	2	*71*	*70*	*1*
sh–wx	257	146	145	1	*124*	*123*	*1*
Total	1648	794	778	16	*794*	*771*	*23*

TABLE 13

Progenies from a back cross (hybrid number, *A-390-2*), between the normal
strain having both heterozygous yellow endosperm and blotched
leaf, and the blotched strain (*A-390-2-bl*) having
heterozygous yellow endosperm[1]

Pedigrees	Ear number	Endosperm character	No. of ob. seeds	Observed no.			Corrected no.			χ^2 for (15 : 1)
				Total	+	bl	Total	+	bl	
A-390-2-1	Tx-6	Y_1	223	31	29	2	*48*	*45*	*3*	
		y_1	124	42	38	4	*25*	*23*	*2*	*0.0447*
		Total	347	73	67	6	*73*	*68*	*5*	
	Tx-7	Y_1	195	20	20	0	*25*	*25*	*0*	
		y_1	142	22	18	4	*17*	*14*	*3*	*0.0571*
		Total	337	•42	38	4	*42*	*39*	*3*	
	Tx-8	Y_1	242	32	32	0	*35*	*35*	*0*	
		y_1	30	15	11	4	*12*	*9*	*3*	*0.0014*
		Total	272	47	43	4	*47*	*44*	*3*	
	Tx-9	Y_1	217	34	34	0	*38*	*38*	*0*	
		y_1	147	29	24	5	*25*	*21*	*4*	*0.0011*
		Total	364	63	58	5	*63*	*59*	*4*	
	Tx-10	Y_1	173	39	39	0	*55*	*55*	*0*	
		y_1	122	54	45	9	*38*	*32*	*6*	*0.0065*
		Total	295	93	84	9	*93*	*87*	*6*	
	Total	—	1615	318	290	28	*318*	*297*	*21*	0.0679
A-390-2-2	Tx-18	Y_1	170	55	45	10	*40*	*33*	*7*	
		y_1	108	11	11	0	*26*	*26*	*0*	*(7:1)* *0.2164*
		Total	278	66	56	10	*66*	*59*	*7*	

1) The back-cross pollination was made by the use of mixed pollen from sister plants
of the *A-390-2-bl* strain.