Theor Appl Genet (1986) 71:585–594

The allotetraploidization of maize

4. Cytological and genetic evidence indicative of substantial progress *

G. G. Doyle

U.S. Department of Agriculture, Agricultural Research Service, and the Agronomy Department, University of Missouri, Columbia, MO 65211, USA

Received February 12, 1985; Accepted September 23, 1985
Communicated by K. Tsunewaki

Summary. Allotetraploidization is the creation of synthetic allotetraploids. The allotetraploidization of maize can be accomplished by concentrating DPA (differential pairing affinity) factors into stocks by a recurrent selection breeding system. Selection is based on pairing configuration frequencies and altered genetic ratios that reflect DPA. Both an observed decline in the quadrivalent frequency per meiocyte from 8.10 to 7.31 and genetic data disclosing a reduction in the average frequency of recessive waxy (*wx wx*) pollen from *Wx Wx wx wx* plants from 17.48% to 13.35%, indicate considerable progress has been made toward allotetraploidization. A simple model for the effect of DPA on chromosome pairing and genetic ratios is presented.

Key words: Autosyndesis – Cytological diploidization – Tetraploids – Quadrivalents – Experimental evolution

Introduction

Allotetraploids (AABB) have two pairs of dissimilar genomes. The genomes (A and B) are derived from related species or genera that had a common ancestor and whose chromosomes were therefore once homologous. During evolutionary divergence, chromosomal structural rearrangements, mutations in the pairing code, and perhaps mutations of genes affecting the general expression of DPA (differential pairing affinity) have occurred with the result that homologous chromosomes in one genome have become homoeologous to their counterparts in the other genome. Enough DPA has arisen between the genomes so that in the allotetraploid almost all the chromosome pairing is between homologues (or autosyndetic).

Allotetraploidization is the creation of a synthetic allotetraploid. When two diploid species are crossed and the chromosome number of the hybrid is doubled, a natural allotetraploid results if there is enough DPA to give autosyndetic pairing. Maize is a single species (*Zea mays* L.). Therefore, to produce allotetraploid maize it is necessary to convert the maize genome (Z) into a restructured genome (R). The Z and R genomes will be genetically equivalent but their chromosomes will pair autosyndetically in the synthetic allotetraploid, ZZRR. The genome R will be reproductively isolated (no crossing over) from the Z genome and thus the R genome can be regarded as being from a new incipient species. The R genome can be created by concentrating induced or naturally occurring DPA factors into stocks by a recurrent selection breeding system.

It was demonstrated (Doyle 1979a) that DPA factors occur naturally between different races of maize and that they are readily induced by X-rays and chemical mutagens. An extremely complex model was presented (Doyle 1979b) to explain how DPA affects the relative frequencies of various pairing configurations and consequently the genetic ratios expected in segmental allotetraploids. A similar study of the effect of DPA in trisomes was presented in Part 3 of this series (Doyle 1982).

This paper reports on cytological and genetic data that indicate substantial progress toward the allotetraploidization of maize. Also a simple, but workable, model is given to explain the effects of DPA.

Experimental design

A composite tetraploid population, called Synthetic R, was created in 1974. Twenty inbred lines that had been X-rayed (5,000 r to the kernels) for 10 generations, eight untreated exotic races, and 10 ethylmethane

* Contribution from the Agricultural Research Service, U.S. Department of Agriculture, University of Missouri, Missouri Agricultural Experiment Station, Journal Series No. 9806

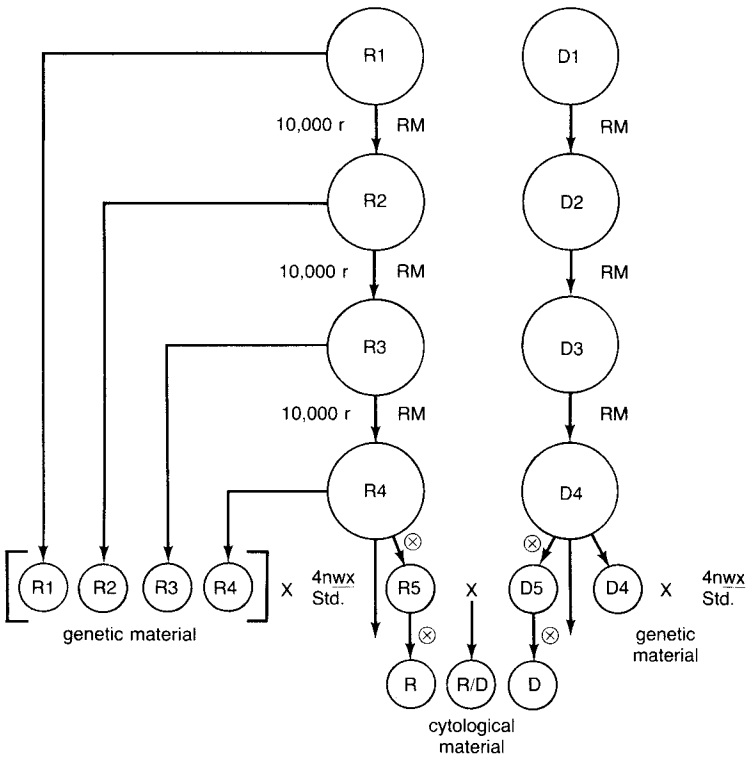

Fig. 1. Experimental design: Two populations of tetraploid maize (Syn R and Syn D) have been maintained by simulated random mating (RM). Plants were grown from reserve stocks of Syn R1, R2, R3, R4, and Syn D4 and crossed with an inbred 4n *wx* line to produce *Wx Wx wx wx* plants for genetic studies. Syn R4 plants were self-fertilized and the progeny were crossed with Syn D # 5 plants to produce Syn R/Syn D hybrids for cytological studies

sulfonate-treated lines (obtained from M. G. Neuffer) were crossed with plants carrying the elongate (*el*) gene. The F2 plants were crossed with tetraploid lines derived from Alexander's Synthetic B (Alexander 1957), which had been subjected to recurrent radiation (along the diploid inbred lines) for ten generations. The F2 plants that were homozygous for *el,* produced diploid eggs which produce tetraploid kernels when fertilized by diploid pollen from a tetraploid. The newly derived 4n material was added together on a 1 : 1 basis with recurrently irradiated 4n Synthetic B families and a few families of recurrently irradiated Argentine Flint (an old 4n line) to form Syn R.

Syn R has been maintained by simulated random mating of 200–300 plants for nine generations (at the time this is written). Twenty kernels from each ear are bulked. A random sample of 900 kernels is taken from this bulked material and is given 10,000 r (150KV at a dose rate of 200 r/min). This material is planted and thinned to a population of about 300 plants. Aside from saving the better seedlings, little selection has been made in the Synthetic R population. It is desirable to keep its gene base as wide as possible.

At the same time another composite tetraploid population, Synthetic D, was created from a variety of tetraploid lines with a large admixture of Alexander's Synthetic B (20–30%), Argentine Flint, and newly *el*-derived 4n material derived from the same (but non-irradiated) inbred lines that went into Syn R. Syn D has been treated in a similar fashion as Syn R (without, of course, the recurrent irradiation). However, additional 4n material has been added over the years. Unlike Syn R, which must be a closed population (to prevent dilution of the effects of recurrent irradiation), new material can be added to Syn D.

Eventually Syn D will provide the Z genomes and Syn R will provide the R genomes to produce a wide variety of allotetraploids (ZZRR).

To determine progress toward allotetraploidization, samples of this material were taken and studied cytologically and genetically. The experimental design is shown in Fig. 1.

Materials and methods

For the cytological studies sporocytes from Syn D, Syn R, and Syn R/Syn D plants were preserved in a 3 ethyl alcohol: 1 propionic acid solution and then examined using the standard acetocarmine squash technique. The pairing configurations were determined at diakinesis. Only those cells that had well-spread figures were scored. Sporocytes with high frequencies of univalents were discarded because they were probably from quasitetraploids such as (4n − 1 + 1). Only a few plants were found of this type. Seedling selection probably eliminated most of the aneuploids in the population.

For the genetic studies mature tassels were taken from *Wx Wx wx wx* Syn R/Std and Syn D/Std hybrids and preserved

in 70% ethyl alcohol with about 1% formaldehyde. Anthers that were about to shed were taken from three flowers and macerated in an aqueous solution of 1% iodine and 1.5% potassium iodide. *Wx Wx* and *Wx wx* pollen cannot be distinguished. They both stain dark blue, while *wx wx* pollen stains reddish brown. From 500 to 700 pollen grains were scored from each sample.

Results

Cytological evidence for progress toward allotetraploidization

The pairing configurations at diakinesis of 5 Syn D, 7 Syn R, and 12 Syn R/Syn D plants are shown in Table 1. In most meiocytes only quadrivalents and bivalents were observed. However, a few trivalents and univalents were found, as indicated at the bottom of the table. These configurations were not included in determining quadrivalent frequencies.

The meiocytes of the five Syn D plants had average quadrivalent numbers ranging from 7.89 to 8.27. These frequencies are not significantly different from the mean quadrivalent number of 8.10, using a X^2 test.

Using the 8.10 quadrivalent frequency of the Syn D plants as a control, six out of seven Syn R and nine of the twelve Syn R/Syn D plants had statistically significant lower frequencies of quadrivalents.

The data indicate that DPA has been induced in the Syn R population. The relationship between DPA and modified pairing-configuration frequencies will be discussed later.

Table 1. Quadrivalent frequencies found in Syn D, Syn R, and Syn D/Syn R tetraploid plants. The χ^2 values were derived using the frequency of 81% quadrivalents (the average of the Syn D plants) as the expected value

Pairing configurations												Total	Ave. No. IV	χ^2	Other[a]
IV	0	1	2	3	4	5	6	7	8	9	10				
II	20	18	16	14	12	10	8	6	4	2	0				
Syn D															
1	0	0	0	0	1	3	13	22	28	22	14	103	7.89	2.86	1 (a)
2	0	0	0	0	0	3	4	19	28	20	7	81	7.98	0.81	0
3	0	0	0	0	2	3	9	20	44	41	12	131	8.08	0.05	1 (b)
4	0	0	0	0	1	1	8	19	30	35	15	109	8.21	0.86	0
5	0	0	0	1	1	0	10	25	37	43	25	142	8.27	2.81	0
Total	0	0	0	1	5	10	44	105	167	161	73	566	8.10		
Syn R															
1	0	0	0	3	3	10	20	28	22	18	7	111	7.16	63.45**	0
2	0	0	0	0	2	8	22	37	29	25	6	129	7.41	39.81**	0
3	0	0	0	0	2	9	23	25	24	25	7	115	7.42	34.82**	0
4	0	0	0	1	1	3	14	30	34	17	8	108	7.60	17.41**	0
5	0	0	0	0	0	6	16	17	31	22	6	98	7.66	12.14**	3 (c)
6	0	0	0	0	1	3	15	27	37	23	13	119	7.82	5.91*	3 (d)
7	0	0	0	0	0	3	4	29	53	32	11	132	8.06	0.13	0
Total	0	0	0	4	9	42	114	193	230	162	58	812	7.60		
Syn D/Syn R															
1	0	0	0	5	9	20	28	25	18	6	0	111	6.23	251.07**	2 (e)
2	0	0	0	3	13	25	30	23	19	8	2	123	6.27	268.15**	0
3	0	0	1	1	8	18	27	31	19	12	3	120	6.63	167.73**	0
4	0	0	0	0	2	21	31	33	30	19	5	141	7.03	105.21**	2 (f)
5	0	0	0	0	3	10	21	23	33	13	4	107	7.03	79.89**	0
6	0	0	0	0	1	8	22	30	28	20	5	114	7.36	39.64**	0
7	0	0	0	0	2	11	16	25	35	25	8	122	7.53	25.50**	0
8	0	0	0	0	1	5	16	25	34	24	12	117	7.76	8.75**	0
9	0	0	0	0	0	4	12	23	30	25	8	102	7.82	5.06*	1 (g)
10	0	0	0	0	0	4	11	26	39	33	11	124	7.96	1.58	0
11	0	0	0	0	1	3	8	19	33	23	14	101	8.03	0.32	0
12	0	0	0	0	1	1	6	23	46	21	16	114	8.10	0.00	0
Total	0	0	1	9	41	130	228	306	364	229	88	1,396	7.31		

[a] Other configurations were (a) 7 IV, 5 II, 2 I; (b) 8 IV, 3 II, 2 I; (c) 2 (7 IV, 1 III, 4 II, 1 I)+(5 IV, 1 III, 8 II, 1 I); (d) 2 (6 IV, 7 II, 2 I), (9 IV 1 II, 2 I); (e) (3 IV, 1 III, 12 II, 1 I)+(5 IV, 1 III, 8 II, 1 I); (f) (6 IV, 7 II, 2 I)+(7 IV, 5 II, 2 I); and (g) 5 IV, 1 III, 8 II, 1 I

* Significant at 0.05, E(Q)=8.10

** Significant at 0.01, E(Q)=8.10

Table 2. The gene segregation for *wx* from different *Wx Wx wx wx* heterozygotes. The adjusted values of *wx* for Syn R1, R2, R3, R4, and Syn D were calculated by multiplying the observed value by 0.1667 (1/6) and dividing the product by 0.1748. The number of plants with significantly lower or higher, or normal (N), frequencies of *wx* pollen were determined by using the χ^2 test with expected values of 17.48%

	No. of plants	No. of grains			% *wx*	Adj. % *wx*	No. of plants with χ^2 significant or not (N)				
		Wx	*wx*	Total			0.01 (–)	0.05 (–)	N	0.05 (+)	0.01 (+)
Control	64	37,695	7,985	45,680	17.48	16.67	1	1	59	3	0
Syn D4	67	34,832	5,911	40,743	14.51	13.83	30	7	25	0	5
Syn R1	65	34,027	6,236	40,263	15.49	14.77	27	1	31	0	6
Syn R2	61	32,127	5,409	37,536	14.41	13.74	27	5	26	1	2
Syn R3	76	39,457	6,214	45,671	13.61	12.97	34	7	33	1	1
Syn R4	83	43,350	6,681	50,031	13.35	12.73	37	6	34	2	4

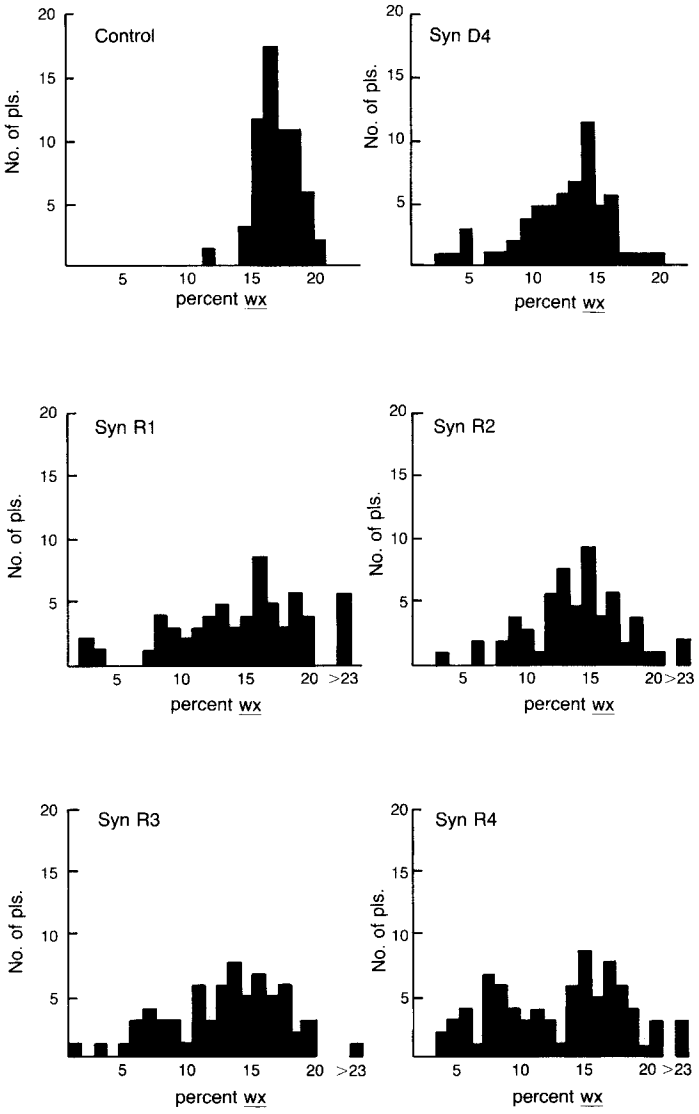

Fig. 2. Histograms showing the distribution of plants with the percentage of *wx* pollen shown on the abscissa

Genetic evidence for progress toward allotetraploidization

Table 2 summarizes the genetic data obtained by observing the frequencies of *wx wx* pollen from Std (standard) 4n *Wx* × Std 4n *wx* plants (control), from the series of Syn crosses (R1 through R4/Std), and from Syn D4/Std (*Wx Wx wx wx*) plants. There is a steady, progressive decrease in the percentage of *wx wx* pollen in the Syn R material from Syn R1 to Syn R4 (15.49% to 13.35%), indicating an increase in DPA. Because the Syn R1 population was derived from diverse and recurrently irradiated material, it had considerable DPA, as indicated by the lowered percentage of *wx wx* pollen (15.49%). Syn D4 also shows the effects of DPA (14.51%). When the data are graphed (Fig. 2) there seem to be bimodal distributions in Syn R3 and Syn R4 populations, and a skewed distribution in the Syn D population.

The relationships between DPA and changed frequencies of pairing configurations and consequent effects on genetic ratios are discussed in the next section.

Discussion

The cytogenetics of autotetraploids is complicated by many factors. Gene segregation in autotetraploids has been examined by many workers (Mather 1935, 1936; Catcheside 1956; Doyle 1973 and others). Space does not permit a review here.

The cytogenetics of segmental allotetraploids is complicated by all the factors found in autotetraploids and several additional ones. A comprehensive theoretical model was presented by Doyle (1979 b). For the purposes of discussion a simplified and workable model will be given here.

The chief simplification is the elimination of the effect of pairing partner switches (as shown in Fig. 3) with chiasmata formation in distal and proximal (interstitial) regions, which would result in the formation of complex types of quadrivalents with 3 or all 4 chro-

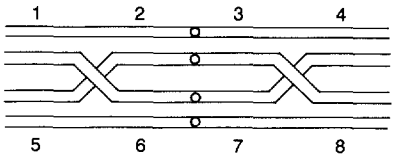

Fig. 3. Pairing switches in a quadrivalent. Chiasmata can arise in any or all of the regions labeled 1 through 8. Ten different types of quadrivalents are possible depending on chiasma distribution

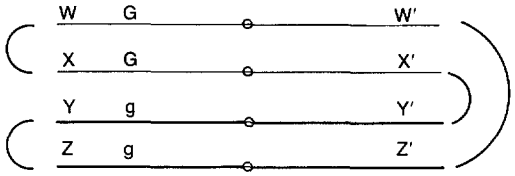

Fig. 4. A model for chromosome pairing in a tetraploid. Each of the four arms can form three different pairs so there are 9 combinations as shown in Fig. 5. The pairing configuration given as an example above would produce a semi-homogenetic quadrivalent, Q–A. *G* and *g* are contrasting alleles at a particular locus

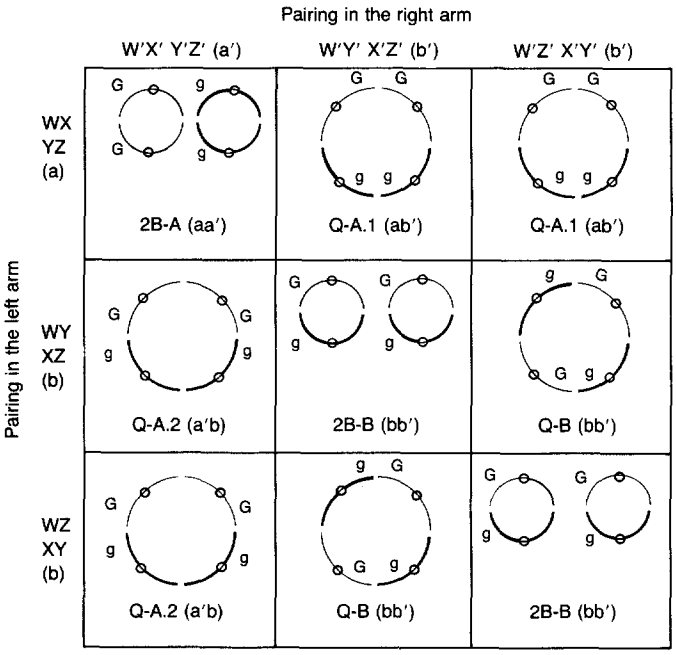

Fig. 5. There are four pairing configurations possible: homogenetic bivalents 2B-A, heterogenetic bivalents 2B-B, heterogenetic quadrivalents Q–B, and semi-homogenetic quadrivalents Q–A. There are two types of semi-homogenetic quadrivalents depending on the arms involved in homologous and non-homologous pairing. They are called Q–A.1 and Q–A.2. The mathematical values in parentheses (aa', ab', etc.) express the expected frequency of the configuration. This assumes that opposite arms of a chromosome pair independently

mosome arms being held together by chiasmata. Also, it is assumed that all arms are paired and thus there will be only two general pairing configurations: two ring bivalents or a ring quadrivalent. A similar model has been presented by Kimber and Alonso (1981), which allows different numbers of chiasmata to be considered.

The basic model is shown in Fig. 4. There are four chromosomes: W–W', X–X', Y–Y', and Z–Z'. Chromosomes W–W' and X–X' are homologous to each other and are homoeologous to chromosomes Y–Y' and Z–Z', which are homologous to each other. Chromosome pairing may be homologous as in WX, W'X', YZ, or Y'Z' or it may be homoeologous as in WY, W'Y', WZ, W'Z', XY, X'Y', XZ, or X'Z'.

DPA factors will affect the relative frequency of homologous versus homoeologous pairing. If it is assumed that chromosome pairing in the left and right arms is independent, then a table can be set up (Fig. 5) to predict pairing configurations of the whole chromosomes. The effect of different frequencies of homologous pairing (a and a') and homoeologous pairing (b and b') can be examined. There are two possible homoeologous pairing-configurations for each arm, so $a+2b=1$, and $a'+2b'=1$. An increase in DPA is expressed as an increase in the value of a or a', or both a and a'. The frequencies of the various configurations with different values of a are shown graphically in Fig. 6.

When ($a=a'$), as shown in Fig. 6, the frequency of homogenetic bivalents, 2B-A, increases as the square of a. When $a=1$ as in an allotetraploid, 2B-A = 100%. Heterogenetic bivalents, 2B-B, and heterogenetic quadrivalents, Q–B, decrease as the square of b. Semi-homogenetic quadrivalents, Q–A, increase temporarily until a becomes greater than 50%. The two types of quadrivalents (Q–A and Q–B) can not usually be distinguished cytologically, so only the total quadrivalent frequency can be observed. The decline in total quadrivalent frequency (Q) is slow at first. Even when $a=0.50$, Q is 62.5%, only a 4.2% reduction from the value of 66.7% expected with random pairing (where $a=0.33$).

If we assume that only one set of four arms (either the left or the right) has DPA and the opposite arms pair at random, then the results are shown in Fig. 7. Here the total quadrivalent frequency (Q) does not change with an increase in a although there is a linear increase in Q–A and a linear decrease to zero of Q–B.

To obtain the reductions in Q shown in Table 1 would require DPA in both arms, according to the model. DPA in one set of arms would not be detectable. However, the model does not consider the effect of pairing partner switching with chiasmata in more

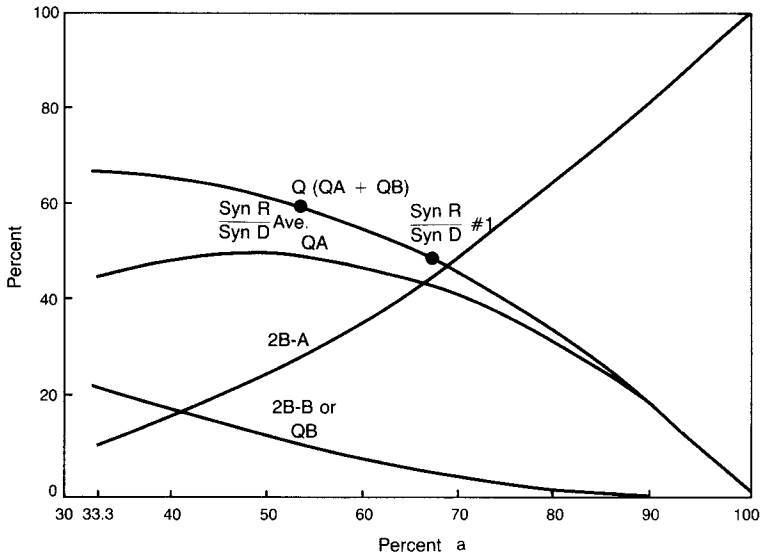

Fig. 6. The frequency of various configurations as related to the values of a (homologous pairing). The lines show changes in pairing configuration frequencies when the amount of DPA is equal in both arms ($a=a'$ and $b=b'$)

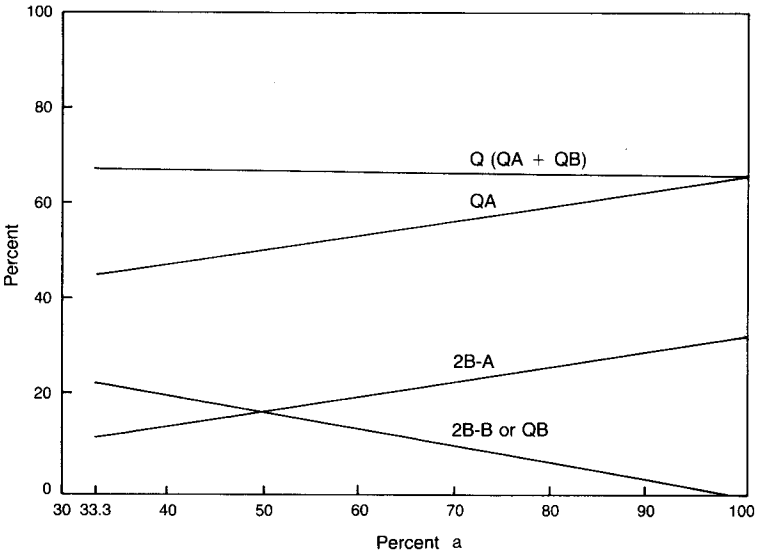

Fig. 7. The frequency of various configurations when it is assumed that only one set of four arms (either the *right* or the *left*) has DPA and the opposite set of arms pairs at random

than one paired region in a set of arms. DPA in one set of arms would lessen the amount of switching and reduce Q. For example, using Fig. 3, if there are chiasmata in regions 1, 4, 5, and 8 then two ring bivalents are formed. If chiasmata form also in regions 2 or 6 or both, then a quadrivalent is formed. The switching of pairing partners could also be inhibited by some sort of genetic control over the general process of pairing. If there were no switching of partners or a limit of one chiasma per arm, and no DPA, Q would be 2/3 or 66.7% and all the quadrivalents would be rings or chains. While the 10 quadrivalent types were not scored it was noted that the non-ring and nonchain quadrivalents were common in Syn R and Syn R/Syn D plants with reduced Q values, thus the general suppression of chiasmata in excess of one per set of arms cannot be the only explanation of reduced Q values and therefore there must have been DPA present.

The value of Q varies with the species. Many species have little pairing partner switching or have chiasmata mostly near the ends of the chromosomes and thus have Q values close to 66.7% (Morrison and Rajhathy 1960). The Q values of maize depend on the homogeneity of material and upon whether it has been selected for fertility. Gilles and Randolph (1951) found that the Q of a line declined from 84.7% to 74.6% after 10 years of selection. Kadam (1944) found that a fertile 4n inbred line had a Q of 85.6% and open-pollinated material had a Q of 75.8%. Shaver (1962) found Q to be 87.1% in an inbred line of 4n maize.

The average Q of the Syn D was 81.0%, which is slightly lower than some of the Q values reported in maize. This probably reflects the presence of DPA, which is indicated by the genetic data. A 4n inbred line would give a better control value for Q.

Selection for vigor and fertility has been found to reduce the Q of raw autotetraploids produced from diploids such as turnips (Swaminathan and Sulbha 1959), grain amaranths (Pal and Pandey 1982), rye (Hilpert 1957), barley (Bender and Gaul 1966) and *Coix* (Venkateswarlu and Rao 1976). The relatively short time span of these experiments makes the creation of any significant new DPA factors unlikely. Perhaps changes in the frequency of genes that affect pairing occur. The mechanisms for quadrivalent reduction are not understood.

In some cases selection for vigor and fertility has not been reflected in a decrease in quadrivalent frequency, as reported by Mastenbroek et al. (1982), who compared Q's of first generation and 22nd generation Synthetic B.

If Q could be reduced to zero by selection, the autotetraploid with all bivalents would produce almost all euploid offspring. However, in an autotetraploid the chromosomes pair at random, therefore a maximum state of heterosis cannot be stabilized as in an allopolyploid where genes in different genomes that give heterotic effects cannot segregate.

A similar study by Gaul and Friedt (1975) using recurrent radiation and chemical mutagens in autotetraploid barley has reduced the average number of quadrivalents from 3.9 to 3.6.

If the Q values in Table 1 are multiplied by 0.667/0.810, then these quadrivalent frequencies may be adjusted to the model and used on the graph. The average quadrivalent frequency of the Syn R/Syn D plants was adjusted from 0.731 to 0.602, and the Q value of the Syn R/Syn D plant No. 1 goes from 0.623 to 0.513. These points have been entered on the graph in Fig. 6. This would estimate the value of autosyndetic pairing (*a*) at 0.53 for the average of the 12 Syn R/Syn D plants and at 0.66 for Syn R/Syn D plant No. 1. If this relatively crude estimate is valid, then allotetraploidization is well advanced.

Changes in the relative frequencies of different pairing configurations will change genetic ratios. The gametic output of each configuration is given in Table 3. Homogenetic bivalents, 2B-A, disjoin to give all *Gg* gametes. Random orientation of the heterogenetic bivalents, 2B-B, on the metaphase plate will give a ratio of 1:2:1 for *GG, Gg* and *gg* gametes. The semi-homogenetic quadrivalent, Q–A, will yield all *Gg* gametes with adjacent-1 or alternate disjunction. Adjacent-2 disjunction (chromosomes with homologous centromeres going to the same poles) gives 1/2 *GG* and 1/2 *gg* gametes. The heterogenetic quadrivalent, Q–B, gives 1/2 *GG* and 1/2 *gg* gametes with alternate disjunction and all *Gg* gametes with adjacent disjunction (adjacent-1 and adjacent-2 disjunction patterns are not relevant to Q–B).

If the three types of disjunction were equal in frequency, the ratio of *GG:Gg:gg* would be 1:4:1. However, if there is directed alternate segregation, then genetic ratios will be affected by the relative frequencies of Q–A's and Q–B's. In maize the frequency of alternate disjunction is one-half in translocation heterozygote quadrivalents (Burnham 1950). Thus, the gametic output of Q–A would be 1/8 *GG*, 3/4 *Gg*, and 1/8 *gg*, and that of Q–B would be 1/4 *GG*, 1/2 *Gg*, and 1/4 *gg*. Under random pairing the ratio of Q–A:Q–B is 2:1 so the combined gametic output will have the 1:4:1 ratio for *GG, Gg*, and *gg*. If the ratio of Q–A: Q–B is $>2:<1$, then the frequency of *gg* gametes arising from the quadrivalents will be less than 1/6, but not less than 1/8. However, the major factor in the decline of *gg* gametes as DPA increases is the quadratic increase of homogenetic bivalents (2B-A) which produce no *gg* gametes.

A graph showing the relationship of DPA in both arms (or in one arm only) to genetic ratios is given in Fig. 8. The formula for determining *gg* is 1/4 (2B-B) + 1/4 (Q–B) + 1/8 (Q–A). The upper line shows the results if DPA is only in one arm and the lower line shows the result of an equal amount of DPA in both arms.

There is a problem with this analysis. Unless the gene locus followed is next to the centromere, the quadrivalent types shown in Fig. 9 need to be considered. The constitution of the chromosomes may be equational (e), *Gg*, or reductional (r), *GG* or *gg*, after crossing-over, depending on whether or not the exchange took place between the gene locus and the centromere.

Their gametic output is shown in Table 3. It may be seen that the effect of equational constitutions results in frequencies of *GG* and *gg* greater than 1/6 (the value

Table 3. The expected gametic output from different pairing configurations with different patterns of chromosome disjunction. The symbols used in the table for chromosome disjunction are explained in the text

Configuration	Type of disjunction	Gametes expected		
		GG	*Gg*	*gg*
2B-A		0	all	0
2B-B		1/4	1/2	1/4
Q-A	adj.-1	0	all	0
	adj.-2	1/4	1/2	1/4
	alt.	0	all	0
Q-B	adj.	0	all	0
	alt.	1/2	1/4	1/2
Q-A.2re	adj.-1	1/8	3/4	1/8
	adj.-2 or alt.	1/4	1/2	1/4
Q-Bre	adj.-a	1/8	3/4	1/8
	adj.-b or alt.	1/4	1/2	1/4
Q-A.2ee or Q-Bee	any	1/4	1/2	1/4

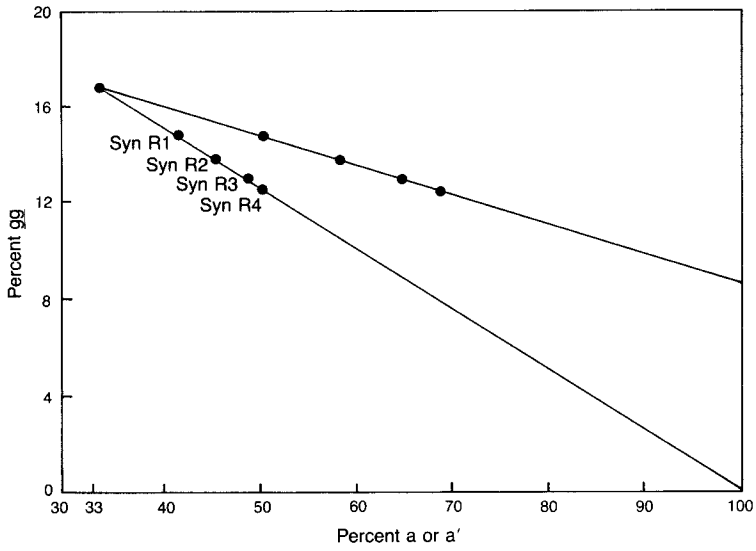

Fig. 8. Graph showing the relationship of the value of a (homologous pairing) and the frequency of recessive gametes (gg) from a douplex heterozygote ($GGgg$). The formula for determining the frequency of gg gametes is 1/4 (2B-B)+1/4 (Q–B)+1/8 (Q A). This assumes that alternate disjunction is 50%

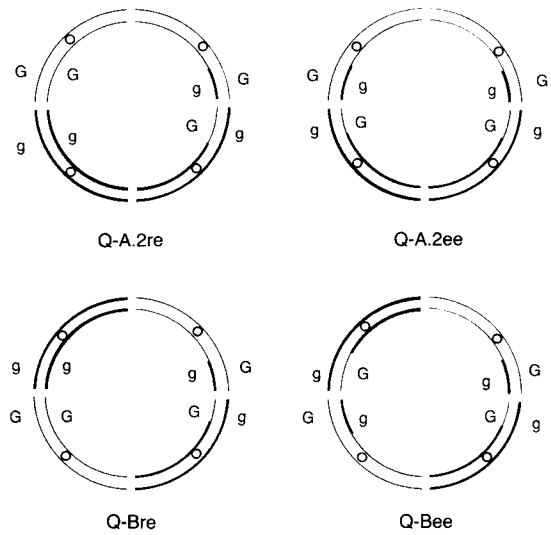

Fig. 9. Four additional types of quadrivalents that result from crossing over between the gene locus and the centromere. These types may produce double-reductional gametes, whose frequency is expressed as α

Table 4. General formulae for the expected frequencies of gametes from different tetraploid genotypes. The duplex (*SSMM*) gametic output is modified by preferential pairing.

Genotype	Frequency	Gametic frequency		
		SS	*SM*	*MM*
SSSS	s^4	all		
SSSM	$4s^3m$	$1/2+\alpha/4$	$1/2-\alpha/2$	$\alpha/4$
SSMM	$6s^2m^2$	$<(1/6+\alpha/3)$	$>(2/3-2\alpha/3)$	$<(1/6+\alpha/3)$
SMMM	$4sm^3$	$\alpha/4$	$1/2-\alpha/2$	$1/2+\alpha/4$
MMMM	m^4			all

expected from random chromosome assortment). Some of these gametes have genes that were on sister chromatids and thus are double reductional. The frequency of double reduction is signified mathematically as α. The value of α in the control material was 2.43% ($1/6+\alpha/3=17.48$).

DPA will reduce the value of α by reducing the frequency of quadrivalents and by decreasing crossing over between homoeologous chromosomes. The data in Table 2 may be adjusted to fit the genetic model (which neglects double reduction) by multiplying the observed values by 0.1667/0.1748. When the adjusted value for Syn R4 is entered on the graph in Fig. 7, the estimated value of a for Syn R4 is 0.49 assuming $a=a'$. This is in reasonable agreement with the value for Syn R5 of 0.53 using the quadrivalent frequency data as shown in Fig. 6.

Of course, individual plants show much greater deviations from the control value and the DPA found in their chromosome 9's (the *wx* locus is on chromosome 9) may be much greater.

Cytological data, such as the quadrivalent frequencies, have the advantage of indicating the DPA level of the whole genome, while genetic data give an indication of DPA for only the chromosomes with gene markers. Several genes will be followed at the same time when multiple recessive stocks have been derived.

Genetic methods of detecting DPA are much more efficient than cytological ones. Large progenies are easily generated in maize. For example, if 100,000 kernels are examined from a testcross of *GGgg*, the course of 100,000 meiotic events can be determined. Every *gg* gamete is the result of homoeologous pairing.

Progress toward allotetraploidization is probably considerably greater than can be demonstrated. In a tetraploid there are three kinds of heterozygotes. If the

Table 5. Frequencies of zygotic genotypes with random mating and the frequencies of gametes if $\alpha=0$, and if $\alpha=1/6$

	Value of S					
	0.99	0.90	0.80	0.70	0.60	0.50
Genotypes						
SSSS	0.9606	0.6561	0.4096	0.2401	0.1296	0.0625
SSSM	0.0388	0.2916	0.4096	0.4116	0.3456	0.2500
SSMM	0.0006	0.0486	0.1536	0.2646	0.3456	0.3750
SMMM	0.0000	0.0036	0.0256	0.0756	0.1536	0.2500
MMMM	0.0000	0.0001	0.0016	0.0081	0.0256	0.0625
Gametes						
if $\alpha=0$						
SS	0.9801	0.8100	0.6400	0.4900	0.3600	0.2500
SM	0.0198	0.1800	0.3200	0.4200	0.4800	0.5000
MM	0.0001	0.0100	0.0400	0.0900	0.1600	0.2500
if $\alpha=1/6$						
SS	0.9817	0.8250	0.6667	0.5250	0.4000	0.2916
SM	0.0165	0.1500	0.2667	0.3500	0.4000	0.4167
MM	0.0017	0.0250	0.0667	0.1250	0.2000	0.2916

standard pairing unit is S and the mutated pairing unit is M, then there are SSSM (simplex), SSMM (duplex) and SMMM (triplex) types of heterozygotes. If there are multiple alleles of the pairing unit they would be designated M1, M2 . . . Mn. With multiple alleles there are other types of genotypes possible such as SSM1M2, SM1M1M2, or M1M1M2M3 (tri-allelics) or SM1M2M3, or M1M2M3M4 (quadri-allelics). The duplex (SSMM) is called a balanced di-allelic and the simplex (SSSM) and the triplex (SMMM) are unbalanced di-allelics.

The Syn R population is a mixture of different genotypes. To simplify the following discussion it will be assumed that there is one mutant pairing allele. The frequency of genotypes under random mating will be $SSSS=s^4$, $SSSM=4s^3m$, $SSMM=6s^2m^2$, $SMMM=4sm^3$ and $MMMM=m^4$, where s is the frequency of the standard unit and m is the frequency of the mutant allele. The gamete production of these genotypes is shown in Table 4. Table 5 shows the frequencies of genotypes and gametes produced by them for different values of s and m. When m is low the frequency of MM gametes is much less than m. If $\alpha=0$ then MM gametes $=m^2$. At the maximum value of α there is a large proportional increase in MM gametes.

These MS and MM gametes will combine with SS gametes from standard 4n maize (SSSS) to form simplex (SSSM) and duplex (SSMM) plants. As discussed in part 2 of this series, preferential pairing is not possible in a simplex. The M-bearing chromosome has no choice; it must pair with an S-bearing chromosome. Since the majority of M chromosomes are present in simplex form, a great amount of potential DPA goes undetected.

If a sample of plants is taken out of the Syn R populations and self-fertilized for several generations the percentage of MMMM plants (that produce only MM gametes) will increase. Also the percentage of SMMM plants (that yield 1/2 MM gametes) will increase temporarily. For example, if s=0.90 and m=0.10, the frequency of MM gametes increases from 0.01 to 0.025 in the first selfed generation (S1) and then to 0.0375, 0.0479, 0.0566, and 0.0638 in subsequent generations. The approach to homozygosity is very slow in tetraploids. This assumes random chromosome assortment that is not disturbed by the presence of DPA. The duplex (SSMM) will probably not have a 1/6 SS:2/3 SM:1/6 MM (neglecting α) ratio, because due to preferential pairing there will be more than 2/3 SM gametes.

The population genetics of a tetraploid population subjected to recurrent irradiation is very complex. One feature, the behavior of reciprocal translocations that are present in the Syn R, has been investigated recently on a theoretical basis (Doyle and Kimber 1983).

Conclusion

Cytological and genetic evidence has been presented that indicates considerable progress toward the allotetraploidization of maize. Progress is probably greater than can be demonstrated because of the heterozgosity of the material. Only the DPA present in duplex condition can be detected and the majority of the DPA is probably in a simplex condition. Also because of the time required to test the material and assess the data (three years at the least) the progress toward allo-

tetraploidization is underestimated. The Syn R stocks used in these experients were from the first to the fifth cycle. The Syn R material is currently in the ninth cycle.

References

Alexander DE (1957) The genetic induction of autotetraploidy. A proposal for its use in corn breeding. Agron J 49:40–43

Bender K, Gaul H (1966) Zur Frage der Diploidisierung autotetraploider Gerste. Z Pflanzenzücht 56:164–183

Burnham CR (1950) Chromosome segregation in translocations involving chromosome 6 in maize. Genetics 35: 446–481

Catcheside DG (1956) Double reduction and numerical nondisjunction in maize. Heredity 10:205–218

Doyle GG (1973) Autotetraploid gene segregation. Theor Appl Genet 43:139–146

Doyle GG (1979a) The allotetraploidization of maize. 1. The physical basis-differential pairing infinity. Theor Appl Genet 54:103–112

Doyle GG (1979b) The allotetraploidization of maize. 2. The theoretical basis – the cytogenetics of segmental allotetraploids. Theor Appl Genet 54:161–168

Doyle GG (1982) The allotetraploidization of maize. 3. Gene segregation in trisomic heterozygotes. Theor Appl Genet 61:81–89

Doyle GG, Kimber G (1983) A computer simulation of the behavior of reciprocal translocations in autotetraploids. Theor Appl Genet 65:53–60

Gaul H, Friedt W (1975) Progress in the diploidization of autotetraploid barley. In: Barley genetics, vol III. Proc 3rd Int Barley Genet Symp, Garching, pp 378–387

Gilles A, Randolph LF (1951) Reduction of quadrivalent frequency in autotetraploid maize during a period of ten years. Am J Bot 38:12–17

Hilpert G (1957) Effects of selection for meiotic behavior in autotetraploid rye. Hereditas 43:318–322

Kadam BS (1944) Chromosome studies in relation to fertility and vigor in inbred and open-pollinated strains of autotetraploid maize. 1. Behavior of 40-chromosome plants. Indian J Genet Plant Breed 4:8–22

Kimber G, Alonso LC (1981) The analysis of meiosis in hybrids. 3. Tetraploid hybrids. Can J Genet Cytol 23:235 to 254

Mastenbroek I, DeWet JMJ, Chieh-Yin Lu (1982) Chromosome behaviour in early and advanced generations of tetraploid maize. Caryologia 35:463–470

Mather K (1935) Reductional and equational separation of the chromosomes in bivalents and tetravalents. J Genet 30:53 to 78

Mather K (1936) Segregation and linkage in autotetraploids. J Genet 32:287–314

Morrison JW, Rajhathy T (1960) Frequency of quadrivalents in autotetraploid plants. Nature 187:528–530

Pal M, Pandey RM (1982) Decrease in quadrivalent frequency over a 10 year period in autotetraploids in two species of grain amaranths. Cytologia 47:795–801

Shaver DL (1962) A comparison of meiosis in perennial teosinte (4n), tetraploid maize and their hybrid. Caryologia 15:53–57

Swaminathan MS, Sulbha K (1959) Multivalent frequency and seed fertility in raw and evolved tetraploids of *Brassica campestris* var. 'toria'. Z Vererbungsl 90:385–392

Ventkateswarlu J, Rao PN (1976) Effect of inbreeding and selection for vigor and fertility on meiotic behavior in autotetraploid Job's Tears, *Coix lacryma-jobi* L. Theor Appl Genet 47:165–169