	PA	Pa	pA	pa
Observed	1696	58	63	1
Calculated (chromosome seg.)	1718.4	49.1	49.1	1.4

Here, the Pa and pA classes are about equal in frequency. The ratio for P vs. p is 1754:64 or 3.53% p; that for A vs. a is 1759:59 or 3.25% a. The expected percentages for chromosome segregation and for maximum equational segregation are 2.8 and 5.19 respectively. The data show the two loci are similarly placed and relatively close to the centromeres.

POLYPLOID RATIOS IN FUNGI. Genetic markers may be used in yeasts and in Neurospora to permit genetic tests for polyploidy. For examples of polyploid segregations in yeast see Leupold and Hottinguer (1954), and Leupold (1956).

Rates of approach to homozygosis in tetraploids vs. diploids.

Haldane (1930), and Bartlett and Haldane (1934) have considered the effects of different types of mating on rates of decrease of heterozygosis in autopolyploids. The proportions of single factor homozygotes expected from selfing two autotetraploid genotypes, as calculated by Greenleaf (1938) for two types of segregation are shown in Table 94. The values for maximum equational segregation (double reduction = 1/6) have been added, since this is the theoretical maximum rather than 1/7 (see pages 186 and 188).

Table 94. Effect of autotetraploidy on percent of homozygosis at a single locus in successive generations of selfing (Greenleaf, 1938, from Table 5, page 463, Jour. Heredity 29, except columns 5 and 8).

		Percent of homozygosis					
		Tetraploid Aaaa			Tetraploid AAaa		
Generations selfed	Diploid Aa	Chromosome seg.	Random Chromatid seg.	Max. equat. seg.*	Chromosome seg.	Random Chromatid seg.	Max. equat. seg.*
1	.5	.25	.2883	.2951	.05	.0918	.0988
2	.75	.38	.4498	.4602	.194	.2714	.2848
3	.875	.4931	.5688	.5811	.3256	.4257	.4420
4	.9375	.5579	.6614	.6743	.4375	.5486	.5658
5	.96875	.6483	.7339	.7467	.5312	.6453	.6623

* Calculated by Neal Tuleen, checked by M. L. Wright.

As shown in Table 94, the rate of approach to homozygosity is expected to be much slower in autopolyploids than in diploids and slower with chromosome than with maximum equational segregation.

Parsons (1959), in considering the effect of different values of α on the rate of approach to homozygosity, has not included the value of 1/6. Geiringer (1949) has presented a mathematical treatment of the expectations from random mating and random chromatid segregation in autopolyploids.

Linkage in autopolyploids

(Fisher 1947, 1948, Gates 1957)

Kinds of genotypes. As stated by Fisher linkage in autopolyploids as compared with diploids is much more complex because of the greater number of possible "gametic geno-

types" and also a greater number of possible "modes of gamete formation". These will be explained later. In diploids the frequencies of the different kinds of gametes can be determined in the progeny of the first backcross. In a diploid heterozygous at two loci there are only two possible heterozygotes, one with the genes arranged in coupling $\underline{AB}$. $\underline{ab}$ and one arranged in repulsion $\underline{Ab}$. $\underline{aB}$. In a tetraploid heterozygous at two loci there are three possible heterozygotes at each locus A^3a, A^2a^2, Aa^3 and B^3b, B^2b^2, Bb^3, making a total of 9 combinations. The general formulas for these are shown in the following double entry table.

	For B vs. b		
For A vs. a	BBBb	BBbb	Bbbb
AAAa	$A^3a\ B^3b$	$A^3a\ B^2b^2$	$A^3a\ Bb^3$
AAaa	$A^2a^2\ B^3b$*	$A^2a^2\ B^2b^2$	$A^2a^2\ Bb^3$*
Aaaa	$Aa^3\ B^3b$	$Aa^3\ B^2b^2$	$Aa^3\ Bb^3$*

* Linkage data from these genotypes were analyzed by de Winton and Haldane (1931).

For each combination, the genes may be arranged on the four members of the tetrasome in more than one way. The $A^2a^2\ B^2b^2$ combination may occur in three different genotypic arrangements on the chromosomes, as follows:

$$\begin{matrix}\underline{A\ B}\\ \underline{A\ B}\\ \underline{a\ b}\\ \underline{a\ b}\end{matrix} \quad \text{or} \quad \begin{matrix}\underline{A\ B}\\ \underline{a\ b}\\ \underline{A\ b}\\ \underline{a\ B}\end{matrix} \quad \text{or} \quad \begin{matrix}\underline{A\ b}\\ \underline{A\ b}\\ \underline{a\ B}\\ \underline{a\ B}\end{matrix}$$

Each of the other eight combinations can be arranged in two different ways on the chromosomes, making a total of 19 possible chromosomal genotypes in all. For example, for the $Aa^3\ Bb^3$ combination the arrangments or genotypes are:

$$\begin{matrix}\underline{A\ B}\\ \underline{a\ b}\\ \underline{a\ b}\\ \underline{a\ b}\end{matrix} \quad \text{or} \quad \begin{matrix}\underline{A\ b}\\ \underline{a\ B}\\ \underline{a\ b}\\ \underline{a\ b}\end{matrix}$$

Many of these genotypes are not easily attainable experimentally.

Fisher's method (modified slightly) of denoting the various genetic constitutions of the four chromosomes in the quadrivalent is based on a double entry table. For example, an AB · Ab · aB · ab genotype is represented as $\frac{1\ 1}{1\ 1}$, in which each of the four positions corresponds to that in the double entry table:

	B	b
A	AB	Ab
a	aB	ab

An AB · $(ab)^3$ genotype is $\frac{1\ \cdot}{\cdot\ 3}$.

In this genotype, the pairing of AB with any of the other three will give recognizable crossover products. The same is true for the following genotypes: $\frac{\cdot\ 3}{1\ \cdot}$, $\frac{\cdot\ 1}{3\ \cdot}$, $\frac{3\ \cdot}{\cdot\ 1}$. Hence these four (all positions of 3-1 in the squares) belong to an "isomorphic" set, the members of which will furnish equivalent information "in the sense that they yield gametic series with the same frequencies." The analysis of more than one member of such a set, except for the reduction in sampling variation, adds no additional information. They may be of value in eliminating disturbances due to differential viability. Those belonging to different isomorphic sets may furnish supplemental information on linkage.

Modes of gamete formation

What is meant by "modes of gamete formation" and "gametic genotypes" may be illustrated for a single locus, then for two linked loci in a tetrasome. For a single locus, there are only two modes of gamete formation, i.e. the two alleles in the gamete may be derived from different chromosomes or from the same chromosome of the parent. In the latter, the two alleles are identical, since they are from sister chromatids and result from the process of double reduction. As to the number of "gametic genotypes" for a single locus, there are 6 combinations of different chromosomes and four of sister chromatids; or a total of ten. For the number of "modes of gamete formation" for a particular genotype involving different numbers of loci in a tetrasomic, Fisher gives the formula: $1/3\ (4^{2h-2} + 4^h + 1)$, where h is the number of heterozygous loci. For h = 1, there are two modes, for h=2 there are $1/3\ (4^2 + 4^2 + 1) = 11$. These 11 possible modes of gamete formation (really combinations of portions from the chromosomes of the parent heterozygote) for a genotype with four different alleles at each locus, e.g. $\underline{a_1b_1}$, $\underline{a_2b_2}$, $\underline{a_3b_3}$, and $\underline{a_4b_4}$ as listed by Fisher, are shown in Table 95. A description of the nature of each mode of formation has been added. This tabulation may be applied to any of the 19 genotypes mentioned on page 197.

Table 95. Modes of gamete formation for two loci in a tetrasome whose chromosomes and genetic constitution are $\underline{a_1\ \ b_1}$ $\underline{a_2\ \ b_2}$ $\underline{a_3\ \ b_3}$ $\underline{a_4\ \ b_4}$

Mode of formation	One typical gamete	Description	No. of gametic genotypes
1	a_1b_1/a_2b_2	Both non C.O., non sisters*	6
2	a_1b_1/a_1b_1	Both non C.O., sisters** at both loci	4
3	a_1b_1/a_2b_3	One " " , one C.O., both non sisters	24
4	a_1b_1/a_1b_2	" " " , " " , sisters for 1 locus	12
5	a_1b_1/a_2b_1	" " " , " " , " for other locus	12
6	a_1b_2/a_3b_4	Both C.O., both non sisters	12
7	a_2b_1/a_3b_1	" " , sisters for one locus (b_1b_1)	12
8	a_1b_2/a_1b_3	" " , sisters for the other locus (a_1a_1)	12
9	a_1b_2/a_2b_3	" " , both non sisters, one gene at one locus and one gene at the other locus from the same chromatid pairs (a_2b_2)	24
10	a_1b_2/a_1b_2	Both C.O., sisters at both loci	12
11	a_1b_2/a_2b_1	" " , non sisters but complementary	6
			136

* from different pairs of chromatids

** from sister chromatids

Note that the two chromosomes in the gamete may be: both non-crossovers, both crossovers, or one a non-crossover the other a crossover; and that both may be non-sisters or sisters, at both loci; or sisters at one locus, not at the other. The number of different gametic genotypes possible for a particular mode of gamete formation is shown in the last column. For example a_1b_1/a_1b_1 is listed as a typical gamete for this mode of formation, #2; but a_2b_2/a_2b_2, a_3b_3/a_3b_3 and a_4b_4/a_4b_4 are exactly analogous, making a total of four gametic genotypes for this mode of gamete formation. Note also that those numbered 4, 5, 7 and 8 in Table 95 are the result of double reduction at one locus, while 2 and 10 are the result of double reduction at both loci. Also note that in 1 and 2 there are no recombinant chromosomes; in 3 to 5 there is one recombinant chromosome, while in the remainder both are recombinants. The relative frequencies of these will depend on the strength of linkage between the genes and between the genes and the centromere. The data may be disturbed to some extent by 3-1 disjunctions.

Methods used for Lythrum

As pointed out earlier, in polyploids the progeny of the first backcross are not sufficient to determine the frequencies of the different kinds of gametes formed by the heterozygote.

The need for a second backcross may be illustrated by the fact that a plant in the first backcross progeny with an AB doubly dominant phenotype may have resulted from any one of five possible gametes, i.e. AB . AB, AB . Ab, AB . aB, AB . ab, or Ab . aB. A second backcross progeny is expected to distinguish them.

An analysis of linkage based on second backcross data in autotetraploid Lythrum salicaria, the wild purple loosestrife, segregating for two linked loci, Rr for Purple vs rosy or pink flower color and Ss for style length was reported by Fisher (1948). Plants with the RS . rs . rs . rs (bisimplex coupling) and Rs . rS . rs . rs (bisimplex repulsion) genotypes were analyzed. From the first backcross of each to the multiple recessive, four phenotypes would be expected and were observed: RS, Rs, rS, and rs. The plants that were RS were backcrossed again and the progeny of each of 148 plants were grown and classified (50 to 100 plants each) to determine the genotypes. Only the corrected proportional frequencies were reported (Table 96).

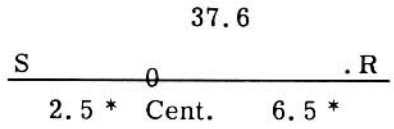
Table 96. Results of second backcross tests of individuals belonging to different phenotypes of the first backcross (Fisher 1948).

Phenotype in 1st backcross	Genotypes as determined by 2nd backcross	Relative numbers observed	
		Coupling	Repulsion
RS	RS/RS	0	0
	RS/Rs	1.135	0.874
	RS/rS	0.567	0.291
	RS/rs	34.054	3.206
	Rs/rS	1.135	19.819
Rs	RR or Rr*	17.724	26.658
rS	SS or Ss*	15.398	28.792
rs	rs/rs	29.986	20.360
	Total	99.999	100.000

* Not separated

Note the differences in relative frequencies of the RS/rs vs. Rs/rS genotypes in the two populations: 34.0 and 1.1 vs. 3.2 and 19.8.

After obtaining the numbers of the different kinds of gametic genotypes contributed by the F_1, the recombination values were determined by matrix analysis (for details of the method, see Gates, 1957). For each genotype the expectations from each mode of formation were set up in matrix form for which sets of simultaneous equations could be derived. Using these with the observed data, Fisher determined the recombination value between the two genes to be 37.6%. He reported that for the R gene $1\frac{1}{2}$% of the progeny were from spores that had received RR. This corresponds to 6% double reduction. For S, the percentage of double reduction was 2.5%. Had the R-s and rS- phenotypes been tested, the percentages of spores that were double reductional for the dominant markers could have been calculated directly from the data. Since simultaneous double reduction for both loci was not observed (no plants from RS/RS gametes), while there was double reduction for R and S independently, he concluded the centromere must be between the two genes. The information was plotted on a map as follows:



* frequency of double reduction

He was able, thus, to plot gene and centromere positions. Recombination values determined by this mode of analysis may be compared with those determined in diploids.

In later tests of plants triplex for the flower color gene, only one plant in 2,057 progeny or .049% was nulliplex, i.e. .19% double reduction (Fisher and Fyfe 1955). Non-random open pollinations of the earlier bisimplex plants were believed to account for the difference. The position of the centromere is probably correct.

Methods used in Primula

A preliminary analysis of linkage in F_2 and reciprocal backcross data from autotetraploid Primula sinensis was reported by de Winton and Haldane (1931). The data were from genotypes that were simplex or duplex at each of two linked loci, or simplex for one and duplex for the other. A few crosses involved three linked loci. They approached the problem of determining recombination values by comparing the observed results with those expected based on several assumptions, including: 1. the chromosomes pair at random as bivalents, 2. crossing over occurs when the chromosomes are single-stranded, and 3. the chromosomes that crossover do not ever pass to the same pole. To determine for two linked loci the frequencies of the various kinds of gametes p and 1-p were used to denote the frequencies of recombinations and parental combinations, respectively, from each bivalent. The sum of the expressions in terms of p for all the possible kinds of bivalents furnished the general expressions for the frequencies of the different kinds of gametes. Sets of expressions were derived for each heterozygous genotype they studied for gametic frequencies and for expectations in F_2. Expressions for gametic frequencies for two genotypes when "only equational segregation", i.e. maximum equational segregation, occurs were listed by Sansome andPhilp, p. 195 (1939). Trial-and-error substitution of p values in these expressions was used to determine the recombination values. Maximum likelihood formulas should furnish a better solution. The method as used by de Winton and Haldane may be of use for autopolyploid species in which multivalents are rare.

The problem of double reduction was mentioned by de Winton and Haldane (1931).

For the loci they studied, crosses with the triplex genotype ($A^3a \times a^4$) gave no recessive offspring. The following recombination values involving three linked loci, and tests in ♂ and ♀ in diploid and tetraploids were reported, in percent with standard errors:

Factors	diploid ♀	diploid ♂	tetraploid ♀	tetraploid ♂
SB	7.35 ± 0.40	12.91 ± 0.50	8.01 ± 0.69	8.41 ± 0.99
SG	33.29 ± 0.74	40.47 ± 0.78	37.58 ± 1.92	38.91 ± 2.23
BG	31.15 ± 0.53	36.24 ± 0.68	35.18 ± 1.85	34.38 ± 2.17

In the ♀, the recombination value between S and G was higher in the tetraploid than in the diploid. Less recombination was observed in the eggs than in the pollen in the diploid, but the values in the tetraploid were essentially the same.

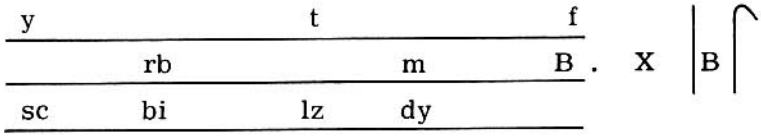
Data have been reported in maize (Murray 1944) for the lg gl_2 B v_4 markers on chromosome 2 which show higher recombination values in the tetraploid than in the diploid in the B-v_4 region near the centromere, but lower values between lg - gl_2 and gl_2 - B. This appears to agree with the Drosophila results. However, data obtained by Welch (1962) showed lower values in the tetraploid for both regions.

Methods applied to Drosophila triploids

The first data on linkage in an autopolyploid were obtained for the X-chromosome in Drosophila triploids by Bridges and Anderson (1925). The genetic markers used and their positions in the linkage map were as follows:

y	sc		bi	rb		t	lz		m	dy		f	B
0.0	0.0+		6.9	7.5		27.5	27.7		36.1	36.2		56.7	57.0
region		1			2			3			4		

Triploid females whose three X-chromosomes carried the genes shown in the following diagram were crossed with Bar males:



Note that in the flies used for the study each region was bounded by two very closely linked markers which marked two of the three chromosomes.

The X-chromosome constitutions of 182 exceptional diploid daughters (their two X-chromosomes coming from the ♀, none from the ♂) were determined by breeding. Only these data were used in the analysis. The recombination values obtained in the triploid and in the diploid check are shown in Table 97.

Table 97. Recombination values in the X chromosomes of Drosophila triploids as compared with those in the diploid check and in the standard map.

Region	3n	2n	standard
1	14.3	6.9	7.5
2	11.3	22.8	20.5
3	3.9	3.9	5.7
4	8.2	16.2	20.8
Total	37.7	56.0	57.0

As shown in Table 97, crossing over in region 1 was twice that in the diploid controls, that in regions 2 and 4 about half as much.

Data on crossing over in chromosomes II and III of Drosophila triploids have been reported by Redfield (1930 and 1932). In her experiments one of the three homologues carried all the recessive markers. The analysis was based on the diploid offspring, using corrections for undetectable crossovers (see her 1930 paper for explanations of the corrections).

The calculated results for chromosome 2 are summarized in Table 98.

Table 98. Recombination values in chromosome 2 of Drosophila triploids as compared with those in the diploid check.

	3n	2n	difference 3n - 2n
al - dp	6.7	6.9	-0.3
dp - b	19.4	26.8	-7.4**
b - pr	9.6	11.3	-1.7*
Cent.pr - c	27.6	20.4	+7.2**
c - px	13.0	21.4	-8.4**
px - sp	6.2	8.4	-2.2*
total	82.5	95.2	-12.7**

* = significant ** highly significant

Near the centromere region, crossing over was higher in the 3n, the 3n/2n value being 1.4. There was a decrease at the ends, but the greatest decrease was in the middle of each arm, 3n/2n being about .7 in one arm and .6 in the other. In general, for the X, II and III chromosomes, regions in which genes are clumped in the diploid linkage map show increased crossing over in the triploid, while regions in which the genes are far apart in the diploid map show decreased crossing over in the triploids. The cross-over values in the triploid, therefore, more nearly indicate the relative physical distances between the genes in the chromosomes. In certain cases, genes that appeared to be completely linked were introduced into triploids in an attempt to obtain the recombination. One example is the yellow scute recombination that was obtained in this way.

Methods applied to trisomic Lycopersicon

Linkage data from a Triplo-A (trisome attached to the nucleolus) cross in tomato involving three linked markers: d_1 Ps/D_1 pS/D_1Ps/ x d_1d_1 ppss diploid were reported by Lesley (1937). The constitution at each locus was of the type AAa. Lesley calculated the results on the basis of the theoretical types of crossovers which should occur, from which an estimate of the actual recombination percent was made. Crossing over between d_1 and s in published tests using the 2n heterozygote as the female parent was 28.3%. In the 2n + 1 it was about 30%.

Special uses and applications of polyploids are discussed in Chapter 9.

CHAPTER 7. AUTOPOLYPLOIDY

Polyploidy in animals. Polyploid larvae (n, 3n, 4n, and 5n, also 2n + 2) occur naturally in the newt (Triturus viridescens) (Fankhauser, 1941). Also triploids were induced by temperature shock treatments immediately after fertilization.

Triploid chickens have been reported; the first one, the result of parthenogenesis, lived for 10 months (Sarvella, 1970). Its sex chromosome constitution was zzW. Triploid chickens have been reported also by Abdel-Hameed and Shoffner (1971).

Haploids. By using cytoplasmic male sterility for pollination control in 4n x 2n crosses, and color markers, numerous haploids were produced in alfalfa (a total of 178 from all sources). The numbers for different seed parents varied from 0 to 10 haploids per 1,000 flowers pollinated. Also the frequency was affected by the pollinator sources (Bingham, 1971).

In barley large numbers of haploids were obtained by culturing embryos from the interspecific hybrids, Hordeum vulgare (2n = 14) x H. bulbosum or its reciprocal (Kao and Kasha, 1969; Lange, 1969; Kasha and Kao, 1970). Progeny were obtained from about 17% of the cultured embryos. All but one of 356 were vulgare type haploids. This results from progressive loss of the bulbosum chromosomes during embryo development, at 3 days 37% of cells with none, at 11 days 94%. If V = the vulgare genome, B = the bulbosum genome; crosses involving tetraploids gave the following results: VV x BBBB gave triploids (VBB = 21 chromosomes; VVVV x BBBB or x BB gave VV = 14 - chromosome plants (Subrahmanyam and Kasha, 1973).

In maize, when plants homozygous for the indeterminate gametophyte factor (ig) are crossed with normal pollen, androgenetic haploids and diploids are obtained (Kermicle, 1973). Among a total of 9256 plants there were 136 (1.47%) with n and 15 (0.16%) with 2n from the male parent and the cytoplasm of the female parent. This offers a method of transferring at one step the male parent genome into the cytoplasm of a different line.

Linkage in autopolyploids. Linkage estimates in autotetraploid maize were made using double and single backcrosses (Bingham, Burnham and Gates, 1968). Matrix procedures for the double-backcross analysis are described and equations derived for calculating estimable modes of gamete formation. Only the double-backcross method was capable of identifying all the original recombinant and double reduction gametes.