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Autotetraploid Gene Segregation[1,2]

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Summary. Autotetraploid gene segregation was studied in *Zea mays* L. using a marking system of two very closely linked genes (A_1 and Sh_2) in the repulsion phase. This system makes it possible to identify many euploid and aneuploid genotypes and enables the estimation of some parameters of autotetraploid gene segregation such as double reduction, numerical nondisjunction, and the relative transmission frequencies of monosomic, disomic, and trisomic gametes. It was found that these three types of gametes did not function at the same rates on the male and female sides. Differences in observed segregation ratios between reciprocal testcrosses were explained by this phenomenon. Estimates of the frequency of double reduction were made for loci used after eliminating the effect of numerical non-disjuction on the segregation ratios. The value of double reduction appears to be the same in the male and female tetrasomic tetraploid. Tetraploids which were disomic for chromosome 3 were not isolated although they might be expected to be common in the progeny of self-fertilized or sib-crossed trisomic tetraploids. Their absence may be explained in part by the low rate of transmission of monosomic gametes from the male parent. Autotetraploid populations which are unstable for chromosome number probably achieve an equilibrium between forces which produce aneuploidy and forces which remove aneuploids from the population.

Introduction

Autotetraploid gene segregation is complicated by many factors not found in diploid gene segregation. Instead of only one kind of heterozygote for a locus as in diploids, there are three in a tetraploid: *AAAa* — triplex, *AAaa* — duplex, and *Aaaa* — simplex. The two homozygotes are called nulliplex (*aaaa*) and quadriplex (*AAAA*). Also, there are many aneuploid genotypes present in populations of autotetraploids which do not breed true for chromosome number. In maize, each of the ten chromosomes may be present in the disomic, trisomic, tetrasomic, pentasomic, or hexasomic condition. Thus there are many types of heterozygotes, one in the disome, two in the trisome, three in the tetrasome, four in the pentasome, and five in the hexasome. Each of these genotypes has its own peculiar segregation ratio. The terminology for the aneuploid types is the same as in the euploid tetrasome. It is based on the number of dominant genes. Thus, *AAa* is a duplex trisome and *AAAaa* is a triplex pentasome.

There are two modes of segregation possible depending on what is considered as the unit of segregation. Muller (1914) used the chromosome as the unit of segregation and devised the scheme shown in Table 1 known as random chromosome segregation. This scheme does not allow for the occurrence of recessive gametes in the progeny of a triplex which was observed by Blakeslee *et al.* (1923) in tetraploid Daturas. It soon became apparent that crossing over takes place at the four strand stage and therefore the unit of segregation is the chromatid. Haldane (1930) calculated the expected gametic ratios on the basis of random chromatid segregation. There are eight chromatids which taken two at a time give 28 different combinations. Four of these 28 pairs are composed of sister chromatids.

A particular sequence of events called double reduction by Mather (1936) is necessary to get genes which are on sister chromatids into the same gamete. A multivalent must be formed where crossing over has occurred between the gene locus used and the centromere to give an equational constitution to one or two pairs of chromosomes. (A more descriptive term might be heteroallelic chromosomes.) These pairs of chromosomes (*Aa* and *Aa*) must go to the same pole at the first division of meiosis. (This is called genetic non-disjunction.) At the second division of meiosis the random orientation of these chromosomes on the metaphase plate one-half of the time will produce double-reductional gametes (*AA* and *aa*).

The frequency of double reduction is expressed mathematically by α, which equals $qea/2$ where q is the quadrivalent frequency, e is the frequency of equational constitutions, and a is the frequency of genetic non-disjunction.

The value of q varies with the species studied. Most species have q values of about 67% (Morrison and Rajhathy 1960). Maize has a rather high quadrivalent frequency: 84.7% (Gilles and Randolph 1951), and 87.1% (Shaver 1962a). Within a species the longer chromosomes probably tend to have higher

[1] This paper is dedicated to Dr. M. M. Rhoades.

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Table 1. *Modes of gene segregation in autotetraploids*

		Triplex AAAa	Duplex AAaa	Simplex AAaaa
Gametes formed by chromosome segregation (Muller 1914)	*AA*	1/2	1/6	0
	Aa	1/2	2/3	1/2
	aa	0	1/6	1/2
Gametes formed by chromatid segregation with double reduction (Fisher and Mather 1943)	*AA*	$(2+\alpha)/4$	$(1+2\alpha)/6$	$\alpha/4$
	Aa	$(1-\alpha)/2$	$(2-2\alpha)/3$	$(1-\alpha)/2$
	aa	$\alpha/4$	$(1+2\alpha)/6$	$(2+\alpha)/4$
Gametes formed by double reduction and numerical nondisjunction (Catcheside 1956)	*AAA*	$x(1+\alpha)/8$	$x\alpha/12$	0
	AAa	$x(3-2\alpha)/8$	$x(3-\alpha)/12$	$x\alpha/8$
	Aaa	$x\alpha/8$	$x(3-\alpha)/12$	$x(3-2\alpha)/8$
	aaa	0	$x\alpha/12$	$x(1+\alpha)/8$
	AA	$(2+\alpha)(1-x)/4$	$(1+2\alpha)(1-x)/6$	$\alpha(1-x)/4$
	Aa	$(1-\alpha)(1-x)/2$	$(2-2\alpha)(1-x)/3$	$(1-\alpha)(1-x)/2$
	aa	$\alpha(1-x)/4$	$(1+2\alpha)(1-x)/6$	$(2+\alpha)(1-x)/4$
	A	$3x/8$	$x/4$	$x/8$
	a	$x/8$	$x/4$	$3x/8$
Gametes formed by double reduction and numerical non-disjunction ($x = t + m$)	*AAA*	$t(1+\alpha)/4$	$t\alpha/6$	0
	AAa	$t(3-2\alpha)/4$	$t(3-\alpha)/6$	$t\alpha/4$
	Aaa	$t\alpha/4$	$t(3-\alpha)/6$	$t(3-2\alpha)/4$
	aaa	0	$t\alpha/6$	$t(1+\alpha)/4$
	AA	$d(2+\alpha)/4$	$d(1+2\alpha)/6$	$d\alpha/4$
	Aa	$d(1-\alpha)/2$	$d(2-2\alpha)/3$	$d(1-\alpha)/2$
	aa	$d\alpha/4$	$d(1+2\alpha)/6$	$d(2+\alpha)/4$
	A	$3m/4$	$m/2$	$m/4$
	a	$m/4$	$m/2$	$3m/4$

quadrivalent frequencies than the shorter chromosomes because of a greater opportunity for pairing partner exchange at zygonema.

The value of e is dependent on the map distance between the gene and the centromere. For short distances where there is only one chiasma, e is twice the recombination frequency. When there are more than one chiasma the situation is complicated. One chiasma gives 100% equational chromosome. Two chiasmata give 50% equational and 50% reductional chromosomes. Three chiasmata give 75% equational and 25% reductional chromosomes. An infinite number of chiasmata would give 66.7% equational and 33.3% reductional chromosomes. For an explanation of this feature the reader is directed to Mather (1935). In organisms where tetrad analysis is possible, e is the same as second-division segregation.

The value of a has not been determined experimentally. Its value is assumed to be 1/3 which is expected from the random disjunction of the chromosomes of a quadrivalent.

Since the frequency of double-reductional gametes varies from one gene locus to another and is rarely equal to 1/6 the frequency of the other gametes, (as would be found in random chromatid segregation), the scheme proposed by Haldane is inadequate. Fisher and Mather (1943) proposed the second scheme in Table 1.

Theoretically if we determine the value of α from one kind of heterozygote we should be able to predict the segregation ratios for the other two kinds of heterozygotes. Sometimes this is not true. Values of α computed from simplex, duplex, and triplex plants heterozygous for the same locus may not agree (Catcheside 1959). One reason for this is that the formulas of Fisher and Mather neglect the presence of monosomic and trisomic gametes which are formed from 3 to 1 disjunctions of the chromosomes of a quadrivalent (numerical non-disjunction).

The effects of numerical non-disjunction have been investigated by Catcheside (1956) who modified the formulas of Fisher and Mather. Catcheside expressed the frequency of numerical non-disjunction as x. He found the value of x for chromosome 4 of maize to be 2.62%.

The formulas of Catcheside make the tacit assumption that monosomic and trisomic gametes are formed with equal frequency. Along with three to one disjunctions there are cases in which chromosomes are not included in the spore nuclei because they were univalents which lagged at the metaphase plate. Morrison (1956) observed a high frequency of micronuclei at the tetrad stage of meiosis in Tetra Petkus rye. Consequently monosomic gametes may be much more common than trisomic gametes.

The last group of formulas in Table 1 are the same as those of Catcheside's except that x has been partitioned into m and t which express the frequencies of monosomic and trisomic gametes respectively. The term $(1 - x)$ becomes d, the frequency of disomic gametes. Thus $m + d + t = 1$.

Aneuploid gametes are very common in tetraploid maize. Table 2 gives the frequencies of plants with chromosome numbers ranging from 36 to 47 found in the progenies of 40-chromosome plants by four different investigators. This table is adapted from Burnham (1962).

Table 2. *Number of plants with chromosome numbers ranging from 36 to 47 found in the progeny of 40-chromosome plants by four different investigators*

Chromosome number												
36	37	38	39	40	41	42	43	44	45	46	47	Total
0	1	3	6	27	12	5	0	0	0	0	0	54[1]
4	1	36	43	261	73	8	3	0	0	0	0	429[2]
2	1	6	4	57	12	11	1	2	1	0	1	98[3]
0	0	3	8	50	13	0	0	0	0	0	0	74[4]
Σ 6	3	48	61	395	110	24	4	2	1	0	1	655
% 0.9	0.5	7.3	9.3	60.3	16.8	3.7	0.6	0.3	0.2	0.0	0.2	

([1] — Randolph 1935; [2] — Kadam 1944; [3] — El Ghawas 1955 and [4] — Catcheside 1956).

Only 60.3% of the plants had 40 chromosomes. Some of these 40-chromosome plants are probably not euploid but are numerically compensated aneuploids such as 4 n − 1 + 1, 4 n − 1 − 1 + 1 + 1, 4 n − 1 − 1 + 2, and others.

Shaver (1962b) found that maize plants with aneuploid chromosome numbers had higher rates of pollen and ovule abortion than euploids. The presence of aneuploids in populations of tetraploid maize accounts for much of their low fertility as compared with diploids and seriously limits the agronomic potential of tetraploid maize.

The presence of aneuploids also creates difficulties in studying gene segregation. Plants with constitutions of *AAaaa*, *AAaa*, and *AAa* have very similar segregation ratios since the ratios are primarily dependent on the number of dominant genes. The use of only 40-chromosome plants does not eliminate the difficulty, because of the common occurrence of 4 n − 1 + 1 plants where there is a 20% probability that the chromosome being studied is in an aneuploid condition. Other numerically compensated types such as 4 n − 1 − 1 + 1 + 1 are very rare and generally may be neglected.

In order to improve the validity of the data, the chromosomes may be identified cytologically as well as counted as was done by Catcheside (1956), but this is a very time consuming procedure and seriously limits the amount of material that can be handled.

A method to determine the constitution of a tetraploid for a particular chromosome is a double marking system. Two genes on the same chromosome in the repulsion phase are followed at the same time. Thus while it may be difficult to distinguish between *AAaaa*, *AAaa*, and *AAa* on the basis of their segregation ratios, it is possible to distinguish between *Ab/Ab/aB/aB/aB*, *Ab/Ab/aB/aB*, and *Ab/Ab/aB*. The ratios of *A* to *a* will be similar but the ratios of *B* to *b* will vary greatly. A very large number of plants can be handled using this method. This paper is based on results from over 2,000 plants whose constitutions for chromosome 3 are definitely known.

Materials and Methods

Two genes A_1 and Sh_2 which are located on the long arm of chromosome 3 were used in this study. Kernels which are homozygous for a_1 have colorless aleurones. Kernels with at least one A_1 allele have colored aleurones in the presence of other dominant complementary genes. Kernels homozygous for sh_2 are shrunken. Kernels with at least one Sh_2 allele are full. (Hereafter the subscripts on these will be dropped.) These two genes are very closely linked, the map distance is 0.2. The frequency of disomic *a sh* gametes is expected to be very low from a *A sh/A sh/a Sh/a Sh* tetraploid. The *A sh* chromosome segments must pair with the *a Sh* chromosome segments (which has a probability of 2/3) crossing over must occur between the loci in both segments (with a probability of .002 × .002) and the two *a sh* chromosomes formed must go to the same gamete (a probability of 1/24). The compound probability is .0000001. The frequency of monosomic *a sh* gametes is $2/3 \times .002 \times 2 \times m$. This is very low also.

For the same of brevity in the tables, genotypes of plants in this paper will be symbolized by three numbers; the first number indicates the number of *A sh* chromosomes, the second number the number of *a Sh* chromosomes, and the third number the number of *a sh* chromosomes. Thus plant genotypes symbolized by 310 and 212 are *A sh/A sh/A sh/a Sh* and *A sh/A sh/a Sh/a sh/a sh*, respectively.

For statistical simplicity all ears which had less than 100 kernels were discarded. Chi squares were derived from expected values produced by contingency tables. When the degrees of freedom exceeded 100 (where the p values are not tabulated) tests for significance were made using the formula devised by Fisher

$$[Z = \sqrt{2x^2} - \sqrt{2\,(\text{d.f.}) - 1}].$$

Results and Discussion

To obtain an estimate of the frequency of numerical non-disjunction and to isolate aneuploids, plants with the constitution 400 (homozygous *A sh*) and 040 (homozygous *a Sh*) were crossed reciprocally.

The F_1's of these crosses were crossed with 004 plants (homozygous *a sh*), and from their segregation ratios their genotypes were determined. (See Table 6.) The results are given in Table 3.

Table 3. *Genotypes found in the progeny of 400 × 040 and 040 × 400 crosses*

Number of plants with genotypes of						
Cross	220	320	230	210	120	Total
400 × 040	213	2	5	1	2	223
040 × 400	549	6	6	22	2	585

Plants with a constitution of 220 were formed without numerical non-disjunction. All of the others are the result of one numerical non-disjunction event per zygote. For example, the two 320 plants from the cross of 400 x 040 arose from a trisomic gamete (*A sh/A sh/A sh*) from the female parent and a regular disomic gamete (*a Sh/a Sh*) from the male parent. It is possible for both gametes which form a zygote to have been aneuploid. Then we would expect genotypes of 310, 130, 330, and 110. They were not found.

The data from Table 3 are rearranged and presented in Table 4 to show the origin of the aneuploid gametes.

Table 4. *Constitutions of gametes producing plants given in Table 3*

Number of gametes which were:							
	Disomic		Trisomic		Monosomic		Total
Male	794	(98.27%)	11	(1.36%)	3	(0.37%)	808
Female	776	(96.04%)	8	(0.99%)	24	(2.97%)	808
Total	1570	(97.15%)	19	(1.18%)	27	(1.67%)	1616

It is apparent that there is a great difference in the rate of formation or transmission of disomic, trisomic, and monosomic gametes. A chi square test for the hypothesis that the frequencies of trisomic and monosomic gametes are equal in both parents gives a chi square of 20.95 which has probability of less than .0005.

Monosomic pollen has an equal or greater frequency than trisomic pollen, therefore monosomic pollen must compete very poorly with trisomic or disomic pollen. On the female side where gametophyte competition is not expected there is a great excess of monosomic gametes. This is probably the result of a high rate of chromosome loss at megasporogenesis. Thus, it appears that the excess of monosomic gametes from the female is compensated somewhat by an excess of trisomic gametes from the male.

This method does not differentiate between the rates of aneuploid spore formation and their rates of transmission. It is further confounded by the likely differential viability of euploid and aneuploid zygotes.

Patterns of aneuploid formation for chromosome 3 may or may not be typical of the other nine chromosomes. Considering all ten chromosomes there are a great number of possible chromosome constitutions. Each of the ten chromosomes may be present in the disomic, trisomic, tetrasomic, pentasomic, or hexasomic condition. Thus, there are 5^{10} or 9,765,625 possible types. Many of them would be very unbalanced and hence inviable. If we consider only those more probable cases where there is a maximum of four numerical non-disjunction events there are 7,961 types which fall into 30 different groups. Table 5 lists these groups with their probable frequencies based upon the expansion of the trinomial $(d + m + t)^{20}$. This mathematical model uses arbitrary values of $d = .970$, $m = .015$ and $t = .015$. [The value of $d = .970$ was chosen because it is the observed value (to two decimal places). Using $d = .970$ also gives an expected frequency of 59.4% 40-chromosome plants which is close to the observed frequency of 60.3% as given in Table 2.]

It may be seen that the frequency of 4 n plants is d^{20} where there are no numerical non-disjunctional events, plus $20\, d^{18}\, m\, t$ where there the trisomic gamete has the same extra chromosome that its monosomic partner lacks. (The rest of the term, $190\, d^{18}\, m\, t$, goes into the 4 n − 1 + 1 group.) A very small contribution to the 4 n group comes from the union of 2 n − 1 − 1 and 2 + 1 + 1, or two 2 n − 1 + 1 gametes where there is complementation. It is expressed by the term $180\, d^{16}\, m^2\, t^2$.

Formulas for the frequencies of 41, 42, 43, and 44 chromosome plants are not given for the sake of brevity. If $t = m$ their frequencies are the same as 39, 38, 37, and 36 chromosome plants, respectively.

The mathematical model does not fit the observed data in some respects. There is an excess of 38-chromosome plants and a deficiency of 39-chromosome plants in the observed data in Table 2. The mathematical model makes the untrue assumption that m, d, and t are exactly the same for all ten chromosomes. Also it neglects the effects of differential zygotic lethality for the different aneuploids and the effects of gametic competition. However, these shortcomings do not help to explain the aforementioned discrepancy.

Nevertheless, the model is useful in obtaining a rough estimate of the probable frequency of certain groups such as the 4 n − 1 + 1 genotype, which is 4.7% of the 40-chromosome class; 8% are not true

Table 5. *The expected frequency of genotypes in the progeny of 4n maize plants*

No. of chromosomes	Constitution	No. of genotypes	Formula		Frequency
40	4 n	1	$d^{20} + 20^{18}\, m\, t + 180\, d^{16}\, m^2\, t^2$		.546389
	4 n − 1 + 1	90	$360\, d^{18}\, m\, t + 5{,}760\, d^{16}\, m^2\, t^2$		.046799
	4 n − 1 − 1 + 1 + 1	1,260	$20{,}160\, d^{16}\, m^2\, t^2$		.000625
	4 n − 2 + 1 + + 1	360	$1{,}440\, d^{16}\, m^2\, t^2$		.000045
	4 n − 1 − 1 + 2	360	$1{,}440\, d^{16}\, m^2\, t^2$		.000045
	4 n − 2 + 2	90	$90\, d^{16}\, m^2\, t^2$		.000003
				Total	.593906
39	4 n − 1	10	$20\, d^{19}\, m + 360\, d^{17}\, m^2\, t$		.168904
	4 n − 1 − 1 + 1	360	$2{,}880\, d^{17}\, m^2\, t$		.005791
	4 n − 2 + 1	90	$180\, d^{17}\, m^2\, t$		.000362
				Total	.175057
38	4 n − 1 − 1	45	$180\, d^{18}\, m^2 + 2{,}880\, d^{16}\, m^3\, t$		.023400
	4 n − 2	10	$10\, d^{18}\, m^2 + 180\, d^{16}\, m^3\, t$		.001301
	4 n − 1 − 1 − 1 + 1	840	$13{,}440\, d^{16}\, m^3\, t$		.000418
	4 n − 2 − 1 + 1	720	$2{,}880\, d^{16}\, m^3\, t$		.000090
				Total	.0250209
37	4 n − 1 − 1 − 1	120	$960\, d^{17}\, m^3$		.001930
	4 n − 2 − 1	90	$180\, d^{17}\, m^3$		.000362
				Total	.002292
36	4 n − 1 − 1 − 1 − 1	210	$3{,}360\, d^{16}\, m^4$		.000104
	4 n − 2 − 1 − 1	360	$1{,}440\, d^{16}\, m^4$		.000045
	4 n − 2 − 2	45	$45\, d^{16}\, m^4$		.000001
				Total	.000150

4 n plants but are numerically compensated aneuploids.

The observed segregation ratios for various euploid and aneuploid genotypes are given in Table 6. Table 7 gives the theoretical gamete production of the aneuploid genotypes. Formulas for the expected frequencies of the three phenotypic classes are given in Table 8 which is derived from tables 1 and 7. In all but one case, the pooled chi-square for each cross in Table 6 is significant. The genetic data are quite variable. However, individual families rarely have significant chi-squares. There are several factors which could produce a generalized variability. First, there is in some cases a deficiency in the *A sh* class. Either, gametes which carry only the *sh* allele are not as viable or compete unfavorably with gametes with the *Sh* allele under certain conditions, or kernels which have the *sh* phenotype are more apt to be lost. It was noted that when the ear got wet prior to harvest the *sh* kernels disintegrated rapidly. If this deficiency in the *A sh* class is not uniform, then we have a component of variability.

Table 6. *Observed segregation ratios for tetrasomic, pentasomic, and trisomic 4 n maize*

Cross	No. of plants	No. of gametes	Percent *A Sh*	Percent *A sh*	Percent *a Sh*	Pooled X^2	Degrees of frd.	p	X^2 ♀ vs. ♂
220 × 004	649	154,022	58.40	20.29	21.31	1524.56**	1296	<.0005	
004 × 220	132	31,721	60.05	19.95	20.00	339.04**	262	<.0020	35.05**
310 × 004	75	15,222	45.96	51.14	2.90	152.80**	148	<.0005	
004 × 310	25	6,598	45.20	51.80	3.00	94.71**	48	<.0005	1.37
130 × 004	90	15,969	44.83	2.45	52.71	202.52**	178	<.0005	
004 × 130	33	10,275	44.91	2.91	52.18	88.58*	64	>.0250	5.12*
320 × 004	17	3,164	70.73	23.10	6.16	79.30**	32	>.0005	
004 × 320	4	1,359	75.94	20.82	3.24	6.64	6	>.3000	21.24**
230 × 004	17	3,748	70.41	5.20	24.39	83.53**	32	>.0005	
004 × 230	6	940	63.62	7.55	28.83	34.39**	10	>.0005	18.38**
210 × 004	50	9,221	29.17	50.63	20.19	125.23*	98	>.0250	
004 × 210	10	2,643	58.99	35.41	5.60	43.77**	18	>.0005	868.37**
120 × 004	34	5,997	29.60	19.36	51.04	111.81**	66	<.0005	
004 × 120	11	1,731	56.73	6.70	36.57	51.58**	20	>.0005	423.86**

* Significant at .05 level
** Significant at .01 level

Table 7. *Theoretical gamete production (trisomic and pentasomic tetraploids)*

Gamete	Genotype	
	AAAaa	AAa
AAA	1/10 t + 2/10 $t\alpha$	—
AAa	6/10 t − 3/10 $t\gamma$	—
Aaa	3/10 t	—
aaa	1/10 $t\alpha$	—
AA	3/10 d + 3/10 dα	1/3 d + 2 dα/3
Aa	6/10 d − 6/10 dα	2/3 d − 4 dα/3
aa	1/10 d + 3/10 dα	2 dα/3
A	—	2 m/3
a	—	m/3

1. Formulas for other genotypes — AAaaa and Aaa may be derived by changing A to a and a to A in the gametes.

Secondly, there is probably an environmental effect on the frequencies of double reduction and numerical non-disjunction.

Thirdly, the chromosomes of an autotetraploid are never completely homologous unless it has been derived from an inbred line. Preferential pairing would take place between the more structurally homologous chromosomes. This would lead to the formation of a higher than random frequency of homogenetic bivalents in the duplex and would result in more heterozygous gametes, which would decrease the rate of double reduction. The lines used in these experiments were related but in all cases there was some non-homology present. Preferential pairing is very common in trisomic racial hybrids of maize, as was shown by Doyle (1969).

Fifthly, when the heterozygous parent is the male, the competitive situation between trisomic, disomic, and monosomic pollen may vary with the amount of pollen used. Light pollinations would allow for a higher rate of transmission of monosomic pollen for example. In practice, the amount of pollen used is very hard to control.

The values of m, d, t, and α in Tables 1, 7, and 8 vary according to the sex of the parent and the number of chromosomes 3 present. Equations to solve for these values may be derived from the formulas in Table 8. Unfortunately they contain two of three unknowns and the equations for each phenotype from a given cross are not independent. They are not solvable using only the data in Table 6.

However, by testing of the progeny of these crosses we can partition the effects of double reduction and numerical non-disjunction. In the cases of the 310 × 004 and 004 × 310 crosses, the *a Sh* kernels were planted and crossed to 004 plants. These *a Sh* plants either had the genotype (012) from a monosomic gamete or the genotype (022) from a double reductional disomic gamete. When 322 plants from crosses of 310 × 004 were tested, it was found that 51 (15.84%) had the genotype 012, and 271 (84.26%) had the genotype 022. When 131 plants from the reciprocal crosses were tested there were 4 (3.05%) 012 genotypes and 127 (96.95%) 022 genotypes. Thus the value of m using the data in Table 6 and the formula in Table 8 (*a Sh* = m/4 + dα/4) is 1.86% for the female (.1585 × .0294 × 4) and 0.35% for the male (.0305 × .0300 × 4). These values should be the same as those given in Table 4. There is good agreement in the case of the male (0.35% vs. 0.37%) and fair agreement for the female data (1.86% vs. 2.97%). The chi squares are 0.01 and 5.60, respectively. The values of α are .0991 (female) and .1163 (male). If the formula of Fisher and Mather (which includes the trisomic cases along with double reduc-

Table 8. *Formulas for computing segregation ratios for trisomic, tetrasomic and pentasomic tetraploids*

Genotype of parent	Frequency of phenotype of progeny in testcross	m	d	t	$d\alpha$	$t\alpha$
220	*A Sh*	= 0	2/3	1	−2/3	−1/3
	A sh	= 1/2	1/6	0	1/3	1/6
	a sh	= 1/2	1/6	0	1/2	1/6
310	*A Sh*	= 0	1/2	3/4	−1/2	−1/4
	A sh	= 3/4	1/2	1/4	1/4	1/4
	a Sh	= 1/4	0	0	1/4	0
320	*A Sh*	= 0	6/10	9/10	−6/10	−3/10
	A sh	= 0	3/10	1/10	3/10	2/10
	a Sh	= 0	1/10	0	3/10	1/10
210	*A Sh*	= 0	2/3	0	−4/3	0
	A sh	= 2/3	1/3	0	2/3	0
	a Sh	= 1/3	0	0	2/3	0

Fourthly, it is possible that somatic non-disjunction may have occurred in a few cases to form a pair of cells with the constitutions of 4 n − 1 and 4 n + 1. Their cell lineages would probably be greater than those of 2 n − 1 and 2 n + 1 cells formed by a similar event in a diploid because of the relatively smaller imbalance on the tetraploid level.

tion cases) is used, the values would be .1176 (female) and .1200 (male).

A better estimate of α in the tetrasomic tetraploid can be obtained by using the data in Table 6 and values of m, d, and t (see Table 9) obtained from the data in Table 4 and the preceding experiment. The m values are averages. The equations now may be solved for α. The α values for the first 6 crosses are .1078, .01035, .0952, .1004, .0864, and .1062. Pooling the data using weighting factors for population size, α — .1042 (female) and .1036 (male). The value of α is probably the same in both sexes.

If we use the α values found in the tetrasomic tetraploid in the analysis of the pentasomic tetraploid segregation ratios, the values of d and t may be determined. (It is assumed that monosomic gametes are not produced by pentasomics.) They are d = = .5018 (female) and .4972 (male); and t = .4982 (female) and .5028 (male). There appears to be no significant competition between disomic and trisomic pollen. No competition is expected on the female side. Progeny tests on the pentasomics are incomplete.

Hexasomic plants have been tentatively identified in the progeny of selfed pentasomics. They have a phenotypic appearance closely resembling trisomic-3 diploids. Their segregation ratios suggest genotypes of 330, 420, 240, 150, and 510. However except for 330, hexasomic plants do not have definitive segregation ratios, 510 is very close to 410, for example. Further progeny testing is required here, also.

In the trisomic tetraploid crosses there is a great difference in the segregation ratios depending on the sex of the heterozygous parent. This is due to a much lower value of m in the male than in the female. Limited data have been obtained from progeny testing the *a Sh* class from the cross 210 × 004. Of 141 *a Sh* plants tested, 123 (87.23%) had the genotype 012 (which arose from monosomic eggs) and 18 (12.77%) had the genotype 022 (which arose from double-reductional eggs). Thus the value of m is .5283 (.8723 × .2019 × 3) and α is .0821 (.1277 × × .2019 × 3 divided by 2 × .4717). The value of .4717 is the value of d, since m + d = 1. The trisomic tetraploid theoretically might form trisomic gametes for the chromosome under study as a result of nondisjunction but this probably very rare.

Unfortunately, progeny tests have not been conducted yet on the reciprocal cross 004 × 210. However if we make the very likely assumption that the value of α is about the same in the male and female trisomic tetraploid, then we can solve for the value of m in the male. If this is done then we get a negative value. Either the data are inaccurate or α has been overestimated. Since α was estimated from a population of only 141 plants the latter is quite possible. Nevertheless, the only way to account for the data is to assume that monosomic gametes function only very rarely in the male. This agrees with the data in Table 4. However the situations are not the same, since a tetrasomic produces only a few monosomic pollen grains while a trisomic produces about 50% monosomic pollen grains.

Table 9 summarizes the preceding discussion. More accurate values will be given in a later report.

Table 9. *Parameters of autotetraploid gene segregation for chromosome 3 in maize*

Constitution		m	d	t	α
Trisome	♀	.5283	.4717	—	.0821
	♂	(.0000)	(1.0424)	—	—
Tetrasome	♀	.0242	.9659	.0099	.1042
	♂	.0036	.9828	.0136	.1036
Pentasome	♀	—	.5018	.4982	—
	♂	—	.4972	.5028	—

The disomic tetraploid should be found in the progeny of self fertilized or sib crossed trisomes. Tables 10 and 11 give the results of such tests. Since only the 110 genotype (with a segregation ratio of 1:1 for *A sh* and *a Sh*) can be recognized only *A Sh* kernels were used.

Table 10. *Self-fertilized trisomic progeny tests*

	Number of plants with genotypes of:						
Parent	220	310	130	210	120	110	Total
210	12	20	3	8	6	0	49
120	30	9	21	5	19	0	84

Table 11. *Sib-crossed trisome progeny tests*

	Number of plants with genotypes of:						
Cross	111 or 112	211	121	210	120	110	Total
102 × 012	141	13	6	0	0	0	160
012 × 102	477	30	29	4	2	0	542

No disomic tetraploids were found. Their expected frequency in the total population is $2/9 \times m^2$ in the case of the selfed trisomes and $1/9 \times m^2$ in the case of the sib crossed trisomes. If m was equal to .5 in both male and female then the frequencies should be 5.56% and 2.78%. These values, multiplied by the reciprocals of the percent of *A Sh* kernels and the product, multiplied by the total population number produce the expected number of disomes. There should have been 8.55 disomes from the selfed trisomes and 75.75 disomes from the sib-crossed trisomes. The value of m in the male parent is very small or disomic tetraploids are inviable. Since monosomic diploids in maize are quite viable (Weber 1971) it seems likely that disomic tetraploids which have a similar genetic imbalance should also be viable.

If pollen monosomic for other chromosomes has the same difficulty in competing with disomic and

trisomic pollen, then the frequency of disomic tetraploids in the population may be expected to be very low. It was seen in Table 2 that disomic progeny had a low expected frequency from eutetraploid. However, the progeny of a selfed trisomic tetraploid (4n−1) would contain around 25% disomes if it were not for the poor transmission of monosomic pollen.

This is one mechanism by which aneuploid genotypes are limited in the population. Another force tending to reduce the frequency of aneuploids in the population is the probable lowered viability or fertility of aneuploids. This remains to be demonstrated when larger populations of aneuploids can be produced.

Conclusions

The genetics of autotetraploids are somewhat complicated. An autotetraploid population of maize is a mixture of just over 50% euploids and very many kinds of aneuploids. There are many possible genotypes which have their own peculiar segregation ratios. These ratios depend on the frequency of double reduction, the relative rates of formation and transmission of monosomic, disomic, and trisomic gametes and the relative viabilities of the resulting euploid and aneuploid zygotes. The expected segregation ratios may be expressed in terms of α (double reduction) and m, d, and t (which express the transmission frequencies of three kinds of gametes) but it must be understood that these values are variable and depend on environmental conditions, methods of pollination, and assume random chromosome pairing, equal viability of all phenotypic classes, and the somatic stability of the chromosome number. Consequently, the estimation of autotetraploid gene segregation parameters is an exercise in theoretical mathematics and not biological reality.

Nonetheless, it is possible to get an understanding of the forces at work in autotetraploid populations without the necessity of quantising them to the second decimal place. Autotetraploid populations which are unstable for chromosome number must achieve an equilibrium between forces which produce aneuploidy and forces which remove aneuploids from the population.

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