Theor. Appl. Genet. 54, 103-112 (1979)

The Allotetraploidization of Maize

Part 1: The Physical Basis – Differential Pairing Affinity*

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Summary. Allotetraploidization is the creation of artificial allotetraploids. Allotetraploidization of maize can be achieved by restructuring a maize genome so that its chromosomes will not pair with those of the normal maize genome. The restructuring can be done by concentrating induced or naturally occurring visible and cryptic chromosome aberrations and qualitatively different genetic material into a single line by a recurrent selection type of breeding program. The basis of allotetraploidization is the presence of differential pairing affinity between normal and restructured chromosomes. Experiments demonstrate that differential pairing affinity factors occur naturally in exotic races and in standard corn belt inbred lines and that they may be readily induced by X-irradiation and chemical mutagens.

Key words: Trisomes – Tetraploids – Preferential pairing – Synapsis – Experimental evolution

Introduction

Allotetraploidization is the creation of artificial allotetraploids. This is very simple when two species (A & B) are available which exhibit enough differential pairing affinity (DPA) to prevent the pairing of their homoeologous chromosomes in the allotetraploid (AABB) produced by doubling the chromosome number of the hybrid (AB).

To produce allotetraploid maize we must work within a single species, *Zea mays* L. If we assign the genome symbol Z to maize, a Z genome must be converted into a new restructured genome which will be called R. To produce this R genome, DPA factors such as induced and naturally occurring visible and cryptic chromosome aberrations and qualitatively different genetic material must be concentrated into a single line.

Because the final product is derived from only one species some cytogeneticists prefer to use the term diploidization rather than allotetraploidization. The product of this process is diploidized only in cytological appearance. It has two pairs of genomes and is structurally equivalent to an allotetraploid. Thus, the term allotetraploidization is preferred.

Allotetraploid maize (ZZRR) would be superior to autotetraploid maize (ZZZZ). An allotetraploid forms only bivalents that are homogenetic (ZZ and RR). These bivalents separate to form (ZR) gametes which restore the (ZZRR) parental form when combined with each other. Thus, allotetraploid maize would be a true breeding hybrid. Also, because virtually no aneuploid gametes are formed, an allotetraploid population will not have the reduction in vigor and fertility due to aneuploidy found in autotetraploid populations.

The idea of allotetraploidization cannot be ascribed to any individual. It has been thoroughly discussed only by Sybenga (1969, 1973). Sybenga focuses his attention on differential pairing affinity. He believes that pairing specificity resides in units called zygomeres and by manipulating these units, synthetic allopolyploids can be produced. Indeed, the critical requirement for allotetraploidization is an understanding of the basis of differential pairing affinity.

Basis of DPA

Differential pairing affinity is the result of many factors. Chromosome pairing consists of three distinct but sequentially dependent processes. These are congressional pairing, synapsis and chiasmatic pairing. Congressional pairing is the movement toward each other of homologous chro-

* Contribution from the Science and Education Administration, U.S. Department of Agriculture, and the Agronomy Department, University of Missouri, Columbia, Missouri, Missouri Agric. Exp. Sta. Journal Series No. 8092

0040-5752/79/0054/0103/£ 2.00

mosomes (or segments thereof) across intracellular distances. This probably occurs premeiotically. Synapsis is the very close alignment of homologous chromosomes which takes place at zygonema. The synaptic force disappears at the start of diplonema and the chromosomes are held together by chiasmata which were produced by crossing over. This chiasmatic pairing disappears at the start of anaphase. The various DPA factors can affect any or all of these processes.

DPA factors fall into three different groups. The first kind is structural nonhomology resulting from chromosome aberrations such as deficiencies, duplications, inversions or reciprocal translocations. It has been demonstrated that inversions create a large DPA factor (Doyle 1963, 1969) where tetraploids or trisomes heterozygous for inversions exhibit strong preferential pairing. Chromosome aberrations so small that they are not visible using the light microscope, called cryptic structural changes, are probably common.

The second type of DPA is the result of qualitative or quantitative differences in the pairing code. It has been suggested that the pairing code resides in certain palindromic segments of DNA (Sobell 1972, 1975; Doyle and Coe 1972; Doyle 1978). The validity of this theory is not established. Nonetheless, whatever the basis of the pairing code, it is subject to mutation. These pairing mutations can be classified as is done with other mutants. Thus, we can have *amorphs* – the loss of any pairing affinity, *hypomorphs* – reduced pairing affinity, *hypermorphs* – increased pairing affinity, *antimorphs* – repulsion rather than attraction and *neomorphs* – a new pairing affinity.

The third type of DPA involves genes which control and modify chromosome pairing. The meiotic process is under genetic control. There are a large number of these genes known. One gene, *Ph*, is known in wheat that prevents the association of homoeologous chromosomes (Sears 1958; Riley and Chapman 1958). The general occurrence of these DPA controlling factors is an interesting possibility.

Effect of DPA

The effect of DPA on types of pairing configurations in diploids, triploids and tetraploids is suggested in Fig. 1. These graphs are not meant to be taken in a rigorous mathematical sense. Obviously, the relative frequencies of the different types of pairing configurations would vary with the chromosome or species involved.

On the left side where there is no DPA, the diploid forms all bivalents. The triploid and the tetraploid form a high frequency of multivalents.

On the right side, where the DPA is very high, the diploid forms all univalents; the triploid forms all homogenetic bivalents, with the odd genome's chromosomes all univalents; and the tetraploid forms all homogenetic bivalents.

In between the two extremes, with increasing DPA there is a decrease in the frequency of multivalents and of heterogenetic bivalents in the polyploids. In the diploid there is a decline in the frequency of bivalents after an initial lag phase due to what may be called 'reserve pairing capacity.' There is also an increase in rod bivalents and a related decline in ring bivalents.

The types of pairing configurations found in polyploids are given in Figs. 2 and 3. These various types are the result of different patterns of pairing-partner exchange and chiasma placement. If DPA is absent, the chromosomes will pair at random and there should be, for exam-

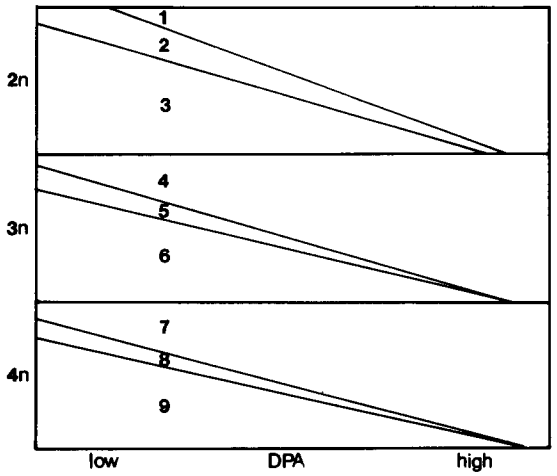

Fig. 1. The numbered areas represent the proportion of pairing configurations in relation to the DPA: 1. Univalents, 2. Rod bivalents, 3. Ring bivalents, 4. Homogenetic bivalents and univalents, 5. Heterogenetic bivalents and univalents, 6. Trivalents, 7. Homogenetic bivalents, 8. Heterogenetic bivalents and 9. Quadrivalents

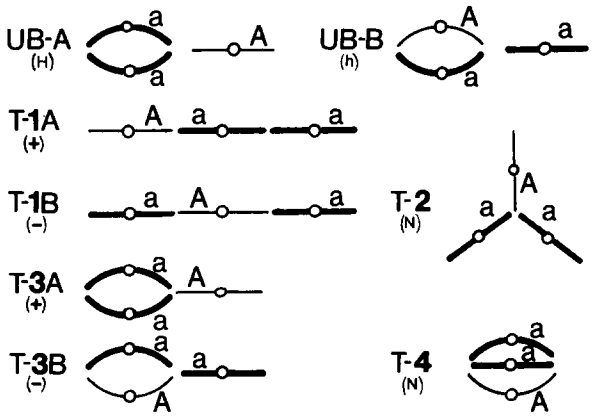

Fig. 2. Pairing configurations in triploids. The symbols in the parentheses indicate nature of pairing. (H) = homogenetic, (h) = heterogenetic, (+) = semi-homogenetic, (–) = semi-heterogenetic and (N) = neutral (Drawings in this paper were done by Ms. Laura Dunham)

ple, one-half the frequency of univalent + homogenetic bivalent (UB-A) as univalent + heterogenetic bivalents (UB-B). If DPA is present there should be more UB-A than if pairing was at random. Multivalents may be classified as semi-heterogenetic, semi-homogenetic or neutral depending on whether the proportion of homologous arm associations is less than, greater than, or equal to one-third, respectively. T-1A, T-3A, and Q-2A are semi-homogenetic, T-1B and T-3B are semi-heterogenetic. T-2 and T-4 are neutral. Only one type of quadrivalent, the ring quadrivalent, is given in both its subtypes. There are nine other types that could be subdivided into semi-homogenetic, semi-heterogenetic or neutral types. These are dealt with in the second paper of this series.

The Assessment of DPA

The graphs in Fig. 1 suggest several ways of assessing DPA. In diploids the relative frequencies of ring and rod bivalents, and univalents could be determined. This method requires trained workers and is not practical on a large scale. Univalent formation leads to the production of hypoploid spores which abort. Large-scale screening is possible by examining rates of pollen abortion. Unfortunately, there is a variable rate of background pollen abortion of 2-5% even in normal inbreds. Also, chromosome aberrations that produce deficient spores would have to be excluded. Furthermore, because of the 'reserve pairing capacity' low levels of DPA could not be detected.

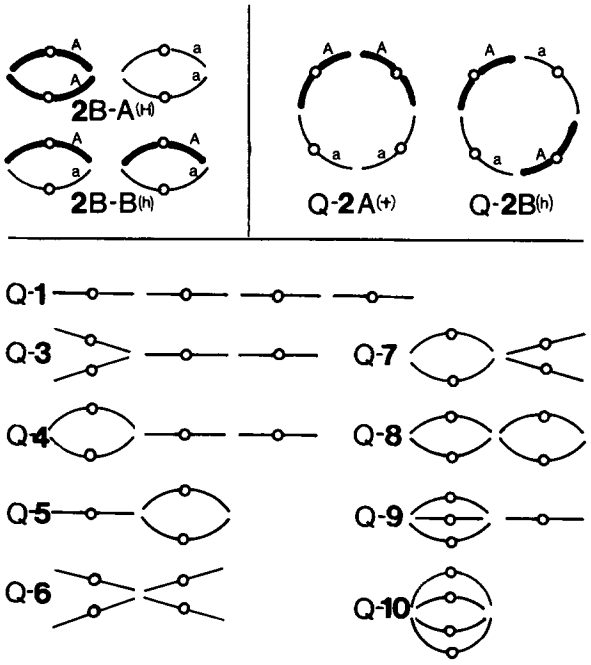

Fig. 3. Pairing configurations in tetraploids. Symbols in parentheses indicate nature of pairing. See legend for Fig. 2

In polyploids, the frequencies of multivalent formation could be determined. Cytological examinations are more difficult to do in polyploids than in diploids and the amount of material that could be studied would be limited. Also, very significant information such as the relative frequencies of homogenetic and heterogenetic bivalents, and the relative frequencies of semi-homogenetic, semi-heterogenetic, and neutral multivalents could not be determined unless cytological markers were available.

The amount of DPA affects the relative proportions of different pairing configurations. Each type of configuration has different expected gametic output. A theoretical analysis of the relationship of DPA and genetic ratios is too lengthy for this paper and will be dealt with in the second paper of this series.

However, the major effect of DPA can be explained briefly. There is a higher than random frequency of homogenetic bivalents (*aa*) in *Aaa* triploids or trisomes and of homogenetic bivalents (*AA* and *aa*) in tetraploids. These configurations lead to the formation of only *a* gametes in the triploids or trisomes and *Aa* gametes in tetraploids.

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 *AAaa*, the course of 100,000 meiotic events can be determined. Every *aa* gamete is the result of homoeologous pairing.

The detection of DPA by the use of genetic data creates poblems. In triploid and tetraploid cultures only euploid plants can be used. This requires chromosome counts. If in tetraploids *AAAA* is crossed with *aaaa*, we will get some *AAAaa*, *AAaaa*, *Aaa* and *AAa* plants along with the desired *AAaa* type.

Aneuploidy also will occur in triploids since one of the parents will be a tetraploid. Triploid kernels are shrunken and difficult to germinate. There are additional problems of the formation of aneuploid gametes whose transmission rates may vary.

If trisomes are used instead of triploids, most of these problems are avoided. There is no need to count the chromosomes of a trisome since its chromosome constitution may be inferred from its phenotype and from the genetic data. If the trisome is used as the male, we need concern ourselves only with the relative frequencies of *n* gamete types since the *n + 1* gametes function only very rarely in fertilization. Thus, from a cross of *aa* × *Aaa*, we will obtain a ratio of *2a : 1A* if pairing is at random and lower frequencies of *A* if the pairing is preferential.

Materials and Methods

Assessment of DPA in trisomic 3 plants was made by studying the gene segregation of *A* and *a*. The gene *A* is located on the long arm of chromosome 3. It is one of a series of complementary factors

controlling the production of anthocyanin in the aleurone layer of the kernel and in other plant tissues. Kernels with at least one *A* gene in the presence of other complementary factors have colored aleurones. Kernels homozygous for *a* have colorless aleurones.

Trisomes homozygous for *a* on standard chromosomes 3 were crossed as the female with plants homozygous for *A* whose chromosomes 3 were different or potentially different because they were in different races or different inbreds, or carried inversions, or had been subjected to X-irradiation or chemical mutagens.

The definition of standard chromosome 3 is arbitrary; it is the chromosome 3 present in the trisomic 3 material obtained from the Maize Genetics Cooperation Stock Center (MGC). Experimentally, it has been determined to be equivalent to the chromosome 3 of inbreds N6 or W23.

The trisome hybrids (*Aaa*) were crossed as the male parents onto *a* testers. All progenies with less than 300 kernels were discarded for the sake of statistical simplicity. At least four crosses were attempted with each individual trisomic male parent. The size of most progenies was around 1,000 kernels.

The exotic races were obtained from MGC and from the Rockefeller Stock Collection Centers in Mexico and Brazil.

Trisomic 3 plants with irradiated chromosomes 3 were produced by treating mature pollen with X-rays at a dose of 500r per minute (150Kv 9ma) and pollinating the standard trisome.

Material treated with chemical mutagens, ethyl methane sulfonate (EMS) and nitrosoguanidine (NG), was provided by the courtesy of M.G. Neuffer. The material was derived by the treatment of pollen in mineral oil with 0.1% EMS for 19 minutes or a saturated solution of NG for 3 minutes (Neuffer and Coe 1977). Plants in the M_2 generation were crossed with the standard trisome.

Assessment of DPA in tetraploids was studied by observing the gene segregation of *Wx* and *wx* in the pollen. Pollen which is *Wx/Wx* or *Wx/wx* contains amylose and amylopectin and stains dark blue in the presence of iodine. Pollen which is *wx/wx* contains only amylopectin and stains red-brown. The iodine solution used was 1% I_2 and 1.5% KI in 70% alcohol. Tassel samples from *Wx/Wx/wx/wx* plants were preserved in 70% alcohol with a little formaldehyde. Pollen was taken from three anthers from three florets. From 600 to 700 pollen grains were counted from each plant.

Irradiated 4n lines were given 5000r (500r per min, 150Kv 9ma) to the kernels for five generations.

Results and Discussion

Trisomics

The results of the studies of the effect of non-random pairing on the genetic ratios found in the progeny of crosses between *aa* testers and trisomic heterozygous pollen parents (*Aaa*) are given in Table 1.

Data from control trisomes in Section 1 corresponded well with the values expected from random pairing. There is no variation that could not be ascribed to chance. We would expect 2.4 cases of significant deviation at the .05 level with 48 tests and there are two.

In Section 2, where the *A* chromosomes were derived from a different race, in all cases there were some plants significantly different statistically from the 33.3% value at the .01 level. There may be one to one segregations for genetic ratios (half $2a : 1A$, half $> 2a : < 1A$) in some of the trisomic hybrids, such as Avati tupi, Chapalote and Reventador. This would be expected since the parents were not inbred lines and could have segregated DPA factors. It would be possible to extract inbred lines from these races and obtain more homogeneous data; note the high interaction chi squares in most cases.

However, when corn belt inbred lines were tested, the data were also found to be heterogeneous (Section 3). The heterogeneity of the data seems to increase with greater deviations from 33.3% transmission of *A* gametes; note the cases on B41, Hy and 38-11. The phenomenon of non-random pairing is probably inherently variable; perhaps it is greatly affected by environmental conditions.

In both Sections 2 and 3, there are many cases in which the transmission of *A* gametes was significantly greater than 33.3%. This will be discussed later in the paper.

In Section 4, the genetic ratios of trisomic inversion-standard heterozygotes are given. The effect of inversions on non-random pairing depends upon their length and their position in the chromosomes. Comparisons between different inversions must be done in the same background. In the case of In3a Kys and In3b Kys we can make a comparison. In3a and In3b are about the same length but In3a is nearer the end of the chromosome (L.40-L.95) than is In3b (L.25-L.75). In3a has a greater effect, supporting the data of Burnham et al. (1972), who found evidence that DPA is more critical at the ends of the chromosome. In3c occupies almost all of the long arm (L.05-L.95) and has a greater effect than In3a, In3b or In3h (L.19-.72), which are all shorter. A comparison of the effects of the two pericentric inversions In3d (S.72-L.42) and In3e (S.39-L.80) shows that the longer the inversion the more effective. In3d is longer than In3a, In3b or In3h and has less effect than any of them because of its internal location. This assumes essentially similar backgrounds.

It is interesting to note that *Kys* DPA factors in In3a trisomes cause a 6.8% reduction of *A* gamete transmission in In/N/N trisomes and a 2.8% reduction in N/N/N trisomes. There are probably synergistic effects with different DPA components.

In Section 5, the effects of using irradiated pollen to produce the trisomic test plants are given. There are many deviations from the transmission rates of the control, and interestingly an equal number above and below in the cases of Std C and Kys. An excess of deviations above 15.17% was found in the Kys In3a 1000r class. It should be noted that the average transmission rates are not greatly different in the controls and the experimental material.

Samples of this irradiated material were reintroduced into the trisomic level by crossing some of the test cross

Table 1. Effect of non-random pairing on genetic ratios from crosses of *a* testers and *Aaa* trisomes

	Source of *A* chromosome	No. of plants tested	No. of gametes tested	%*A*	No. of plants with *A* transmission rates[a]														No. of plants[b]					Inter. X^2	P
					⩽6	9	12	15	18	21	24	27	30	33	36	39	42	45	.01⁻	.05	N	.05⁺	.01		
1	**Controls**																								
	Std. A	14	13,622	32.92	–	–	–	–	–	–	–	–	2	9	3	–	–	–	–	1	13	–	–	10.74	.70-.60
	Std. B	4	7,255	32.53	–	–	–	–	–	–	–	–	1	3	0	–	–	–	–	–	4	–	–	1.82	.70-.60
	Std. C	30	25,117	33.80	–	–	–	–	–	–	–	–	4	16	10	–	–	–	–	–	29	1	–	35.23	.20-.10
	Total Std.	48	45,994	33.34															–	1	46	1	–		
2	**Races**																								
	Arg. popcorn	26	32,052	34.42	–	–	–	–	–	–	–	–	4	8	9	5	–	–	2	1	15	2	6	100.40	<.0005
	Avati Tupi	4	6,978	25.18	–	–	–	–	1	–	1	–	–	1	1	–	–	–	2	–	2	–	–	92.74	<.0005
	Chapalote	7	10,283	30.42	–	–	–	–	–	1	–	3	–	1	2	–	–	–	4	–	3	–	–	91.01	<.0005
	Gaspe flint	6	9,817	33.57	–	–	–	–	–	–	–	–	2	3	1	–	–	–	1	–	4	–	1	23.44	<.0005
	Gourdseed	12	13,099	29.71	–	–	–	–	–	–	–	4	3	5	–	–	–	–	4	–	8	–	–	41.36	<.0005
	Jala	8	6,102	28.88	–	–	–	–	–	–	1	3	3	1	–	–	–	–	4	2	2	–	–	10.57	.20-.10
	Missouri Pipe	4	2,078	39.32	–	–	–	–	–	–	–	–	–	1	–	1	2	–	–	–	1	–	3	11.41	<.005
	Natal W.H.T.	4	3,738	29.64	–	–	–	–	–	–	–	–	3	1	–	–	–	–	3	–	1	–	–	2.79	.50-.40
	Nnara	17	14,644	32.70	–	–	–	–	–	–	–	1	7	6	3	–	–	–	2	1	12	2	–	42.89	<.0005
	Papago flour	17	12,443	30.22	–	–	–	–	–	–	–	2	11	4	–	–	–	–	4	4	9	–	–	24.40	.10-.05
	Reventador	9	8,162	32.08	–	–	–	–	–	–	–	–	5	–	4	–	–	–	2	1	5	1	–	34.94	<.0005
	Tepite	8	5,182	27.50	–	–	–	–	1	1	–	3	1	2	–	–	–	–	4	1	3	–	–	62.11	<.005
	Zapalote gr.	12	8,712	31.65	–	–	–	–	–	–	1	–	5	3	3	–	–	–	2	–	10	–	–	31.20	<.001
3	**Inbred lines**																								
	B2	10	7,906	32.75	–	–	–	–	–	–	–	2	2	3	2	1	–	–	–	3	6	–	1	26.87	<.5000
	B41	27	26,133	25.14	–	–	–	2	–	3	9	9	3	1	–	–	–	–	25	–	2	–	–	210.76	<.0005
	C103	17	20,490	35.01	–	–	–	–	–	–	–	1	3	5	4	2	2	–	1	2	8	1	5	122.08	<.0005
	CI7	20	22,010	30.59	–	–	–	–	–	–	1	5	4	6	3	1	–	–	7	2	9	1	1	186.36	<.0005
	CI21E	15	13,205	32.15	–	–	–	–	–	–	2	1	1	8	3	–	–	–	3	–	11	1	–	90.05	<.0005
	Hy	43	43,037	24.07	–	–	–	1	8	9	5	8	9	3	–	–	–	–	36	–	7	–	–	557.26	<.001
	K6	7	7,705	31.89	–	–	–	–	–	–	–	2	2	1	2	–	–	–	2	–	3	1	1	51.29	<.0005
	K55	19	19,491	34.80	–	–	–	–	–	1	1	2	1	4	6	3	1	–	4	–	10	1	4	191.04	<.001
	K64	8	8,315	34.01	–	–	–	–	–	–	–	–	1	3	4	–	–	–	1	–	5	–	2	82.12	<.0005
	Kys	37	38,394	29.56	–	–	–	–	–	–	1	10	21	2	3	–	–	–	14	9	14	–	–	67.85	<.05-.025
	M14	22	21,765	34.23	–	–	–	–	–	–	–	1	3	6	10	2	–	–	1	3	15	1	2	65.97	<.0005
	Mo5	14	17,532	35.78	–	–	–	–	–	–	–	–	1	1	10	2	–	–	–	1	6	4	3	22.73	.05-.025
	N6	22	20,731	33.59	–	–	–	–	–	–	–	–	4	11	6	1	–	–	–	–	19	–	3	70.55	<.0005
	Tr	3	2,553	39.71	–	–	–	–	–	–	–	–	–	–	–	2	1	–	–	–	–	–	3	1.72	.50-.40
	W23	10	12,212	33.93	–	–	–	–	–	–	–	–	–	7	3	–	–	–	–	–	9	–	1	14.17	.20-.10
	38-11	40	39,866	25.06	–	–	–	–	1	4	6	10	15	2	2	–	–	–	37	1	2	–	–	285.44	<.0005

Table 1. (Continuation)

	Source of *A* chromosome	No. of plants tested	No. of gametes tested	% *A*	No. of plants with *A* transmission rates[a]														No. of plants[b]					Inter.	P
					≤6	9	12	15	18	21	24	27	30	33	36	39	42	45	.01⁻	.05	N	.05⁺	.01	X^2	
4	**Inversions**																								
	In3a(L.40-.95)																								
	Std.	13	10,062	21.95	–	–	–	–	2	3	1	1	–	–	–	–	–	–	13	–	–	–	–	23.95	.025-.010
	Kys	12	7,791	15.17	–	1	1	5	4	1	–	–	–	–	–	–	–	–	12	–	–	–	–	48.98	<.0005
	In3b(L.25-.75)	16	11,043	20.23	–	–	–	1	6	7	2	–	–	–	–	–	–	–	16	–	–	–	–	37.71	.025-.010
	In3c(L.05-.95)	31	28,206	14.21	–	5	7	12	3	3	1	–	–	–	–	–	–	–	31	–	–	–	–	290.05	<.0005
	In3e(5.39-L.80)	12	13,888	17.03	–	–	1	4	4	1	2	–	–	–	–	–	–	–	12	–	–	–	–	74.21	<.0005
	In3n(L.19-L.72)	15	17,306	14.99	–	1	3	5	5	1	–	–	–	–	–	–	–	–	15	–	–	–	–	81.00	<.0005
	In3d(S.72-L.42)	19	18,613	23.88	–	–	1	2	2	5	6	2	–	–	–	–	–	–	19	–	–	–	–	230.80	<.0005
5	**X-ray Treatments**																								
	Std. C Control	30	25,117	33.80	–	–	–	–	–	–	–	–	4	16	10	–	–	–	–	–	29	1	–	35.23	.20-.10
	Std. C 1000r	32	26,373	32.44	2	–	–	–	2	–	–	2	5	8	7	5	1	–	5	1	19	4	3	141.62	<.0005
	Kys Control	37	38,394	29.56	–	–	–	–	–	–	1	10	21	2	3	–	–	–	1	2	31	2	1	67.85	<.0005
	Kys 500r	38	41,617	30.22	–	–	–	–	–	1	–	11	20	5	1	–	–	–	1	1	32	0	4	96.11	<.0005
	Kys 1000r	36	34,183	28.30	–	1	–	–	–	–	4	8	11	9	3	–	–	–	5	3	26	2	2	557.91	<.0005
	Kys 2000r	13	11,997	28.56	1	–	–	–	–	1	1	5	2	3	–	–	–	–	3	–	8	1	1	141.62	<.0005
	Kys In3a C.	12	7,791	15.17	–	1	1	5	4	1	–	–	–	–	–	–	–	–	1	1	8	–	2	48.98	<.0005
	Kys In3a 1000r	59	76,358	16.00	3	3	10	14	15	9	5	–	–	–	–	–	–	–	14	–	17	3	25	1384.56	<.0005
6	**Restituted X-ray Trisomes**																								
	Std. C																								
	A (2.97)	11	8,928	32.78	–	–	–	–	–	–	–	–	4	5	1	1	–	–	–	–	10	1	–	16.04	.10-.05
	B (15.90)	6	6,558	35.18	–	–	–	–	–	–	–	–	–	3	1	2	–	–	–	–	3	3	–	12.13	.05-.025
	C (17.14)	6	3,363	35.00	–	–	–	–	–	–	–	–	–	4	1	–	–	–	–	–	5	–	1	14.12	.025-.01
	D (18.54)	2	743	32.44	–	–	–	–	–	–	–	–	–	2	–	–	–	–	–	–	2	–	–	0.04	.90-.80
	E (27.91)	2	2,534	33.70	–	–	–	–	–	–	–	–	–	2	–	–	–	–	–	–	2	–	–	0.32	.80-.70
	F (28.44)	7	5,116	36.67	–	–	–	–	–	–	–	1	1	1	2	1	1	–	1	4	2	1	2	72.90	<.0005
	G (36.84)	9	8,344	33.92	–	–	–	–	–	–	–	–	1	4	4	–	–	–	–	1	8	–	–	9.29	.40-.30
	H (38.31)	8	7,433	31.83	–	–	–	–	–	–	–	–	4	3	1	–	–	–	1	1	5	1	–	24.49	<.0005
	I (40.85)	16	13,184	35.13	–	–	–	–	–	–	–	–	7	7	2	–	–	–	–	–	12	2	2	23.18	.10-.05
	Kys																								
	A (8.99)	3	2,668	35.72	–	–	–	–	–	–	–	–	–	–	3	–	–	–	–	–	–	–	3	0.57	.80-.70
	B (20.48)	13	11,522	34.82	–	–	–	–	–	–	–	–	5	3	1	3	1	–	–	–	5	1	7	76.80	<.0005
	C (32.47)	4	4,034	36.32	–	–	–	–	–	–	–	–	1	2	–	1	–	–	–	–	–	–	4	17.53	<.0005
	D (33.07)	2	2,098	36.65	–	–	–	–	–	–	–	–	–	–	1	1	–	–	–	–	–	–	2	0.58	.50-.40
	E (34.39)	4	3,384	31.29	–	–	–	–	–	–	–	–	3	1	–	–	–	–	–	–	3	–	1	3.95	.30-.20
	F (34.90)	4	2,861	36.94	–	–	–	–	–	–	–	–	–	–	3	1	–	–	–	–	–	–	4	2.69	.50-.40
	In3a																								
	A (10.23)	10	8,789	14.84	–	1	4	2	1	1	1	–	–	–	–	–	–	–	1	3	3	–	3	175.76	<.0005
	B (11.51)	3	2,580	16.43	–	–	–	2	–	1	–	–	–	–	–	–	–	–	–	–	2	–	1	11.50	.005-.001
	C (18.14)	10	10,722	12.86	–	2	4	3	1	–	–	–	–	–	–	–	–	–	6	–	4	–	–	36.78	<.0005

Table 1. (Continuation)

	Source of *A* chromosome	No. of plants tested	No. of gametes tested	%*A*	No. of plants with *A* transmission rates[a]														No. of plants[b]					Inter.	P
					≤6	9	12	15	18	21	24	27	30	33	36	39	42	45	.01⁻	.05	N	.05⁺	.01	X^2	
7	**Restituted exotic trisomes**																								
	Arg. popcorn																								
	(36.78)♀	3	3,232	34.10	–	–	–	–	–	–	–	–	–	1	2	–	–	–	–	–	3	–	–	3.94	.20-.10
	(38.18)♀	2	1,598	31.85	–	–	–	–	–	–	–	–	1	–	1	–	–	–	1	–	1	–	–	11.45	.025-.01
	Chapalote																								
	(21.86)♂	5	5,355	31.22	–	–	–	–	–	–	–	1	2	2	–	–	–	–	2	–	3	–	–	10.32	.02-.01
	(25.76)♂	8	7,432	25.15	–	–	–	–	–	–	5	2	1	–	–	–	–	–	7	1	–	–	–	15.87	.02-.01
	Gaspé flint																								
	(37.32)♀	6	7,930	34.04	–	–	–	–	–	–	–	–	1	3	1	–	–	–	–	–	5	1	–	9.51	.20-.10
	Gourdseed																								
	(25.54)♂	5	7,366	32.96	–	–	–	–	–	–	–	–	1	3	1	–	–	–	–	–	4	1	–	9.36	.10-.05
	(26.65)♀	2	3,276	24.21	–	–	–	–	–	–	2	–	–	–	–	–	–	–	2	–	–	–	–	0.76	.40-.30
	(27.70)♀	7	6,372	28.45	–	–	–	–	–	2	–	3	1	1	–	–	–	–	4	2	1	–	–	63.37	<.0005
	Nnara																								
	(28.29)♂	12	9,552	34.32	–	–	–	–	–	–	–	–	4	3	3	1	1	–	–	1	9	–	2	68.34	<.0005
	(36.49)♂	2	2,557	37.00	–	–	–	–	–	–	–	–	–	1	1	–	–	–	0	–	1	–	1	10.95	<.0005
	Tepite																								
	(17.44)♂	14	13,040	32.91	–	–	–	–	–	1	–	3	1	2	5	1	1	–	4	1	5	2	2	125.60	<.0005
	Zapalote gr.																								
	(25.11)♂	6	4,460	36.42	–	–	–	–	–	–	–	–	–	2	2	2	–	–	–	–	4	1	1	6.40	.30-.20
8	**Chemical mutagens**																								
	EMS																								
	A	12	13,253	19.16	–	1	1	1	5	2	–	2	–	–	–	–	–	–	12	–	–	–	–	170.36	<.0005
	B	5	6,412	25.67	–	–	–	–	–	1	–	4	–	–	–	–	–	–	5	–	–	–	–	10.69	.05-.025
	C	4	4,616	30.85	–	–	–	–	–	–	–	1	1	2	–	–	–	–	2	–	2	–	–	8.08	.05-.025
	D	12	14,275	32.14	–	–	–	–	–	–	–	1	3	6	2	–	–	–	2	1	9	–	–	30.42	<.0005
	E	8	9,073	32.18	–	–	–	–	–	–	–	–	3	4	1	–	–	–	1	–	7	–	–	14.04	.05-.025
	F	12	15,519	33.75	–	–	–	–	–	–	–	2	–	4	6	–	–	–	2	–	6	–	4	74.01	<.0005
	G	13	21,090	34.31	–	–	–	–	–	–	–	–	1	7	4	1	–	–	1	–	10	–	2	29.77	.025-.010
	H	11	14,252	34.80	–	–	–	–	–	–	–	–	–	5	4	2	–	–	–	–	8	1	2	15.14	.20-.10
	I	8	8,943	35.51	–	–	–	–	–	–	–	–	–	2	4	2	–	–	–	–	5	2	1	8.66	.30-.20
	J	6	7,450	36.11	–	–	–	–	–	–	–	–	–	1	4	–	1	–	–	–	3	–	3	12.35	.05-.025
	NG																								
	A	6	6,442	31.90	–	–	–	–	–	–	–	1	1	4	–	–	–	–	1	1	4	–	–	10.24	.10-.05
	B	4	4,536	31.90	–	–	–	–	–	–	–	–	2	2	–	–	–	–	–	–	4	–	–	1.39	.40-.30
	C	7	6,335	33.94	–	–	–	–	–	–	–	–	–	2	2	3	–	–	–	2	4	1	–	32.66	<.0005
9	**Hybrid**																								
	Hy	43	43,751	24.11	–	–	–	1	8	9	5	9	9	3	–	–	–	–	36	–	7	–	–	557.26	<.0005
	38-11	40	39,866	25.06	–	–	–	1	4	6	10	15	2	2	–	–	–	–	37	1	2	–	–	285.44	<.0005
	Hy/38-11	13	15,171	19.31	–	–	–	4	2	4	2	1	–	–	–	–	–	–	13	–	–	–	–	108.72	<.0005

[a] The numbers below are the midpoints of classes of percent *A* transmission (33 = 31.51%-34.50%)

[b] The number below .01(-), .05(-), .05(+), .01(-) indicate the cases of significant deviations at the .01 and .05 levels from the expected transmission rate. The deviations may be (-) or (+). N indicates normal expected rates

progeny with standard trisomic 3. The results are given in Section 6. The percentages in the parentheses are the transmission rate of the testcross progeny parent. The DPA induced by irradiation is irregular in its inheritance. Plus deviations may become minus deviations or vice versa, or the deviations may disappear.

A similar study was conducted using the progeny of trisomic racial hybrids and is shown in Section 7. Here some of the trisomes were derived through the female from crosses of *Aaa* × *aa*. These cases are marked (♀).

We would expect that in derived trisomes the *A* locus could become separated from DPA factors by crossing over. There are two chances for crossing over to occur when the derived trisome has the odd chromosome transmitted through the male. The first is in the initial testcross (*aa* × *Aaa* = *Aa*). Since the *A* chromosome could not be a univalent and be transmitted, it must have crossed over with an *a* chromosome. The second opportunity for crossing over occurs in the next cross (*aaa* × *Aa*). When the derived trisome is through the female (*Aaa* × *aa* = *Aaa*), it is possible that the A chromosome was a univalent and no crossing over took place. This probably explains the greater correspondence of the *A* transmission rates in the female-derived trisomes. Unfortunately female-derived trisomes with irradiated chromosomes were not used. Section 8 deals with the effects of chemical mutagens on nonrandom pairing. Each family, as indicated by a letter, was derived from an M_2 plant crossed with the standard trisome. Here we see a very strong effect when EMS is used. The data from NG are inadequate. There are significant deviations below and above 33% as found in other types of trisomic heterozygotes.

Section 9 shows an attempt to combine DPA factors from the chromosomes 3 of 38-11 and Hy. A hybrid of these two lines was used. Although there is no transgressive segregation, the mean of *A* transmission from the hybrid-derived material is much reduced.

The interpretation of the data presented in Table 1 presents many problems. It is easy to explain transmission frequencies of *A* lower than 33% by conventional hypotheses. The weaker congressional forces, or weaker synaptic forces, or lower crossing over rates between the odd chromosome and the standard chromosomes resulted in a lower than random frequency of heterogenetic bivalents and semi-heterogenetic trivalents and a consequent reduction in the frequency of monosomic *A* gametes.

However, in all classes of trisomes except the controls there are a significant number of cases where the frequency of *A* gametes was greater than 33%. Only recently has the investigator devised a reasonable hypothesis to explain these results. Certain segments of a chromosome may act as synaptic initiators. These segments may have highly repeated pairing units and the number of these units determines the pairing strength. The number may fluctuate because of unequal crossing over or unequal exchange between sister chromatids which may be induced by X-irradiation or chemical mutagens, thus producing hypomorphic or hypermorphic mutants.

Tetraploids

Two studies of the effect of DPA were made in tetraploids. The first experiment was to survey the amount of DPA present in various tetraploid stocks. Twenty 4n *Wx* plants were crossed with twenty 4n *wx* plants. These 4n *Wx* and 4n *wx* plants were derived from various genetic stocks and accessions such as Argentine Flint and Alexander's Synthetic B. They cannot be given pedigrees relating them to standard diploid material. Gene segregation was observed in F_1 plants by using the *wx* locus. The results are given in Table 2 and are presented in graphic form in Fig. 4. There is considerable variation in the percent of *wx* pollen. It is not possible to obtain control data where the chromosomes would pair at random. This would require isogenic 4n stocks of *Wx* and *wx* which are not available. If we use the mean frequency of *wx* in the total population as the expected value then there are 77 out of 344 cases (22.4%) which have significant deviations from the mean. Also, interaction chi squares indicate a great amount of heterogeneity in the whole population and in most families.

Some of this heterogeneity may be the result of including *Wx/Wx/wx/wx/wx* and *Wx/Wx/wx* plants along

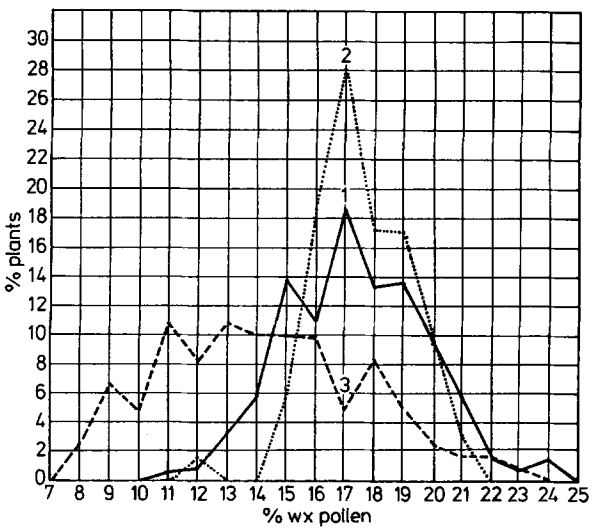

Fig. 4. The data from Tables 2 and 3 are shown here. Line 1 is the population from Table 2, line 2 is the control population, and 3 is the irradiated population from Table 3

Table 2. The effect of DPA on the frequency of *wx* pollen in *Wx Wx wx wx* plants from 20 families

Family	No. of plants	No. of pollen	% *wx*	No. of plants with n% wx (P)[a]	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	No. of plants .01⁻	.05	N	.05⁺	.01	Inter. X^2	P	Total X^2	P
1	20	14,798	15.58	–	–	1	1	1	2	5	4	2	3	1	–	–	–	–	–	3	1	16	–	–	42.91	.005-.001	34.72	<.0005
2	10	7,183	16.44	–	–	–	–	1	–	3	–	4	1	–	–	1	–	–	–	1	–	8	1	–	23.55	.01-.005	4.72	.05-.025
3	21	15,060	16.55	–	–	–	–	2	3	4	2	3	–	4	2	1	–	–	–	1	3	16	1	–	63.04	<.0005	7.74	.01-.005
4	17	12,268	16.67	–	–	–	–	2	3	1	1	4	2	3	1	–	–	–	–	1	4	12	–	–	39.74	.0001-.0005	4.72	.05-.025
5	11	7,878	16.69	–	–	–	–	1	2	1	–	4	1	1	1	–	–	–	–	1	2	8	–	–	23.69	.01-.005	2.85	.10-.05
6	19	14,320	16.71	–	–	–	–	1	3	2	2	3	4	3	1	–	–	–	–	2	2	14	1	–	40.00	.005-.001	4.91	.05-.025
7	22	15,577	16.75	–	–	1	1	1	–	6	2	4	1	2	3	–	–	–	1	3	1	16	1	1	90.84	<.0005	4.78	.05-.025
8	19	13,857	16.93	–	–	–	–	1	–	4	3	5	2	3	–	–	–	1	–	1	1	16	–	1	40.40	.005-.001	2.25	.20-.10
9	23	16,569	16.96	–	–	–	–	1	2	2	4	5	5	2	2	–	–	–	–	1	2	19	1	–	38.86	.025-.01	2.38	.20-.10
10	11	7,825	16.96	–	–	–	–	–	2	2	2	1	1	1	–	2	–	–	–	–	2	8	–	1	32.84	.001-.0005	1.13	.30-.20
11	19	13,549	17.00	–	–	–	–	–	–	5	3	3	4	2	2	–	–	–	–	–	–	18	1	–	46.04	<.0005	1.57	.30-.20
12	20	14,418	17.17	–	–	–	1	–	1	2	3	5	2	2	2	2	–	–	–	1	1	16	1	1	55.56	<.0005	0.58	.50-.40
13	12	8,687	17.21	–	–	–	–	–	–	2	2	4	–	2	1	1	–	–	–	–	–	10	1	1	33.29	.025-.01	0.25	.70-.60
14	10	6,940	17.41	–	–	–	–	–	–	2	2	–	2	3	–	1	–	–	–	–	–	9	–	1	19.50	.025-.01	0.00	<.9995
15	21	15,216	17.44	–	–	–	–	–	2	1	4	3	5	3	1	1	1	–	–	–	1	19	–	1	55.56	<.0005	0.00	<.9995
16	15	10,955	18.47	–	–	–	–	–	–	2	1	3	2	2	2	1	1	1	–	–	–	11	2	1	35.06	.005-.001	8.45	.005-.001
17	18	13,254	18.52	(1)	–	–	–	–	–	1	1	4	3	4	2	2	1	–	–	–	–	14	1	3	33.29	.025-.01	11.19	.001-.0005
18	16	11,201	18.75	(1)	–	–	–	–	–	1	1	1	4	2	5	2	–	–	–	–	–	13	2	1	22.04	.20-.10	13.88	<.0005
19	20	15,026	18.88	–	–	–	–	–	–	1	1	4	2	5	3	2	2	–	–	–	–	15	3	2	32.26	.025-.01	22.49	<.0005
20	20	14,761	20.63	–	–	–	–	–	–	–	–	2	2	2	5	4	–	1	4	–	–	9	4	7	41.62	.0005-.001	106.11	<.0005
Total	344	249,342	17.41	(2)	–	2	3	11	20	47	38	64	46	47	33	20	5	3	5	15	20	267	20	22	847.80	<.0005		

[a] Pentasomic plants

Table 3. The effect of DPA induced by X-irradiation on frequencies of *wx* pollen from *Wx Wx wx wx* tetraploids

	No. of plants	No. of Pollen	% *wx*	(P)[a]	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	No. of plants .01⁻	.05	N	.05⁺	.01	Inter. X^2	P	Total X^2	P
Control																														
1	10	7,004	16.56	(2)	–	–	–	–	1	–	–	–	3	3	2	1	–	–	–	–	–	1	–	9	–	–	12.56	.20-.10	4.12	.05-.025
2	16	11,391	17.43		–	–	–	–	–	–	–	–	4	5	3	2	–	2	–	–	–	–	–	14	2	–	28.31	.025-.01	0.02	.90-.80
3	18	12,879	17.68	(1)	–	–	–	–	–	–	–	2	2	4	3	4	3	–	–	–	–	–	1	17	–	–	21.27	.10-.05	0.34	.60-.50
4	20	14,592	17.79		–	–	–	–	–	–	–	1	3	6	3	4	3	–	–	–	–	–	–	19	1	–	20.26	.40-.30	0.95	.40-.30
Total	64	45,680	17.48	(3)	–	–	–	–	1	–	–	3	12	18	11	11	6	2	–	–	–	1	1	59	3	–	86.78	.05-.025	–	–
																						0.6	2.6	57.6	2.6	0.6				
Treated																														
1	16	11,433	11.13	(1)	–	4	2	3	2	4	–	–	1	–	–	–	–	–	–	–	–	15	–	1	–	–	320.34	<.0005	320.34	<.0005
2	20	14,736	12.15	–	1	2	2	5	1	3	2	2	2	–	–	–	–	–	–	–	–	16	1	3	–	–	67.48	<.0005	290.14	<.0005
3	20	14,695	12.55	–	1	2	–	2	6	3	4	–	1	–	–	1	–	–	–	–	–	15	3	2	–	–	76.36	<.0005	248.08	<.0005
4	16	11,864	12.70	–	1	1	2	3	–	1	3	3	2	–	–	–	–	–	–	–	–	10	–	6	–	–	60.44	<.0005	188.00	<.0005
5	20	14,611	15.39	–	–	–	–	–	1	2	3	5	5	1	2	1	–	–	–	–	–	6	1	13	–	–	35.97	.025-.01	44.57	<.0005
6	17	11,894	17.69	(1)	–	–	–	–	–	–	–	2	1	4	6	2	1		1	–	–	–	–	16	–	1	20.67	.20-.10	0.35	.70-.60
7	11	7,679	19.90	–	–	–	–	–	–	–	–	–	–	1	2	2	2	2	1	1	–	–	–	7	2	2	16.20	.10-.05	31.04	<.0005
Total	120	86,912	14.14	(2)	3	9	6	13	10	13	12	12	12	6	10	6	3	2	2	1	–	62	5	48	2	3	847.80	<.0005	533.95	<.0005
																						1.2	4.8	108	4.8	1.2				

[a] P = Pentasomic plants

with the desired type *Wx/Wx/wx/wx*. Two plants had 8.4% and 6.0% *wx* pollen and were assumed to be *Wx/Wx/Wx/wx/wx* (pentasomic). These were not used in computing the mean. This type of aneuploid should be about as frequent as the aforementioned types.

The second experiment was to observe the effects of radiation on DPA in tetraploids. Isolations of 4n *Wx* and 4n *wx* from Alexander's Synthetic B were made. Lines of 4n *wx* were irradiated (5000r to the kernels) every generation for 5 years. Likewise lines of 4n *Wx* and the 4n *wx* were self-fertilized for 5 generations. Then the irradiated and control 4n *Wx* lines were crossed with 4n *wx* lines and the percent of *wx* pollen was determined in the F_1. The results are given in Table 3 and are shown graphically in Fig. 4.

Five of the seven families in the irradiated group had significant decreases in the percent of *wx* pollen, one had a significant increase. X-irradiation seems to be very effective in producing DPA.

Conclusions

It has been shown that DPA factors occur naturally in different lines of maize. A segmental allotetraploid could be made from the Hy, B41 or 38-11 and standard maize genome if their chromosomes 3 are representative of the DPA level of the other chromosomes. Likewise, if irradiation has affected the other chromosomes in the tetraploid material as it has with chromosome 9 we would have a segmental allotetraploid. To allotetraploidize maize, it is necessary to improve our understanding of the patterns of pairing and gene segregation in segmental allotetraploids as it is affected by DPA. Also an efficient, practical method is needed to concentrate DPA factors into single lines. These topics will be dealt with in later papers in this series.

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Received September 20, 1978
Communicated by P.L. Pfahler

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