

# DOUBLE AND SINGLE BACKCROSS LINKAGE ESTIMATES IN AUTOTETRAPLOID MAIZE<sup>1</sup>

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LINKAGE estimates in autotetraploids are more complicated than in diploids for three reasons outlined by FISHER (1949): 1) The multiplicity of segregating genotypes—for two loci with two alleles at each there are 19 doubly heterogenic genotypes in an autotetraploid, compared with only two in a diploid. 2) The eleven possible modes of gamete formation, compared with only two in diploids—tetraploid gametes may contain all combinations of recombinant and non-recombinant chromatids, whereas diploids may be only recombinant or non-recombinant. 3) The frequencies of different gametic genotypes cannot be identified by a single backcross to a tester. In this study, for example, where the linked genes were shrunken and waxy on chromosome 9, doubly dominant progeny of the first backcross could have been produced by five gametic genotypes,  $ShWx/ShWx$ ,  $ShWx/Shwx$ ,  $ShWx/shWx$ ,  $ShWx/shwx$ , and  $Shwx/shWx$ . Three of the gametes had recombinant chromatids, but all gametes were indistinguishable in the first backcross. The gametic series may be determined, however, if these progeny of the first backcross are subjected to a second backcross. Each gamete is then recognized, not by the appearance of one individual, but by the frequency distribution observed in a second-backcross family from that individual.

FISHER's monumental paper (1947) set forth the general theory of polysomic linkage and accounted for all the complexities. He then applied this theory to tetrasomic linkage analysis in *Lythrum salicaria* (1949), using double-backcross data. The second backcross, although laborious, is an extremely powerful tool in unravelling the complex gametic segregation and providing unbiased recombination estimates.

The time and space required to raise and identify the progeny of a second backcross are almost insurmountable problems in higher plants. Hence, most linkage estimates in autotetraploids have been computed from single-backcross data. These include DEWINTON and HALDANE (1931) with *Primula*, SANSOME, (1933) with *Solanum*, and WELCH (1962) with maize. Single-backcross linkage can be estimated comparatively easily using maximum likelihood (MATHER 1938). However, a single backcross cannot directly identify the recombinant

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chromatids in doubly dominant gametes, as mentioned above, and the estimates computed from them always contain some bias. MATHER (1936) demonstrated that single-backcross estimates of closely linked loci will contain less bias than estimates of more than 15%. FRANKLIN (1967) proposed that single-backcross data from several selected genotypes can be combined to minimize the bias.

Linkage between shrunken and waxy in autotetraploid maize is estimated in this study from both double- and single-backcross data. The double-backcross estimate is based on bisimplex coupling and bisimplex repulsion genotypes and FISHER's theory of polysomic linkage (1947, 1949). This linkage estimate is compared with those from single backcrosses and with the diploid value. Information is also presented on the genetic position of the centromere in chromosome 9 of maize.

#### MATERIALS AND METHODS

Autotetraploid maize stocks were furnished by L. F. RANDOLPH, Botany Department, Cornell University, and D. E. ALEXANDER, Agronomy Department, University of Illinois. Genes involved were shrunken and waxy on chromosome 9. Shrunken phenotypes were identified visually, and waxy by the potassium iodide-iodine test for starch. Double recessive autotetraploid testers were always used as pollen parents in the backcrosses, hence, recombination was estimated in the female parents.

Seven bisimplex coupling and two bisimplex repulsion genotypes were identified by crossing heterozygous stocks with shrunken-waxy testers and classifying the progeny. The backcross used to identify the respective genotypes constituted the first backcross in this study. Doubly dominant progeny of this first backcross were then used as females in a second backcross. Seedling blight reduced the size of all families and one coupling family was completely lost. Kernels on the ears of first backcross progeny constituted the second backcross progeny. About 100 kernels per ear were classified and the segregation identified as fitting that expected for bisimplex coupling, bisimplex repulsion, double reduction for shrunken, waxy, or both. This revealed the type of gamete received by that individual in the first backcross. Frequencies of the different gametic genotypes entering the first backcross were used for FISHER's analysis. Segregations from the progeny of all gametes identified were used for the single-backcross analysis.

#### RESULTS

*Double backcross linkage analysis:* This analysis was similar to that used by FISHER (1949), in that recombination estimates were based on gametic segregations from bisimplex coupling and bisimplex repulsion, and only individuals with doubly dominant phenotypes were subjected to the second backcross. Use of only doubly dominant plants eliminated disturbances due to inequality of the ratio of dominants to recessives. A comparison of how well gametic frequencies obtained from only doubly dominant plants fitted those expected from all plants will be given at the end of this section. The logic and mathematics of this analysis were presented by FISHER (1947, 1949). A detailed interpretation of FISHER's papers was made by GATES (1957), and an abbreviated description is given here.

The first step was to determine the frequencies of the five different doubly dominant gametes which entered the progeny of the first backcross (Table 1). These gametes, coded  $a$  through  $e$  for those from bisimplex coupling and  $a'$  through  $e'$  for those from bisimplex repulsion, were identified by the segregations

TABLE 1

Number and relative frequencies of shrunken-waxy gametes identified from six bisimplex coupling and two bisimplex repulsion families

| Gametes            | Bisimplex |           |          |           |           |           |
|--------------------|-----------|-----------|----------|-----------|-----------|-----------|
|                    | Coupling  |           |          | Repulsion |           |           |
|                    | Number    | Frequency | Code     | Number    | Frequency | Code      |
| <i>ShWx/ShWx</i> * | 3         | .65       | <i>a</i> | 0         | 0.0       | <i>a'</i> |
| <i>ShWx/Shwx</i> * | 17        | 3.70      | <i>b</i> | 2         | 1.87      | <i>b'</i> |
| <i>ShWx/shWx</i> * | 0         | 0.0       | <i>c</i> | 0         | 0.0       | <i>c'</i> |
| <i>ShWx/shwx</i> * | 169       | 36.74     | <i>d</i> | 2         | 1.87      | <i>d'</i> |
| <i>Shwx/shWx</i> * | 5         | 1.09      | <i>e</i> | 19        | 17.76     | <i>e'</i> |
| <i>Shwx/- wx</i> † | 40‡       | 8.69      | <i>f</i> | 30‡       | 28.03     | <i>f'</i> |
| <i>shWx/sh-</i> †  | 46‡       | 10.00     | <i>g</i> | 35‡       | 32.71     | <i>g'</i> |
| <i>shwx/shwx</i> † | 180‡      | 39.13     | <i>h</i> | 19‡       | 17.76     | <i>h'</i> |
| Totals             | 460       | 100.00    |          | 107       | 100.00    |           |

\* Identified by second backcross.

† Identified by first backcross, not separated into genotypes.

‡ Adjusted downward in each family in proportion to *ShWx*-gametes identified in second backcross.

of their first-backcross progeny when subjected to a second backcross to a homozygous recessive tester. The observed segregation data used for the identifications are summarized in Table 7. For example, first-backcross progeny that received gametes coded *a* were *ShWx/ShWx/shwx/shwx* and their segregation in the second backcross fitted that expected for biduplex coupling. Similarly, progeny that received gametes coded *b* or *b'* had second backcross segregations which fitted duplex-simplex coupling.

Although the second backcross provides an accurate determination of the gametic output from a given genotype, it does not supply the frequencies of the 11 possible modes of gamete formation (Table 2). This is because several gametic genotypes may be produced by more than one mode of gamete formation from a given parental genotype. Hence, to find which gametes are competent to estimate respective modes, gametic matrices are employed (Tables 3 and 4). Each matrix has 11 rows corresponding to the modes of gamete formation, and 10 columns for the 10 possible gametic genotypes. The frequencies given in the matrices are based on normal tetraploid segregation. For example, for mode 1 in coupling (Table 3), both chromatids in the gamete must be non-recombinant and non-sisters (see Table 2), and gametic frequencies will be  $\frac{1}{2}$  *ShWx/shwx* and  $\frac{1}{2}$  *shwx/shwx*.

In bisimplex coupling, only three of the six possible pairings of the chromosomes will give crossovers which can be recognized experimentally, and in bisimplex repulsion, only one of the six pairings gives recognizable crossovers. REDFIELD (1932) discussed this problem in relation to crossing over in triploid *Drosophila*. However, this discrepancy is eliminated by basing the gametic frequencies in the matrices (Tables 3 and 4) on the theoretical situation where each

TABLE 2

*Eleven modes of gamete formation from autotetraploid genotype:  $a_1b_1/a_2b_2/a_3b_3/a_4b_4$*

| Mode | Frequency | One typical gamete | Recombinant chromatids per gamete | Number of gametic genotypes | Genetic description of chromatids                                     |
|------|-----------|--------------------|-----------------------------------|-----------------------------|---|
| 1    | $f_1$     | $a_1b_1/a_2b_2$    | 0                                 | 6                           | Both non-recomb., non-sisters.*                                       |
| 2    | $f_2$     | $a_1b_1/a_1b_1$    | 0                                 | 4                           | Both non-recomb., sisters† at both loci.                              |
| 3    | $f_3$     | $a_1b_1/a_2b_3$    | 1                                 | 24                          | One non-recomb., one recomb., both non-sisters.                       |
| 4    | $f_4$     | $a_1b_1/a_1b_2$    | 1                                 | 12                          | One non-recomb., one recomb., sisters at A locus.                     |
| 5    | $f_5$     | $a_1b_1/a_2b_1$    | 1                                 | 12                          | One non-recomb., one recomb., sisters at B locus.                     |
| 6    | $f_6$     | $a_1b_2/a_3b_4$    | 2                                 | 12                          | Both recomb., both non-sisters, each gene from a different chromatid. |
| 7    | $f_7$     | $a_2b_1/a_3b_1$    | 2                                 | 12                          | Both recomb., sisters at B locus.                                     |
| 8    | $f_8$     | $a_1b_2/a_1b_3$    | 2                                 | 12                          | Both recomb., sisters at A locus.                                     |
| 9    | $f_9$     | $a_1b_2/a_2b_3$    | 2                                 | 24                          | Both recomb., both non-sisters.                                       |
| 10   | $f_{10}$  | $a_1b_2/a_1b_2$    | 2                                 | 12                          | Both recomb., sisters at both loci.                                   |
| 11   | $f_{11}$  | $a_1b_2/a_2b_1$    | 2                                 | 6                           | Both recomb., non-sisters but complementary.                          |
|      |           |                    | Total                             | 136                         |   |

\* From different pairs of chromatids.  
 † From sister chromatids (double reduction).

TABLE 3

*Gametic matrix for bisimplex coupling genotype  $ShWx/shwx/shwx/shwx$  ( $a_1b_1/a_2b_2/a_3b_3/a_4b_4$ )*

| Mode of Gamete Formation* | Gametic genotype |        |        |        |        |        |        |        |        |        |
|---------------------------|------------------|--------|--------|--------|--------|--------|--------|--------|--------|--------|
|                           | $ShWx$           | $ShWx$ | $ShWx$ | $ShWX$ | $Shwx$ | $Shwx$ | $Shwx$ | $shWx$ | $shWx$ | $shwx$ |
|                           | $ShWx$           | $Shwx$ | $shWx$ | $shwx$ | $shWx$ | $Shwx$ | $shwx$ | $shWx$ | $shwx$ | $shwx$ |
| 1. $a_1b_1/a_2b_2$        | 0                | 0      | 0      | 1/2    | 0      | 0      | 0      | 0      | 0      | 1/2    |
| 2. $a_1b_1/a_1b_1$        | 1/4              | 0      | 0      | 0      | 0      | 0      | 0      | 0      | 0      | 3/4    |
| 3. $a_1b_1/a_2b_3$        | 0                | 0      | 0      | 1/4    | 0      | 0      | 1/4    | 0      | 1/4    | 1/4    |
| 4. $a_1b_1/a_1b_2$        | 0                | 1/4    | 0      | 0      | 0      | 0      | 0      | 0      | 1/4    | 1/2    |
| 5. $a_1b_1/a_2b_1$        | 0                | 0      | 1/4    | 0      | 0      | 0      | 1/4    | 0      | 0      | 1/2    |
| 6. $a_1b_2/a_3b_4$        | 0                | 0      | 0      | 0      | 0      | 0      | 1/2    | 0      | 1/2    | 0      |
| 7. $a_2b_1/a_3b_1$        | 0                | 0      | 0      | 0      | 0      | 0      | 1/2    | 1/4    | 0      | 1/4    |
| 8. $a_1b_2/a_1b_3$        | 0                | 0      | 0      | 0      | 0      | 1/4    | 0      | 0      | 1/2    | 1/4    |
| 9. $a_1b_2/a_2b_3$        | 0                | 0      | 0      | 0      | 1/4    | 0      | 1/4    | 0      | 1/4    | 1/4    |
| 10. $a_1b_2/a_1b_2$       | 0                | 0      | 0      | 0      | 0      | 1/4    | 0      | 1/4    | 0      | 1/2    |
| 11. $a_1b_2/a_2b_1$       | 0                | 0      | 0      | 0      | 1/2    | 0      | 0      | 0      | 0      | 1/2    |

\* One typical gamete listed for each mode.

TABLE 4

*Gametic matrix for bisimplex repulsion genotype*  
*Shwx/shWx/shwx/shwx (a<sub>1</sub>b<sub>1</sub>/a<sub>2</sub>b<sub>2</sub>/a<sub>3</sub>b<sub>3</sub>/a<sub>4</sub>b<sub>4</sub>)*

| Mode of<br>Gamete Formation*                                     | Gametic genotype |             |             |             |             |             |             |             |             |             |
|--|------------------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|
|  | <i>ShWx</i>      | <i>ShWx</i> | <i>ShWx</i> | <i>ShWX</i> | <i>Shwx</i> | <i>Shwx</i> | <i>Shwx</i> | <i>shWx</i> | <i>shWx</i> | <i>shwx</i> |
|  | <i>ShWx</i>      | <i>Shwx</i> | <i>shWx</i> | <i>shwx</i> | <i>shWx</i> | <i>Shwx</i> | <i>shwx</i> | <i>shWx</i> | <i>shwx</i> | <i>shwx</i> |
| 1. <i>a<sub>1</sub>b<sub>1</sub>/a<sub>2</sub>b<sub>2</sub></i>  | 0                | 0           | 0           | 0           | 1/6         | 0           | 1/3         | 0           | 1/3         | 1/6         |
| 2. <i>a<sub>1</sub>b<sub>1</sub>/a<sub>1</sub>b<sub>1</sub></i>  | 0                | 0           | 0           | 0           | 0           | 1/4         | 0           | 1/4         | 0           | 1/2         |
| 3. <i>a<sub>1</sub>b<sub>1</sub>/a<sub>2</sub>b<sub>3</sub></i>  | 0                | 0           | 0           | 1/12        | 1/6         | 0           | 1/4         | 0           | 1/4         | 1/4         |
| 4. <i>a<sub>1</sub>b<sub>1</sub>/a<sub>1</sub>b<sub>2</sub></i>  | 0                | 1/12        | 0           | 0           | 0           | 1/6         | 0           | 0           | 5/12        | 1/3         |
| 5. <i>a<sub>1</sub>b<sub>1</sub>/a<sub>2</sub>b<sub>1</sub></i>  | 0                | 0           | 1/12        | 0           | 0           | 0           | 5/12        | 1/6         | 0           | 1/3         |
| 6. <i>a<sub>1</sub>b<sub>2</sub>/a<sub>3</sub>b<sub>4</sub></i>  | 0                | 0           | 0           | 1/6         | 1/6         | 0           | 1/6         | 0           | 1/6         | 1/3         |
| 7. <i>a<sub>2</sub>b<sub>1</sub>/a<sub>3</sub>b<sub>1</sub></i>  | 0                | 0           | 1/6         | 0           | 0           | 0           | 1/3         | 1/12        | 0           | 5/12        |
| 8. <i>a<sub>1</sub>b<sub>2</sub>/a<sub>1</sub>b<sub>3</sub></i>  | 0                | 1/6         | 0           | 0           | 0           | 1/12        | 0           | 0           | 1/3         | 5/12        |
| 9. <i>a<sub>1</sub>b<sub>2</sub>/a<sub>2</sub>b<sub>3</sub></i>  | 0                | 0           | 0           | 1/6         | 1/12        | 0           | 1/4         | 0           | 1/4         | 1/4         |
| 10. <i>a<sub>1</sub>b<sub>2</sub>/a<sub>1</sub>b<sub>2</sub></i> | 1/12             | 0           | 0           | 0           | 0           | 1/6         | 0           | 1/6         | 0           | 7/12        |
| 11. <i>a<sub>1</sub>b<sub>2</sub>/a<sub>2</sub>b<sub>1</sub></i> | 0                | 0           | 0           | 1/6         | 0           | 0           | 1/3         | 0           | 1/3         | 1/6         |

\* One typical gamete listed for each mode.

chromatid is marked and each pairing in both bisimplex coupling and repulsion yields detectable crossovers.

As only progeny of doubly dominant gametes (Table 3, columns 1 through 5) were being subjected to a second backcross, it is clear from Table 3 that frequencies of modes 6, 7, 8, and 10 could not be estimated if only bisimplex coupling were used. Likewise if only bisimplex repulsion were used (Table 4), the frequency of mode 2 could not be estimated. FISHER (1947) pointed out that these genotypes supplement each other in the information they furnish. Both genotypes are, therefore, used in the analysis. However, both matrices are still incapable of estimating all 11 frequencies of modes of gamete formation because each shares in common two degrees of indeterminateness (FISHER 1949). This indeterminateness may be obviated by replacing the 11 unknown mode frequencies,  $f_1, f_2, \dots, f_{11}$ , which add to unity, by linear functions of these which also add to unity. This may be done for bisimplex coupling and repulsion by deleting the first and last rows of the gametic matrices and ascribing compound frequencies to the third, sixth, and ninth rows (Table 5). The compound frequencies are appropriate, since gametes produced by modes 1 and 3 are experimentally indistinguishable, as are those produced by modes 9 and 11.

The reduced matrix for coupling, Table 5, yields the following series of equations:

$$\begin{aligned} f_2/4 &= a & (2f_1 + f_3)/4 &= d \\ f_4/4 &= b & (f_2 + 2f_{11})/4 &= e \\ f_5/4 &= c & & \end{aligned}$$

where  $a, b, c, d,$  and  $e$  are observed gametic frequencies given in Table 1. These equations are solved for the unknown frequencies:

TABLE 5

*Gametic matrices condensed to show information available from second backcross experiment*

| Frequencies<br>to be estimated | Bisimplex        |             |             |             |             |                   |             |             |             |             |
|--------------------------------|------------------|-------------|-------------|-------------|-------------|-------------------|-------------|-------------|-------------|-------------|
|                                | Coupling gametes |             |             |             |             | Repulsion gametes |             |             |             |             |
|                                | <i>ShWx</i>      | <i>ShWx</i> | <i>ShWx</i> | <i>ShWx</i> | <i>Shwx</i> | <i>shWx</i>       | <i>ShWx</i> | <i>ShWx</i> | <i>ShWx</i> | <i>Shwx</i> |
|                                | <i>ShWx</i>      | <i>Shwx</i> | <i>shWx</i> | <i>shwx</i> | <i>shWx</i> | <i>ShWx</i>       | <i>Shwx</i> | <i>shWx</i> | <i>shwx</i> | <i>shWx</i> |
| $2f_1 + f_3$                   | 0                | 0           | 0           | 1/4         | 0           | 0                 | 0           | 0           | 1/12        | 1/6         |
| $f_2$                          | 1/4              | 0           | 0           | 0           | 0           | 0                 | 0           | 0           | 0           | 0           |
| $f_4$                          | 0                | 1/4         | 0           | 0           | 0           | 0                 | 1/12        | 0           | 0           | 0           |
| $f_5$                          | 0                | 0           | 1/4         | 0           | 0           | 0                 | 0           | 1/12        | 0           | 0           |
| $f_6 - f_1 - f_{11}$           | 0                | 0           | 0           | 0           | 0           | 0                 | 0           | 0           | 1/6         | 1/6         |
| $f_7$                          | 0                | 0           | 0           | 0           | 0           | 0                 | 0           | 1/6         | 0           | 0           |
| $f_8$                          | 0                | 0           | 0           | 0           | 0           | 0                 | 1/6         | 0           | 0           | 0           |
| $f_{10}$                       | 0                | 0           | 0           | 0           | 0           | 1/12              | 0           | 0           | 0           | 0           |
| $f_9 + 2f_{11}$                | 0                | 0           | 0           | 0           | 1/4         | 0                 | 0           | 0           | 1/6         | 1/12        |
| Totals                         | <i>a</i>         | <i>b</i>    | <i>c</i>    | <i>d</i>    | <i>e</i>    | <i>a'</i>         | <i>b'</i>   | <i>c'</i>   | <i>d'</i>   | <i>e'</i>   |

$$f_2 = 4a$$

$$f_4 = 4b$$

$$f_5 = 4c$$

$$2f_1 + f_3 = 4d$$

$$f_9 + 2f_{11} = 4e.$$

Following the same procedure for the reduced repulsion matrix, Table 5, the remaining unknown frequencies are obtained:

$$f_7 = 6c' - 2c$$

$$f_8 = 6b' - 2b$$

$$f_{10} = 12a' \quad f_6 - f_1 - f_{11} = 3(d' + e' - d - e).$$

Derivations of these equations for the unknown frequencies using the repulsion matrix are given in the Appendix.

Using these equations and the observed values *a* through *e* and *a'* through *e'* (Table 1), direct estimation is now possible for the frequencies of the nine linear functions derived from the modes of gamete formation (Table 6). The sum of the nine unreduced frequencies was greater than unity and was a reflection of the excess of double dominants in the first-backcross progenies, as pointed out by FISHER (1949). The estimates are, therefore, reduced to sum to unity by dividing each by the unreduced total.

To obtain the estimated total recombination fraction, 29.22%, the frequencies of recombinant chromatids contributed by all modes of gamete formation are added. Each mode is weighted by 0, 1/2, or 1, depending on whether it contained zero, one, or two recombinant chromatids, respectively. As the significance of the negative value, -46.29, for compound frequency  $f_6 - f_1 - f_{11}$  is perhaps not intuitive, the algebraic derivation of the formula for estimating its frequency is given in the Appendix. It can be stated, however, that the negative value adjusts for the proportion of non-recombination in the compound mode  $2f_1 + f_3$ . As listed in Table 2, mode  $f_1$ , for example, contains no recombinant chromatids.

Double reduction for each locus was estimated by summing modes which con-

TABLE 6

*Frequencies of modes of gamete formation, recombination, and double reduction for shrunken-waxy, based on double backcrosses*

| Modes of gamete formation | Estimated frequencies (percent) |          | Proportion of recombinant chromatids | Contribution to percent recombination | Double reduction for |           |
|---------------------------|---------------------------------|----------|--------------------------------------|---------------------------------------|----------------------|-----------|
|                           | Unreduced                       | Reduced* |                                      |                                       | <i>Sh</i>            | <i>Wx</i> |
| $2f_1 + f_3$              | 146.96                          | 124.60   | 1/2                                  | 62.30                                 | ....                 | ....      |
| $f_2$                     | 2.60                            | 2.20     | 0                                    | 0.0                                   | 2.20                 | 2.20      |
| $f_4$                     | 14.80                           | 12.55    | 1/2                                  | 6.27                                  | 12.55                | ....      |
| $f_5$                     | 0.0                             | 0.0      | 1/2                                  | 0.00                                  | 0.00                 | 0.00      |
| $f_6 - f_1 - f_{11}$      | -54.60                          | -46.29   | 1                                    | -46.29                                | ....                 | ....      |
| $f_7$                     | 0.00                            | 0.00     | 1                                    | 0.00                                  | ....                 | 0.00      |
| $f_8$                     | 3.82                            | 3.24     | 1                                    | 3.24                                  | 3.24                 | ....      |
| $f_{10}$                  | 0.00                            | 0.00     | 1                                    | 0.00                                  | 0.00                 | 0.00      |
| $f_9 + 2f_{11}$           | 4.36                            | 3.70     | 1                                    | 3.70                                  | ....                 | ....      |
| Totals                    | 117.94                          | 100.00   |                                      | 29.22                                 | 17.99                | 2.20      |

\* See text.

tained sister chromatids at respective loci. The value thus computed is analogous to *alpha* (frequency of double reduction) as defined by FISHER and MATHER (1943). Mode 2 required double reduction at both loci and indicated both *sh* and *wx* were on one side of the centromere, with *wx* being genetically close to the centromere (*alpha* = 2.20%). No gametes were observed which were double reduction for *wx* alone (Mode 5). Double reduction for *sh* (*alpha* = 17.99%) exceeded the theoretical maximum of 16.67% for equational segregation (MATHER 1936; BURNHAM 1962). This excess was probably within the experimental error, but no standard error could be computed as data from all families were pooled in the analysis. This value is of interest since *sh* is not the most distal gene on the short arm of 9. If additional tests confirm a value of about 1/6, the practice of testing for goodness of fit to the 1/7 value for what has been termed "random chromatid segregation" may be of doubtful value. Genes closer to the centromere than *sh* would be expected to give lower double reduction values, some of which should approximate 1/7. More data are needed for *sh*, for genes between *sh* and *wx*, and for genes distal to *sh*.

As only doubly dominant plants were used in the analysis, it is desirable to know how well gametic frequencies from these (Table 1) fitted frequencies that could have been expected if all plants had been used. The expected frequencies *a* to *h*, and *a'* to *h'* may be derived by multiplication of the reduced frequencies of modes (Table 6) by the original gametic matrices (Tables 3 and 4 as condensed in Table 5) (GATES 1957). For example, for the *Sh-wx wx* phenotype in coupling the expression for the expected frequency is:

$$\frac{2f_1 + f_3}{4} + \frac{f_5}{4} + \frac{f_6 - f_1 - f_{11}}{2} + \frac{f_7}{2} + \frac{f_8}{4} + \frac{f_9 + 2f_{11}}{4} + \frac{f_{10}}{4}$$

then substituting the reduced frequency values in Table 6, this is:

TABLE 7

Single backcross segregations and percent recombination for shrunken-waxy, based on four genotypes

| Number in family                   | Code‡ | Phenotype of progeny (observed numbers) |                   |                   |                     | Percent recombination |
|------------------------------------|-------|---|-------------------|-------------------|---------------------|-----------------------|
|                                    |       | Sh-Wx-                                  | Sh-wxwx           | shshWx-           | shshwxwx            |                       |
| Biduplex coupling/ratio of progeny |       |   |                   |                   |                     |                       |
|                                    |       | 5-2p+p <sup>2</sup> †                   | 2p-p <sup>2</sup> | 2p-p <sup>2</sup> | 1-2p+p <sup>2</sup> |                       |
| 3                                  | a     | 180                                     | 32                | 36                | 37                  | 32.98 ± 3.45          |
| Duplex-simplex coupling            |       |   |                   |                   |                     |                       |
|                                    |       | 3-p                                     | 2+p               | p                 | 1-p                 |                       |
| 17                                 | b     | 795                                     | 575               | 98                | 274                 | 24.60 ± 1.98          |
| 2                                  | b'    | 89                                      | 59                | 13                | 38                  | 23.50 ± 2.01          |
| Total                              |       | 884                                     | 634               | 111               | 312                 | 24.35 ± 1.95          |
| Bisimplex coupling                 |       |   |                   |                   |                     |                       |
|                                    |       | 1-p                                     | p                 | p                 | 1-p                 |                       |
| 1*                                 | ..    | 80                                      | 18                | 32                | 86                  | 23.15 ± 5.18          |
| 1*                                 | ..    | 144                                     | 27                | 25                | 132                 | 15.85 ± 2.02          |
| 1*                                 | ..    | 90                                      | 17                | 22                | 89                  | 17.89 ± 2.59          |
| 1*                                 | ..    | 73                                      | 29                | 24                | 76                  | 26.24 ± 3.09          |
| 1*                                 | ..    | 88                                      | 20                | 24                | 95                  | 19.38 ± 2.62          |
| 1*                                 | ..    | 106                                     | 27                | 25                | 87                  | 21.22 ± 2.61          |
| 1*                                 | ..    | 126                                     | 44                | 43                | 118                 | 26.23 ± 2.40          |
| 28                                 | d     | 1156                                    | 250               | 296               | 1131                | 19.27 ± 1.42          |
| 62                                 | d     | 2445                                    | 470               | 455               | 2427                | 15.95 ± 0.97          |
| 29                                 | d     | 1087                                    | 296               | 283               | 1004                | 21.68 ± 1.47          |
| 19                                 | d     | 824                                     | 160               | 156               | 734                 | 16.86 ± 1.72          |
| 8                                  | d     | 239                                     | 87                | 69                | 278                 | 23.18 ± 2.93          |
| 9                                  | d     | 347                                     | 56                | 64                | 349                 | 14.71 ± 2.57          |
| 2                                  | d'    | 77                                      | 24                | 23                | 77                  | 23.35 ± 2.89          |
| Total                              |       | 6882                                    | 1525              | 1541              | 6683                | 18.44 ± 0.39          |
| 9                                  | d§    | 465                                     | 86                | 83                | 227                 | .....                 |
| 5                                  | d§    | 116                                     | 39                | 26                | 215                 | .....                 |
| Bisimplex repulsion                |       |   |                   |                   |                     |                       |
|                                    |       | 1+p                                     | 2-p               | 2-p               | 1+p                 |                       |
| 1*                                 | ..    | 37                                      | 55                | 56                | 35                  | 18.00 ± 10.85         |
| 1*                                 | ..    | 44                                      | 68                | 78                | 42                  | 11.19 ± 9.51          |
| 15                                 | e'    | 254                                     | 428               | 473               | 280                 | 11.60 ± 3.82          |
| 4                                  | e'    | 54                                      | 98                | 90                | 58                  | 12.02 ± 8.38          |
| 5                                  | e     | 118                                     | 147               | 151               | 107                 | 28.98 ± 5.82          |
| Total                              |       | 507                                     | 796               | 848               | 522                 | 14.95 ± 5.11          |
| Weighted average of all genotypes  |       |   |                   |                   |                     | 18.79 ± 1.05          |

\* First backcross segregations from 7 bisimplex coupling and 2 bisimplex repulsion parental plants.

† p is the crossover percentage with chromosome segregation.

‡ Indicates the source and type of gamete (Table 1, Columns 4 and 7), identified by performing a second backcross on Sh-Wx- progeny of first backcross.

§ Abnormal segregations which were not included in the single-backcross linkage estimate.

$$\frac{124.60}{4} + \frac{0}{4} + \frac{(-46.29)}{2} + \frac{0}{2} + \frac{3.24}{4} + \frac{3.70}{4} + \frac{0}{4} = 9.74.$$

When the observed coupling and repulsion frequencies (Table 1) were fitted to the expected, the Chi-squares were 2.27 ( $P = .90-.95$ ), and 4.11 ( $P = .70-.80$ ), respectively. Therefore, use of only doubly dominant plants was adequate in this experiment.

*Single backcross linkage analysis:* Recombination was estimated by the method of maximum likelihood based on chromosome segregation (Table 7). Estimates were based on chromosome rather than chromatid segregation as there was a slight deficiency of recessives for both shrunken and waxy. The maximum likelihood method of estimating the recombination value and its standard error was outlined by MATHER (1938).

Single-backcross segregations were from the first backcross of bisimplex coupling and repulsion, and from the second backcross of the *Sh-Wx*- progeny of the first backcross. The second backcross identified the gametes from the first backcross and constituted a single backcross of the progeny of these gametes. Most of the progeny were bisimplex coupling and repulsion, but two other genotypes were also identified, viz., duplex-simplex coupling (produced by double reduction for *Sh*), and biduplex coupling (produced by double reduction for both *Sh* and *Wx*). Percent recombination was  $18.44 \pm 0.39$  for bisimplex coupling,  $14.95 \pm 5.11$  for bisimplex repulsion,  $24.35 \pm 1.95$  for duplex-simplex coupling, and  $32.98 \pm 3.45$  for biduplex coupling. The average over all genotypes was  $18.79 \pm 1.05$ , weighted by the number classified for each.

Estimates based on bisimplex coupling are implicitly more reliable than those from other genotypes as the chromosome carrying the dominant genes must always pair with one carrying recessives. Thus preferential pairing, if it occurs, can have no effect on recombination. In addition, most of the recombinant chromatids are directly identifiable in the recombinant "p" classes. In the other genotypes, recombinant and nonrecombinant chromatids are confounded with each other and with experimental variation in each class.

#### DISCUSSION

R. A. FISHER's method of estimating linkage in autopolyploids was invaluable in this study. It accounted for all the complexities in genetic segregation and as linkage could be estimated from only doubly dominant progeny, discrepancies due to inviability of recessives were eliminated. Also, the double backcrosses permitted identification of recombinant and double reductional gametes which were indistinguishable among dominant progeny of single backcrosses. CATCHESIDE (1956) proposed that numerical nondisjunction—chromosome separation at the first meiotic division which is not two from two—was a normal component of variation in autotetraploid segregation. However, recombination estimates based on simplex parents are not affected by numerical nondisjunction if all of the gametes function. The double-backcross estimate was, therefore, as unbiased as possible.

The double-backcross recombination estimate of 29.22% between shrunken and waxy was larger than the average single-backcross estimate of 18.79%. This was to be expected since recombinant chromatids among dominants could not be identified by a single backcross. However, the average single-backcross estimate was indicative of the general degree of linkage and approximated the average diploid value of 21% based on two-point test crosses (EMERSON, BEADLE and FRASER 1935). Other single-backcross estimates in maize (WELCH 1962) were also comparable with diploid values, with only one exception. Single-backcross estimates require much less time and space and should be adequate for preliminary linkage tests and most breeding research with autotetraploids. However, precise autotetraploid linkage estimates and maps should be based on double-backcross data.

Several factors could contribute to a larger recombination estimate in autotetraploids than in diploids. SVED (1964) concluded that multiple crossing-over and random partner exchange among chromatids may lead to a higher autotetraploid recombination value than the corresponding value in diploids, without the mean frequency of crossing-over being increased. If complete randomness is assumed, and no allowance made for reductional segregation of the centromeres at division I of meiosis, the maximum recombination frequency in autotetraploids is theoretically 75%. This can be calculated from FISHER's Table 3 (1949), Table 2 in this paper, and was discussed by SVED (1964). Using SVED's calculations, a diploid recombination value of 21% could rise to about 28% in an autotetraploid with multiple crossing-over. However, absence of double reductional gametes for waxy alone, gametic code *c* (Table 1) indicated that multiple crossing-over in the shrunken-waxy region was rare. Production of this gamete would have required simultaneous cross-overs between the centromere and waxy, and between shrunken and waxy. Three other factors which could increase the autotetraploid value are preferential pairing, increase in crossing-over *per se*, and non-random quadrivalent separation. Preferential pairing, however, could not have influenced recombination in bisimplex coupling. If the increase in autotetraploid recombination cannot be attributed to multiple crossing-over or preferential pairing, then it could reflect an increase in crossing-over or non-random quadrivalent separation.

Double reduction frequencies (Table 6) indicated that waxy and shrunken were in the same chromosome arm and genetically proximal and distal to the centromere, respectively. These genes in diploid maize have previously been located in the short arm of chromosome 9 (McCLINTOCK 1931). LEVINGS and ALEXANDER (1966) reported that waxy was closely linked to the centromere based on the frequency of double reduction.

#### SUMMARY

Recombination between the genes shrunken and waxy in autotetraploid maize was estimated using double- and single-backcross data. Parental genotypes were bisimplex coupling and bisimplex repulsion. Doubly dominant single-backcross

progeny were subjected to a second backcross to reveal the initial frequency of parental gametes carrying recombinant chromosomes which could not be identified in the first backcross. Matrix procedures for the double-backcross analysis are described and equations derived for calculating estimable modes of gamete formation. Experimental gametic frequencies were used to estimate the frequencies of the modes and obtain the double-backcross linkage estimate of 29.22%. This estimate was larger than the average single-backcross estimate of 18.79%, based on maximum likelihood, and the previously reported average diploid value of 21%. Double reduction values indicated shrunken and waxy were in the same arm of chromosome 9, with waxy being genetically proximal and shrunken distal to the centromere.—Only the double-backcross method was capable of identifying all the original recombinant and double reduction gametes and is recommended for precise linkage studies.

## APPENDIX

*Derivation of unknown gametic frequencies from repulsion*

Table 5 yields the following series of equations for repulsion:

$$\begin{aligned} f_{10}/12 &= a' & (2f_1+f_3)/12 + (f_6-f_1-f_{11})/6 + (f_9+2f_{11})/6 &= d' \\ f_4/12+f_8/6 &= b' & (2f_1+f_3)/6 + (f_6-f_1-f_{11})/6 + (f_9+2f_{11})/12 &= e' \\ f_5/12+f_7/6 &= c'. \end{aligned}$$

Hence:

$$\begin{aligned} f_{10} &= 12a' & f_7 &= 6c' - f_5/2 \\ f_8 &= 6b' - f_4/2 & &= 6c' - 2c \\ &= 6b' - 2b. \end{aligned}$$

Adding the two equations involving the compound frequencies gives:

$$(2f_1+f_3)/4 + (f_6-f_1-f_{11})/3 + (f_9+2f_{11})/4 = d' + e'.$$

So

$$\begin{aligned} f_6-f_1-f_{11} &= 3d' + 3e' - 3(2f_1+f_3)/4 - 3(f_9+2f_{11})/4 \\ &= 3d' + 3e' - 3(4d)/4 - 3(4e)/4 \\ &= 3(d' + e' - d - e). \end{aligned}$$

Q.E.D.

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