

### 7. Double reduction at the waxy locus.

Counts of waxy pollen grains from 27 autotetraploid maize plants were used to study double reduction at the waxy locus. All pollen samples were stained with the same KI concentration and above-stage lighting was used. Counts for simplex, duplex, and triplex were made randomly in time, and on a hemocytometer slide. Although root tip counts to screen for aneuploidy were not made, microsporocytes from some tillers appeared to be euploid at diakinesis. Alpha values were computed using a maximum likelihood formula.

Information about the numbers of pollen grains counted for each class, observed and expected percentages of waxy, and alpha values are presented below. With no double reduction (D.R.), alpha equals 0, and with maximum D.R., alpha equals  $1/6$  or 0.167.

Class	<u>No. of grains</u> total      per plt.		<u>obs.</u>	<u>Percent waxy</u>		Computed alpha values
				<u>no</u> D.R.	<u>max.</u> D.R.	
simplex	2270	324	50.2	50.0	54.0	0.007
duplex	4812	437	20.8	16.7	22.2	0.13
triplex	3214	357	1.5	0.0	4.2	0.06
simplex + duplex + triplex						0.079

Percentages of waxy and alpha values from the three genotypes are clearly inconsistent. The observed duplex percentage is close to that expected for random chromatid assortment, whereas simplex and triplex classes indicated low and intermediate amounts of double reduction, respectively. Correspondingly, alpha values indicated waxy is almost 50 map units from the centromere in duplex, very near the centromere in simplex, and intermediate in triplex. The alpha value based on all three classes is also intermediate, but meaningless due to the variation among classes.

As non-waxy autotetraploid pollen often exhibited different degrees of staining, dosage effects resulting in mis-classification of heterozygous grains offer one possible explanation for the discrepancies. Other factors such as numerical non-disjunction, and variable quadrivalent formation and separation could also have affected segregation.

Previously, Dempsey (1956 Maize News Letter) reported 50.1 and 48.8 percent waxy for simplex and 18.7 percent for duplex segregations. Levings (1963 Thesis, Illinois) reported about 17 percent waxy segregation for duplex and computed an alpha value of 0.0097 for duplex.

As the differences between the percentages expected for no double reduction and for maximum double reduction are not great, alpha values, and consequently map units from centromere, vary considerably for even small percentage changes. Hence, double reduction and alpha values determined in this way may be of little value in mapping the centromere.

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#### 8. Bivalent pairing in autotetraploids.

In autotetraploid maize and alfalfa, it is not known whether the bivalent pairing that occurs is preferential or at random. Consequently, a linkage method has been devised to determine which type of pairing actually occurs. Although single locus segregations are affected by the type of pairing, differences will be much greater where linkage is involved, and the linkage test is more discriminating.

Autotetraploid alfalfa produces mostly bivalents at meiosis, and the test was conceived for use on alfalfa. However, the principles also apply to putative allotetraploids such as 4n maize X 4n teosinte hybrids, and to lines of 4n maize which consistently produce some bivalents. Many 4n maize lines observed at Minnesota produced two or more bivalents per cell, probably chromosome 10, and presumably the number will increase in time through 'diploidization'.

The technique in its simplest form requires:

- 1) two linked genes in a biduplex X binulliplex testcross,
- 2) phenotypic classification of progeny, and
- 3) chi-square tests of observed to expected segregations for random or preferential pairing.

The three possible modes of bivalent pairing for four 'homologous' chromosomes are given below. A biduplex repulsion genotype is illustrated.