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A STUDY OF ANEUPLOIDY IN AUTOTETRAPLOID MAIZE¹

MASTER

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An increase in both male and female sterility invariably accompanies the induction of tetraploidy in maize (Randolph, 1935) and other crops (Müntzing, 1936, 1961; et al.). By selection in $4n$ maize, Randolph developed both fertile and sterile lines. These were rather extensively studied by Fischer (1939). His findings showed clearly that the difference between the fertile and sterile lines was mainly genically controlled. Moreover, meiotic analysis (Kadam, 1944) revealed that gametogenesis was essentially similar in both sterile and fertile lines.

However, Alexander (1957), on theoretical grounds, has calculated that as much as 39% sterility of tetraploid maize could be accounted for by aneuploidy of gametes. This calculation was essentially a comparison of the range and frequency of various aneuploid types with their expected frequency from determination of chromosome number of microspores by Punyasingh (1947). Alexander attributed the difference between observed and expected to abortion of chromosomally inviable embryos or gametophytes.

In $4n$ *Lactuca* (Einset, 1947) and in $4n$ *Hordeum* (Rommel, 1961) it

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has been clearly shown that aneuploids have markedly reduced fertility by comparison with euploids. These results do not actually conflict with those of Fischer (1939), but a disagreement is implied; that aneuploidy may not contribute importantly to sterility in maize, while it so clearly does in other forms.

Because of the ever increasing importance of polyploidy in cultivars, and because agronomically useful populations are likely to be mixtures of euploids and aneuploids, it seemed important to study the effects of aneuploidy not only upon sterility, but also upon the degree of aneuploidy in succeeding generations.

MATERIALS AND METHODS

A tetraploid Argentine Flint maize strain was chosen for study which is a long-time tetraploid. Prior to this study it was maintained for 3 generations by sibbing, coupled with selection for fertility. It is likely that selection for fertility was also practiced for several generations prior to these.

356 seedlings were grown from sibbed seed, and chromosome numbers were determined from root tip preparations. After the selection of about 20 plants of each of the common chromosome numbers, 39, 40 and 41, the remaining seedlings were screened rapidly for plants having the more unusual chromosome numbers. The seedlings were then transplanted to the Botany Garden while still very small. Growing conditions were favorable throughout the remainder of the season.

At flowering time, ovules of 39 chromosome plants were fertilized with pollen from 39 chromosome plants, ovules of 40 chromosome plants with

pollen of 40 chromosome plants, etc. In all cases, pollen was collected each day from all plants of a given chromosome number and bulked. Each ear was pollinated daily until no fresh silk remained.

In the determination of female fertility, the tips and butts of each ear were sliced off, and discarded, since these are areas of frequent "natural" abortion. The cylindrical center section of each ear was then shelled and the kernels counted. The rachis or cob was then scraped free of chaff (glumes) and the number of original ovules counted. Female fertility was then expressed as a percent of original ovules which produced a mature kernel. Completely barren ears were disregarded on the assumption that this expression may be independent of the partial sterility typical of tetraploids.

Male fertility was determined from killed and fixed sections of the male inflorescence, harvested the day before anthesis would have occurred. Pollen was removed from the male florets, stained in IKI solution, and examined microscopically. For each plant, 100 pollen grains from one anther taken from each of 3 different florets were scored for pollen abortion. Pollen grains which were empty, partially empty, or were of grossly abnormal size were scored as aborted grains.

In the determination of the effects of aneuploidy of one generation upon the next, the seeds obtained by the bulked pollination technique described above were bulked according to the chromosome number of the parents. Random samples of this seed were germinated, and somatic counts made from root tip preparations.

EXPERIMENTAL RESULTS

Difficulty was encountered in obtaining measures of female fertility in plants with extreme chromosome numbers. Of the four 37-chromosome plants, and the two plants with 44 chromosomes, all failed entirely to set seed. Moreover, only two of the six 38-chromosome plants produced seed, and only one of the three 43-chromosome plants produced seed. Consequently, few data were obtained for plants with chromosome numbers beyond the 39 - 42 range.

Ovule fertility

Figure 1 shows the percent of fertile ovules in each ear harvested. As shown in Table I, the 40 chromosome class was significantly more fertile than each of the aneuploid classes, except for the scanty 38-chromosome class. None of the comparisons between aneuploid classes are at the level of significance.

Pollen fertility

Figure 2 shows the percent of normal-appearing pollen for each plant selected for pollination. As shown in Table II, average pollen fertility for the different chromosome classes was not as widely different as was ovule fertility. However, tests indicate that the euploid class is significantly more fertile than each aneuploid class. Comparisons between aneuploid classes are not significant.

The effects of aneuploidy upon the succeeding generation

Figures 3 through 7 show the distribution of chromosome numbers in the progenies of five chromosome number classes. Table III shows that the mean chromosome number of each of the progenies is significantly (or nearly so) different from that of each of the other progenies. None of the t values

for skewness of the distributions of the five progenies was significant, but that of the 43-chromosome progeny was nearly significant:

39-chromosome progeny	t = +.4931
40-chromosome progeny	t = +.6993
41-chromosome progeny	t = -.7651
42-chromosome progeny	t = +.5780
43-chromosome progeny	t = -1.8410

However, as shown in Table III, the mean chromosome number of each progeny of aneuploid parents more nearly euploid than that of its parents.

Table IV shows the coefficient of correlation values for the relationship of male and female fertility of individual plants. Only two correlations are significant including that of the 42-chromosome progeny. The fact that the coefficient for the overall population is highly significant has little real meaning, since sterility due to aneuploidy is confounded with the correlation within plants. However, the fact that all of the r values are positive may indicate a general correlation between male and female fertility within plants, but one which is usually too weak to be detected at the population sizes used in this experiment.

DISCUSSION

The limits of aneuploidy in tetraploid maize

The results obtained by intercrossing plants within groups having both high and low aneuploid numbers of chromosomes show clearly that the range of aneuploidy in $4n$ maize is rigidly set at 37 - 44. It is conceivable that these limits are set by the failure of gametophytes which, if they functioned,

would give rise to embryos beyond the 37 - 44 range. However, the findings of Puryasingh (1947) indicate that little or no selection exists against aneuploid gametophytes on diploid silks. It therefore seems warranted to conclude that the limits of aneuploidy in $4n$ maize are set by abortion of embryos outside this range. It is a necessary corollary that aneuploidy per se is an important cause of reduced seed set in $4n$ maize.

This conclusion raises still another point: If embryo development is incompatible with numbers beyond the 37 - 44 range, for apparently quantitative reasons, it seems likely that many embryos would be chromosomally abortive within the 37 - 44 range if the same chromosome were in excess or deficient more than once. This idea is supported by the reduced fertility of the 39 and 41 chromosome classes, which, except for newly arisen non-disjunction, should not be expected to produce embryos beyond the tolerable limits. Moreover, it may become important to distinguish qualitatively for the degree of tolerance to aneuploidy among the different maize chromosomes, and to interactions between unbalanced combinations of specific chromosomes. This point may be particularly important, as Dr. G. G. Doyle points out, since $2n$ maize is probably an ancient allotetraploid, and thus would have varying degrees of homeology between different chromosomes.

In order to make an approximation of the full degree of sterility which could be attributed to chromosomal causes, it is clear that a study of the chromosome numbers in the gametes of aneuploid plants is necessary. Unfortunately, analysis of AI or later meiotic and microspore division stages was not possible in the material used in this research. Nevertheless, the present work does support the rationale for the calculation of Alexander (1957)

that as much as 39% of sterility in $4n$ maize can be attributed to chromosomal, rather than genetic causes. It is also indicated that Alexander's figure may be a minimal, rather than a maximal approximation. However, the fact that there was much overlapping of both ovule and pollen fertility (Figs. 1 and 2) indicates that all sterility cannot be chromosomal in cause, and that much is genetic (Fischer, 1939). While it could be argued that some 40 -chromosome plants are actually compensated aneuploids, this phenomenon appears to be too infrequent (Catcheside, 1956; Shaver, 1960) to be of major bearing on these results.

Pollen abortion

In view of the fact that gametophytes of diploid maize are so intolerant of even minor excesses or deficiencies of chromatin, it is surprising that there was so little difference between pollen fertility in euploid and aneuploid classes. This indicates that the efficient screening mechanism against aneuploid (especially male) gametophytes at the diploid level is chiefly swamped at the $4n$ level. As yet it is impossible to say whether this diminution of screening effect is due merely to the fact that additions or subtractions of chromatin are relatively less drastic, or whether some further screening device is rendered inoperative by induction of tetraploidy. It is clear, however, that the induction of tetraploidy diminishes the restraint upon genomic divergence imposed upon diploids by gametophyte selection. This release may be the major factor in the apparently superior evolutionary capability of polyploids (Swanson, 1957).

Correlation of pollen and ovule fertility

The weakness of the correlation between pollen and ovule fertility within plants was expected, because the method of measuring ovule fertility confounds female gametophyte abortion with embryo abortion. Since pollen abortion bears little relationship to male gametophyte aneuploidy, it seems reasonable that abortion of the female gametophyte per se has even less relation to female gametophyte aneuploidy. The major component of ovule sterility, then, must be embryo, rather than female gametophyte abortion. This line of reasoning leads to the conclusion that in tetraploids, embryo abortion is the major screening device against intolerable chromosome complements, while in diploids, the major device is male gametophyte abortion.

Dr. Randolph has pointed out that the present work would not distinguish between embryo abortion and inability of male gametophytes to function on aneuploid styles (silks). This factor appeared to be a major cause of sterility in autotetraploid lettuce (Einset, 1947). While the results of Punyasingh (1947) make it seem less likely that this is the case in $4n$ maize, nevertheless, this point should be elucidated by further appropriate cytological studies.

SUMMARY

Classes of aneuploid maize plants with 38, 39, 41, and 42 chromosomes were found to have a significantly lower ovule fertility than the euploid 40 chromosome class, but comparisons between different aneuploid classes were not significant. Similar results were obtained from pollen abortion studies, though differences were much smaller. Correlations between ovule

sterility and pollen abortion within plants were usually not significant. Since there was much overlapping in the expression of ovule and pollen fertility between ploidy classes, it is concluded that genetic causes of sterility are also of major importance in the reduction of fertility of 4n maize. Study of the chromosome numbers of the 5 progenies obtained by sibbing within ploidy classes shows that parental ploidy is reflected in altered chromosome number distribution in the progenies. All comparisons between means of sibling progenies were significant or near-significant. Since siblings of even the extreme ploidy classes do not have chromosome numbers beyond the range of 37 to 44, it is likely that these limits are set by abortion of embryos having numbers outside this range.

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TABLE I

Ovule Fertility Among Chromosome Number Classes of 4n Argentine Flint Maize

Chromosome #	# of plants	Aver. % fertile	All possible t tests
38	2	30.7	38 vs. 39 t = 2.09
39	12	60.6	38 vs. 40 t = 3.33
40	29	76.6	38 vs. 41 t = 2.58
41	15	67.4	38 vs. 42 t = 1.08
42	9	48.0	39 vs. 40 t = 4.42** 39 vs. 41 t = 1.60 39 vs. 42 t = 1.43 40 vs. 41 t = 3.22** 40 vs. 42 t = 3.51** 41 vs. 42 t = 1.68

** Indicates significance at the 1% level in all Tables.

TABLE II

Pollen Fertility Among Chromosome Number Classes of 4n Argentine Flint Maize

Chromosome #	# of plants	Aver. % fertile	All possible t tests
38	5	91.9	38 vs. 39 t = .18
39	8	91.6	38 vs. 40 t = 5.25**
40	32	96.1	38 vs. 41 t = .18
41	14	91.6	38 vs. 42 t = .96
42	11	89.6	39 vs. 40 t = 2.73*
			39 vs. 41 t = .00
			39 vs. 42 t = .61
			40 vs. 41 t = 4.81**
			40 vs. 42 t = 3.24**
			41 vs. 42 t = .81

* Indicates significance at the 5% level in all Tables.

TABLE III

Average Chromosome Numbers of the Progenies of Parents
with Different Chromosome Numbers¹

Parental chromosome #	# of parental plants	Average chrom. # of progeny	# of prog. plants counted	All possible t tests ²
38	6	38.3	6	39 vs. 40 t = 6.024**
39	15	39.2	68	39 vs. 41 t = 7.980**
40	32	40.2	75	39 vs. 42 t = 8.887**
41	20	40.7	55	39 vs. 42 t = 11.250**
42	15	41.1	41	40 vs. 41 t = 2.839**
43	3	41.9	38	40 vs. 42 t = 4.599**
				40 vs. 43 t = 7.692**
				41 vs. 42 t = 1.817
				41 vs. 43 t = 4.800**
				42 vs. 43 t = 2.819**

¹ The distribution of chromosome number in these progenies is shown in Figs. 3-7.

² Because of its small size, the 38 chromosome class is excluded.

TABLE IV

Coefficients of Correlation Between Male and Female
Fertility of Individual Plants

Correlation	
Within the 39-chromosome class:	$r = +.329$
Within the 40-chromosome class:	$r = +.252$
Within the 41-chromosome class:	$r = +.289$
Within the 42-chromosome class:	$r = +.632^*$
Overall disregarding classes:	$r = +.458^{**}$

Figure 1. Distribution of ovule fertility in plants with specified chromosome numbers. Each circle represents one plant.

Figure 2. Distribution of pollen fertility in plants with specified chromosome numbers. Each circle represents one plant.

Figure 3-7. Distribution of chromosome numbers in the sibling progenies of parents having 39, 40, 41, 42, and 43 chromosomes.



