THE CYTOLOGICAL IDENTIFICATION OF THE CHROMOSOME ASSOCIATED WITH THE *R-G* LINKAGE GROUP IN *ZEA MAYS*

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INTRODUCTION

Genetic investigations with *Zea mays* have established ten linkage groups. Likewise, cytological investigations have revealed the presence of ten morphologically identifiable chromosomes composing the haploid complement (McCLINTOCK 1929b). It is the aim of the present investigation to correlate a particular linkage group with a particular chromosome.

The method employed has been to obtain 2n+1 plants trisomic for different members of the haploid complement, and then, by means of trisomic inheritance, to determine which chromosome carries a particular group of genes.

The 2n+1 plants have been obtained from the progeny of one original triploid (McCLINTOCK 1929a). The chromosome number in the female gametes of a triploid varies from ten to twenty. Trisomic individuals were obtained directly from the F_1 of a cross triploid × diploid and from the 2n+1 progenies of F_1 individuals with more than one extra chromosome.

Inheritance data obtained from the 2n+1 plants of culture 131 suggested that in this culture the *r-g* carrying chromosome was present in triplicate. An effort was therefore made to test the validity of this interpretation and to verify the genetic inference that the nine other linkage groups are independent of the *r-g* chromosome.

The evidence indicates that the smallest chromosome of the haploid complement carries the genes of the *r-g* linkage group. Thus, 2n+1 plants of culture 131 showing trisomic inheritance for *r* have the smallest chromosome in duplicate in their 11-chromosome microspores. Likewise, 2n+1 plants of other cultures which show the smallest chromosome in duplicate in 11-chromosome microspores have given trisomic inheritance for *r* in later genetic investigations.

METHODS

Root-tips were fixed in a chromic-acetic-formalin mixture and sectioned in paraffin. The aceto-carmine smear method was used for sporocytes which previously had been fixed in an acetic-absolute alcohol mixture. The microspores were fixed in an acetic-absolute alcohol mixture, stained with carmine and cleared with chloral hydrate.

For morphological studies of the chromosomes, late prophase stages of the division of the microspore nucleus were found most valuable, since the chromosomes at this stage are longer, their constrictions are more obvious and the relative length of their arms is more readily determined than in the contracted metaphase stage. From comparative studies of the most easily distinguishable chromosomes of the complement it is clear that the morphology as shown by the prophase and metaphase microspore figures is essentially similar to that shown in the root tips. The presence of only the haploid complement and the ease of observation in the microspore favored the use of this stage for cytological studies.

OBSERVATIONS ON TRISOMIC INHERITANCE FOR *R*

As has been stated, the 2n+1 individuals of culture 131 were trisomic for the *r-g* chromosome. This culture arose from a 2n+1 plant which had been selfed. Of 61 individuals examined, 39 (or 63.7 percent) were 2n, 21 (or 34.4 percent) were 2n+1 and 1 (or 1.6 percent) was 2n+2. It can be safely assumed that all the 2n+1 plants of this culture were trisomic for the same chromosome, since the normal rate of non-disjunction in *Zea mays* is very low.

As a result of the distribution of the members of the trivalent at meiosis, 11-chromosome carrying and 10-chromosome carrying gametes are formed. In a normal pollination, the extra chromosome carrying pollen grains do not function well in competition with the n-carrying pollen grains (see table 1). For genetic investigations, therefore, it is necessary to

TABLE 1

Percent of 2n+1 individuals resulting from the cross 2n ♀ ×2n+1 ♂.

2n ♀ ×2n+1 ♂	2n	2n+1	Percent 2n+1
$192_1 \times 224_3$	261	3	1.13
$192_8 \times 225_3$	83	2	2.35

consider the functioning of 11-chromosome carrying gametes only in the case of the female. On the basis of random distribution of the three similar chromosomes at meiosis one should expect half of the eggs to carry the extra chromosome, but actually only about one-third of the eggs carried it (see above and table 2). This discrepancy can be explained on the basis of irregularities at meiosis. Nine bivalents and one trivalent are found at metaphase I only in approximately two-thirds of the sporocytes; in the other sporocytes there are ten bivalents and one univalent. When the extra chromosome appears thus as a univalent its

TABLE 2

Number 2n+1: 2n individuals from the cross 2n+1 ♀ ×2n♂.

CULTURE	2n	2n+1
166	11	4
168	1	1
176	5	2
224	2	2
225	14	5
229	10	10
230	14	4
231	17	2
232	9	11
Totals	83	41 33.06 percent 2n+1

behavior is very irregular (McCLINTOCK 1929a). It may not go into the spindle figure but remain in the cytoplasm. It may be found in an abnormal position in the spindle. Again, the univalent may lag in the central part of the spindle with or without showing evidence of a separation of its split halves. If the halves should be included in the two telophase I nuclei, they would probably lag in the second meiotic mitosis. In all of these cases a loss of the univalent will occur in meiosis, with the formation of all n-carrying nuclei instead of half n-carrying and half n+1. This phenomenon could account for the increased ratio of n to n+1 gametes. The difference in many cases is probably not due to lack of viability of n+1 gametes or 2n+1 plants, since in many ears of *Zea mays* the regularity of row and kernel position allows undeveloped kernels to be readily detected. Some 2n+1 plants bore almost perfectly filled ears. It is possible, also, that the lowest megaspore, if it contains the extra chromosome, does not function to produce the embryosac but, in a certain percent of the cases, one of the megaspores that contains the haploid complement functions instead.

The trisomic individuals of culture 131 were crossed so that their progenies were heterozygous for at least one factor of every linkage group. Backcross and F_2 ratios were obtained to determine which factors were inherited on a trisomic and which on a disomic basis. Abundant evidence for trisomic inheritance of r in the 2n+1 progenies of culture 131 (cultures 189, 209, 224, 225, 229, 231) was obtained.

Simple ratios may best be considered first. When the *R* factor for red aleurone is duplex (*RRr*) the gametic ratio expected from random distribution of the extra chromosome is 2*R*:2*Rr*:1*RR*:1*r*, or a total of 5*R*:1*r*. Since only the n-carrying pollen grains need be considered, the functioning male gametic ratio is 2*R*:1*r*. In the 2n+1 progenies of culture 131 duplex for *R* the backcross ratios through the pollen were 646*R*:355*r* (table 3),

TABLE 3

rr ♀ × *RRr* ♂	COLORED	COLORLESS
$192_8 \times 225_3$	240	91
$192_9 \times 209_{49}$	198	141
$192_{10} \times 224_2$	208	123
Totals	646	355

a fair approximation to 2:1. On the same basis, crosses of 2n *Rr* ♀ × 2n +1*RRr* ♂ should give 5*R*:1*r*. Table 4 shows a total count of 2102*R*:435*r*. A sib cross between two heterozygous 2n individuals gave 290*R*:88*r*, or the expected 3:1 ratio (table 7).

TABLE 4

Rr ♀ × *RRr* ♂	COLORED	COLORLESS
$225_{11} \times 224_2$	265	74
$225_{14} \times 224_2$	235	49
$231_7 \times 231_9$	382	61
$231_{14} \times 231_9$	419	82
$231_{16} \times 231_9$	357	82
$231_{17} \times 231_9$	444	87
Totals	2102	435

When *R* is simplex (*Rrr*) the expected gametic ratio is 1*R*:2*Rr*:1*rr*:2*r*. With elimination of the n+1 carrying pollen grains the functioning male gametic ratio is $1R:2r$. In the progenies of culture 131 only one individual tested was so constituted and gave a backcross ratio through the pollen of $110R:234r$ (table 5). Conversely a 2:1 ratio is expected in crossing a 2n *Rr* with this 2n+1 of constitution *Rrr*; actual counts showed $348R:182r$ (table 5).

TABLE 5
Crosses involving the simplex (Rrr) individual, 224₃.

rr ♀ × *Rrr* ♂	COLORED	COLORLESS
$192_1 \times 224_3$	110	234
Rr ♀ × *Rrr* ♂		
$224_4 \times 224_3$	182	97
$224_4 \times 224_3$ second ear	166	85
Totals	348	182
Rrr ♀ × *Rr* ♂		
$224_3 \times 224_4$	160	57

The extra chromosome having thus been shown to carry a factor for the *r-g* linkage group, cytological examinations were made in order to determine which of the ten chromosomes of the haploid complement it was. Since the ten chromosomes are all morphologically distinguishable, it was only necessary to examine the 11-chromosome carrying microspores and see which chromosome was present in duplicate. Observations on diakinesis had already indicated that the chromosome involved was either the smallest or the next to the smallest.

The methods devised at the time of the investigation for the observation of the late prophase chromosome made it somewhat difficult to obtain figures with all of the chromosomes lying perpendicular to the optical axis. Some good figures were obtained, however (figure 1). Many figures were found with all but one or two chromosomes lying flat. Since the differences in size between the four smallest and the six largest chromosomes are obvious in the later prophase stage almost regardless of the position of the chromosomes in the nucleus, it is comparatively easy to know when the four smallest chromosomes are lying flat, and hence to obtain accurate figures (figure 2). In the 11-chromosome microspores in which one chromosome is present in duplicate it is easy to determine whether it belongs to the group of four small chromosomes or to the group of six large ones. When it belongs to the former group one observes five small instead of four small chromosomes, and the frequency of figures with all five chromosomes lying in the desired plane is sufficiently high to make accurate comparisons of the chromosomes for the purpose of determining which of them is present in duplicate. Such a case is shown in figure 3.

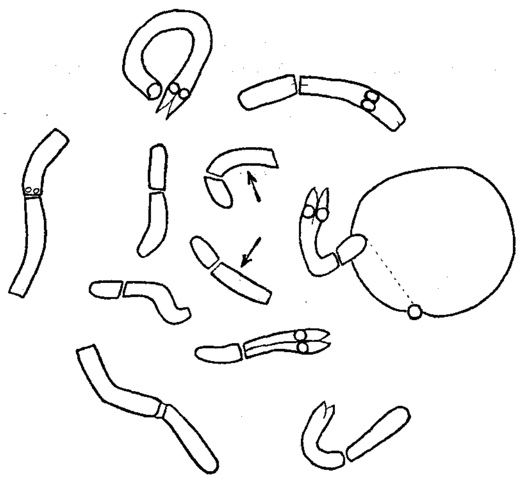

FIGURE 1.—Late prophase chromosomes in an 11-chromosome (n+1) microspore. The arrows indicate the duplicated chromosomes of the haploid complement; these are the *r-g* carrying chromosomes. At this stage the chromosomes are frequently very angular. ×2150.

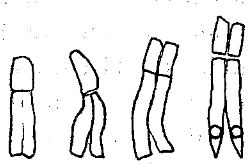

FIGURE 2.—The four smallest chromosomes from a normal (n) microspore; late prophase ×2150.

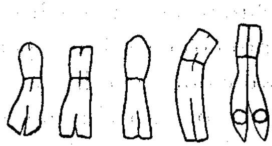

FIGURE 3.—The five smallest chromosomes from an 11-chromosome (n+1) microspore. Note that the chromosome in duplicate is the smallest chromosome of the set. Compare with figure 2. ×2150.

It is clear that the extra chromosome which carries the genes for the *r-g* linkage group is the smallest chromosome of the haploid complement.

The presence in triplicate of the smallest chromosome does not markedly affect the growth or appearance of the plant. One is unable to detect even a moderate difference in comparing 2n+1 with 2n individuals. On the average, the 2n+1 individuals are somewhat weaker than the 2n individuals. Since genetic material of *Zea mays* is very heterozygous for growth and morphological characters, it is possible that the morphological changes produced by the presence of this extra chromosome would be recognizable only in fairly homozygous material. In consequence of the inability to recognize 2n+1 plants with certainty in the field, every plant must be examined cytologically or tested genetically to detect the presence of the extra chromosome.

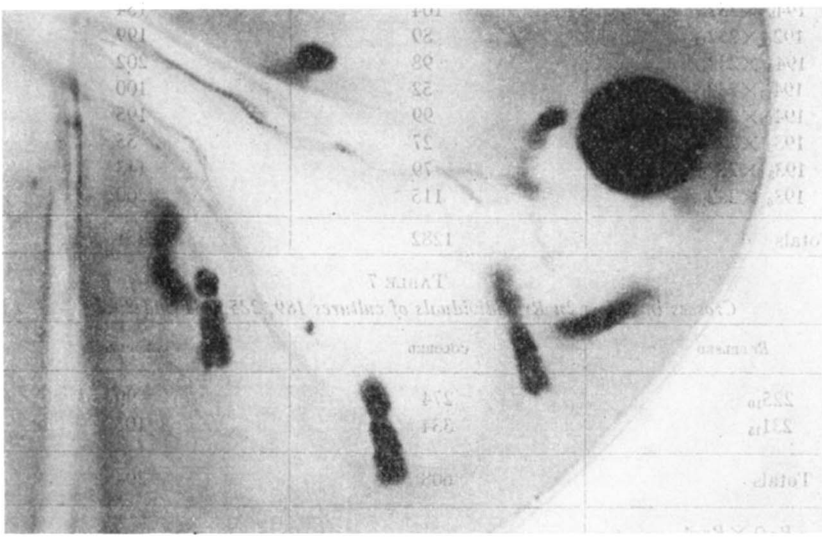

FIGURE 4.—Photomicrograph of microspore showing late prophase chromosomes of first nuclear division. The smallest chromosome of the complement, that which carries the genes of the *R-G* linkage group, is the second chromosome from the left. It is the only chromosome in focus throughout its entire length. ×1800.

Another 2n+1 plant (144_1) not directly related to culture 131, an F_2 individual from the cross triploid×diploid, was examined cytologically. The examination showed clearly the presence of the smallest chromosome in triplicate. It was therefore necessary to prove that this plant or its 2n+1 progeny would show trisomic inheritance for *r*. Colored kernels from the cross 144_1 (2n+1)×*ACr* (2n) were grown. The 2n and 2n+1 F_1 individuals were backcrossed to *r* testers (*ACr*). Pollen of 2n+1 plants placed on *r* testers gave a total count of 1282 colored to 2451 colorless kernels, a simple trisomic ratio of 1:2 (table 6). Plant 144_1 probably had the constitution *Rrr*, since each of the 2n+1 offspring was *Rrr*. The 2n sibs (culture 232, table 7) showed, in contrast, a good disomic 1:1 ratio.

TABLE 6

rr ♀ × Rrr ♂	COLORED	COLORLESS
$194_{12} \times 232_2$	99	178
$194_{14} \times 232_2$	33	75
$194_{3a} \times 232_4$	82	153
$194_{15} \times 232_4$	79	184
$194_3 \times 232_6$	97	140
$194_{13} \times 232_6$	78	174
$194_7 \times 232_8$	82	167
$194_1 \times 232_{11}$	69	132
$194_5 \times 232_{11}$	104	154
$192_{12} \times 232_{12}$	89	199
$194_4 \times 232_{12}$	98	202
$194_{16} \times 232_{13}$	52	100
$194_{19} \times 232_{13}$	99	195
$193_2 \times 232_{16}$	27	55
$193_3 \times 232_{16}$	79	143
$193_6 \times 232_{16}$	115	200
Totals	1282	2451

TABLE 7

Crosses involving 2n Rr individuals of cultures 189, 225, 231 and 232.

Rr SELFED	COLORED	COLORLESS
225_{10}	274	99
231_{15}	334	105
Totals	608	204
Rr ♀ × Rr ♂		
$225_{17} \times 225_{16}$	290	88
Rr ♀ × rr ♂		
$225_{12} \times 192_4$	184	219
$225_{13} \times 192_4$	177	187
$232_1 \times 194_{15}$	172	195
$232_5 \times 192_6$	113	104
$232_7 \times 192_6$	221	198
$232_{14} \times 192_6$	182	168
$232_3 \times 192_6$	70	73
$232_{10} \times 194_{13}$	20	30
$232_{18} \times 192_6$	22	22
Totals	1161	1196
rr ♀ Rr ♂		
$192_7 \times 189A_{50}$	132	135

Still another unrelated trisomic plant ($219A_5$) was found which possessed the smallest chromosome in triplicate. Its genic constitution with respect to *r* was unknown, but its pollen was used on *r* testers with the thought that it might be heterozygous. The total backcross ratio from the three ears obtained (table 8) was 295*R*:131*r*, indicating a duplex (*RRr*) condition in this plant.

TABLE 8

rr ♀ × *RRr* ♂	COLORED	COLORLESS
$192_{12} \times 219A_5$	192	94
$193_1 \times 219A_5$	15	11
$194_{11} \times 219A_5$	88	26
Totals	295	131

Thus far only ratios through the pollen have been considered. The ratios are simple and agree with the expectations. The trisomic ratios as expressed through the female resulting from the cross 2n+1 ♀ ×2n ♂ are complicated by the presence of an excess of 2n over 2n+1 individuals. Possible explanations for this have been given on page 177. As was stated there, if there were random assortment of the three homologous chromosomes at meiosis 50 percent of the gametes should be n and 50 percent n+1. Actually, only about one-third of the eggs carry the extra chromosome. This condition materially changes the expected ratio.

In a simplex plant (*Rrr*) the gametic ratio, on the basis of random assortment of the three homologous chromosomes would be 1*R*:2*Rr*:1*rr*:2*r* which would give on backcrossing to *rr* plants, a phenotypic ratio of 1*R*:1*r*. If, however, lagging and loss of one of the three chromosomes occur and at random (or if there is also some selection against n+1 megaspores in favor of n) the gametic ratio produced as a result of these occurrences would be 1*R*:2*r* and all the gametes would be n. The total gametic ratio, therefore should fluctuate between these two extremes depending upon the relative amounts of random distribution of the three homologous chromosomes as compared to lagging and loss that have occurred. It is therefore necessary to know what proportion of the female gametes were produced after random assortment and what proportion after loss.

If a 2n+1 simplex plant (*Rrr*) is pollinated with pollen from a 2n *rr* plant the proportion of *R*-carrying and *r*-carrying gametes may be directly determined. The amount of lagging, or of lagging plus megaspore selection, then, may be computed from the distortion of the 1:1 ratio (expected with no lagging) in the direction of a 1:2 ratio (expected with 100 percent lagging). An approximate measure of the total amount of lagging and loss that has occurred during megasporogenesis in the ovules of the 2n+1 plants of culture 232 (table 9) has been obtained in this manner.

TABLE 9

Rrr ♀ × *rr* ♂	COLORED	COLORLESS
$232_4 \times 192_4$	137	178
$232_6 \times 194_{15}$	130	149
$232_8 \times 192_6$	115	150
$232_9 \times 192_6$	137	178
$232_{16} \times 192_6$	160	181
Totals	679	836

The 2n+1 plants of culture 232 were all *Rrr* (see table 6). They were pollinated with pollen from 2n *r*-testers (*ACr*). Each ear obtained showed a deviation from a 1:1 ratio expected with random assortment of the three homologous chromosomes toward the 1:2 ratio expected from lagging and loss, or from lagging and loss plus megaspore selection. Of the 1515 kernels obtained 679 were colored (dominant) and 836 were colorless (recessive).

1*R*:1*r* = gametic ratio due to random assortment of three homologous chromosomes.

1*R*:1*r*:1*r* = gametic ratio due to lagging and loss.

When these two gametic ratios are placed one above the other, it is seen that 679 represents the number of *R*-carrying gametes produced by both types of distribution, and similarly, 836 the number of *r*-carrying gametes produced. If 679 represents the number of *R*-carrying gametes produced, then 679 can be given to a similar proportion of *r*-carrying gametes. The total number (679+679) is 1358. The difference between 1515, the total number of gametes, and 1358 is 157, which is the number of the remaining recesives gametes (or kernels). It is easy to see by this method that this number (157) represents one-third of the total number of gametes produced as the result of lagging and loss (or lagging and loss plus megaspore selection). The number 157 is also the amount by which the recessives exceed the dominants. Thus, in a population composed of individuals, some of which, taken as a group, represent a 1:1 ratio and others of which, similarly, represent a 1:2 ratio, the number by which the recessives in the total population exceed the dominants should equal one-third of the individuals representing the 1:2 ratio, or, in the present instance, one-third of the individuals produced as a result of lagging.

The number of colorless kernels (recessives) exceeds the number of colored kernels (dominants) by 157; hence, the total number of gametes produced after loss should be three times 157, or 471. This number represents approximately 30 percent of the total number of kernels. Assuming that the data represent a sufficiently uniform random sample, it can be concluded that approximately 30 percent of the gametes were produced after loss of the extra chromosome through lagging at the first or second meiotic mitosis, or possibly through lagging and loss plus any loss that may have occurred through selective viability of megaspores.

If 30 percent of the gametes were produced through such loss, then loss occurred in 30 percent of the ovules. From the remaining 70 percent of the ovules half, or 35 percent, should possess n+1 female gametes and consequently 35 percent of the plants from the cross 2n+1 ♀ ×2n ♂ should carry the extra chromosome. This, in turn, can well explain the deviation from the expected ratio of 2n+1 to 2n individuals in the cross 2n+1×2n (see page 177 and table 2). It is expected that both cytological and genetical evidence will be obtained which will indicate more definitely how much of the deviation is actually due to lagging and loss, and how much may be due to a possible selective viability of n+1 gametes or 2n+1 embryos.

Assuming this same amount of loss, deviations from the 5*R*:1*r* ratio expected in the cross *RRr*×*rr* (table 10) can be interpreted. Each in-

TABLE 10

RRr ♀ × *rr* ♂	COLORED	COLORLESS
$209_{41}\times192_{9}$ (first ear)	130	33
$209_{41}\times192_{8}$ (second ear)	207	56
$209_{41}\times193_{3}$ (tiller ear)	193	52
$209_{58}\times192_{6}$	289	72
Totals	819	213

dividual cross shows a deviation from the 5:1 ratio in an increase in the number of colorless kernels over expectancy. The total count of 819 colored:213 colorless represents a ratio of 3.84:1 instead of 5:1. A ratio of 3.61:1 would be expected if it were assumed that 30 percent of the female gametes were produced as the result of loss. The ratio due to loss is $2R:1r$, as contrasted with the ratio of $5R:1r$ produced through random assortment of the three homologous chromosomes.

These same assumptions are utilized in explaining the deviations observed in selfing an individual of the constitution *RRr*. On the basis of random assortment and no lagging of the three homologous chromosomes, the female gametic ratio should be $2R:2Rr:1RR:1r$, the male, because of the elimination of the extra chromosome carrying pollen, $2R:1r$. One would expect, therefore, a 17:1 ratio in F_2. On the basis of 30 percent of the gametes being produced as the result of loss of the extra chromosome a 12.8:1 ratio would be expected instead. Table 11 shows the results obtained.

TABLE 11

AACCRRr SELFED	COLORED	COLORLESS
231_9 first ear	396	41 $X^2=2.47$, $P=0.10+$
231_9 second ear	188	24
Totals	584	65 $X^2=7.42$, $P= >0.01$

Determinations of goodness of fit by means of the χ^2 method indicate a significant deviation on the total counts. A full ear of well developed kernels of unmistakable classification resulted upon selfing the first ear. A χ^2 determination on this ear alone gives a fit well within the probability. The second ear on this plant produced by selfing was poorly filled, with many kernels underdeveloped. It is possible that in this ear the color in some of the kernels did not develop. This possibility is supported by the fact that some kernels on this ear showed the presence of color by only a slight degree of mottling. It is possible, also, that some $2n+1$ embryos did not develop fully on this particular ear.

The description of trisomic inheritance of *r* given above shows the nature of trisomic inheritance in *Zea mays* with regard to the smallest chromosome of the haploid set.

INDEPENDENCE OF THE *R-G* LINKAGE GROUP

The method of trisomic inheritance is a convenient means of determining with certainty the independence of linkage groups. Evidence obtained from both cytological and genetical observations indicates that the *r-g* linkage group is independent of all the other nine linkage groups established genetically. At least one factor of each linkage group has been tested (c and w_x, s_u, b, y, g_{l1}, p_r, f, d and a). 2n+1 individuals heterozygous for these genes have been selfed and backcrossed.

TABLE 12

Results of crosses involving c-w_x, s_u, b, y, g_{l1}, p_r, d and a among 2n+1 individuals trisomic for the r-g chromosome. For explanation see page 189.

I. Disomic inheritance of c and w_x (see appendix).		
2n+1 Cc [C or c]×2n AcR	C	c
131_{33}×$B346_1$	78	69
2n AcR×2n+1 Cc [C or c]		
104_2×88_1	194	149
$B346_1$×131_5	172	184
	W_x	w_x
2n+1 W_xw_x [W_x] pollen counts	535	549
2n W_xw_x pollen counts	633	594
2n w_xw_x×2n+1 W_xw_x [W_x]		
192_8×225_3	152	179
II. Disomic inheritance of s_u.		
2n+1 S_us_u [s_u] selfed	S_u	s_u
220_3	282	110
220_{14}	279	97
220_{14}	174	53
220_{15}	223	68
220_{17}	261	90
220_{17}	221	86
231_9	318	102
Totals	1758	586
2nS_us_u×2n+1 S_us_u [s_u]		
220_{11}×$_{18}$	228	98
225_{14}×$_2$	208	73
231_7 ×$_9$	324	112
231_{16}×$_9$	316	126
231_{17}×$_9$	359	139
Totals	1495	548
2n s_us_u×2n+1 S_us_u [s_u]		
188×$189B_{18}$	190	169
2n S_us_u selfed		
220_5	288	99
220_8	269	77
220_{12}	352	112
225_{10}	278	95
231_5	420	138
231_{15}	327	112
Totals	1934	633

TABLE 12—(*continued*)

III. Disomic inheritance of *b* (see page 189).		
2n+1 *Bb*[*b*] selfed	*B*	*b*
88_1	54	19
IV. Disomic inheritance of *y* (see appendix).		
2n+1 *Yy* [*Y* or *y*] selfed	*Y*	*y*
176_6	94	36
2n *Yy*×2n+1 *Yy* [*y*]		
$194_{12}\times 232_2$	139	37
$194_3a\times 232_4$	113	40
$194_{15}\times 232_4$	136	46
$194_3\times 232_6$	102	38
$194_{13}\times 232_6$	128	46
$194_7\times 232_8$	127	40
$194_{19}\times 232_{13}$	143	52
Totals	888	299
2n *yy*×2n+1 *Yy* [*y*]		
$194_1\times 232_{11}$	95	89
$194_5\times 232_{11}$	85	63
$194_{16}\times 232_{13}$	77	72
$193_3\times 232_{16}$	66	74
Totals	323	298
V. Disomic inheritance of g_{l1}.		
2n+1 $G_{l1}g_{l1}$ [G_{l1}]×2n $g_{l1}g_{l1}$	G_{l1}	g_{l1}
$229_7\times 201_2$	113	105
VI. Disomic inheritance of p_r.		
2n+1 P_rp_r [P_r] selfed	P_r	p_r
220_3	149	45
220_{14}	287	89
220_{14}	170	57
220_{17}	228	87
220_{17}	196	78
Totals	1030	356
2n+1 P_rp_r [P_r]×2n p_rp_r		
$220_{15}\times 203_1$	129	120
$220_{19}\times 203_8$	110	108
Totals	239	228
2n P_rp_r×2n+1 P_rp_r [P_r]	P_r	p_r
$220_{11}\times 220_{18}$	306	80
2n P_rp_r selfed		
220_5	271	116
220_8	249	97
220_{12}	362	102
Totals	882	315
2n P_rp_r×2n p_rp_r		
$220_{13}\times 203$	194	207

TABLE 12—(*continued*)

VII. Disomic inheritance of d (see appendix).

	D	d
2n+1 Dd [D] selfed		
231_9	129	44
2n dd×2n+1 Dd [D]		
205×231_9	119	118
2n+1 Dd [D]×2n dd		
231_{12}×205	170	140

VIII. Disomic inheritance of a (see appendix).

	A	a
2n+1 Aa [A or a] selfed		
88_1	73	19
2n+1 Aa [A or a]×2n aa		
131_5×R511_3	80	53
2n aa×2n+1 Aa [A or a]		
206_6×189B_{60}	144	173

It is needless to discuss every cross represented in table 12, for the results are self explanatory. The crosses involving the recessive sugary gene (s_u) can be used as a single example (see table 12, section II). A 2n+1 plant of culture 131, homozygous for sugary, was crossed with pollen from a 2n starchy plant (S_uS_u). The F_1 2n and 2n+1 individuals were selfed and backcrossed to test for trisomic or disomic inheritance. In each section the type of cross is indicated. The symbol of the gene placed in brackets indicates how the genic constitution of the 2n+1 individual would have differed had it been trisomic for this gene. On selfing 2n+1 plants heterozygous for sugary a total of 1758 S_u to 586 s_u kernels were obtained, precisely a 3:1 ratio. Diploid (2n) sibs upon selfing gave a total count of 1934 S_u:633 s_u kernels. The two ratios are similar and disomic. If these 2n+1 plants were trisomic for sugary ($S_us_us_u$), an approach to a 2:1 ratio would be expected. Similarly, a 2:1 ratio would be expected in the sib crosses 2n (S_us_u)×2n+1. Here a ratio of 1495 S_u:548 s_u kernels was obtained, a 3:1 instead of a 2:1 ratio. It is therefore concluded that the linkage group including s_u is independent of the r-g linkage group and must be associated with another chromosome. The data on factors c, w_x, y, g_l, p_r and d are sufficiently numerous to need no further explanation.

In the case of b, genetic data are hardly necessary, since the b-l_g linkage group has been associated with another chromosome.

The data on the a factor are few but indicate a disomic instead of a trisomic inheritance. In the case of a 2n+1 heterozygous a plant selfed the results (73A:19a) indicate neither a duplex (AAa) 17−:1 ratio nor a simplex (Aaa) 2:1 ratio, but better, a disomic 3:1 ratio. Further, the cross of a heterozygous 2n+1 plant×2n aa would have given, if duplex, 5A:1a

or if simplex, $1A:1+a$. $80A:53a$ probably represents a 1:1 disomic ratio. In the case of 2n $aa\times$2n+1 heterozygous a, a simplex constitution would have given a 1:2 ratio and a duplex constitution a 2:1 ratio. $144A:173a$ approaches neither of these but probably represents a 1:1 disomic backcross ratio.

The factor for fine stripe (f) did not segregate sharply in the seedling stage so that backcross counts were not sufficiently reliable. Cytological evidence from Doctor BRINK'S material indicates that the f-b_r linkage group is carried by a long chromosome.

SUMMARY

1. A 2n+1 plant of *Zea mays* resulting from the cross diploid$\times$triploid and its 2n+1 progenies were found to give trisomic inheritance for r.

2. In these plants the smallest chromosome is present in triplicate.

3. Two unrelated 2n+1 individuals were found to be trisomic for the smallest chromosome of the haploid set. These plants, upon later testing, gave trisomic inheritance for r.

4. In 2n+1 individuals one-third of the eggs carry the extra chromosome. In a normal pollination the extra chromosome-carrying pollen grains function only in a small percentage of the cases.

5. Plants trisomic for the r-g linkage group have given disomic inheritance for c, w_x, s_u, b, y, g_{l1}, p_r, d and a.

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LITERATURE CITED

MCCLINTOCK, B., 1929a A cytological and genetical study of triploid maize. Genetics 14: 180–222.

1929b Chromosome morphology in *Zea mays*. Science 69: 629.

APPENDIX

During the time this paper was in press the following linkage groups were found to be associated with chromosomes other than the r-g carrying chromosome: $C-s_h-w_x$, $Y-P_l$, $A-d_1-c_r$. By the method of association of linkage groups with particular chromosomes the independence of six of the ten linkage groups ($C-s_h-w_x$, $R-g$, $B-l_g$, $Y-P_l$, $P-b_r$, $A-d_1-c_r$) has been definitely established.