LINKAGE IN AUTOTETRAPLOID MAIZE[1]

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THE study of linkage in diploid organisms is comparatively simple. At the first division of meiosis four chromatids take part in the formation of each bivalent and, since four potential gametes result from each meiosis, these strands pass to different gametes and may, genetically, be dealt with separately. The only concern, therefore, is with the proportions of each type of strand resulting from crossing-over and their possible combinations in the zygotes. Allopolyploids, such as wheat, show the same general type of genetical behavior since multivalent formation is rare.

In autopolyploids, on the other hand, more than four chromatids are concerned in the formation of the various configurations which occur at the first division of meiosis, and, since there are still only four gametes formed, each gamete receives more than one strand from each configuration. Hence with autopolyploids there is the additional consideration of the ways in which the chromatids may be combined in the gametes themselves. This leads to a number of complications in the analysis of linkage in autopolyploids.

MULLER (1914) calculated the monofactorial segregations to be expected from autotetraploids if the two chromatids of each chromosome acted as one at the first meiotic division, and HALDANE (1930) extended the work to include several higher autopolyploids. This mode of inheritance is called chromosome segregation. HALDANE (1930) also established the segregations to be expected if the two chromatids from each chromosome acted entirely independently of one another. Random chromatid segregation is the term applied to this method. SÖMME (1930) reported some linkage studies in autotetraploid *Primula sinensis* and DE WINTON and HALDANE (1931) in a more comprehensive investigation of the subject, using the same plant, gave the linkage formulae applicable to the chromosome type of segregation. SANSOME (1933), working with the tomato, presented the corresponding linkage formulae on the basis of random chromatid segregation. In addition to these cases of linkage studies in autopolyploid plants, investigations on crossing-over in triploid *Drosophila melanogaster* have been reported by BRIDGES and ANDERSON (1925) and REDFIELD (1930, 1932). Later workers (MATHER 1936) have recognized that these segregation and linkage expectations are in the nature of limiting types, and that the true segregations to be expected

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from autopolyploid organisms will actually lie somewhere between the anticipated chromosome and chromatid values.

The object of the work reported here was to determine whether the linkage intensity in autotetraploid maize was the same as in the diploid.

MATERIALS AND METHODS

The linked genes used in this study are located in the chromosome 2 linkage group of maize and are shown, together with their assigned loci, in Figure 1 (RANDOLPH 1941b). The characters produced by these genes are distinguishable in the seedling stage and may be described as follows (EMERSON, BEADLE and FRASER 1935; FRASER 1939): lg_1—liguleless leaf-1; ligule absent in the seedling and throughout the life of the plant; leaves show a tendency to grow upright and the plant has a characteristic appearance even in the seedling stage; classification good; viability normal. gl_2—glossy seedling-2; seedling leaves glossy, when sprinkled with water from a fine-spray watering can the moisture adheres in large droplets; classification easy in the early stages, but usually impossible in the mature plant; viability good. *B*—plant color intensifier; in the seedling stage the distinction between *B* and *b* can only be made in the presence of the super-*g* allele of the *R-r* pair for aleurone color; classification in mature plants usually made without difficulty; viability good. v_4—virescent seedling-4; seedlings yellowish-green, turning green slowly; classification good; viability normal.

Autotetraploid strains of $lg_1\ gl_2\ b^{\backslash g}\ v_4$ stocks originally developed by PROFESSOR L. F. RANDOLPH as a result of the heat treatment method (RANDOLPH 1932) were used in this investigation as a source of linked genes. (The symbols $B^{\backslash g}$ and $b^{\backslash g}$ are used to indicate that either the R^g or r^g gene is involved.) Similarly produced material carrying the dominant alleles was also employed.

Classification of the lg_1 character in autotetraploid plants in the seedling stage was found to be considerably more difficult than in closely related diploid stocks. The effect of the Lg_1 gene, normal liguled leaf, varied since plants differed in the degree of ligule present, the structure ranging in size from a mere vestige to the type ordinarily found in diploids. Careful examination of the second leaf when the seedlings were in the third-leaf stage apparently resulted in accurate classification. Plants which possessed an auricle but no visible ligule were grouped with the Lg_1 segregants. In the mature plant stage the upright growing habit of the leaves of lg_1 plants provided an easy method to separate them from their normal sibs.

The classification of gl_2 and v_4 was just as simple as in diploid stocks.

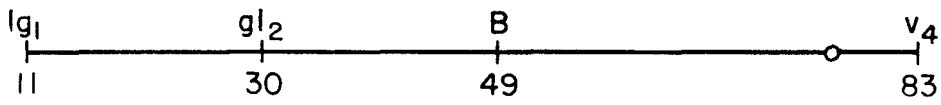

FIGURE 1.—Maize chromosome 2 linkage group genes used in this study and their assigned loci (RANDOLPH 1941b).

It was impossible to identify the B^{g} plants in the seedling stage when grown in the greenhouse during the winter. Mature plants in the field ranged in color from pronounced sun red to typical green and were therefore grouped as follows: "B1"—glumes, stalk, husks and roots well colored. "B2"—glumes, husks and roots well colored; stalk slightly colored compared to "B1." "B3"—glume receptacles of several tassel flowers usually colored, color spreading into glumes; some color in stalk particularly at base; often color in husks; roots colored (typical dilute sun red appearance). "B4"—glume receptacles of some tassel flowers may or may not be colored, color spreading little if any into glumes; often some color in stalk near ear or at base; usually faint splash of color in husks and usually none in roots. "b"—all green, no color showing, although plants with a very slight trace of possible color in some roots were placed in this class. One of the reasons for the variability in plant color was the apparent introduction of the super-*r* allele of the *R-r* genes into the plants grown in the field which were also segregating for R^{g} or r^{g}. This meant that *b* plants could be either dilute sun red, "B3," or green. Plants in the above "B1" and "B2" groups were placed in the *B* phenotypic class and those in "B3," and "B4" and "b" in the *b* class. Records seemed to indicate that the "B1" plants possessed two doses of the *B* gene compared to one in the "B2" plants. Stocks known to be $lg_1\ gl_2\ b^{g}\ v_4$ contained occasional plants which showed the characteristics described for "B4" indicating that these plants in segregating progenies were correctly designated when placed in the *b* class.

The data are based entirely on backcross populations. Most crosses were made in both directions so that crossing-over in the male parent could be compared with the female.

EXPERIMENTAL RESULTS

Diploid organisms can be only one of three possible genotypes with respect to a single pair of alleles *X* and *x*, namely *XX*, *Xx* or *xx*. Autotetraploid zygotes, on the other hand, may be classified into the following five groups: *XXXX*, quadruplex; *XXXx*, triplex; *XXxx*, duplex; *Xxxx*, simplex; and *xxxx*, nulliplex.

Table 1 shows the breeding behavior of these autotetraploid types (MULLER 1914; HALDANE 1930; and DE WINTON and HALDANE 1931).

TABLE 1

Theoretical autotetraploid single gene ratios

Genetic constitution of autotetraploid	Zygotic type	Phenotypic ratio (*X*:*x*)			
		After selfing		After backcross to *xxxx*	
		Chromosome segregation	Random chromatid segregation	Chromosome segregation	Random chromatid segregation
XXXX	quadruplex	1:0	1:0	1:0	1:0
XXXx	triplex	1:0	783:1	1:0	27:1
XXxx	duplex	35:1	21:1	5:1	11:3
Xxxx	simplex	3:1	559:225	1:1	13:15
xxxx	nulliplex	0:1	0:1	0:1	0:1

Single gene ratios: Table 2 gives the ratios obtained when plants triplex for the gene *B*, duplex for the genes Lg_1 Gl_2 *B* and V_4, and simplex for the genes Lg_1 and *B* were crossed with the nulliplex, lg_1 gl_2 b^g v_4. The results, which include observations on 21,108 plants, are not independent due to linkage.

All dominant strains were believed to be quadruplex for the Lg_1, Gl_2 and V_4 genes, but analysis of the single factor ratios revealed that some stocks, used as a source of dominant genes in part of the progenies grown in the field, were duplex for Lg_1. This explains the occurrence of simplex as well as duplex ratios for this gene in the field data. The other dominant stocks proved to be quadruplex for Lg_1 and all were quadruplex for Gl_2 and V_4 since they gave duplex ratios when their F_1's were backcrossed to the nulliplex. A few exceptions due to numerical nondisjunction occurred, however, resulting in modified ratios. These cases will be discussed under the next heading.

None of the *B* dominant strains were homozygous for this gene except possibly one which was used as a source of dominant genes in part of the progenies grown in the third planting in the greenhouse. The proof of this condition will not be known until backcross populations can be studied under optimum conditions for the expression of this character. The family showing triplex segregation for *B* apparently arose as the result of an ovule of a *BBbb* plant being self-pollinated instead of crossed with *bbbb*. This produced a *BBBb* "F_1" which was then crossed with *bbbb* giving the triplex segregation indicated in Table 2.

Families not showing clear duplex or simplex segregation for Lg_1 and *B*, but instead giving figures lying between these two types, were subjected to the standard error test for significance ($\sigma_{xn} = \sqrt{xyn}$; MATHER 1938). After statistical analysis of families which were showing, by observation, neither definite duplex nor simplex segregation of Lg_1, none exhibited significant departure from the two types of ratios. The *B* segregation in one pedigree, however, did deviate significantly from that expected on both the duplex and simplex bases, and was not, therefore, included in the table of single gene backcross ratios.

A few families which should have shown duplex segregation tended to display simplex ratios for certain genes. This was the case with gl_2 in two pedigrees which departed significantly from both duplex and simplex expectations, and the segregation of lg_1 in another culture also approached the simplex type. These three sets of figures were not included in the single factor data. The omission of this material does not have the justification it does with the *B* data above since the latter family was expected to segregate in either a duplex or simplex fashion. However, the inclusion of these figures may have distorted the true single gene ratios. The excess of lg_1 plants in the last-mentioned culture, grown in the greenhouse, was most likely due to classification before the third leaf was fairly well-developed resulting in some of the Lg_1 plants being placed in the lg_1 class. This difficulty was overcome in subsequent pedigrees of planting 3. Some cultures also required more time for the classification of this character than others even at later growth stages.

Table 2 shows that lg_1 tended to fit the expectations based on random chromatid segregation more closely than those founded on chromosome segregation. No

significant deviations from random chromatid segregation were found in plantings 2 and 3 in the duplex × nulliplex class, planting 2 in the nulliplex × duplex class, and planting 2 in the nulliplex × simplex class. Planting 2 in the simplex × nulliplex class showed no significant deviation from the expectation of chromosome segregation, and planting 2 in the nulliplex × simplex class in addition to showing no significant variation from random chromatid segregation likewise exhibited no significant difference with chromosome segregation. The probability of obtaining as large or larger deviation by chance in the case of random chromatid segregation was, however, considerably greater. The four remaining groups varied significantly from both chromosome and random chromatid segregation, two approaching the former and two the latter.

The segregation of gl_2, like lg_1, approached the random chromatid expectation more closely than it did the figures calculated on the basis of chromosome segregation. Plantings 1 and 3 in the duplex × nulliplex class, and planting 2 in the nulliplex × duplex class expressed no significant difference between the observed gl_2 segregation and that expected with random chromatid segregation. Three of the four remaining groups, although expressing significant deviations from both the chromosome and random chromatid expectations, closely approached the latter. Segregation in the fourth category was almost halfway between the two types, being a little closer to the figures calculated for the chromosome type.

The *B* ratios tended more toward chromosome segregation than to random chromatid segregation. Each of the four combined groups of families exhibited no significant departure from the figures expected on the basis of chromosome segregation. The two smaller groups, however, also fit the random chromatid expectation, one of them giving the same probability in both cases. Further proof that random chromatid segregation of this gene is also taking place is the appearance of *b* plants in the progeny of the triplex × nulliplex.

The v_4 segregation approximated the chromosome expectation, although the only case of no significant deviation from these figures was in planting 1 in the duplex × nulliplex class. All other groups, except one, more nearly agreed with chromosome segregation than with random chromatid segregation, but significant deviations were expressed. The exception was planting 3 in the nulliplex × duplex class.

DE WINTON and HALDANE (1931) found that the three linked genes they worked with in tetraploid *Primula sinensis* showed chromosome segregation. These authors believed that the most likely explanation of this situation is that the genes involved are located rather near the centromere. In the tetraploid tomato SANSOME (1933) reported that three linked factors each probably exhibited random chromatid segregation, and presented data to indicate that random chromatid segregation is very likely shown by some genes and chromosome segregation by others. The maize plant has an advantage over the above species for studies of this kind because in half of the ten chromosomes the centromere position is known with a fair degree of accuracy although in the remaining half the location is less definite (RANDOLPH 1941b). The centromere position in chromosome 2 belongs to the more certain group. The furthest gene from the

TABLE 2

Observed single factor backcross ratios of the lg_1, gl_2, B *and* v_4 *genes*

Parents	Zygotic types	Planting number and where grown*	Number of families	Observed			Expected							
							Chromosome segregation				Random chromatid segregation			
				Dominant	Recessive	Total	Dominant	Recessive	d	$\frac{d}{\sigma}$	Dominant	Recessive	d	$\frac{d}{\sigma}$
$(Lg_1)2(lg_1)2$	duplex	1 GH	†	594	215	809	674.1	134.9	80.1	7.56	635.6	173.4	41.6	3.56
×	×	2 F	17	1813	482	2295	1912.4	382.6	99.4	5.57	1803.2	491.8	9.8	0.50‡
$(lg_1)4$	nulliplex	3 GH	32	6425	1823	8248	6873.1	1374.9	448.1	13.24	6480.5	1767.5	55.5	1.49‡
		total	§	8832	2520	11352	9459.6	1892.4	627.6	15.80	8919.3	2432.7	87.3	2.00
$(lg_1)4$	nulliplex	2 F	5	291	75	366	305.0	61.0	14.0	1.96	287.6	78.4	3.4	0.43‡
×	×	3 GH	33	5793	1265	7058	5881.4	1176.6	88.4	2.82	5545.5	1512.5	247.5	7.18
$(Lg_1)2(lg_1)2$	duplex	total	38	6084	1340	7424	6186.4	1237.6	102.4	3.19	5833.0	1591.0	251.0	7.10
$(Lg_1)(lg_1)3$	simplex													
×	×													
$(lg_1)4$	nulliplex	2 F	17	623	643	1266	633.0	633.0	10.0	0.56‡	587.8	678.2	35.2	1.98
$(lg_1)4$	nulliplex													
×	×													
$(Lg_1)(lg_1)3$	simplex	2 F	9	375	407	782	391.0	391.0	16.0	1.14‡	363.1	418.9	11.9	0.85‡
$(Gl_2)2(gl_2)2$	duplex	1 GH	†	627	182	809	674.1	134.9	47.1	4.44	635.6	173.4	8.6	0.74‡
×	×	2 F	34	2825	652	3477	2897.4	579.6	72.4	3.29	2731.9	745.1	93.1	3.85
$(gl_2)4$	nulliplex	3 GH	33	6689	1740	8429	7023.9	1405.1	334.9	9.79	6622.7	1806.3	66.3	1.76‡
		total	§	10141	2574	12715	10595.4	2119.6	454.4	10.81	9990.2	2724.8	150.8	3.26
$(gl_2)4$	nulliplex	2 F	13	847	205	1052	876.6	175.4	29.6	2.45	826.6	225.4	20.4	1.53‡
×	×	3 GH	33	5621	1437	7058	5881.4	1176.6	260.4	8.32	5545.5	1512.5	75.5	2.19
$(Gl_2)2(gl_2)2$	duplex	total	46	6468	1642	8110	6758.1	1351.9	290.1	8.64	6372.0	1738.0	96.0	2.60

TABLE 2—Continued

Observed single factor backcross ratios of the lg_1, gl_2, B *and* v_4 *genes*

Parents	Zygotic types	Planting number and where grown*	Number of families	Observed			Expected							
							Chromosome segregation				Random chromatid segregation			
				Dominant	Recessive	Total	Dominant	Recessive	d	$\frac{d}{\sigma}$	Dominant	Recessive	d	$\frac{d}{\sigma}$
$(B)3b$ × $(b)4$	triplex × nulliplex	2 F	1	123	2	125	125.0	0	. . .	. . .	120.5	4.5	2.5	1.21‡
$(B)2(b)2$ × $(b)4$	duplex × nulliplex	2 F	7	277	71	348	290.0	58.0	13.0	1.87‡	273.4	74.6	3.6	0.47‡
$(b)4$ × $(B)2(b)2$	nulliplex × duplex	2 F	5	304	71	375	312.5	62.5	8.5	1.18‡	294.6	80.4	9.4	1.18‡
$B(b)3$ × $(b)4$	simplex × nulliplex	2 F	13	523	526	1049	524.5	524.5	1.5	0.09‡	487.1	561.9	35.9	2.22
$(b)4$ × $B(b)3$	nulliplex × simplex	2 F	9	387	386	773	386.5	386.5	0.5	0.04‡	358.9	414.1	28.1	2.02
$(V_4)2(v_4)2$	duplex	1 GH	†	661	148	809	674.1	134.9	13.1	1.24‡	635.6	173.4	25.4	2.18
×	×	2 F	35	2993	546	3539	2949.0	590.0	44.0	1.98	2780.6	758.4	212.4	8.70
$(v_4)4$	nulliplex	3 GH	33	6873	1556	8429	7023.9	1405.1	150.9	4.41	6622.7	1806.3	250.3	6.64
		total	§	10527	2250	12777	10647.1	2129.9	120.1	2.85	10038.9	2738.1	488.1	10.52
$(v_4)4$	nulliplex	2 F	14	985	163	1148	956.6	191.4	28.4	2.25	902.0	246.0	83.0	5.97
×	×	3 GH	33	5673	1385	7058	5881.4	1176.6	208.4	6.66	5545.5	1512.5	127.5	3.70
$(V_4)2(v_4)2$	duplex	total	47	6658	1548	8206	6838.1	1367.9	180.1	5.33	6447.5	1758.5	210.5	5.66

* GH indicates greehouse-grown plants and F field-grown plants.

† The number of families involved in this material is not known since the seed was bulked and planted by PROFESSOR L. F. RANDOLPH for a purpose in which this information was not necessary.

‡ Deviation not significant. Deviation to standard error ratios of 1.96, i.e., having a probability of 0.05–1 in 20, and higher are considered significant.

§ The total number of families is unknown because of the situation in planting 1 (see footnote †).

centromere is lg_1 which is approximately 65 units to the left of this organ, and the closest gene, v_4, is about seven units to the right of the centromere (ANDERSON and RANDOLPH 1945). The maize data show that as the loci of the genes become progressively closer to the centromere there is a corresponding shift from nearly random chromatid segregation, as was the case with lg_1, to figures approaching those expected on the basis of chromosome segregation as, for instance, was found with v_4.

Genetical evidence of numerical nondisjunction: The production of 2n + 1 and 2n − 1 gametes, involving an extra or a deficient chromosome 2, by quadruplex plants can be determined by the type of ratio in families involving quadruplex × nulliplex F_1 plants backcrossed to the nulliplex. When disjunction of the four homologous number 2 chromosomes in the quadruplex is two from two, the F_1 with the nulliplex is $XXxx$ and upon backcrossing to the recessive gives $5X{:}1x$ with chromosome segregation. If numerical nondisjunction takes place in the quadruplex resulting in three from one separation of chromosome 2, most F_1 plants involving these gametes will be either $XXXxx$ or Xxx. When backcrossed to the nulliplex the chromosome ratios will be $19X{:}1x$ and $1X{:}1x$, respectively, which are easily detected from the duplex segregation of $5X{:}1x$.

Table 3 shows the single gene backcross ratios in three families exhibiting numerical nondisjunction. Pedigree WG41–56 seems to have had an F_1 parent with the constitution $(Lg_1\ Gl_2\ V_4)3{\cdot}(lg_1\ gl_2\ v_4)2$. This type of plant would arise by the combination of a $(Lg_1\ Gl_2\ V_4)3$ gamete from the quadruplex with a $(lg_1\ gl_2\ v_4)2$ gamete from the nulliplex. Upon backcrossing this F_1 to the recessive, chromosome segregation would give 19 dominant:1 recessive and random chromatid segregation would result in 11.05 dominant:1 recessive. Table 3 indicates that in pedigree WG41–56 the lg_1 ratio did not deviate significantly from the random chromatid expectation and the same was true for gl_2 and v_4 with chromosome segregation.

Pedigrees W41–22 and W41–47 apparently were derived from F_1's containing only one dominant gene instead of the usual two. Their F_1 genotypes were, therefore, assumed to be $lg_1\ Gl_2\ B\ V_4 \cdot (lg_1\ gl_2\ b\ v_4)2$ and $Lg_1\ Gl_2\ b\ V_4 \cdot (lg_1\ gl_2\ b\ v_4)2$, respectively. When backcrossed to the nulliplex the resulting families should give 1 dominant:1 recessive with chromosome segregation and 7 dominant:8 recessive with random chromatid segregation. The observed lg_1 and gl_2 ratios in all cases in these two families were not significantly different from either the chromosome or random chromatid expectations. The B segregation in W41–22 fits the chromosome, but not the random chromatid figures; W41–47 did not involve the B gene. The v_4 ratios, except W41–47 which fits chromosome segregation, showed significant deviations from both types of expectation.

The single factor backcross segregation of the four genes in these three aberrant families, including 543 plants, is similar to that shown in Table 2 for families derived from F_1 plants possessing two of each of the dominant genes, and for pedigrees segregating in a simplex manner for Lg_1 and B due to the heterozygous nature of their parents. That is, genes located relatively far from the centromere

TABLE 3

Single gene backcross ratios in families showing numerical nondisjunction

Assumed genotypes of parents	Zygotic types	Planting number and where grown	Pedigree	Observed			Expected							
							Chromosome segregation				Random chromatid segregation			
				Dominant	Recessive	Total	Dominant	Recessive	d	$\frac{d}{\sigma}$	Dominant	Recessive	d	$\frac{d}{\sigma}$
$(lg_1)4$	nulliplex	3												
×	×													
$(Lg_1)3(lg_1)2$	"triplex"	greenhouse	WG41–56	213	19	232	220.4	11.6	7.4	2.23	212.7	19.3	0.3	0.07*
$(Lg_1)(lg_1)2$	"simplex"	2												
×	×													
$(lg_1)4$	nulliplex	field	W41–47	45	53	98	49.0	49.0	4.0	0.81*	45.7	52.3	0.7	0.14*
$(gl_2)4$	nulliplex	3												
×	×													
$(Gl_2)3(gl_2)2$	"triplex"	greenhouse	WG41–56	221	11	232	220.4	11.6	0.6	0.18*	212.7	19.3	8.3	1.98
$(Gl_2)(gl_2)2$	"simplex"	2	W41–22	112	101	213	106.5	106.5	5.5	0.75*	99.4	113.6	12.6	1.73*
×	×		W41–47	46	52	98	49.0	49.0	3.0	0.61*	45.7	52.3	0.3	0.06*
$(gl_2)4$	nulliplex	field	Total	158	153	311	155.5	155.5	2.5	0.28*	145.1	165.9	12.9	1.47*
$B(b)2$	"simplex"	2												
×	×													
$(b)4$	nulliplex	field	W41–22	119	94	213	106.5	106.5	12.5	1.71*	99.4	113.6	19.6	2.69
$(v_4)4$	nulliplex	3												
×	×													
$(V_4)3(v_4)2$	"triplex"	greenhouse	WG41–56	223	9	232	220.4	11.6	2.6	0.78*	212.7	19.3	10.3	2.45
$(V_4)(v_4)2$	"simplex"	2	W41–22	124	89	213	106.5	106.5	17.5	2.40	99.4	113.6	24.6	3.38
×	×		W41–47	58	40	98	49.0	49.0	9.0	1.82*	45.7	52.3	12.3	2.49
$(v_4)4$	nulliplex	field	Total	182	129	311	155.5	155.5	26.5	3.00	145.1	165.9	36.9	4.19

* Deviation not significant. Deviation to standard error ratios of 1.96, i e., having a probability of 0.05–1 in 20, and higher are considered significant.

tend to give random chromatid segregation and those situated close approach the expectations of chromosome segregation.

Unlike the quadruplex, the detection of numerical nondisjunction in the nulliplex is impossible by genetical methods. A *XXxxx* plant when backcrossed would give $4X{:}1x$ and a *XXx* plant $5X{:}1x$ with chromosome segregation, whereas the ordinary duplex *XXxx* would also give $5X{:}1x$ with chromosome segregation.

It was observed by KADAM (RANDOLPH 1941a) in the tetraploid maize lines he worked with that approximately one half of the plants have the balanced chromosome number and the remainder have one or a few chromosomes more or less than the balanced number of 40. If we assume that 50 percent of the quadruplex gametes are unbalanced, we would expect on a random basis that only ten percent of these would involve chromosome 2. The theoretical expectation of numerical nondisjunction is, therefore, five percent. CATCHESIDE (1956) also stated that the amount of numerical nondisjunction for each chromosome in maize is probably of the order of about five percent. All quadruplex gametes which do not have two number 2 chromosomes can easily be detected when the families backcrossed to the F_1's involving these gametes are studied. Planting 3 included the backcross progeny derived from 47 different F_1 plants and, therefore, represents 47 quadruplex gametes. Only one of these backcross families, WG41–56, produced a ratio that was not of the duplex type indicating that one unbalanced quadruplex gamete in 47, or 2.1 percent, functioned to produce an F_1 plant with the nulliplex. The percentage of numerical nondisjunction in the quadruplex stocks involved in plantings 1 and 2 could not be calculated because plant numbers were not recorded.

The difference between the theoretically expected five percent of numerical nondisjunction with any one chromosome and the observed value of 2.1 percent involving chromosome 2 in the quadruplex may be due to the relatively small number of plants upon which the latter figure is based. It is also quite possible that $2n + 1$ and $2n - 1$ gametes are largely eliminated.

It is emphasized that even though the dominant constitution of F_1 plants is accurately detectable, the complete genotype is not known owing to the little or no effect the addition or omission of recessive genes has on genetic ratios. This influences linkage. However, the proportion of F_1 plants containing more than two and less than two number 2 chromosomes carrying recessive genes is theoretically only five percent.

Theory of linkage in autotetraploids: Diploid organisms heterozygous for two linked genes can exhibit only the gametic series characteristic of coupling or repulsion. In the autotetraploid, on the other hand, there are seven possible gene arrangements with chromosome segregation and an additional six possessing the ability to segregate on the basis of random chromatid segregation making a total of 13 different combinations (DE WINTON and HALDANE 1931; SANSOME 1933). These types are presented in Table 4.

The gametic series produced with chromosome segregation in the various arrangements of two linked factors possessing the ability to segregate were developed by DE WINTON and HALDANE (1931). As the mode of segregation indi-

cates, the formulae of these workers do not consider the possibility of sister chromatids entering the same gamete, but instead suppose they always go into different gametes. Their single gene ratios in *Primula sinensis* uphold this assumption. These investigators believed that crossing-over between one pair of the four homologous chromosomes is independent of crossing-over between the remaining pair. SÖMME (1930), also working with autotetraploid *Primula sinensis*, found that crossing-over may take place between any two of the four chromosomes, a fact which substantiates DE WINTON and HALDANE's view. The chromosome segregation formulae were also developed with the stipulation that after two chromosomes have paired they must proceed to different poles. If this were not the case there would be a conversion of coupling into repulsion, a situation which would be shown by some of the XY segregants of $XY \cdot (xy)3 \times (xy)4$ when backcrossed to $(xy)4$. The theory of these formulae is further based on crossing-over involving only two chromosomes. The zygote $XYZ/xyz/xyz/xyz/$ with the four homologous chromosomes A, B, C and D, respectively, can be used to explain this belief. Chromosome A may pair with B, and crossing-over twice give XyZ/xyz and xYz/xyz gametes, or it may pair with both B and C giving xYz/xyz and Xyz/xyZ gametes. The first type of crossing-over is called recurrent and the second progressive. BRIDGES and ANDERSON (1925) found both these forms of crossing-over in the triploid *Drosophila melanogaster*. Progeny of the Xyz/xyZ gamete with $(xyz)4$ would exhibit repulsion of X and Z, a situation which is rare in *Primula sinensis* if it occurs at all. Under these conditions XyZ and xYz gametes should be produced more commonly by the zygote $XYZ \cdot (xyz)3$ than by $XYZ \cdot xyz$, provided the crossover values are the same in both. DE WINTON and HALDANE found that double crossovers are not more common in the tetraploid, not at least to any significant extent, and therefore consider only recurrent crossing-over in their formulae.

The random chromatid gametic series in Table 4 were developed by SANSOME (1933). The conditions for random chromatid segregation require that the locus of the gene be independent of the centromere in disjunction, and in tetraploids quadrivalent formation is normally required. Genetical nondisjunction will, therefore, occur in 50 percent of the first divisions, except perhaps in a region close to the centromere. With random chromatid segregation it is possible for sister chromatids to enter the same gamete by a process which MATHER (1936) calls double reduction. It was shown that the two chromatids from the same chromosome would only reach the identical gamete as a result of crossing-over between the locus and the centromere, leading to equational separation at the locus, followed by genetical nondisjunction at first anaphase, i.e. the two chromosomes which crossed over going to the same pole at first anaphase. This would allow the two sister strands to be in the same interphase nucleus, but joined to different centromeres. Double reduction leads to an excess of recessives as compared to cases where this phenomenon does not exist.

MATHER (1935) established a new segregation for the occurrence of completely equational separation. Random chromatid segregation was shown to be the result of a combination of reductional separation, which leads to chromosome segrega-

TABLE 4

Arrangements in an autotetraploid of two linked genes possessing the ability to segregate and their respective gametic series

Arrangement	Type of zygote	Types of gametes	Gametic series: Chromosome segregation	Gametic series: Random chromatid segregation
Single coupling	$XY \cdot (xy)3$	XY	$1-p$*	$2.6 - p$
		Xy	p	p
		xY	p	p
		xy	$1-p$	$3 - p$
Single repulsion	$Xy \cdot xY \cdot (xy)2$	XY	$1+p$	$1 + p$
		Xy	$2-p$	$2.25- p$
		xY	$2-p$	$2.25- p$
		xy	$1+p$	$1.5 + p$
Asymmetrical coupling	$XY \cdot Xy \cdot (xy)2$	XY	$3-p$	$13 -2p$
		Xy	$2+p$	$7 +2p$
		xY	p	$2p$
		xy	$1-p$	$6 -2p$
Asymmetrical repulsion	$(Xy)2 \cdot xY \cdot xy$	XY	$2+p$	$7 + p$
		Xy	$3-p$	$9.5 - p$
		xY	$1-p$	$2.75- p$
		xy	p	$.75+ p$
Double coupling	$(XY)2 \cdot (xy)2$	XY	$5-2p+p^2$	$33-6p+p^2$
		Xy	$2p-p^2$	$6p-p^2$
		xY	$2p-p^2$	$6p-p^2$
		xy	$1-2p+p^2$	$9-6p+p^2$
Double repulsion	$(Xy)2 \cdot (xY)2$	XY	$4 +p^2$	$24 +p^2$
		Xy	$1 -p^2$	$9 -p^2$
		xY	$1 -p^2$	$9 -p^2$
		xy	p^2	p^2
Coupling and repulsion	$XY \cdot Xy \cdot xY \cdot xy$	XY	$8+ p-p^2$	$51+ p-p^2$
		Xy	$2- p+p^2$	$15- p+p^2$
		xY	$2- p+p^2$	$15- p+p^2$
		xy	$p-p^2$	$3+ p-p^2$
Triplex single coupling	$XY \cdot (Xy)2 \cdot xy$	XY	†	‡
		Xy		
		xY		
		xy		
Triplex single repulsion	$(Xy)3 \cdot xY$	XY	†	‡
		Xy		
		xY		
		xy		
Triplex double coupling	$(XY)2 \cdot Xy \cdot xy$	XY	†	‡
		Xy		
		xY		
		xy		

TABLE 4—Continued

Arrangements in an autotetraploid of two linked genes possessing the ability to segregate and their respective gametic series

Arrangement	Type of zygote	Types of gametes	Gametic series: Chromosome segregation	Gametic series: Random chromatid segregation
Triplex double repulsion	$XY \cdot (Xy)2 \cdot xY$	XY Xy xY xy	†	‡
Triplex coupling	$(XY)3 \cdot xy$	XY Xy xY xy	†	‡
Triplex coupling and repulsion	$(XY)2 \cdot Xy \cdot xY$	XY Xy xY xy	†	‡

* p is the crossover value.
† The production of xy gametes is impossible in this case with chromosome segregation.
‡ SANSOME (1933) did not publish the gametic series expected with this gene arrangement.

tion, and equational separation in the random proportions of one-seventh reductional and six-sevenths equational separation. MATHER (1935, 1936) furthermore showed that a ratio approaching that expected on the basis of random chromatid segregation should be considered as due to a combination of the two types of separation in the correct proportions rather than to the occurrence of real random chromatid segregation. FISHER (1947) showed in detail the combinatorial and statistical problems involved in the theoretical analysis of linkage in polysomic inheritance, and included an elaboration on the multiplicity of the modes of gamete formation.

The preceding discussion of the gametic series possible with two linked genes in autotetraploids on the basis of both chromosome and random chromatid segregation, and the limitations of these formulae is intended to serve as a background for the analysis of linkage in autotetraploid maize.

Linkage: Most of the observed data were obtained from duplex × nulliplex progenies which gave the double coupling gametic series, because the duplex plants employed were derived from quadruplex × nulliplex crosses. However, since two of the three dominant stocks used in forming the duplex plants for planting 2 were heterozygous for lg_1, some backcross families in this planting showed simplex segregation for lg_1 and, therefore, exhibited asymmetrical coupling. No stocks carrying the dominant alleles were homozygous for B so this gene, like lg_1 in planting 2, also showed asymmetrical as well as double coupling. Results on B are available only from planting 2. This gene was not involved in planting 1, and its segregation could not be determined in planting 3 since the

plants were grown in the greenhouse during the winter and classified in the seedling stage. Under these conditions several families in planting 2 showed simplex segregation for both lg_1 and B and were, therefore, representing either the single coupling or single repulsion gametic series. The distance between these genes resulted in relatively large crossover classes so in almost all families it was impossible to determine which of the two gametic series was being exhibited. These data, therefore, are not included in the following observed results.

By substituting the diploid crossover value for p in the linkage formulae shown in Table 4, it is possible to determine whether the observed results are significantly different from those expected if the crossover values are the same in the diploid and autotetraploid.

Table 5 includes the linkage data found by FRASER (1939) for the lg_1, gl_2, B,

TABLE 5

Diploid backcross segregation of the linked genes lg_1, gl_2, b *and* v_4 *and their crossover values (after* FRASER *1939)*

Genes *XY*	Observed numbers					Recombinations	
	XY	*Xy*	*xY*	*xy*	Total	Number	Percent
$Lg_1\ Gl_2$	1825	442	433	1787	4487	875	19.5
$Lg_1\ B$	1485	782	712	1508	4487	1494	33.3
$Lg_1\ V_4$	1232	1035	1044	1176	4487	2079	46.3
$Gl_2\ B$	1742	516	455	1774	4487	971	21.6
$Gl_2\ V_4$	1349	909	927	1302	4487	1836	40.9
$B\ V_4$	1491	706	785	1505	4487	1491	33.2

and v_4 genes in the diploid. The $lg_1\ gl_2\ b\backslash^g\ v_4$ stocks used in his studies were related to the corresponding autotetraploid strains employed in the present investigation. FRASER did not present his data in the manner shown in Table 5, since he was interested in illustrating the use of this material in locating gene loci. F_1 plants apparently were used both as female and male parents, so if crossing-over varies in the two sexes the results represent an approximate average of crossing-over in the female and male.

Observed and expected numbers: Table 6 includes asymmetrical coupling data on 2,324 plants and Table 7 shows the double coupling results which involve 20,825 plants. All families that exhibited asymmetrical coupling also segregated in a double coupling manner for some pairs of genes, so the latter category includes both types of segregation. The total number of different plants represented in the two tables, however, is 20,983, since the double coupling data in two families, including 158 plants, showing asymmetrical coupling were not used due to the approximate simplex segregation of gl_2.

The asymmetrical coupling x^2 values for the expected numbers with random chromatid segregation shown in Table 6 are not included in several cases, since in every instance, except one, they were considerably larger than the corresponding values calculated on the chromosome segregation basis. The data in Table 6 indicate that when the diploid crossover values are substituted in the gametic

series expected with chromosome segregation in an autotetraploid there are, in most cases, no significant differences between the observed and expected segregations. This suggests that the strength of linkage of these genes is the same in the diploid and autotetraploid.

The results on double coupling presented in Table 7 are not as simple as those of asymmetrical coupling. In almost all instances the observed figures do not even approximate the segregations expected. In five cases in planting 2, however, the χ^2 values with chromosome segregation were not significant. The groups of families from which these data came include the smallest number of plants of any shown in Table 7 and, therefore, should receive less concern than the segregations in the plantings containing larger numbers. Unfortunately, most of these nonsignificant χ^2 values involved the *B* gene on which there is no information in plantings 1 and 3.

The χ^2 figures for random chromatid segregation, like those with asymmetrical coupling, were considerably larger than the corresponding values for chromosome segregation. In almost every case the Xy class contained less plants than the complementary xY group. The order of the genes in chromosome 2 and their loci are shown in Figure 1. These genes were paired in studying linkage—$Lg_1\ Gl_2$, $Lg_1\ B$, $Lg_1\ V_4$, $Gl_2\ B$, $Gl_2\ V_4$ and BV_4—so that the gene on the left is always the one farther from the centromere. Crossing-over between unlike chromosomes in the region between these two genes followed by genetical nondisjunction, which is to be expected with genes situated at some distance from the centromere since they tend to show random chromatid segregation, leads to an excess of xY gametes over Xy gametes. This situation is diagramed in Figure 2. Crossing-over between the two genes followed by genetical nondisjunction should actually lead to an increase in the XY class and decreases in the Xy and xy classes. The gametes with normal disjunction are $5XY{:}1Xy{:}1xY{:}1xy$, whereas with genetical nondisjunction they are $7XY{:}1xY$. The observed XY data were less than that expected with chromosome segregation in every case, the Xy results were not always less than those expected with chromosome segregation, but varied on both sides of these figures, and the xy numbers were larger than those expected with chromosome segregation in every instance except three. In cases involving v_4, where the centromere is between the linked genes, a crossover between the gene on the left and the centromere gives the same results as those shown in the diagram. A crossover between the centromere and the v_4 locus would result in an excess of Xy gametes as compared to the xY class. This occurrence, however, would be much less frequent due to the short distance involved. It is obvious that the data cannot be interpreted solely on the basis of crossing-over between unlike chromosomes in the region between two genes followed by genetical nondisjunction. Double reduction will account for an excess of recessives where the two genes are located on the same arm, allowing crossing-over to take place between the centromere and the gene nearest to it. It is interesting to note in double coupling that in cases where the genes are far apart the expected chromosome segregation crossover classes are considerably larger than the recessive parental type. This is a decided contrast to a diploid backcross population.

TABLE 6

Asymmetrical coupling

Parents	Observed and expected numbers	Number of families	XY	Xy	xY	xy	Total	χ^2
			Lg_1Gl_2	Lg_1gl_2	lg_1Gl_2	lg_1gl_2		
$Lg_1Gl_2.lg_1Gl_2.(lg_1gl_2)2$	Observed	16	561	35	418	190	1204	
×	Expected with chromosome seg.		562.9	39.1	440.4	161.6	1204.0	6.566*
$(lg_1gl_2)4$	Expected with random chromatid seg.		583.9	18.1	342.2	259.8	1204.0	†
$(lg_1gl_2)4$	Observed	8	319	17	240	110	686	
×	Expected with chromosome seg.		320.7	22.3	250.9	92.1	686.0	5.222*
$Lg_1Gl_2.lg_1Gl_2.(lg_1gl_2)2$	Expected with random chromatid seg.		332.7	10.3	195.0	148.0	686.0	†
			Lg_1B	Lg_1b	lg_1B	lg_1b		
$Lg_1B.Lg_1b.(lg_1b)2$	Observed	2	89	72	8	38	207	
×	Expected with chromosome seg.		92.0	80.5	11.5	23.0	207.0	11.844
$(lg_1b)4$	Expected with random chromatid seg.		98.2	61.0	5.3	42.5	207.0	4.697*
$(lg_1b)4$	Observed	3	85	69	11	32	197	
×	Expected with chromosome seg.		87.6	76.6	10.9	21.9	197.0	5.490*
$Lg_1B.Lg_1b.(lg_1b)2$	Expected with random chromatid seg.		93.5	58.1	5.0	40.4	197.0	11.764
$Lg_1B.lg_1B.(lg_1b)2$	Observed	5	99	18	95	29	241	
×	Expected with chromosome seg.		107.1	13.4	93.7	26.8	241.0	2.390*
$(lg_1b)4$	Expected with random chromatid seg.		114.3	6.2	71.0	49.5	241.0	†
$(lg_1b)4$	Observed	3	100	12	64	30	206	
×	Expected with chromosome seg.		91.6	11.4	80.1	22.9	206.0	6.239*
$Lg_1B.lg_1B.(lg_1b)2$	Expected with random chromatid seg.		97.7	5.3	60.7	42.3	206.0	12.279

TABLE 6—Continued

Asymmetrical coupling

Parents	Observed and expected numbers	Number of families	XY	Xy	xY	xy	Total	χ^2
			Lg_1V_4	Lg_1v_4	lg_1V_4	lg_1v_4		
$Lg_1V_4.lg_1V_4.(lg_1v_4)2$	Observed	17	537	86	531	112	1266	
×	Expected with chromosome seg.		535.3	97.7	519.7	113.3	1266.0	1.667*
$(lg_1v_4)4$	Expected with random chromatid seg.		587.9	45.1	385.9	247.1	1266.0	†
$(lg_1v_4)4$	Observed	9	337	38	334	73	782	
×	Expected with chromosome seg.		330.6	60.4	321.0	70.0	782.0	9.085
$Lg_1V_4.lg_1V_4.(lg_1v_4)2$	Expected with random chromatid seg.		363.2	27.8	238.4	152.6	782.0	†
			Gl_2B	Gl_2b	gl_2B	gl_2b		
$Gl_2B.Gl_2b.(gl_2b)2$	Observed	11	405	279	29	121	834	
×	Expected with chromosome seg.		387.0	308.0	30.0	109.0	834.0	4.921*
$(gl_2b)4$	Expected with random chromatid seg.		403.2	238.4	13.8	178.6	834.0	42.240
$(gl_2b)4$	Observed	8	325	226	24	102	677	
×	Expected with chromosome seg.		314.1	250.0	24.4	88.5	677.0	4.747*
$Gl_2B.Gl_2b.(gl_2b)2$	Expected with random chromatid seg.		327.3	193.5	11.2	145.0	677.0	†
			BV_4	Bv_4	bV_4	bv_4		
$BV_4.bV_4.(bv_4)2$	Observed	13	476	47	422	104	1049	
×	Expected with chromosome seg.		466.5	58.0	407.7	116.8	1049.0	4.184*
$(bv_4)4$	Expected with random chromatid seg.		497.8	26.7	309.2	215.3	1049.0	†
$(bv_4)4$	Observed	9	353	34	312	74	773	
×	Expected with chromosome seg.		343.8	42.7	300.5	86.0	773.0	4.132*
$BV_4.bV_4.(bv_4)2$	Expected with random chromatid seg.		366.8	19.7	227.9	158.6	773.0	†

* Deviation not significant. When the probability is 0.05, 1 in 20, the χ^2 value with 3 degrees of freedom is 7.815; therefore, χ^2 values this high and higher are considered as indicating a significant deviation. MATHER (1938) includes a table of χ^2.

† χ^2 calculations not completed since the value would be considerably greater than the χ^2 for chromosome segregation.

TABLE 7

Double coupling

Parents	Observed and expected numbers	Planting number	Number of families	XY $Lg_1\,Gl_2$	Xy $Lg_1\,gl_2$	xY $lg_1\,Gl_2$	xy $lg_1\,gl_2$	Total	χ^2
$(Lg_1Gl_2)2.(lg_1\,gl_2)2$ × $(lg_1\,gl_2)4$	Observed	1	*	562	32	65	150	809	
	Expected with chromosome seg.			626.7	47.5	47.4	87.4	809.0	63.110
	Expected with random chromatid seg.			613.8	21.8	21.8	151.6	809.0	94.768
	Observed	2	16	1616	105	148	301	2170	
	Expected with chromosome seg.			1681.0	127.3	127.3	234.4	2170.0	28.708
	Expected with random chromatid seg.			1646.5	58.5	58.5	406.5	2170.0	201.835
	Observed	3	32	5776	649	774	1049	8248	
	Expected with chromosome seg.			6389.5	483.9	483.8	890.8	8248.0	317.403
	Expected with random chromatid seg.			6258.3	222.3	222.3	1545.1	8248.0	†
	Observed	Total	‖	7954	786	987	1500	11227	
	Expected with chromosome seg.			8697.2	658.6	658.6	1212.6	11227.0	320.020
	Expected with random chromatid seg.			8518.6	302.6	302.6	2103.2	11227.0	2530.574
$(lg_1\,gl_2)4$ × $(Lg_1Gl_2)2.(lg_1\,gl_2)2$	Observed	2	5	267	24	21	54	366	
	Expected with chromosome seg.			283.5	21.5	21.5	39.5	366.0	6.586‡
	Expected with random chromatid seg.			277.7	9.9	9.9	68.5	366.0	†
	Observed	3	33	5178	615	443	822	7058	
	Expected with chromosome seg.			5467.6	414.1	414.0	762.3	7058.0	119.511
	Expected with random chromatid seg.			5355.4	190.2	190.2	1322.2	7058.0	†
	Observed	Total	38	5445	639	464	876	7424	
	Expected with chromosome seg.			5751.2	435.5	435.5	801.8	7424.0	120.125
	Expected with random chromatid seg.			5633.0	200.1	200.1	1390.8	7424.0	†

TABLE 7—Continued

Double coupling

Parents	Observed and expected numbers	Planting number	Number of families	XY	Xy	xY	xy	Total	χ^2
				Lg_1B	Lg_1b	lg_1B	lg_1b		
$(Lg_1B)2.(lg_1b)2$	Observed	2	1	18	1	5	5	29	
×	Expected with chromosome seg.			21.5	2.7	2.7	2.1	29.0	7.604‡
$(lg_1b)4$	Expected with random chromatid seg.			21.5	1.3	1.3	4.9	29.0	11.172
$(lg_1b)4$	Observed	2	2	123	14	17	15	169	
×	Expected with chromosome seg.			125.2	15.7	15.6	12.5	169.0	0.849‡
$(Lg_1B)2.(lg_1b)2$	Expected with random chromatid seg.			125.2	7.6	7.6	28.6	169.0	†
				Lg_1V_4	Lg_1v_4	lg_1V_4	lg_1v_4		
$(Lg_1V_4)2.(lg_1v_4)2$	Observed	1	*	503	91	158	57	809	
×	Expected with chromosome seg.			578.2	96.0	95.9	38.9	809.0	58.675
$(lg_1v_4)4$	Expected with random chromatid seg.			586.2	49.4	49.4	124.0	809.0	†
	Observed	2	16	1501	220	337	112	2170	
	Expected with chromosome seg.			1550.9	257.4	257.4	104.3	2170.0	32.224
	Expected with random chromatid seg.			1572.5	132.5	132.5	332.5	2170.0	†
	Observed	3	32	5312	1113	1415	408	8248	
	Expected with chromosome seg.			5895.1	978.2	978.3	396.4	8248.0	§
	Expected with random chromatid seg.			5977.1	503.4	503.5	1264.0	8248.0	†
	Observed	Total	‖	7316	1424	1910	577	11227	
	Expected with chromosome seg.			8024.2	1331.6	1331.6	539.6	11227.0	§
	Expected with random chromatid seg.			8135.9	685.3	685.3	1720.5	11227.0	†

TABLE 7—Continued

Double coupling

Parents	Observed and expected numbers	Planting number	Number of families	XY	Xy	xY	xy	Total	χ^2
				Lg_1V_4	Lg_1v_4	lg_1V_4	lg_1v_4		
$(lg_1v_4)4$	Observed	2	5	253	38	61	14	366	
×	Expected with chromosome seg.			261.6	43.4	43.4	17.6	366.0	8.828
$(Lg_1V_4)2.(lg_1v_4)2$	Expected with random chromatid seg.			265.2	22.3	22.4	56.1	366.0	†
	Observed	3	33	4765	1028	908	357	7058	
	Expected with chromosome seg.			5044.6	837.1	837.1	339.2	7058.0	65.970
	Expected with random chromatid seg.			5114.8	430.8	430.8	1081.6	7058.0	†
	Observed	Total	38	5018	1066	969	371	7424	
	Expected with chromosome seg.			5306.2	880.5	880.5	356.8	7424.0	64.193
	Expected with random chromatid seg.			5380.0	453.2	453.1	1137.7	7424.0	†
				Gl_2B	Gl_2b	gl_2B	gl_2b		
$(Gl_2B)2.(gl_2b)2$	Observed	2	7	249	24	28	47	348	
×	Expected with chromosome seg.			267.6	22.3	22.4	35.7	348.0	6.400‡
$(gl_2b)4$	Expected with random chromatid seg.			263.1	10.4	10.3	64.2	348.0	†
$(gl_2b)4$	Observed	2	5	279	17	25	54	375	
×	Expected with chromosome seg.			288.4	24.1	24.1	38.4	375.0	8.770
$(Gl_2B)2.(gl_2b)2$	Expected with random chromatid seg.			283.5	11.2	11.1	69.2	375.0	23.820
				Gl_2V_4	Gl_2v_4	gl_2V_4	gl_2v_4		
$(Gl_2V_4)2.(gl_2v_4)2$	Observed	1	*	539	88	122	60	809	
×	Expected with chromosome seg.			586.4	87.7	87.8	47.1	809.0	20.687
$(gl_2v_4)4$	Expected with random chromatid seg.			591.6	44.0	44.1	129.3	809.0	†
	Observed	2	34	2510	315	436	216	3477	
	Expected with chromosome seg.			2520.4	377.1	377.1	202.4	3477.0	20.383
	Expected with random chromatid seg.			2542.6	189.3	189.3	555.8	3477.0	†

TABLE 7—Continued

Double coupling

Parents	Observed and expected numbers	Planting number	Number of families	XY	Xy	xY	xy	Total	χ^2
				Gl_2V_4	Gl_2v_4	gl_2V_4	gl_2v_4		
$(Gl_2V_4)2.(gl_2v_4)2$	Observed	3	33	5706	983	1167	573	8429	
×	Expected with chromosome seg.			6110.0	914.2	914.1	490.7	8429.0	115.663
$(gl_2v_4)4$	Expected with random chromatid seg.			6163.9	458.9	458.9	1347.3	8429.0	†
	Observed	Total	‖	8755	1386	1725	849	12715	
	Expected with chromosome seg.			9216.8	1379.0	1379.0	740.2	12715.0	125.980
	Expected with random chromatid seg.			9298.1	692.3	692.3	2032.3	12715.0	†
$(gl_2v_4)4$	Observed	2	13	755	92	154	51	1052	
×	Expected with chromosome seg.			762.6	114.1	114.1	61.2	1052.0	20.009
$(Gl_2V_4)2.(gl_2v_4)2$	Expected with random chromatid seg.			769.3	57.3	57.3	168.1	1052.0	†
	Observed	3	33	4724	897	949	488	7058	
	Expected with chromosome seg.			5116.2	765.5	765.4	410.9	7058.0	111.162
	Expected with random chromatid seg.			5161.3	384.3	384.3	1128.1	7058.0	†
	Observed	Total	46	5479	989	1103	539	8110	
	Expected with chromosome seg.			5878.8	879.6	879.5	472.1	8110.0	107.071
	Expected with random chromatid seg.			5930.6	441.6	441.5	1296.3	8110.0	†
				BV_4	Bv_4	bV_4	bv_4		
$(BV_4)2.(bv_4)2$	Observed	2	7	240	37	44	27	348	
×	Expected with chromosome seg.			257.9	32.1	32.1	25.9	348.0	6.449‡
$(bv_4)4$	Expected with random chromatid seg.			257.8	15.6	15.6	59.0	348.0	†
$(bv_4)4$	Observed	2	5	269	35	51	20	375	
×	Expected with chromosome seg.			277.9	34.6	34.6	27.9	375.0	10.300
$(BV_4)2.(bv_4)2$	Expected with random chromatid seg.			277.8	16.8	16.8	63.6	375.0	†

* The number of families involved in this material is not known since the seed was bulked and planted by PROFESSOR L. F. RANDOLPH for a purpose in which this information was not necessary.

† χ^2 calculations not completed since the value would be considerably greater than the χ^2 for chromosome segregation.

‡ Deviation not significant. When the probability is 0.05, 1 in 20, the χ^2 value with 3 degrees of freedom is 7.815; therefore, χ^2 values this high and higher are considered as indicating a significant deviation.

§ χ^2 value very large; therefore, calculations not completed.

‖ The total number of families is unknown because of the situation in planting 1 (see footnote *).

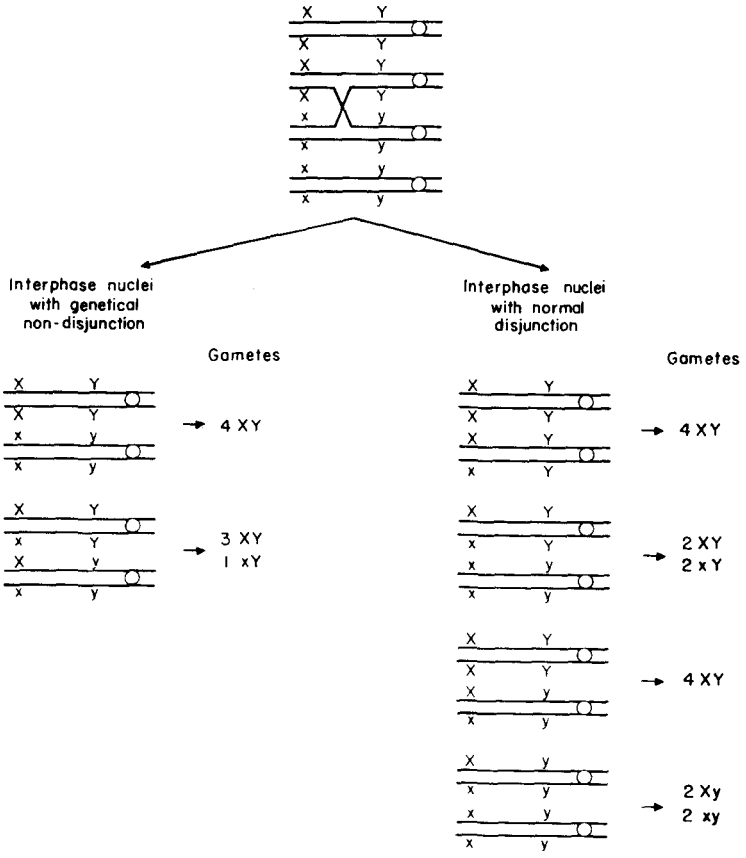

FIGURE 2.—Effect on gamete production of crossing-over between unlike chromosomes in the region between two genes followed by genetical nondisjunction.

The problem of segregation in the duplex is complicated by the fact that any chromosome may pair either with a like, an unlike, or both kinds of other chromosomes. In the simplex at any level the odd chromosome must be paired with an unlike one. MATHER (1936) found that pachytene partner exchanges should result in the duplex showing a greater increase in the number of recessive gametes over 1 in 6 than it does over 4 in 8 in the simplex. The excesses would be alike if there is no partner exchange. This situation appears to be the most probable explanation for the lack of agreement between the observed and expected double coupling data. Pachytene partner exchange in the asymmetrical coupling results, on the other hand, would not have such a great tendency to distort the observed figures.

Estimation of linkage intensity: In asymmetrical coupling the observed data which did not vary significantly from the numbers expected with chromosome segregation can be used to calculate crossover values. These nonsignificant deviations indicate that the gametic series proposed by DE WINTON and HALDANE

(1931) is correct providing the linkage intensity is the same in the diploid and autotetraploid.

The asymmetrical coupling chromosome segregation gametic series is $3-p$ XY:$2+p$ Xy:p xY:$1-p$ xy if the zygote is $XY \cdot Xy \cdot (xy)2$ and $3-p$ XY:p Xy:$2+p$ xY:$1-p$ xy if the zygote is $XY \cdot xY \cdot (xy)2$ (Table 4). The Lg_1 Gl_2 data in Table 6 where the female parent was the heterozygous one may be used to illustrate the method of calculating the crossover value, p. The numbers observed were as follows:

Phenotypes	Lg_1 Gl_2	Lg_1 gl_2	lg_1 Gl_2	lg_1gl_2	Total
Gametic series	$3-p$	p	$2+p$	$1-p$	
Observed numbers	561	35	418	190	1204

By arranging the individual terms in the gametic series in descending order—$3-p$:$2+p$:$1-p$:p—and letting a, b, c, and d represent the observed numbers in descending order, respectively, the method of maximum likelihood (DE WINTON and HALDANE 1931) shows that p is a root of

$$\frac{a}{p-3}+\frac{b}{p+2}+\frac{c}{p-1}+\frac{d}{p}=0.$$

Clearing fractions and factoring reduces this expression to $(a+b+c+d)p^3-(-a+4b+c+2d)p^2-(2a-3b+6c+5d)p+6d=0$. Since the gametic series is the same for all pairs of genes exhibiting asymmetrical coupling, this formula may be used with this type of data in all cases by merely substituting the appropriate observed numbers. The Lg_1 Gl_2 results previously mentioned give the following expression: $1204p^3-1371p^2-1183p+210=0$, where $a=561$, $b=418$, $c=190$, and $d=35$.

A cubic equation has three roots so the next point is to determine where the first significant digits of these roots lie. This can be done by assigning various values to x, obtaining the corresponding values for y and then drawing the curve. Every time y changes in sign the curve crosses the x axis which indicates that a root of the equation lies between the corresponding values of x (HART 1931). Since the crossover value, p, was known to be between 0 and 0.5, only the first digit of the roots situated in this range was determined by the graph method. The curves in all cases were sharply defined indicating that only one root lay between 0 and 0.5 and, therefore, the location of the other roots was not necessary. It is possible to be unable to detect roots that are very close together by this method, but with the number of values assigned to x together with the distinct slope of all curves this is highly improbable.

After the first significant digit of the root was obtained Horner's method (MELLOR 1909) was used to carry the approximation to four decimal places.

Now that an estimate of p has been determined it is necessary to have some measure of the confidence which can be placed in this statistic. The variance and standard error are measures of the spread of the distribution of the estimate around its true value, and so are measures of the precision with which the estimate is made. The formula employed in calculating the standard error of p (s_p) was developed from a method shown by MATHER (1938). The validity of the procedure was not proven mathematically, but seems logical since both DE WIN-

TON and HALDANE's (1931) and MATHER's (1938) formulae were derived by the method of maximum likelihood and particularly because of the way in which the former investigators arranged their equation. The expression used is as follows, where V_p is the variance—the standard error squared—and n is the total number of observations:

$$-\frac{1}{V_p} = -\frac{n}{2}\left(\frac{1}{p-3}+\frac{1}{p+2}+\frac{1}{p-1}+\frac{1}{p}\right)$$

$$= -\frac{n\,(2p^3-3p^2-5p+3)}{p\,(p^3-2p^2-5p+6)}$$

$$s_p = \sqrt{V_p}$$

The general formula for s_p, where p is derived from a diploid backcross population, is $\sqrt{\frac{p\,(1-p)}{n}}$ (MATHER 1938).

Table 8 shows the diploid crossover values taken from Table 5 with their standard errors which were calculated by the formula just mentioned. The diploid values were computed from backcross data published by FRASER (1939). This investigator did not present his results in such a way that the linkage values could be determined for each sex separately, since he was interested in other phases of the data. The diploid crossover values, however, are believed to represent an approximate average of the amount of crossing-over in the two sexes if a difference actually exists. Examination of FRASER's records showed that recent pollinations, at least, were made with F_1 plants both as female and male parents. The autotetraploid crossover values shown in Table 8 were calculated from pairs of genes showing asymmetrical coupling in planting 2. This type of gametic series was obtained in this planting because some of the quadruplex stocks used in forming the duplex plants were heterozygous for lg_1 and B, resulting in some backcross families exhibiting asymmetrical coupling of these genes with Gl_2 and V_4. Under these conditions no progenies showed asymmetrical coupling of the Gl_2 and V_4 genes. Only the groups of families showing deviations which were

TABLE 8

*Comparison of diploid crossover values and their standard errors both in percent with the corresponding autotetraploid values**

Genes	Diploid	Tetraploid female	Tetraploid male
$Lg_1\ Gl_2$†	19.5 ± 0.59	15.4 ± 1.75	13.5 ± 2.14
$Lg_1\ B$‡	33.3 ± 0.70	§	25.4 ± 6.16
$Lg_1\ B$†	33.3 ± 0.70	39.2 ± 9.39	25.0 ± 5.94
$Lg_1\ V_4$†	46.3 ± 0.74	44.3 ± 5.74	§
$Gl_2\ B$‡	21.6 ± 0.61	17.8 ± 2.31	17.7 ± 2.55
$B\ V_4$‡	33.2 ± 0.70	31.8 ± 3.31	32.0 ± 3.89

* The autotetraploid crossover values were calculated from pairs of genes showing the asymmetrical coupling gametic series, and from family groups of these genes which did not exhibit significant deviations from the numbers expected with chromosome segregation (Table 6).

† Lg_1 is simplex.

‡ B is simplex.

§ Observed segregation varied significantly from the numbers expected with chromosome segregation.

not significantly different from those expected with chromosome segregation are included in Table 8.

The significance of the differences between these crossover values was determined by the conventional formula for the standard error of a difference, i.e., $\sqrt{(s_{p1})^2 + (s_{p2})^2}$, where s_{p1} is the standard error of one of the crossover values being compared and s_{p2} the standard error of the other. Difference to standard error of the difference ratios of 1.96, having a probability of 0.05, and higher were considered significant. The autotetraploid crossover values in the Lg_1–Gl_2 region in both the female and male were significantly less than that of the diploid. The probabilities of obtaining as great, or greater differences by chance are approximately 0.03 and 0.01, respectively. All other possible combinations of the crossover values for a single pair of genes showed differences which were not significant.

DE WINTON and HALDANE (1931) compared the linkage intensities of three pairs of genes in the diploid *Primula sinensis* with the corresponding ones in the autotetraploid. The diploid female crossover values were lower than the male values, whereas in the tetraploid differences in the two sexes were absent or very slight. In each case the tetraploid values were approximately intermediate between those found in the diploid female and male. The differences between the diploid and autotetraploid, however, were not always large compared with their standard errors, and apparently the only significant difference was between the crossover values for the genes *S* and *B* on the male side of the plant. DE WINTON and HALDANE summarize their work on linkage intensity in *Primula sinensis* by saying that the crossover values are nearly, but not quite, the same in the diploid and autotetraploid. The intensity of linkage is identical in the female and male in the tetraploid.

DE WINTON and HALDANE's autotetraploid crossover values were calculated from backcross data showing the single coupling gametic series, and the maize values were computed from similar results exhibiting asymmetrical coupling. The use of simplex data in analyzing linkage groups in autotetraploids is more desirable than duplex results on two grounds: (1) there is more even segregation, i.e., the chances of obtaining recessives are greater than in the duplex and (2) one has not to consider the effect of two like chromosomes pairing as would be necessary in the duplex (MATHER 1936). The fact that a significant difference was found in only one case between the maize diploid and autotetraploid crossover values, therefore, is not conclusive evidence of the relationship of these values, since the autotetraploid linkage intensities were calculated from asymmetrical coupling data.

DISCUSSION

It is obvious that simplex data are more satisfactory than duplex results in a study of linkage in autotetraploids. MATHER (1936) showed that sister chromatids reach the same gamete as a result of double reduction. This process is dependent upon two variables: (1) the genetic distance of the locus from the centromere, this with crossing-over determining the frequency of equational

separation at the locus, and (2) the frequency of nondisjunction of the equationally separating chromosomes. Double reduction leads to an excess of recessives, and for monofactorial segregation of the simplex and duplex types MATHER (1936) derived a method for the estimation of these excess recessives. These values were termed the indices of separation and symbolized α in the simplex and β in the duplex. It was shown that there is numerical evidence for the expectation that changes of partner among the chromosomes at pachytene should result in β being greater than α; therefore, a significantly greater β than α value for the same factor pair gives genetical evidence of partner exchanges between the locus of the gene and the centromere. Subsequently, FISHER and MATHER (1943) found this assumption to be incorrect. They stated that α is sufficient to specify all segregations and that the difference in value of the indexes estimated from simplex and duplex segregations must have some other explanation.

FISHER (1944) gave a method for calculating genotype frequencies in tetrasomic inheritance which takes double reduction into account. LITTLE (1945, 1958) reviewed the literature on gene segregation in autotetraploids and in the later paper presented the values of α for the lg_1 gl_2 B v_4 genes in autotetraploid maize which he had calculated from the single gene data of WELCH (1942, 1943). The results were as follows:

Gene locus	α	Crossover units from centromere
lg_1	0.228 ± 0.017	65
gl_2	0.215 ± 0.017	46
B	0.109 ± 0.050	27
v_4	0.086 ± 0.016	7

The order of magnitude of double reduction, shown by the values of α, is the same as the order of magnitude of the crossover distances from the centromere. LITTLE (1958) did not state whether the α values were calculated from simplex or duplex data and whether he used the formulae of MATHER (1936) or those of FISHER and MATHER (1943). It appears that LITTLE (1958) used the method introduced by MATHER (1936). These α values are double the magnitude of those computed by the FISHER and MATHER (1943) method.

Prior to the work of CATCHESIDE (1956), the effect of numerical nondisjunction had not been separated from double reduction. This investigator, working with maize, showed that the duplex index should exceed the simplex index by a definite amount based on the frequency of numerical nondisjunction for the locus. CATCHESIDE (1959) also found that the differences in indexes of double reduction (α) calculated from simplex and duplex data for various loci in the tomato and potato could be accounted for by the complicating effects of numerical nondisjunction.

Since DE WINTON and HALDANE's (1931) formulae are based entirely on chromosome segregation and, therefore, do not consider double reduction, their use with genes situated relatively far from the centromere is inaccurate. With double coupling the linkage intensity of two genes both located at some distance from the centromere and also themselves rather widely separated would be

further affected by pachytene partner exchanges. The crossover values calculated from asymmetrical coupling data by the DE WINTON and HALDANE method do not give as poor an estimate as the values computed from double coupling results, assuming there are partner exchanges, because the excess recessives in the former are proportionately less. In addition to the disproportionate increase in excess recessives in the duplex compared with the simplex as a result of pachytene partner exchanges, there is another disadvantage with duplex data, i.e., that segregation is less even than in the simplex.

MATHER (1936) derived formulae for the calculation of the linkage intensity from single coupling and single repulsion data which take double reduction into account. Attention was confined in the development of these expressions to the case of close linkage where the occurrence of double crossing-over and pachytene partner exchanges are rare enough to be neglected. These formulae, in general, give higher crossover values than the expressions of DE WINTON and HALDANE.

There seems to be no simple formulae to use in place of DE WINTON and HALDANE's (1931) for the estimation of large recombination values, because the number of variables is too great to handle. Hence it would appear that one can place very little faith in the accuracy of estimation of linkage values of over about 15 percent, on account of the possibility of the occurrence of double crossing-over, so introducing the question of interference, and of partner exchanges of the chromosomes at pachytene, which profoundly affect the relations of recombination and crossing-over. While the strands taking part in any number of chiasmata involving detectable crossing-over cannot show more than 50 percent recombination, there can, as a result of pachytene partner exchanges, be more than 50 percent recombination gametes in the case where the factors are in single coupling, but not in the case of single repulsion (MATHER 1936). Unfortunately, the DE WINTON and HALDANE method is the only one available for estimating linkage intensities of widely separated genes.

Where there is a change of partner there will be less double crossing-over than in the diploid. However, there is a further point, the result of these changes of partner being limited in number. In calculating coincidence values the formula $\frac{xn}{ab}$, where x is the number of double crossovers, a and b the number of singles in the two regions and n the number of individuals, is used. This formula, if applied to tetraploids, implies that crossing-over in any one region involves the four chromosomes at random with relation to the other region. Since the number of changes of partner of the chromosomes at pachytene is limited, it follows that crossing-over in one region is more likely to involve the same pair of chromosomes than would be the case if pairing were at random. Hence the frequency of double crossover strands will be greater as compared with the frequency of single crossovers than would be the case if pairing were at random. The coincidence value obtained will be high and may even exceed 1, and as such seems to lose much of its meaning in the case of autotetraploids (MATHER 1936).

Studies on linkage intensity in autotetraploids should be made with single coupling data, because pachytene partner exchanges have less effect in increasing

the excess recessives than they do with duplex data. There is also more even segregation with single coupling results, thus allowing smaller numbers to yield accurate data. No formulae have been devised which take pachytene partner exchanges completely into account. MATHER's (1936) method, however, was developed for closely linked genes showing the single coupling gametic series and considers double reduction, while the corresponding DE WINTON and HALDANE (1931) formula is based entirely on chromosome segregation. In spite of the fact that the procedure of the latter investigators takes neither partner exchanges nor double reduction into account there is no other method which can be used for genes having large recombination values. With present formulae, therefore, accurate crossover values in autotetraploids can be obtained only from genes showing less than 15 percent recombination.

Although each of the three regions in autotetraploid lg_1 gl_2 b v_4 maize is more than 15 units in length, single coupling data would permit the calculation of α, the index of double reduction. However, it is doubtful whether even single coupling results analyzed by MATHER's (1936) method would give precise crossover values, due to double crossing-over and pachytene partner exchanges between the respective pairs of genes under consideration.

FISHER (1947) demonstrated the combinatorial and statistical problems involved in the theoretical analysis of linkage in polysomic inheritance. In studying linkage in tetrasomics, FISHER (1949) showed the value of using the offspring of the first backcross to perform a second backcross to the recessive and presented the crossover and double reduction results, based on second backcross progenies, for two loci in *Lythrum salicaria*.

It would appear that a highly desirable combination of genes, from a spacing standpoint, with which to study linkage in autotetraploid maize could be selected in chromosome 5. The chief difficulty would be the time required in developing a good tetraploid stock. Before attempting an analysis of linkage it would be advisable to inbreed both the mutant and normal tetraploid strains for several generations. Maternal diploid stocks could be acquired which would be identical with the tetraploids, except that they would contain two sets of chromosomes instead of four. An opportunity would then be available to determine accurately the linkage intensities in autotetraploid maize, and to compare them with the corresponding diploid values. Pollinations should be made in such a way that crossing-over could be studied separately in the female and male.

SUMMARY

Autotetraploids have no strict segregation expectations for any gene, since segregation is dependent on the way in which the eight chromatids of the first meiotic division separate into four pairs during gamete formation. This leads to a number of complications in the analysis of linkage in autotetraploids.

The linked genes employed in this investigation are located in the chromosome 2 linkage group of maize; their symbols and loci in the diploid are as follows: lg_1 11, gl_2 30, B 49 and v_4 83. The centromere is located approximately seven units to the left of v_4.

The lg_1 and gl_2 single gene ratios each tended to fit the expectations based on random chromatid segregation, and the B and v_4 results approached chromosome segregation.

Numerical nondisjunction was exhibited by 2.1 percent of the quadruplex gametes studied in one experiment compared with a theoretical expectation of five percent.

Asymmetrical coupling data showed that in most cases there were no significant differences with chromosome segregation between the observed and expected numbers. This suggests that the strength of linkage of these genes is the same in the diploid and autotetraploid.

The asymmetrical coupling data which did not vary significantly from the numbers expected with chromosome segregation were used to calculate crossover values. The autotetraploid crossover values in the Lg_1—Gl_2 region in both the female and male were significantly less than that of the diploid. All other possible combinations of the crossover values for a single pair of genes showed differences which were not significant.

Weaknesses of the formulae available for the estimation of large recombination values in autotetraploids are discussed.

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