were extremely uniform, but succeeding generations were less and less uniform, probably as the result of mutations. As a check on their mode of origin, East used hybrids of Fragaria vesca which were heterozygous for two pairs of genes, R vs. r for red vs. white fruit and P vs. p for pink vs. white flower. These were pollinated by F. chiloensis or F. virginiana (2n=56). Maternals of the RP, Rp, rP and rp phenotypes were obtained, all with the diploid chromosome number and homozygous. This is indisputable evidence that their origin is from haploid cells followed by chromosome doubling. In corn, the frequency appears to be very low, but if it could be increased in some manner, the problem of the low fertility of haploid plants would be circumvented. East pointed out the potential value of such behavior to agriculture.

The two brown (androgenic) monoploids listed in Table 82 were from a seed with twin embryos (Gerrish 1956). These are of interest because they have the ♀ cytoplasm along with the complete set of genes from the male parent. Conversion of inbred lines to cytoplasmic sterile lines could be accomplished in one step (Chase 1951a).

The intense sun red diploids were probably the result of mutations of Pl to pl. The purple diploids were probably the result of misclassification for green vs. purple roots in the seedlings. The brown diploids may have been mutants of A to a; or androgenic diploids (page 180).

Of the 328 monoploids that reached tasseling stage, 91 were selfed, of which 18 set seed (1 to 10 seeds per ear); 165 were tassel sterile and were crossed as the ♀ parent. Forty-four of these 165 pollinations set seed with 1 to 46 seeds per ear. Of the 67 open pollinated monoploids, 42 set seed with 1 to 27 seeds per ear.

From the eight monoploids having self seed in one experiment, five homozygous diploids were established. These represent about 2% of the original seedlings with green roots. The plants were grown in the field. This may account for the low rate.

One might expect the survival of only those monoploids having gene combinations which result in the greatest vigor. As a result of this natural selection, one might expect diploid lines established from haploids to be superior to inbreds established from the same material by conventional methods. In extensive tests of homozygous diploids in sweet and field corn established by Chase, and of unselected inbreds from the same material, Thompson (1954) found no significant differences in topcross yields, also the homozygous diploids were not superior in root or stalk characters. No comparative tests have been made on haploids and inbreds derived from open pollinated material.

The production of haploids and of diploids from haploids has applications to basic problems as well. In tomatoes and in Datura, diploid lines have been produced from haploids, and tetraploids in turn from those diploid lines. As Chase has stated it "As tools for experimental research monoploids offer many possibilities: in the cytological field for studies of the meiotic distributions of unpaired chromosomes, non-homologous synaptic relations of the chromosomes and mechanics of mutational effects, measurement of mutation rates, studies of cytoplasmic effects, and biochemical investigations; in the agronomic field for the production of diploid, homozygous stocks directly from the monoploids." A study of the progressive change from great uniformity to greater and greater variability should be possible utilizing diploid lines established in this manner. Multiple interchange stocks might be an aid in analyzing the changes.

Theoretical genetic ratios for a single locus

As noted in Chapter 6, the additional homologous chromosomes in trisomics and tetrasomics increase the number of possible heterozygous genotypes and also affect the

expected segregation ratios for genetic characters. In an autotriploid every chromosome is expected to be present as a trisome; in an autotetraploid, every one as a tetrasome. The ratios for individuals heterozygous at a single locus in autotriploids should be similar to those for trisomics; except for the possible effect of higher sterility in triploids. The same should be true for the autotetraploids. Trisomic ratios were discussed in the previous chapter in relation to the identification of each linkage group with the chromosome on which it is carried. Here, the theoretical ratios will be considered, along with methods of calculating them.

Segregation types

Three types of segregations in autopolyploids have been proposed or recognized, i.e. chromosome segregation (Muller 1914), random chromatid segregation (Haldane 1930), and maximum equational segregation (termed complete equational by Mather 1935, and Sansome and Philp 1939, Table 26, p. 181). The term "maximum equational" has been proposed as a more descriptive term than complete equational (Burnham and Cortazar in MS). Chromosome segregation is the type in which the chromatids derived from a particular multivalent belong to separate chromosomes in that multivalent. For example, in an autotetraploid with the triplex AAAa genotype, only AA and Aa gametes are formed and in a ratio of 1:1. If either of the other two types of segregation occurs, gametes with aa are expected also, derived from sister chromatids. The manner in which this probably occurs is illustrated by the diagrams in Fig. 51.

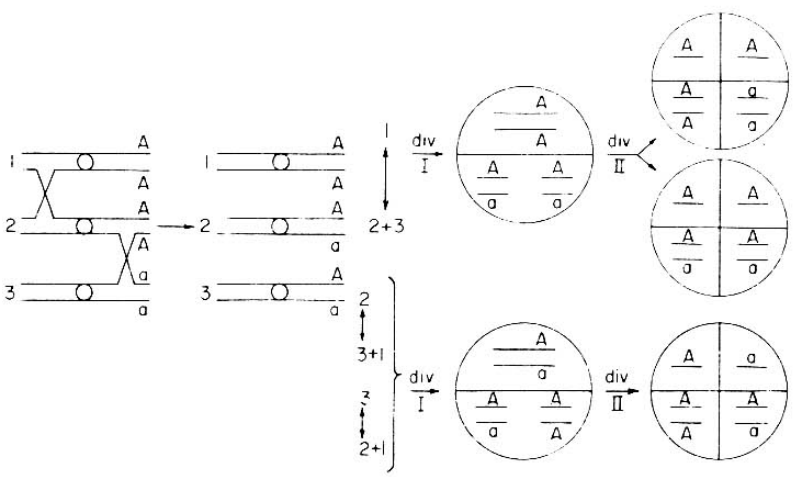

FIG. 51. Crossovers and segregations in a triploid (AAa or duplex genotype), which lead to the production of spores receiving two alleles derived from sister chromatids (double reduction), one of which is aa. Note that the division II which produces aa also produces AA derived from sister chromatids.

The evidence in Drosophila, Zea, and Neurospora is that with few if any exceptions, the first division is reductional at the centromere. If crossing over occurs between chromatid pairs numbered 2 and 3 in Figure 51, and these then pass to the same pole, gametes that are aa and AA and derived from sister chromatids are produced. This behavior has been termed double reduction, post reduction, or equational segregation at the locus. Bridges (1916) termed it "equational non-disjunction". It involves chromatid segregation. The terms double reduction and equational segregation will be used here. Note that one of the two homologous chromosomes in such a double reductional gamete is a non-crossover, the other a crossover between the locus and the centromere. This is the type of gamete utilized by Anderson (1925) and Bridges and Anderson (1925) in their demonstration that crossing over occurs when each chromosome is double-stranded, i.e. "four-strand" crossing over.

Requirements for double reduction

The cytological behavior necessary in a tetrasome to permit double reduction at a given locus, as discussed by Mather (1935, 1936) and Little (1945, 1958) is the following:

1. Quadrivalent formation. If no quadrivalents are formed, and crossing over occurred when the chromosomes were paired as bivalents, no double reduction is expected.

2. Crossing over between the gene and the centromere. Only from meiocytes with a crossover between the gene locus and the centromere is double reduction expected.

3. The two pairs of chromatids resulting from such crossing over must pass to the same pole in anaphase I. That is, sister alleles have a chance of being included in the same gamete only if the products of crossing over pass to the same pole as in Fig. 51. There is evidence from Drosophila and from maize that chromsomes that crossover pass to opposite poles. In a multivalent if the crossover nearest the centromere determines the disjunction of those centromeres, this requirement for a locus distal to the second crossover can be met as shown in Fig. 51.

4. Random separation of the chromatids at anaphase II. For the two pairs of chromatids that passed to the same pole at division I, four equally frequent combinations of chromatids would be expected. Certain of these will be of the type resulting in double reduction.

Conceivably, the frequency of double reduction might be affected also by the behavior of chiasmata subsequent to crossing over. For example, in certain species the three types of segregation from a quadrivalent may occur at random; in other species alternate and only one of the two possible adjacent types or a 2:1:1 ratio. A study of crossing over in proximal and distal regions is needed in autotetraploids for both types of species.

Ratios for the theoretical extremes

AUTOTETRAPLOIDS. An "index of separation" proposed by Mather was shown to be dependent on the parameters $\underline{a}$ and $\underline{e}$, $\underline{a}$ being the frequency with which equationally separating chromosomes pass to the same interphase nucleus at the end of the first meiotic division, and $\underline{e}$, the mean frequency of equational separation. If quadrivalents are never formed, $\underline{a} = 0$. If quadrivalents are always formed, $\underline{a}$ can reach its maximum value of 1/3. If there is no crossing over between the locus and the centromere $\underline{e} = 0$. If there is at least one crossover in that region, $\underline{e}$ can reach its maximum value of 1. Hence the maximum value for the index of separation is 1/3. Since at this maximum only half the resulting gametes are double reductional for the locus considered, the maximum frequency of double reduction, i.e. maximum equational segregation, is 1/3 x 1/2 or 1/6.

This maximum frequency should be attainable when multivalents are always formed and there is always one effective crossover between the gene locus and the centromere. Each centromere in the multivalent will then carry a crossover and a non-crossover chromatid. The multivalents may appear in various forms but the expected gametes and their frequencies for an autotetraploid are most easily arrived at by means of a 4-armed configuration scheme used by R. E. Clausen (Burnham and Cortazar in MS) and shown in Fig. 52.

For the quadrivalent, the four alleles at the single locus are designated a, b, c, and d. There are three possible different arrangements of the four alleles, one of which is shown in Fig. 52. For each arrangement, equal frequencies of alternate, and the two

types of adjacents are expected. For example, for the arrangment shown in the diagram, the expectations are:

At end of Div. I	Segregation types: alternate	adjacent	adjacent
centromere disjunctions	1 + 4 / 2 + 3	1 + 3 / 2 + 4	1 + 2 / 3 + 4
chromatid pairs at the poles	ab, cd / cd, ab	ab, ab / cd, cd	ab, cd / ab, cd
at end of Div. II			
gametes or spores	2(ac+bd+ad+bc)	aa+bb+2ab+ cc+dd+2cd	2(ac+bd+ad+bc)

Note that the aa, bb, cc and dd double reductional combinations were formed by one of the adjacent segregations.

If the gametes from the other two arrangements of the four loci are determined in a similar manner, the total gametic series is:
(1) 10 ab + 10 ac + 10 ad + 10 bc + 10 bd + 10 cd + 3 aa + 3 bb + 3 cc + 3 dd. The total of the last four combinations which have sister chromatids is 12, which is 12/72 or 1/6 of the total; the same as the maximum value for double reduction calculated previously.

The above gametic series may be used also to determine the frequencies of the gametic genotypes from any of the different heterozygous genotypes, e.g., for AAAa, corresponding to a, b, c, and d respectively, for maximum equational segregation there are 39 AA + 30 Aa + 3 aa gametes which reduces to 13:10:1. The gametic ratios for chromosome and maximum equational segregations from the various heterozygous genotypes in tetrasomes are summarized in Table 83.

The differences in frequencies of aa gametes in the two extremes, chromosome, and maximum equational segregation, are not very great, about 4% for the AAAa and Aaaa genotypes and 5.5% for AAaa. These differences would be expected in the first backcross progeny.

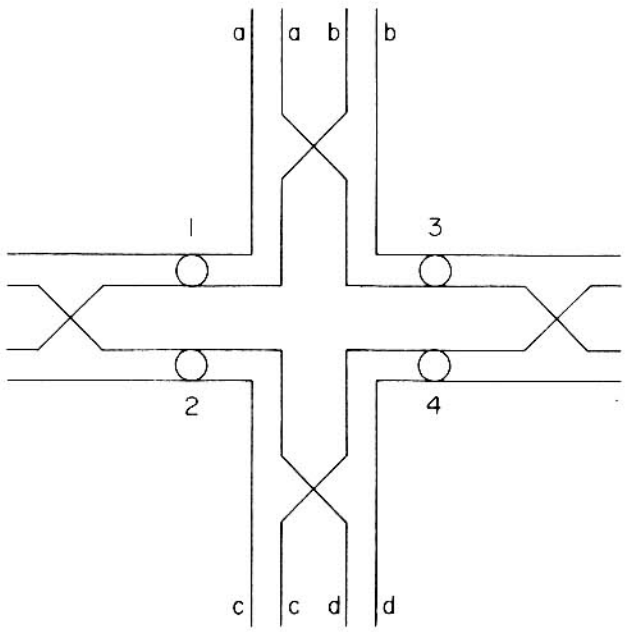

FIG. 52. Scheme for determining the theoretical maximum frequency of double reduction at a single locus in an autotetraploid. One of the three equally probable possible arrangements of the four alleles at one locus, designated a, b, c, and d, is shown together with crossovers between the locus and the centromeres, numbered 1 to 4. By assuming equal frequencies of alternate and two adjacent segregations of the centromeres, (1 + 4)/(2 + 3), (1 + 3)/(2 + 4), (1 + 2)/(3 + 4), respectively, for each of the three arrangements, the kinds of chromatids and their frequencies may be determined, as described in the text.

What are the theoretical extreme values for double reduction? One extreme is zero, shown by genes genetically completely linked with the centromere. For them there are no double reductional gametes. A common assumption has been that an infinite number

Table 83. Gametic types and frequencies expected from chromosome and maximum equational segregation in tetrasomics or tetraploids heterozygous at a single locus.

Genotype	Chromosome segregation	% aa	Maximum equational seg.	% aa
AAAa	AA+Aa	0	13AA+10Aa+aa	4.2
AAaa	AA+4Aa+aa	16.7	2AA+5Aa+2aa	22.2
Aaaa	Aa+aa	50.0	AA+10Aa+13aa	54.2

of crossovers between the centromere and a locus results in random chromatid segregation. Of the 28 possible combinations, four or 1/7 have sister chromatids (double reduction). Since this is smaller than the 1/6 derived above, it is not the theoretical maximum value for double reduction. Also since 1/7 is a lower value, it can be attained when fewer than every cell has one effective crossover between the locus and the centromere (Mather 1936). Even if the observed double reduction were 1/7, it could not be interpreted as indicating that random chromatid segregation had occurred, as pointed out by Mather (1936) and by Little (1958). This makes it pointless to use "random chromatid" segregation as one of the possible types to which observed data are tested for goodness of fit. The gametic ratios expected for random chromatid segregation have been omitted from the tables. Only its ease of calculation is in its favor. The general expressions (1) above, and (2) to be derived below for trisomics, remove this obstacle to the calculation of expected gametic ratios for maximum equational segregation.

It should be pointed out and emphasized that maximum equational segregation is not likely to be observed experimentally, because of the extreme requirements for it to occur. Some intermediate value is undoubtedly more realistic, but it is unfortunate that 1/7 has been chosen.

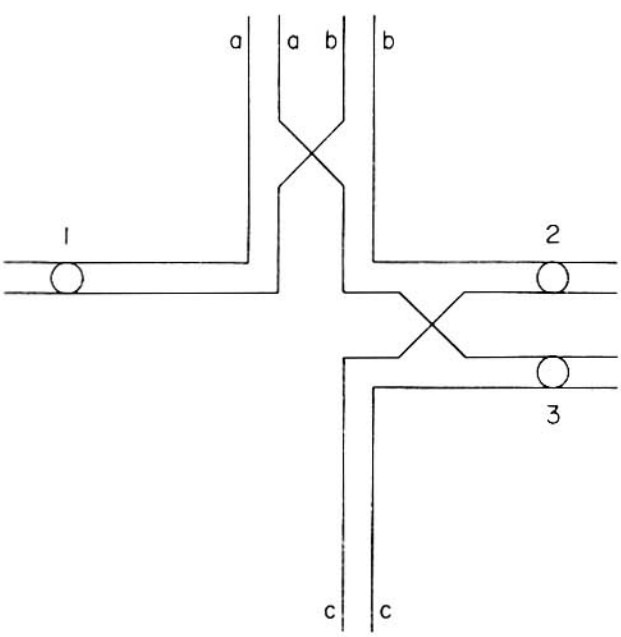

FIG. 53. Scheme for determining the theoretical maximum frequency of double reduction at a single locus in an autotriploid, as described in Fig. 52 and in the text.

AUTOTRIPLOIDS. The expected gametic ratios for the trisomes in an autotriploid or in a trisomic may be derived in a similar manner, assuming two chromosomes from the trivalent pass to one pole, one chromosome to the other pole. The three alleles at the locus are a, b, and c. As before, equal frequencies of alternate and each of the two adjacent segregations are assumed.

One of the three possible arrangements of the three loci, together with the assumed crossovers, is shown in Fig. 53.

The expected gametic types and frequencies from the arrangement shown in Fig. 53. are as follows.

At end of Div. I	1 alternate	1 adjacent	1 adjacent
Centromere disjunctions	1 + 3 / 2	1 + 2 / 3	2 + 3 / 1
Chromatid pairs at the poles	ab, ac / bc	ab, bc / ac	bc, ac / ab
n + 1 gametes	aa + bc + ac + ab	ab + bc + bb + ac	ab + cc + ac + bc
n gametes	2 b + 2 c	2 a + 2 c	2 a + 2 b

Note that in the aa, bb, and cc n + 1 or 2-chromatid gametes the two alleles are from sister chromatids, i.e. from "double reduction". Only one of them arises from each of the three types of segregation. If the gametes from the other two arrangements are determined in a similar manner, the total gametic series is:

(2) 9 ab + 9 ac + 9 bc + 3 aa + 3 bb + 3 cc; and 12 a + 12 b + 12 c for the n + 1 and n spores or gametes.

The total of the "double reductional" combinations is 25% of the n + 1 gametes.

This gametic series may be used to determine the expected gametes and their frequencies for maximum equational segregation for any of the different heterozygous genotypes. For example, for AAa corresponding to a, b, and c respectively, the gametes are: 15AA + 18Aa + 3aa + 24A + 12a or 5AA + 6Aa + aa + 8A + 4a. If unequal numbers of n + 1 and n spores are functional, the gametic ratio may be adjusted. For example, if there are only 25% of 2n + 1 in the offspring from 2n + 1 ♀ x 2n, an assumed gametic ratio of 1 (n+1): 3 (n) would give this result. Thus, for maximum equational segregation the expected ratio would be 1 (5AA + 6Aa + aa) + 3 (8A + 4a). The expected gametic ratios from the AAa and Aaa genotypes for chromosome and maximum equational segregations are summarized in Table 84.

Table 84. Gametic types and frequencies expected from chromosome and maximum equational segregations in trisomics or triploids, heterozygous at a single locus.

Genotypes	Chromosome seg.			Maximum equational seg.		
50% n + 1	n + 1	n	% aa + a	n + 1	n	% aa+a
AAa	AA+2Aa	2A+a	16.7	5AA+6Aa+aa	8A+4a	20.8
Aaa	2Aa+aa	A+2a	50.0	AA+6Aa+5aa	4A+8a	54.2
25% n+1						
AAa	AA+2Aa	6A+3a	25.0	5AA+6Aa+aa	24A+12a	27.0
Aaa	2Aa+aa	3A+6a	58.3	AA+6Aa+5aa	12A+24a	60.4

The difference in percentage of a + aa gametes (a phenotypes in backcrosses) for the two extremes, chromosome and maximum equational segregations, is only about 4% for the AAa and Aaa gentoypes if 2n + 1 and 2n individuals cannot be distinguished. The difference is less if the frequency of 2n + 1 is low. The difference is greater within the 2n + 1 classes.

The ratios expected in F_2 from trisomics or autotriploids are summarized in Table 85.

Table 85. Expected trisomic F_2 ratios for chromosome and maximum equational segregation.

Genotype	Type of segregation	Ratio 2n+1:2n	Genotypes of 2n+1 offspring AAA	AAa	Aaa	aaa	2n offspring AA	Aa	aa	% a in 2n+1	2n	Total Ratio A:a	% a
AAa	chromosome	1:1	2	5	2	0	4	4	1	0.0	11.1	17:1	5.6
"	max. eq.	1:1	10	17	8	1	16	16	4	2.8	11.1	67:5	6.9
"	chromosome	1:3	2	5	2	0	12	12	3	0.0	11.1	11:1	8.3
"	max. eq.	1:3	10	17	8	1	48	48	12	2.8	11.1	131:13	9.0
Aaa	chromosome	1:1	0	2	5	2	1	4	4	22.2	44.4	2:1	33.3
"	max. eq.	1:1	1	8	17	10	4	16	16	27.8	44.4	46:26	36.1
"	chromosome	1:3	0	2	5	2	3	12	12	22.2	44.4	22:14	38.9
"	max. eq.	1:3	1	8	17	10	12	48	48	27.8	44.4	86:58	40.3

If the trisomic individuals can be distinguished from those that are 2n, 2n + 1 individuals that are aaa in the progeny of a duplex AAa individual are the result of double reduction, and indicate that crossing over has occurred between the centromere and the a locus.

The ratios expected in F_2 from tetrasomics or autotetraploids are summarized in Table 86.

Table 86. Expected tetrasomic F_2 ratios for chromosome and maximum equational segregation. Random 2-2 segregation of the chromosomes in the tetrasomes is assumed.

Genotype	Type of segregation	A^4	A^3a	A^2a^2	Aa^3	a^4	Ratio A:a	% a
$AAAa = A^3a$	Chromosome	1	2	1			all A	0.0
"	Max. eq.	169	260	126	20	1	575:1	0.174
$AAaa = A^2a^2$	Chromosome	1	8	18	8	1	35:1	2.8
"	Max. eq.	4	20	33	20	4	77:4	5.19
$Aaaa = Aa^3$	Chromosome			1	2	1	3:1	25.0
"	Max. eq.	1	20	126	260	169	407:169	29.03

As shown in Tables 85 and 86, F_2 data are not very effective in measuring frequencies of double reduction.

The results of first and second backcrosses for trisomics and tetrasomics are summarized in Table 87.

Table 87. Ratios of dominant:recessive in first backcross and second backcross progeny from trisomics and tetrasomics. For the trisomics only the 2n + 1 offspring are considered.

Genotypes	First backcross progeny Chromosome seg.	% rec.	Max. eq. seg.	% rec.	Second backcross test Chromosome seg.	% AA	Max. eq. seg.	% AA
AAa 2n+1:	all A-	0.0	11A-:1a	8.3	1AA:2Aa	33.3	5AA:6Aa	~~54.5~~ 45.5
Aaa 2n+1:	2A-:1a	33.3	7A-:5a	41.7	all Aa	0.0	1AA:6Aa	14.3
AAAa	all A-	0.0	23A:1a	4.2	1AA:1Aa	50.0	13AA:10Aa	56.3
AAaa	5A-:1a	16.7	7A:2a	22.2	1AA:4Aa	20.0	2AA:5Aa	28.6
Aaaa	1A-:1a	50.0	11A:13a	54.2	0:all	0.0	1AA:10Aa	9.1

For the trisomics, the second backcross or self of the progeny with the A-phenotype from the first backcross should separate 2n from 2n + 1 individuals, and to separate the different trisomic genotypes. As shown in Table 87, the second backcross should furnish more reliable information on distances of genes from the centromeres; particularly for the trisomics if the trisomic segregates in the first backcross progeny are used. Trisomics should be preferable over triploids because of their higher fertility.

In tetrasomics, the second backcross should distinguish quadruplex (AAAA) from triplex (AAAa) individuals; also individuals that are pentasomic vs. trisomic for the locus. The latter genotypes are expected when three of the four chomosomes pass to one pole and one to the other.

Ratios in terms of double reduction frequency (α)

Fisher's and Mather's approach to the ratios expected in a polysomic is based on a consideration of what he terms the laws of gametic output. For a single locus in any trisomic or tetrasomic, only two modes of gamete formation are distinguishable, i.e. the two genes (alleles) in any n + 1 gamete may be derived from different chromosomes of the parent multivalent or from the same chromosome. In the latter case they are identical at that locus since they are from sister chromatids, and result from double reduction as described earlier. Fisher and Mather (1943) have derived special expressions for the frequencies of the different kinds of gametes in terms of alpha (α), the total frequency of double reduction. Alpha is no longer used for the "index of separation". How these expressions are derived is shown below, based on an explanation furnished personally by Dr. Fisher. For a tetrasome in which the four homologous alleles at one locus are designated a, b, c, and d, the possible kinds of gametes in which the two alleles were derived from sister chromatids would be aa, bb, cc, dd; i.e. four possible combinations. For the other mode of gamete formation in which the two alleles are derived from different chromosomes, there are six possible combinations, ab, ac, ad, bc, bd and cd. The relative frequencies of these two groups is unknown, but if α is assumed to be the total frequency of the group in which the gametes carry alleles from sister chromatids, i.e. double reduction, then $1-\alpha$ is the total frequency for the other group. Since the four double reductional combinations are expected to be equally frequent, the probability of each is $\frac{\alpha}{4}$. Likewise the frequency of each combination in the other group is $\frac{1-\alpha}{6}$. These relations are summarized in Table 88, along with the genetic constitutions of the corresponding gametes expected from a tetrasomic which is triplex at the Aa locus, i.e. AAAa.

Table 88. Kinds of gametes from a tetrasomic classified as to the two modes of gamete formation and their frequencies in terms of α, which is the total frequency of double reduction. The second and fifth columns are the genetic genotypes for each combination from an AAAa tetrasomic.

Group 1 — The two alleles from the same chromosome			Group 2 — The two alleles from different chromosomes in the quadrivalent		
Combinations	Gene constitution from AAAa	Frequency	Combinations	Gene constitution from AAAa	Frequency
aa	AA	$\frac{\alpha}{4}$	ab	AA	$\frac{1-\alpha}{6}$
bb	AA	"	ac	AA	"
cc	AA	"	ad	Aa	"
dd	aa	"	bc	AA	"
			bd	Aa	"
			cd	Aa	"
	Total	α		Total	$1-\alpha$