DOUBLE REDUCTION IN AUTOTETRAPLOID MAIZE[1]

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A unique aspect of autopolyploid genetics is double reduction, the process by which sister factors of a chromosome enter the same gamete at the second division of meiosis. The phenomenon affects all properties of a population which are dependent upon segregation. The present study was undertaken to detect double reduction and to estimate its magnitude for various genes in autotetraploid maize. In addition, an evaluation was made of autotetraploid data as a tool for genetic analysis.

THEORY

MATHER, in a series of papers (1935a, 1935b, 1936) which dealt in part with autotetraploids, discussed segregation in the light of cytological events. He pointed out that chromosomal and chromatid segregations were limiting types and that the actual segregation would in most instances fall between the two extremes. He formulated the indices alpha and beta to measure double reduction and described in detail the necessary cytological events. The quantity alpha, as MATHER defined it in his 1936 publication, is twice the proportion of gametes showing double reduction at a specified locus. FISHER and MATHER (1943) used alpha to represent the frequency itself. In recent literature, alpha has been exclusively used in the latter context and will be used in this manner throughout the remainder of this paper. Under this circumstance, alpha is equal to one seventh when chromatid segregation occurs.

One of the interesting aspects of autotetraploid genetics is the location of the centromere in a linkage group since alpha is determined, in part, by the amount of crossing over between gene and centromere. Numerous workers (DE WINTON and HALDANE 1935; EL-GHAWAS 1955; LITTLE 1958; WELCH 1962) have used the method to determine the position of the centromere. Genes remote to the centromere are expected to have high alpha values while genes near to the centromere have low values.

There are a number of variables influencing the value of alpha. For a gamete to receive sister alleles, a crossover or crossovers must occur between the centromere and gene. A single crossover between a gene and the centromere results in equational separation of chromatids distal to the point of crossing over. With

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equation separation, sister alleles are joined to different centromeres at first division. If no crossing over occurs, reductional separation of chromatids follows and at second division sister chromatids must separate to different gametes. This event precludes double reduction. Since crossing over contributes to double reduction only when it causes equational separation at first division, some multiple crossovers will not contribute to double reduction. Genetic and environmental factors which influence crossing over will likewise alter the frequency of double reduction.

Even if equational separation occurs, double reductional gametes will not result unless genetic nondisjunction takes place. Genetic nondisjunction occurs when crossover chromatids move to the same interphase nucleus at the end of the first meiotic division. This event is conditioned by a number of factors. First, quadrivalents or trivalents must be formed during prophase I. Since trivalents are anomalous and often result in numerical nondisjunction, they will be dealt with later. In autotetraploids, pairing involves two of the four homologous chromosomes at any one point although different chromosomes may pair with other homologues at different points. The postpachytene association of four homologous chromosomes held together by chiasmata produces quadrivalents at diplotene. When the pairing in autotetraploids is such that bivalents result, parts of sister chromatids cannot enter the same gamete. However, when quadrivalents are present, the chromosomes separate two by two. If this event occurs at random, there will be one chance in three that any two chromatids that have engaged in crossing over will enter the same interphase nucleus. Therefore, quadrivalents are necessary for genetic nondisjunction.

The coorientation of the centromeres on the spindle at metaphase I determines the segregation of chromosomes at anaphase I. Two arrangements are recognized for ring quadrivalents, convergent (alternate) and parallel (adjacent). The convergent type results in crossover chromatids passing to opposite poles, therefore it prevents genetic nondisjunction. The parallel arrangement results in two types of disjunction. One type results in crossover chromatids moving to opposite poles, while the other allows them to pass to the same pole. The latter type results in genetic nondisjunction. In addition. quadrivalents may have a straight chain configuration in which case three different coorientations are possible. These orientations may or may not result in genetic nondisjunction. A few other types of configuration can occur, e.g. double rings and variations in chains, but their frequencies are small.

If equational separation and genetic nondisjunction have occurred in the first division, one final obstacle to double reduction remains in the second division. Parts of sister chromatids are attached to different centromeres at second division by virtue of previous crossing over. These centromeres divide and the attached chromatids move to opposite poles. Since homologous parts of sister chromatids are attached to different centromeres, they pass to the poles independently. Under these circumstances, homologous parts of sister chromatids have a probability of one half of entering the same gamete.

Variables making up the alpha value can be summarized in the formula,

$$\alpha = \varepsilon qgs \tag{1}$$

The quantity ε refers to the amount of equational separation, q the frequency of quadrivalent formation, g the frequency of genetic nondisjunction and s the frequency at second division with which homologous parts of sister chromatids moving to the same pole.

CATCHESIDE (1956) has stated that numerical nondisjunction cannot be neglected in autotetraploid maize because a substantial porportion of the progeny are aneuploids. When numerical nondisjunction occurs, chromosomal separation at the first meiotic division is not two by two. In euploids such an event may result when trivalents and univalents are formed instead of quadrivalents or bivalents, or when indifferent coorientation occurs. Aneuploid individuals must always exhibit numerical nondisjunction because gametes inevitably must be produced that are deficient or duplicate for one or more chromosomes. Although some unbalanced gametes do function, they are undoubtedly at a selective disadvantage. CATCHESIDE (1956) has derived the expected gametic proportions for a duplex backcross in terms of alpha and numerical nondisjunction, x. The duplex backcross expectations for the recessive and dominant phenotypes are $(2 + x + 4\alpha - 3\alpha x)/12$ and $(10 - x - 4\alpha + 3\alpha x)/12$, respectively, when dominance is complete. Using these proportions, general formulae for alpha and its standard error were derived by the maximum likelihood method where z is the percent recessive individuals in the population

$$\alpha = \frac{12z - x - 2}{4 - 3x}$$

$$S_{\alpha} = \frac{12}{4 - 3x}\sqrt{\frac{z(1-z)}{n}}$$

If appropriate values of x and z are substituted into formulae, alpha and its standard error can be determined.

Since double reduction is functionally related to the amount of crossing over between a gene and centromere, it is possible to determine the recombination fraction when alpha and certain cytological variables are known. CATCHESIDE (1956) has estimated α for the su_1 locus and used this information for determining recombination between the gene and centromere. He gives the formula for α to be $\alpha = cqg$, when our notations are used. The c refers to the amount of crossing over and he states that the half chance of having the necessary disjunction at division II of meiosis, s of our notation, is offset by the double chance of the necessary crossover in each cell. His c is equivalent to $\varepsilon/2$, where ε is the frequency of equational separation used in equation (1). DEMARLY (1958) has given the relationship between equational separation and crossing over in autotetraploids,

$$\varepsilon_i = \frac{6}{7}\left[1 - \left(\frac{5}{12}\right)^i\right] \tag{2}$$

where i refers to the number of crossovers. From this equation it can be seen that the relationship $c = \varepsilon/2$ holds for 0 or 1 crossovers, but does not hold for multiple crossovers. Therefore, CATCHESIDE's method for determining the recombination frequency will not be precise when multiple crossovers occur.

In the development which follows formulae are derived which take into account the discrepancy between crossing over and equational separation due to multiple crossovers. Let us assume that chromosome strands are paired at all points and any strand is equally likely to be paired with any other strand at a given point. Alternative models are recognized where the number of changes in pairing partners is restricted. These models are perhaps more realistic, but without definite information on pairing relationships, the random change of partner model was selected. In addition, a complete noninterference model is postulated which requires no chromosome (chiasma) or chromatid interference. If crossing over occurs at random, the POISSON distribution of crossovers may be assumed. Thus the frequency of i crossovers is $(e^{-c}\, c^i)/i!$, where c is the mean number of crossovers. Then substituting in equation (2), the frequency of equational separation ε, is

$$\varepsilon = \frac{6}{7} \sum_{i=0}^{\infty} \left[1 - \left(\frac{5}{12}\right)^i \right] \frac{e^{-c}\, c^i}{i!} = \frac{6}{7} \left(1 - e^{-7c/12}\right). \tag{3}$$

After solving equation (1) for ε and setting it equal to equation (3), we obtain the solution to c, the mean number of crossovers occurring between a locus and its centromere,

$$c = -\frac{12}{7} \ln \left(1 - \frac{7\alpha}{6qgs}\right). \tag{4}$$

With appropriate estimates of alpha and the cytological variables, the mean number of crossovers between a locus and its centromere can be determined. Recombinations, R_T, between a gene and its centromere is then provided by substituting the mean number of crossovers into the formula (Model 2, SVED 1964):

$$R_T = \frac{3}{4}\left(1 - e^{-c/3}\right).$$

After substitution for c,

$$R_T = \frac{3}{4}\left[1 - exp\left(\frac{4}{7} \ln \left(1 - \frac{7\alpha}{6qgs}\right)\right)\right]. \tag{5}$$

It should be noted that the recombination value, R_T, obtained from the above equation, is a tetraploid recombination value and should not be expected to be the same as a diploid recombination value. It has been shown by SVED (1964) that multiple crossing over affects diploid and tetraploid recombination frequencies in different ways. If the mean frequency of crossing over per strand is the same in the diploid and tetraploid, the mean number of crossovers in the tetraploid would be twice that in the diploid. In this event, substitution of $\frac{1}{2}\, c$, the mean number of crossovers, from equation (4) into HALDANE's (1919) equation

TABLE 1

Duplex backcross data for the g_1, su_1, y_1 *and* wx_1 *loci*

Locus	Total	Dominants	Recessives	Ratio
g_1	6348	5181	1167	4.44:1
su_1	20885	17072	3813	4.48:1
y_1	30465	24998	5467	4.57:1
wx_1	1089	904	185	4.89:1

for the recombination frequency of diploids, would give the diploid recombination fraction, R_D.

$$R_D = \frac{1}{2}\left(1 - e^{-c}\right)$$

After substitution for one half of c,

$$R_D = \frac{1}{2}\left[1 - exp\left(\frac{6}{7}\ln\left(1 - \frac{7\alpha}{6qgs}\right)\right)\right]. \qquad (6)$$

Finally, it should be noted that if no multiple crossovers occur between the gene and centromere, then $\varepsilon/2$, R_T and R_D should estimate the same quantity. It is only because multiple crossing over affects these three parameters differently that they do not estimate exactly the same quantity.

RESULTS AND DISCUSSION

Duplex backcross segregation ratios were obtained for four loci—su_1, (Sugary endosperm-1) on chromosome 4, y_1, (yellow endosperm-1) on 6, wx_1, (Waxy endosperm-1) on 9 and g_1, (Golden-1) on 10. Classification of these characters in segregating populations was carried out in the usual manner with no apparent difficulty. Segregation ratios for the four loci are presented in Table 1. For su_1 and y_1, data were obtained in more than one year, but no significant difference was found between ratios obtained in different years. If no double reduction occurs, the duplex backcross should give a 5:1 ratio.

The segregation ratios differ for the four loci. This, of course, is in marked contrast to the diploid situation where all genes have the same segregation expectation. In autotetraploids, each locus has a unique segregation expectation which is dependent upon its location on the chromosome and such cytological events as

TABLE 2

Alpha and its standard error when numerical nondisjunction is zero and 0.0258

	$X=0$		$X=0.0258$	
Locus	α	Standard error	α	Standard error
g_1	.0514	± .0146	.0458	± .0149
su_1	.0478	± .0080	.0422	± .0082
y_1	.0385	± .0066	.0327	± .0067
wx_1	.0097	± .0341	.0033	± .0348

quadrivalent frequency, numerical nondisjunction and genetic nondisjunction. Genes near the centromere should exhibit wide segregation ratios (like chromosomal segregation). As genes become remote from the centromere, segregation ratios should narrow until a limit is reached (chromatid segregation). Waxy-1 is a good example of a locus close to the centromere. Its 4.89:1 duplex backcross ratio is very close to the 5:1 expected of chromosomal segregation. The values for g_1, su_1, and y_1 lie between chromosomal and chromatid segregation expectations. The majority of genes in maize would fit into this category.

In Table 2 alpha values and their standard errors are presented for the four loci. In the first case, no numerical nondisjunction ($x = 0$) was assumed, while in the second, a value of 0.0258 [an average of KADAM's (1944) and CATCHESIDE's (1956) observations] was used although it was realized that the values are likely to be different for each chromosome. The effect of numerical nondisjunction on alpha is to overestimate it, consequently, the correction for numerical nondisjunction reduces the alpha value. Nevertheless, there was not a significant difference between the corrected and uncorrected alpha values. The alpha values associated with the loci g_1, su_1, and y_1 are significantly different from zero and one seventh. Alpha values of zero and one seventh are expected from chromosomal segregation and random chromatid segregation, respectively. These three loci are, therefore, not segregating independently of the centromeres, nor are they completely linked with the centromeres. However, the alpha value for the waxy-1 locus is not significantly different from zero. Its segregation ratio nearly approximates chromosomal segregation and it must be considered very closely linked to the centromere. Therefore, a crude estimate of a gene's location with respect to the centromere can be determined by the magnitude of double reduction.

Recombination percentages between a gene and its centromere were determined for the four loci (Table 3). In order to solve equations (5) and (6), estimates of the cytological variables q, g, and s were necessary. A value of .783 was used for quadrivalent frequency, q. This frequency represents the mean when the values of CATCHESIDE (1956) and GILLES and RANDOLPH (1951) are combined. It is significant that half the parentage of CATCHESIDE's material and all the material of this study were obtained from a common source, L. F. RANDOLPH. A value of one third was selected for the frequency of genetic nondisjunction, g.

TABLE 3

Recombination and map distance between a locus and its centromere derived from 4n and 2n data

	Percent recombination estimated from 4n data						Map distance estimated from 2n data
	R_T		R_D		$\varepsilon/_2$		R_D
Locus	$X=0$	$X=.0258$	$X=0$	$X=.0258$	$X=0$	$X=.0258$	
g_1	22.3	19.5	20.5	18.2	19.7	17.5	15.0
su_1	20.5	17.8	19.0	16.7	18.3	16.2	9.0 or more
y_1	16.1	13.5	15.2	12.8	14.7	12.5	5.0–10.0
wx_1	3.8	1.3	3.7	1.3	3.7	1.3	2.0– 3.6

CATCHESIDE used the value (0.373) (0.5) for the frequency of g. The 0.373 value is the frequency of parallel (adjacent) disjunction of ring quadrivalents as determined by VENKATESWARLU (cited by CATCHESIDE 1956) and the 0.5 is the expected value for the appropriate type of parallel disjunction leading to genetic nondisjunction. His value of g might be very accurate if only ring quadrivalents were obtained; however, this value is unlikely to be applicable to chain quadrivalents or other configurations. For this reason, it was decided to assume that disjunction of tetraploid quadrivalents was largely random. Finally, s is one-half, the frequency at second division with which homologous parts of sister chromatids moving to the same pole. Alpha values from Table 2 were used in the computations where the frequency of numerical nondisjunction was zero and 0.0258.

In Table 3, R_T is determined from equation (5) using values of alpha corresponding to zero and 0.0258 values of numerical nondisjunction. Since the effect of numerical nondisjunction is to reduce the estimate of double reduction, the recombination percentage is similarly decreased. This same effect is shown for R_D, the diploid estimate of recombination. R_D was computed from equation (6) in which case the mean number of crossovers was halved. A comparison of R_T and R_D shows the recombination percentage to be lower for R_D except for the wx_1 locus. Differences between R_T and R_D are due to multiple crossovers. Since wx_1 is closely linked to the centromere with little chance for multiple crossovers, the equal or nearly equal values of R_T and R_D for this locus are expected.

The $\varepsilon/2$ columns of Table 3 give the recombination frequency between gene and centromere using the method of CATCHESIDE (1956). A comparison of R_D and $\varepsilon/2$ values indicates only small differences. Therefore, the effects of multiple crossovers are not very important on recombination frequencies of the order of 20%. With larger recombination frequencies a more significant difference between R_D and $\varepsilon/2$ would be expected. The last column of Table 3 presents the approximate position of the centromeres in relation to the four loci (ANDERSON and RANDOLPH 1945). The results were based on linkage studied in diploid maize where the centromeres were marked with translocations. Reliability of these estimates depends upon how close the translocation point is to the centromere. Since a translocation is unlikely to mark the centromere precisely, estimates tend only to narrow down the possible position of the centromere. In addition, translocations may suppress crossing over to some extent which would add to the discrepancy. Since the values given in the last column are map distances, some of the masking effects of multiple crossovers has been removed. For this reason, a comparison between recombination and map distance is not precise. However, when the distances are short, the discrepancy between a recombination value and a map distance is small. Columns 4, 5, 6, and 7 versus 8 provide a comparison of the recombination frequency between a locus and its centromere derived from tetraploid data and the map distance obtained from diploid data. Recombination estimated from the tetraploid data is somewhat greater than the diploid estimate for the g_1, su_1 and y_1 loci. It is approximately the same for the wx_1 locus.

It is not possible from this study to determine which estimate of recombination between gene and centromere is the more accurate, those based on tetraploid or

diploid data. Both methods have their faults. Accuracy of the diploid estimate is dependent upon a translocation adequately marking a centromere and yet not influencing crossing over. Recombination derived from the tetraploid has a number of undesirable features. Certain cytological variables, numerical nondisjunction, quadrivalent frequency and genetic nondisjunction, need to be accurately determined. These observations are both difficult and tedious to obtain. In the case of R_D, it was necessary to make assumptions regarding chromosome pairing relationships and interference. Likewise in this connection, the frequency of crossovers was taken as being twice as great in tetraploids as in diploids. It should be noted, however, that this conversion for purposes of comparison with diploid data would be unnecessary if tetraploid data of this sort were available. Obviously, errors in determining the cytological variables or an invalidation of the assumptions would bias the results. It is perhaps surprising then that the differences between the diploid and tetraploid recombination percentages are small.

SUMMARY

Duplex backcross segregation ratios of autotetraploid maize are presented for four loci, g_1, su_1, y_1, and wx_1. The frequency of double reduction, alpha, was computed for the loci. Alpha values determined for g_1, su_1, and y_1 were significantly different from zero and one seventh. Values of zero and one seventh are expected of genes completely linked to the centromere or segregating independently of the centromere. The alpha value for wx_1 was not different from zero; therefore, it is assumed to be closely linked to the centromere. A method was introduced for determining recombination between a gene and its centromere which takes into account multiple crossovers. The method utilizes information on the values of alpha and certain cytological variables in conjunction with an assumed crossover distribution theory. Recombination values were derived from tetraploid data for the four loci and compared with map distances estimated from diploid data based on translocation studies. The comparison showed small differences between the tetraploid and diploid estimates.

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