PREFERENTIAL PAIRING IN STRUCTURAL HETEROZYGOTES
OF ZEA MAYS

GREGORY G. DOYLE

Received March 5, 1963

ONE of the interesting problems of cytogenetics is the establishment of a quantitative relationship between the degree of homology of two chromosomes and their pairing affinity at meiosis. The understanding of this relationship would aid in the interpretation of two natural phenomena—the partial or complete failure of chromosome pairing in some hybrids and preferential pairing in allopolyploids.

It is generally assumed that in the course of evolutionary divergence, gene mutations and chromosome structural changes occur in isolated populations of a species with the result that new species are formed which have a common ancestor. When these divergent species are crossed, the decrease in chromosome homology is reflected in a loss of pairing affinity which may be manifested by the presence of varying numbers of univalents at the diakinesis and metaphase stages of meiosis.

In a majority of interspecific hybrids and in some intergeneric hybrids a decrease in pairing affinity is not readily discernible from a study of diakinesis configurations. It is possible to discover the extent to which pairing affinity has been reduced, however, by doubling the chromosome number of the hybrid (AB) to form the allotetraploid (AABB) and testing for the occurrence of preferential pairing. In a polyploid where a chromosome has more than one possible pairing partner, it will tend to pair preferentially with the chromosome with which it has the greatest affinity, i.e., the chromosome which most resembles it. In an allotetraploid, the homologous chromosomes of like genomes will tend to pair preferentially with each other rather than with their homoeologous counterparts in the unlike genome.

There is evidence that in some cases preferential pairing is under genetic control. OKAMOTO (1957), SEARS (1958), RILEY (1960), and RILEY et al. (1961) have discovered that chromosome V of wheat has an influence on the mode of...
chromosome pairing in wheat allopolyploids. In strains which lack chromosome V, multivalents are found; some pairing occurs between homoeologous chromosomes. Thus, differentiation of the genomes alone will not explain preferential pairing. Preferential pairing will be manifested in two ways. In a stable allotetraploid, the differential pairing affinity is so great that only homogenetic bivalents and no heterogenetic bivalents or multivalents are formed. The terms “homogenetic” and “heterogenetic” were proposed by Stebbins (1950) and refer to the structural homology or nonhomology of the chromosomes of a bivalent. Thus in an allotetraploid AABB we would expect a lower quadrivalent frequency than in either of the two autotetraploid forms, AAAA and BBBB. The reduction in quadrivalent frequency is a measure of the pairing affinity of the A and B chromosomes. This criterion is not always reliable, since in some genera quadrivalents are rarely formed even in autotetraploids.

The second manifestation of preferential pairing in allotetraploids, the high frequency of homogenetic bivalents, may be detected by its effect on intergenomic gene segregation. If the two homologous chromosomes of the A genome are marked with a dominant gene (G) and the corresponding pair in the B genome are marked with the recessive allele of this gene (g), gametes with the constitution of gg will be produced only when heterogenetic bivalents or multivalents are formed. Homogenetic bivalents lead to the production of Gg gametes. Since preferential pairing results in the formation of fewer heterogenetic bivalents and quadrivalents than would be expected if pairing were at random, a reduction in the frequency of the recessive class of gametes will be found.

Sarvena (1958) found a consistent direct relationship between the frequency of multivalents and the percentage of recessive segregants in synthetic allopolyploids of Gossypium. Gerstel and Phillips (1958) have studied gene segregation in many synthetic allopolyploids of Gossypium and Nicotiana. They found ratios from 4.3:1 to virtually 1:0 in Gossypium and from 3:1 to 1:0 in Nicotiana. They assumed that the more distantly related the genomes combined in the allopolyploid were, the lower was the frequency of recessive segregants. Shaver (1962a, b) studied preferential pairing in tetraploid hybrids of perennial teosinte (Zea perennis (Hitchc.) Reeves and Mangelsdorf) and Zea mays L. A high degree of preferential pairing was found.

We have seen how it is possible to determine relative degrees of pairing affinity between chromosomes. However, before a quantitative relationship could be established between the degree of homology of two chromosomes and their pairing affinity at meiosis, it would be necessary to define in quantitative terms all the differences in chromosome structure and genetic material which have arisen during the course of evolutionary divergence. This is impossible to do completely. Only gross structural differences can be detected by examining pachytene configurations. Small inversions, duplications, deletions, and translocations would go undetected. Indeed in most organisms, unlike maize, pachytene analysis is impossible because this stage is unfavorable for examination.

There is no way in which the genetic differences between two genomes can be stated in quantitative terms. Only for a few characters can the inheritance pattern be determined to be disomic or tetrasomic.
Even if all the structural and genetic differences between two genomes were known, it would not be possible to distinguish between the effects of each component of nonhomology on the decrease in pairing affinity.

An experimental approach to this problem involves the synthesis of polyploids which are heterozygous for known structural rearrangements and the determination of the magnitude of preferential pairing in these polyploids.

RHOADES (1957) synthesized maize triploids which were heterozygous for two different inversions—inversion 3a and inversion 3b. He found that gene segregation in the triploid inversion heterozygotes was significantly different from that in control triploids (with all normal chromosomes).

Preferential segregation of second and third chromosomes in triploid Drosophila was reported by KOZHEVNIKOV (1940) and preferential segregation of the first chromosome was mentioned by SCHULTZ and REDFIELD (1951). In all these cases one chromosome of a triploid carried inversions. Recent work by GRELL (1961) with a series of sequentially derived rearranged second chromosomes in Drosophila melanogaster triploids has demonstrated that the more complex the rearrangement of the inversion-bearing chromosome, the greater the degree of preferential segregation.

This paper concerns the demonstration of preferential pairing in trisomes, triploids, and tetraploids which were heterozygous for inversion 3a.

**MATERIALS AND METHODS**

Inversion 3a (In 3a) is a paracentric inversion in the long arm of chromosome 3. This inversion has been studied intensively by RHOADES and DEMPSEY (1953), who found that the break points were at .4 and .95 (distance from centromere). A knob or an enlarged chromomere is just distal to the proximal break point; (see Figures 1 and 2.) By studies on crossing over between a normal (N) chromosome and duplicate-deficient chromosomes (derived from In 3a/N 3 plants) RHOADES and DEMPSEY were able to place the a1 locus 46 crossover units from the proximal break point and 15 units from the distal break point.

There are several methods of detecting preferential pairing. The three used in the work to be presented are:

1. Modifications of gene segregation in inversion heterozygotes from that found in controls (plants with all normal chromosomes were used as controls). The locus a1 was followed in the genetic experiments on all three levels of polyploidy. The dominant allele A1 is one of a series of complementary genes controlling the production of anthocyanin pigment in the plant. Kernels which are homozygous for a1 have colorless aleurones; kernels with at least one A1 allele have colored aleurones in the presence of the other dominant complementary factors. In the trisomes the sh2 locus was followed as well. Kernels homozygous for sh2 are shrunken. The presence of one or more Sh2 alleles produces a full kernel. Sh2 is very closely linked to A1; the map distance is 0.25 units. Hereafter, for the sake of convenience the subscripts on these symbols will be dropped.

2. Modification of the trivalent frequency of the trisomic inversion heterozygotes from that found in control trisomes. Microsporocytes at the diakinesis stage
FIGURES 1 and 2.—Normal chromosome 3 (above). Inversion 3a (below).

were examined and a tabulation was made of the trivalent and bivalent-univalent frequencies.

3. Modification of the anaphase bridge frequencies of trisomic and duplex (In/In/N/N) tetraploid inversion heterozygotes as compared with that of diploid and simplex (In/N/N/N) tetraploid inversion heterozygotes.

The rationale behind these methods will be presented later.

Trisomic inversion heterozygotes and control trisomes were made up by crossing suitable pollen parents (homozygous In 3a and homozygous N 3 plants) with trisome 3 plants obtained from Maize Cooperative.

Triploid controls and triploid inversion heterozygotes were obtained by RHOADES by crossing plants homozygous for N 3, A and the gene ameiotic (am) with pollen parents of the constitution a/a or a*/a* (an asterisk as a superscript following the gene symbol will be used in this paper to indicate that the gene
is on an In 3a chromosome). Plants which are homozygous for am do not undergo meiosis; instead they form a few diploid eggs which, when fertilized by haploid sperm yield triploids which are all euploid (RHOADES 1956; SINHA 1962).

Tetraploid controls were produced by crossing appropriate stocks. The synthesis of tetraploid inversion heterozygotes poses a more difficult problem. Since it is difficult to produce polyploidy in maize by colchicine treatment, genetic means of introducing gene markers and chromosome rearrangements into tetraploid strains may be used. Diploid plants with constitution of $a^*/a^*$, am/am produced diploid eggs, which when fertilized by diploid sperm from quadruplex $(A/A/A/A)$ tetraploids yielded tetraploid duplex inversion heterozygotes $(A/A/a^*/a^*)$. Duplex inversion heterozygotes of another type $(A^*/A^*/a^*/a^*)$ as well as simplex inversion heterozygotes $(A^*/a^*/a^*/a^*)$ were produced with use of the gene asynaptic (as) (BEADLE 1930). Plants homozygous for as undergo an aberrant form of meiosis resulting in the formation of a few diploid eggs. Thus plants of the constitution $A^*/A^*$, as/as and $A^*/a^*$, as/as were crossed with nulliplex $(a/a/a/a)$ pollen parents. The tetraploid pollen parent stocks used were derived from a 4n stock produced by RANDOLPH (1932) by heat-shock.

Autotetraploids in general do not breed true for chromosome number, and maize is no exception to this rule. Pooled data of RANDOLPH (1935b), KADAM (1944), and CATCHESIDE (1956) show that out of a total of 557 plants in the progeny of 40-chromosome plants only 60.7 percent of them have 40 chromosomes. The rest are known to be hypoploid or hyperploid for one or more chromosomes.

It is mandatory in a study of tetraploid genetics to use only plants which are tetrasomic for the chromosome being followed with genetic markers. For example, plants with the constitutions of $AAa$ (trisomic duplex), $AAaa$ (tetrasomic duplex), and $AAaaa$ (pentasomic duplex) cannot be readily distinguished, as their phenotypic ratios in backcross progenies are very similar. In a study of preferential pairing it is particularly important that only tetrasomic plants are used, since the phenomenon of preferential pairing can be expected to be quite different in trisomic or pentasomic tetraploid plants.

Therefore, chromosome counts were made in mitotically dividing root tip cells. Only 40-chromosome plants were used in this study. The root tips were placed in either saturated paradichlorobenzene oracenaphthene solutions for a few hours to shorten the chromosomes. The root tips were then fixed in Crafsolution, embedded in paraffin, sectioned, mounted, and stained using the method of RANDOLPH (1935a).

Sporocytes were fixed in a one part propionic acid to three parts 95 percent alcohol solution. Cytological examinations were made using the standard acetocarmine technique.

RESULTS

Genetic evidence for preferential pairing in trisomes and triploids: In the absence of preferential pairing, a trisomic or triploid plant of a duplex $(AAa)$ constitution would produce progeny when backcrossed to a homozygous recessive
pollen parent in a ratio of 5A:1a if it were not for two factors—double reduction, and the loss of one of the A bearing chromosomes during meiosis. Double reduction refers to the production of disomic gametes containing alleles which were on sister chromatids. If these gametes were as frequent as the other kinds, then we would have what is called chromatid segregation, and the expected ratio would be 4A:1a provided no chromosomes are lost. Double reduction depends on a series of events. A multivalent, in this case a trivalent, must be formed and crossing over must take place between the gene and the centromere to give an equational (Aa) constitution. The chromosomes participating in the crossover must undergo genetic nondisjunction, i.e., they must pass to the same pole at the first division.

The frequency of double reduction is expressed mathematically by the Greek letter alpha (α). The value of α for any particular gene locus is dependent on the map distance between that locus and the centromere. Since the α locus is approximately 65 units from the centromere, a considerable amount of double reduction can be expected. The value of α cannot be derived from genetic data without determining the frequency of another event which also decreases the 5:1 ratio expected on the basis of random chromosome segregation. When a bivalent and a univalent are formed, the univalent is frequently lost. The frequency of this event may be determined by counting the number of plants which are disomic and trisomic for chromosome 3 (in this case) in the progeny of trisomic or triploid seed parent. Einset (1943) found percent of a total of 247 maize plants in the progenies of trisome 3 plants to be trisomic. Thus 61 percent of the megaspores are monosomic and 39 percent are disomic. Since the ratio of A:a from AAa is 2:1 in the monosomic spores and from 1:0 to 14:1 (depending on the frequency of double reduction) in the disomic spores, an increase in the recessive class may be expected with a decrease in the proportion of disomic spores.

When a AAa trisome is used as the pollen parent in a backcross, a ratio very close to 2:1 may be expected as disomic pollen functions only rarely in fertilization. Triploids were not used as pollen parents in this work. Here, the expected ratio would be somewhat higher since disomic pollen is functional to a considerable extent. The use of the trisome as the pollen parent gives more easily interpretable data, since the data are not influenced by double reduction (which results in nonfunctional disomic spores) and by the loss of a chromosome at meiosis. It is immaterial whether a chromosome is lost or not, since if it was not lost it would be included in a nonfunctional disomic spore.

The discussion has dealt with one type of trisome, AAa, which is termed a duplex trisome. Another type of trisome, AAA—a simplex trisome—was used as well. A simplex trisome used as the pollen parent in a backcross yields a ratio close to 1A:2a. As the seed parent in a backcross, the simplex trisome would give a 1:1 ratio if it were not affected by double reduction and the loss of a chromosome in meiosis.

Preferential pairing in trisomic and triploid inversion heterozygotes with the constitution of A*A*a or AAa* may be expected to result in a lower frequency of recessive gametes than found in the corresponding controls for the following reasons.
1. When a bivalent and a univalent are formed, the bivalent will be hetero-
genetic with a frequency less than the random two thirds. The reduction in the ran-
dom frequency may be designated by a preferential pairing factor \( (p) \). Thus the
frequencies with which a bivalent is homogenetic or heterogenetic may be ex-
pressed by the terms, \( \frac{1}{3} + p \) and \( \frac{1}{3} - p \), respectively. The gametic output
from these types of pairing is shown in Table 1. It may be seen that if \( p \) has a posi-
tive value, this will result in a lower frequency of recessive gametes.

2. When a trisomic or triploid inversion heterozygote is used as the seed par-
ent, the loss of one of the chromosomes will tend to decrease the relative fre-
quency of recessive gametes as compared with the controls. When a homogenetic
bivalent is formed, the loss of the univalent has no effect on the progeny ratios.
When a heterogenetic bivalent is formed, loss of the univalent will lead to the
production of one half recessive gametes instead of one fourth. Since the fre-
quency of heterogenetic bivalents is less than two thirds in an inversion heter-
ozygote, chromosomal loss will have less effect in reducing the frequency of re-
cessive gametes than in the control.

3. The double reductional gametes occur in the ratio of 2 \( AA \): 1 \( aa \). The result
of double reduction is to increase the frequency of recessive gametes from that
expected on the basis of chromosome segregation. In the inversion heterozygote
we may expect the frequency of double reduction to be lower than in the controls
because of the presence of the inversion which decreases crossing over and lowers
the frequency of trivalent formation.

In the case of \( A*aa \) trisomes preferential pairing will act to increase the fre-
quency of recessive gametes.

The gametic output of inversion heterozygotes is given in Table 1. Using Table
1, we may set up equations in which the expected frequency of recessive progeny
in a backcross of a trisome is expressed. For the sake of brevity only equations for
the more useful pollen parent data will be given.

\[
\begin{align*}
A^*A^a \text{ or } AAa^* & : \\
R &= \frac{1}{2} \left( \frac{1}{3} - p \right) (1 - t) + \frac{1}{2} t \\
A^*aa & : \\
R &= \left( \frac{1}{3} + p \right) (1 - t) + \frac{1}{2} \left( \frac{1}{3} - p \right) (1 - t) + \frac{1}{2} t
\end{align*}
\]

where \( R \) is the frequency of recessive progeny, \( p \) is the preferential pairing factor
and \( t \) is the trivalent frequency. Disjunction from the trivalents is assumed to be
at random. Furthermore, it is assumed that disomic spores do not function in
fertilization.

The preceding discussion has neglected a factor which is not directly associated
with preferential pairing but which will also modify the genetic data. The use
of a large inversion allows for the frequent formation of deficient chromosomes
resulting from crossing over in the inversion loop. Gametes which contain only
deficient chromosomes are not functional in the pollen and may not be func-
tional in the megagametophyte (RHOADES and DEMPSEY 1953). Before a true pic-
ture of the effects of preferential pairing can be obtained, the genetic data will
have to be corrected for this factor. Anticipating a later discussion, it may be
stated that the effect of the formation of deficient chromosomes is small in com-
parison with the effect of preferential pairing.
Effects on the gametic ratio of various pairing configurations in three types of inversion heterozygotes

<table>
<thead>
<tr>
<th>Inversion heterozygote</th>
<th>Pairing configuration</th>
<th>AA</th>
<th>Dicomic</th>
<th>Gametes produced</th>
<th>Monosomic</th>
</tr>
</thead>
<tbody>
<tr>
<td>$A^*A^<em>a, AAa^</em>$</td>
<td>Bivalent + Univalent (1-t)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Homogenetic (1/3+p)</td>
<td>0</td>
<td>1/2</td>
<td>0</td>
<td>1/2</td>
</tr>
<tr>
<td></td>
<td>Heterogenetic (2/3-p)</td>
<td>1/4</td>
<td>1/4</td>
<td>0</td>
<td>1/4</td>
</tr>
<tr>
<td>$A^*aa$</td>
<td>Bivalent + Univalent (1-t)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Homogenetic (1/3+p)</td>
<td>0</td>
<td>1/2</td>
<td>0</td>
<td>1/2</td>
</tr>
<tr>
<td></td>
<td>Heterogenetic (2/3-p)</td>
<td>1/4</td>
<td>1/4</td>
<td>1/4</td>
<td>1/4</td>
</tr>
<tr>
<td>$A^*A^<em>a, AAa^</em>$</td>
<td>Trivalent (t)</td>
<td>1/6++</td>
<td>1/3-</td>
<td>$a/3$</td>
<td>1/3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>$2a/3$</td>
<td></td>
<td></td>
<td>1/6</td>
</tr>
<tr>
<td>$A^*aa$</td>
<td>Trivalent (t)</td>
<td>$a/3$</td>
<td>1/3-</td>
<td>1/6++</td>
<td>1/3</td>
</tr>
</tbody>
</table>

$*$ Genes marked with an asterisk are in an inverted chromosome.

Table 2 gives the gene segregation data of trisomic and triploid controls and inversion heterozygotes. The differences between the ratios of inversion heterozygotes and corresponding controls are all highly significant. The lowest chi-square, 20.1, was found in comparing the triploids. The data conform to theoretical expectations. The only way in which the data can be explained is by assuming that the frequency of homogenetic bivalents is much greater than would be ex-

<table>
<thead>
<tr>
<th>Cross</th>
<th>No. of plants</th>
<th>No. of ears</th>
<th>No. of gametes</th>
<th>Percentage</th>
<th>Interaction $\chi^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>$A Sh$</td>
<td>$a Sh$</td>
</tr>
<tr>
<td>Trisome:</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$a sh/a sh \times A Sh/a Sh/a sh$</td>
<td>8</td>
<td>48</td>
<td>1398+</td>
<td>32.7</td>
<td>33.9</td>
</tr>
<tr>
<td>$a sh/a sh \times A^* Sh*/a Sh/a sh$</td>
<td>10</td>
<td>15</td>
<td>4288+</td>
<td>21.7</td>
<td>39.2</td>
</tr>
<tr>
<td>$aa \times Aaa$</td>
<td>12</td>
<td>22</td>
<td>5603</td>
<td>33.4</td>
<td>66.6</td>
</tr>
<tr>
<td>$aa \times A^*aa$</td>
<td>13</td>
<td>31</td>
<td>7543</td>
<td>22.0</td>
<td>78.0</td>
</tr>
<tr>
<td>$aa \times A Aaa$</td>
<td>18</td>
<td>33</td>
<td>11373</td>
<td>67.1</td>
<td>32.9</td>
</tr>
<tr>
<td>$aa \times A^*A^*a$</td>
<td>9</td>
<td>21</td>
<td>3932</td>
<td>78.6</td>
<td>21.4</td>
</tr>
<tr>
<td>$AAAa \times aa$</td>
<td>20</td>
<td>20</td>
<td>2790</td>
<td>79.8</td>
<td>20.0</td>
</tr>
<tr>
<td>$A^*A^*a \times aa$</td>
<td>7</td>
<td>7</td>
<td>1487</td>
<td>90.7</td>
<td>9.3</td>
</tr>
<tr>
<td>$AAaa^* \times aa$</td>
<td>1</td>
<td>1</td>
<td>163</td>
<td>92.0</td>
<td>8.0</td>
</tr>
<tr>
<td>Triploid:</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$AAaa \times aa$</td>
<td>11</td>
<td>11</td>
<td>1482</td>
<td>79.3</td>
<td>20.7</td>
</tr>
<tr>
<td>$AAaa^* \times aa$</td>
<td>41</td>
<td>44</td>
<td>6715</td>
<td>84.1</td>
<td>15.9</td>
</tr>
</tbody>
</table>

$*$ Genes marked with asterisk are in an inverted chromosome.

† Two A sh gametes were found.
‡ One A sh gamete was found.
§ Significant at 5 percent level.
++ Significant at 1 percent level.
pected on a random basis. An estimate will be made of the magnitude of this preferential pairing after certain cytological data have been presented.

Interaction chi-squares between plants were computed to test the homogeneity of the data. In a few cases the chi-square values were significant. The source of this variability is unknown.

Genetic evidence for preferential pairing in tetraploids: The genetics of tetraploids is complicated by a number of factors. For a discussion, the reader is referred to papers by Muller (1914), Haldane (1930), Mather (1936), Fisher and Mather (1943), and Catche.

The effect of preferential pairing on gene segregation in duplex tetraploids with the constitution of \(A^*A^*aa\) or \(AAa^*a^*\) is to lower the frequency of recessive gametes. The formation of heterogenetic bivalents will lead to the formation of \(\frac{1}{4}aa\), \(\frac{1}{2}Aa\) and \(\frac{1}{4}AA\) gametes. When quadrivalents are formed, the expected ratio is \((1 + 2a) / 6 aa\): \((2 - 2a) / 3 Aa\): \((1 + 2a) / 6 AA\) (Fisher and Mather 1943). This neglects numerical nondisjunction, which is the three to one disjunction of the chromosomes of a quadrivalent at the first division of meiosis. The effect of numerical nondisjunction has been analyzed theoretically by Catche (1956). Since the presence of the inversion interferes with crossing over and the quadrivalent frequency is probably reduced in tetraploid inversion heterozygotes, the value of \(a\) (the frequency of double reduction) is probably lower than in control tetraploids. This is probably of minor importance as compared with the lowered frequency of heterogenetic bivalents in reducing the percentage of recessive progeny.

As with the trisomic inversion heterozygotes, the frequencies with which bivalents are homogenetic or heterogenetic may be designated as \((\frac{1}{3} + p)\) and \((\frac{2}{3} - p)\) respectively. Here also the use of an inversion introduces a complication. When anaphase bridges are formed the dominant gene may be lost in the acentric fragment. Thus gametes which would have been \(Aa\) produce \(a\) progeny.

An equation may be set up in which the expected frequency of recessive gametes from a backcrossed duplex is expressed. For \(A^*A^*aa\) or \(AAa^*a^*\), \(R = \frac{1}{4} (\frac{2}{3} - p) (1 - q) + cq\), where \(R\) is the frequency of recessive gametes, \(p\) is the preferential pairing factor, \(q\) is the quadrivalent frequency, \(1 - q\) is the bivalent frequency, and \(c\) is the contribution of quadrivalents to the recessive class of gametes. The value of \(c\) is dependent on the frequencies of double reduction and numerical nondisjunction.

Preferential pairing is not possible in simplex \((In/N/N/N)\) or triplex \((In/In/In/N)\) inversion heterozygotes. The odd chromosome in both cases must pair with a structurally different chromosome.

Table 3 gives the gene segregation data for tetraploid inversion heterozygotes and corresponding controls. Table 4 gives chi-squares found in testing for significance of the differences in phenotypic ratios of pairs of crosses. The differences in the ratios of the control and inversion heterozygotes in the case of the duplex are highly significant. The two kinds of duplexes, \(A^*A^*aa\) and \(AAa^*a^*\), gave gametic ratios which were significantly different when the heterozygotes were used as the female parent. It is believed that the magnitude of preferential pair-
TABLE 3
Gene segregation in tetraploid inversion heterozygotes and corresponding controls

<table>
<thead>
<tr>
<th>Crosses</th>
<th>No. of plants</th>
<th>No. of ears</th>
<th>No. of gametes</th>
<th>Percent a</th>
<th>Ratio A:a</th>
<th>Interaction χ² between plants</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aaaa × 4n a</td>
<td>6</td>
<td>6</td>
<td>983</td>
<td>54.63</td>
<td>1:1.20</td>
<td>2.17</td>
</tr>
<tr>
<td>A*Aaaa × 4n a</td>
<td>7</td>
<td>7</td>
<td>922</td>
<td>54.92</td>
<td>1:1.12</td>
<td>2.57</td>
</tr>
<tr>
<td>4n a × Aaaa</td>
<td>6</td>
<td>22</td>
<td>2950</td>
<td>51.12</td>
<td>1:1.05</td>
<td>3.68</td>
</tr>
<tr>
<td>4n a × A*Aaaa</td>
<td>6</td>
<td>15</td>
<td>1885</td>
<td>51.62</td>
<td>1:1.07</td>
<td>3.04</td>
</tr>
<tr>
<td>AAaa × 4n a</td>
<td>20</td>
<td>20</td>
<td>4413</td>
<td>20.83</td>
<td>3.82:1</td>
<td>17.22</td>
</tr>
<tr>
<td>A*A+a × 4n a</td>
<td>17</td>
<td>17</td>
<td>3038</td>
<td>10.80</td>
<td>8.26:1</td>
<td>28.76†</td>
</tr>
<tr>
<td>AAa<em>a</em> × 4n a</td>
<td>37</td>
<td>37</td>
<td>6360</td>
<td>12.56</td>
<td>6.74:1</td>
<td>45.54</td>
</tr>
<tr>
<td>4n a × AAaa</td>
<td>15</td>
<td>60</td>
<td>8321</td>
<td>19.79</td>
<td>4.05:1</td>
<td>42.64‡</td>
</tr>
<tr>
<td>4n a × A*A+a</td>
<td>11</td>
<td>44</td>
<td>4295</td>
<td>13.43</td>
<td>6.44:1</td>
<td>15.34</td>
</tr>
<tr>
<td>4n a × AAa<em>a</em></td>
<td>22</td>
<td>55</td>
<td>9406</td>
<td>14.03</td>
<td>6.13:1</td>
<td>73.30‡</td>
</tr>
</tbody>
</table>

* Genes marked with an asterisk are in an inverted chromosome.
+ Significant at .05 level.
‡ Significant at .01 level.

ing is the same in both types but the dominant allele is lost more frequently in AAa*a* tetraploids. This will be discussed in detail in a later section. The significant differences between the male and female gametic ratios in inversion heterozygotes of the same constitution remain to be explained.

In a few instances significant chi-squares were found in testing the homogeneity of the data obtained from different plants of the same genotype. The source of this variability is unknown.

Cytological evidence for preferential pairing: A. The effect of preferential pairing on the trivalent frequency at the trisome level. When there are three chromosomes, two of which are structurally alike and one which is different, there will be a tendency for the like chromosomes to form a bivalent while the odd chromo-

TABLE 4
Chi-squares found in testing for significance of the difference in phenotypic ratios of pairs of crosses

<table>
<thead>
<tr>
<th>Comparison</th>
<th>Chi-square</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aaaa × 4n a vs. A<em>a</em>aaa × 4n a</td>
<td>0.55</td>
</tr>
<tr>
<td>4n a × Aaaa vs. 4n a × A<em>a</em>aaa</td>
<td>0.12</td>
</tr>
<tr>
<td>AAaa × 4n a vs. A<em>A</em>aa × 4n a</td>
<td>128.04‡</td>
</tr>
<tr>
<td>AAa<em>a</em> × 4n a vs. A<em>Aa</em>a* × 4n a</td>
<td>129.94‡</td>
</tr>
<tr>
<td>4n a × AAaa vs. 4n a × A<em>Aa</em>a*</td>
<td>78.89‡</td>
</tr>
<tr>
<td>A<em>A</em>aa × 4n a vs. AAa<em>a</em> × 4n a</td>
<td>105.22‡</td>
</tr>
<tr>
<td>4n a × A<em>A</em>aa vs. 4n a × AAa<em>a</em></td>
<td>6.06†</td>
</tr>
<tr>
<td>AAaa × 4n a vs. 4n a × AAaa</td>
<td>1.61</td>
</tr>
<tr>
<td>A<em>A</em>aa × 4n a vs. 4n a × A<em>A</em>aa</td>
<td>11.93‡</td>
</tr>
<tr>
<td>AAa<em>a</em> × 4n a vs. 4n a × AAa<em>a</em></td>
<td>7.04‡</td>
</tr>
</tbody>
</table>

* Genes marked with an asterisk are in an inverted chromosome.
† Significant at .05 level.
‡ Significant at .01 level.
some is represented as a univalent. As a result, the trivalent frequency should be less than when all three chromosomes are alike. The trivalent frequency in control trisomes was found to be 68.9 percent. Sporocytes from four plants, and a total of 591 cells, were examined. In seven In/N/N trisomes, out of a total of 427 cells, only 56.6 percent had trivalents. This difference, 12.3 percent, is statistically significant ($x^2 = 15.9; P < .0005$).

It seems likely that there is a similar reduction in the trivalent frequency on the triploid level, as well as a decrease in the quadrivalent frequency in tetraploid inversion heterozygotes. Since chromosome 3 is not readily distinguishable from the other chromosomes at diakinesis, it is not possible to make an accurate analysis in triploids and tetraploids.

B. The effect of preferential pairing on the anaphase bridge frequencies of trisomic (In/N/N) and duplex tetraploid (In/In/N/N) inversion heterozygotes.

The frequency with which anaphase bridges are formed in meiocytes of an inversion heterozygote is a product of the frequency of heterosynapsis (the pairing of inverted and standard chromosome segments at the pre-diplotene stages of meiosis) and the probability that crossing over which will give rise to anaphase bridges will occur in the heterosynaptically paired region.

In a diploid inversion heterozygote the frequency of heterosynapsis must be 100 percent. In a trisomic or duplex tetraploid inversion heterozygote, however, the frequency of heterosynapsis may be from 66.7 percent to zero percent, depending on the magnitude of preferential pairing. It would be possible to determine the degree of heterosynapsis in a polyploid inversion heterozygote from the frequency of anaphase bridges if the rate of crossover events leading to bridge formation from a heterosynaptically paired region were known. It would be inadvisable to use the bridge frequency of a diploid inversion heterozygote for this purpose. The crossover potential may not be the same in diploid and tetraploid plants. Also, in polyploids when a multivalent is formed, two chromosomes with a potential bridge may go to the same pole at the first division. This bridge may or may not be resolved at the second division. If the dyads separate randomly at the second division, this bridge will be resolved one half of the time.

In simplex tetraploids (In/N/N/N) the inverted region can only pair with the normal segment. Therefore, the frequency of heterosynapsis must be 100 percent and we have a fairly good "control" for the frequency with which an anaphase bridge is formed following heterosynapsis in a duplex tetraploid (In/In/N/N). The behavior of one pair of heterosynaptic segments should not differ greatly from that of two pairs. There is one difficulty, however; the quadrivalent frequency of the simplex may be greater than that of the duplex, in which case more chromatid bridges will be resolved at the first anaphase in the duplex than in the simplex.

There is no adequate control for the trisomic inversion heterozygote.

Table 5 gives the anaphase bridge frequencies of diploid, trisomic, simplex tetraploid, and duplex tetraploid inversion heterozygotes.

Using the bridge frequency of the simplex as an estimate of the frequency with which bridges are formed in a duplex inversion heterozygote following hetero-
synapsis, we can calculate the frequency of heterosynapsis in the duplex. If there were no preferential pairing, then the anaphase bridge frequency of the duplex should be twice that of the simplex, times two-thirds (the expected frequency of heterosynapsis in a duplex when the chromosomes associate at random).

The bridge frequency at the first anaphase in the simplex \( B_s \) is 33.7 percent, that of the duplex \( B_d \) is 15.4 percent. Double bridges are given the same value as single bridges for this purpose. It may be seen that \( 2 \times B_s \times \frac{2}{3} = B_d \), that is \( 2 \times 33.7\% \times \frac{2}{3} = 44.9\% \). This is greater than 15.4 percent. Thus frequency of heterosynapsis must be less than the random \( \frac{2}{3} \) value.

An equation may be set up and the frequency of heterosynapsis \( (h) \) solved for:

\[
2 \times B_s \times h = B_d. \quad 2 \times 33.7\% \times h = 15.4\%, \text{ and } h = 22.8\%. \]

This means that inverted and normal segments are synapsed in 22.8 percent of the meiocytes of a duplex inversion heterozygote, instead of in 66.7 percent as would be the case if synapsis were at random.

DISCUSSION AND CONCLUSIONS

It has been stated previously that there is an effect on the genetic data resulting from the formation of deficient and duplicate-deficient chromosomes and the loss of the dominant marker in theacentric fragment. The percentage of spores with deficient chromosomes may be estimated from the data on bridge formation presented in Table 5. In the trisome it is 10.1 percent (\( \frac{1}{2} \times 14.2\% + 0.4\% + 2.6\% \)). When an anaphase bridge breaks, two deficient chromosomes are formed. Chromatid bridges are formed between a normal and an inversion chromosome. Therefore, in trisomic compounds of \( A^*A^*a, AAa^* \) or \( A^*aa \), monosomic pollen which does not function because of the presence of a deficient chromosome represents the loss of \( A \) and \( a \) gametes in equal frequencies. The genetic data from a trisome backcrossed as the pollen parent may be corrected by the use of the formula: \( R_o = (R' - \frac{1}{2}d)/(1 - d) \), \( R' = R_o (1 - d) + \frac{1}{2}d \), where \( R' \) is the frequency of recessive gametes expected if no deficient chromosomes were formed,

### TABLE 5

**Anaphase configurations of diploid, trisomic, simplex tetraploid, and duplex tetraploid inversion heterozygotes**

<table>
<thead>
<tr>
<th>Inversion heterozygote</th>
<th>Anaphase 1 (number of cells)</th>
<th>Anaphase 2 (number of cells)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No bridge</td>
<td>Single bridge</td>
</tr>
<tr>
<td>Diploid</td>
<td>667</td>
<td>394</td>
</tr>
<tr>
<td>(In/N)</td>
<td>61.8%</td>
<td>36.5%</td>
</tr>
<tr>
<td>Trisomic</td>
<td>935</td>
<td>156</td>
</tr>
<tr>
<td>(In/N/N/N)</td>
<td>85.4%</td>
<td>14.2%</td>
</tr>
<tr>
<td>Simplex tetraploid</td>
<td>656</td>
<td>324</td>
</tr>
<tr>
<td>(In/N/N/N/N)</td>
<td>66.3%</td>
<td>32.8%</td>
</tr>
<tr>
<td>Duplex tetraploid</td>
<td>754</td>
<td>103</td>
</tr>
<tr>
<td>(In/In/N/N)</td>
<td>86.1%</td>
<td>11.8%</td>
</tr>
</tbody>
</table>
$R_0$ is the observed frequency of recessive gametes, and $d$ is the frequency of deficient chromosomes.

The corrected genetic data from the trisomic inversion heterozygotes are given in Table 6.

It is not possible to correct the genetic data when a trisome or triploid is used as the female parent since there are complicating factors, one of which is that some deficient chromosomes can be transmitted through the megaspores.

On the tetraploid level, the formation of deficient chromosomes is not important; however, the loss of the dominant allele in the acentric fragment will affect the genetic data to a slight degree. Only rarely will a spore from a tetraploid contain only deficient chromosomes and any lethality from this source may be neglected.

The loss of the dominant allele in the acentric fragment decreases the frequency of dominant gametes, and it therefore works counter to the effects of preferential pairing. This event accounts for the different gametic ratios from the two types of duplex inversion heterozygotes, $A^*A^*aa$ and $AAa^*a^*$. The $a$ locus is not centrally located in the inverted segment; it is 46 crossover units from the proximal break point and 15 units from the distal break point. The consequences of this are shown in the Figure 3.

The total observed frequency of events (from Table 5) giving rise to acentric fragments is 26.2 percent $(11.8 + 2(0.7) + 2(1.5) + 2(5.0))$. The frequency of anaphases in which the acentric fragment carries the dominant allele should be approximately $1/4 \times 26.2$ percent in $A^*A^*aa$ tetraploids and $3/4 \times 26.2$ percent in $AAa^*a^*$. Such anaphases arising from crossovers in region I result in 12.5 percent more recessive gametes than do anaphases of other types following heterogenetic pairing. Crossovers in region II or non crossover events lead to the formation of 25 percent recessive gametes. Crossovers in region I lead to 37.5 percent. It is immaterial whether a chromosome is deficient as long as it contains the $A$ locus. Thus the excess in recessive gametes due to the loss of the dominant gene is 0.82 percent $(1/4 \times 26.2\% \times 12.5\%)$ and 2.45 percent $(3/4 \times 26.2\% \times 12.5\%)$ for the two types of tetraploids. The difference, 1.63 percent, corresponds well with 1.8 percent, the observed difference in the frequency of $a$ gametes when the two types of tetraploids were used as the female parent. When they were used as the male parent, the difference was only 0.6 percent.

Consequently, the observed genetic data should perhaps be corrected for this

### Table 6

Percentages of dominant ($D$) and recessive ($R$) gametes from trisomic inversion heterozygotes used as the pollen parent, as observed and as corrected for the nonfunctioning of pollen with deficient chromosomes

<table>
<thead>
<tr>
<th>Cross</th>
<th>$D_0$</th>
<th>$R_0$</th>
<th>$D'$</th>
<th>$R'$</th>
</tr>
</thead>
<tbody>
<tr>
<td>$a\text{sh}/a\text{sh} \times A^<em>\text{Sh}^</em>/a\text{Sh}/a\text{sh}$ (A)</td>
<td>21.7</td>
<td>78.3</td>
<td>24.6</td>
<td>75.4</td>
</tr>
<tr>
<td>$a/a \times A^*/a/a$</td>
<td>22.0</td>
<td>78.0</td>
<td>24.8</td>
<td>75.2</td>
</tr>
<tr>
<td>$a/a \times A^<em>/A^</em>/a$</td>
<td>78.6</td>
<td>21.4</td>
<td>75.7</td>
<td>24.3</td>
</tr>
</tbody>
</table>
Tetraploid type

Chromosome constitution

A*A*aa

A

A

A

AAa*a*

A

A

One pair of chromosomes in inversion loop

Anaphase configurations (with relative frequency)

c.o. in I  c.o. in II

(1/4)  (3/4)

(1/4)  (3/4)

FIGURE 3.—Consequences of crossing over in two types of tetraploid inversion heterozygotes.

phenomenon. This complication can be avoided by following gene loci which are in the normal arm of an inverted chromosome. Since the quadrivalent frequency in the tetraploids is not known, the equation $R = \frac{1}{4} \left( \frac{2}{3} - p \right) \left( 1 - q \right) + cq$ is not solvable. However, we can assume that $p$ has a large value.

In trisomics, however, the trivalent frequency has been cytologically determined; using the corrected values of $R$ ($R'$) we can solve the equations previously given for the value of $p$, and thus determine the frequency of heterogenetic bivalents.

\[
(A^*A^*a): R' = \frac{1}{2} \left( \frac{2}{3} - p \right) (1 - t) + \frac{1}{2} t \\
(A^*aa): R' = \left( \frac{1}{3} + p \right) (1 - t) + \frac{1}{2} \left( \frac{2}{3} - p \right) (1 - t) + \frac{1}{2} t
\]

The values are: $R'$ $(A^*A^*a) = .243, R'$ $(A^*aa) = .752$, and $t = .567$. We shall assume that there is no preferential disjunction of the chromosomes of a trivalent. This assumption is not necessarily justified. Solving these equations for $p$ we get values of .416 and .394, respectively. The difference between these two estimates is not statistically significant. It is noteworthy that the magnitude of preferential pairing is the same whether the constitution of the trisome is $(In/N/N)$ or $(In/In/N)$.

The average $p$ value is .405. If we accept this as a good estimate, then the frequency of heterogenetic bivalents is 26.2 percent ($\frac{2}{3} - p$) and the frequency of homogenetic bivalents is 73.8 percent ($\frac{1}{3} + p$). This is a very high degree of preferential pairing.

The studies of RILEY and others on the genetic control of preferential pairing in wheat are perhaps pertinent. RILEY (1960) believes "that chromosome V ap-
PREFERENTIAL MEIOTIC PAIRING

pears to control pairing by reducing attraction to a level below which homoeologous pairing cannot be completed. The chromosome V gene inhibits positively acting processes, responsible for pairing, which are determined elsewhere in the genotype." Also, he believes that this type of genetic control is active in other genera and is responsible for the diploidisation of raw allotetraploids in nature.

It is possible that some of the heterogeneity (interaction $\chi^2$) found between plants in this study could be genetic in origin. In any event, preferential pairing is a complex phenomenon consisting of genomic differentiation and of pairing mechanisms which allow a chromosome to distinguish between homologous and homoeologous pairing partners. Several hypotheses could be advanced which would help to explain preferential pairing. These hypotheses are intimately associated with the unknown mechanisms of chromosome pairing. A number of very important questions would first have to be answered—are long range forces active, is synapsis reversible, are the chromosomes polarized at the onset of meiosis, etc.?

Additional studies on preferential pairing are in progress and any conjectures will be deferred until they are completed.

SUMMARY

A quantitative relationship between the homology of two chromosomes and their pairing affinity at meiosis may be established by measuring the degree of preferential pairing in polyploids which are heterozygous for known chromosomal structural rearrangements. Preferential pairing can be detected by its effects on gene segregation, multivalent frequency, and on the frequency of anaphase bridge formation. The known structural rearrangement used in this study was $In\ 3a$, a paracentric inversion in the long arm of chromosome 3 of maize. The inversion occupies about one-third of the total chromosome length (3L.4-.95). The gene segregation data of normal controls and of inversion heterozygotes were found to be significantly different and in conformity with theoretical expectations. The trivalent frequency of control trisomes (68.9 percent) was found to be significantly higher than that of trisomic inversion heterozygotes (56.7 percent). By using the cytologically determined trivalent frequency of trisomic inversion heterozygotes and genetic data obtained from them (which were corrected for the effects of deficient chromosome formation), the frequency of heterogenetic bivalents was estimated at 26.2 percent instead of the random 66.7 percent value. It was not possible to make an estimate of the frequency of heterogenetic bivalents in triploids or tetraploids because the frequency of multivalent formation is not determinable with any degree of accuracy. However, in the tetraploids it was possible to estimate the value of heterosynapsis to be 22.8 percent by the use of data on the anaphase bridge frequency of simplex and duplex tetraploids.

ACKNOWLEDGMENT

The author wishes to thank Dr. M. M. Rhoades for his encouragement and unfailing interest, and also for the triploid progenies used in this study and for
Figure 1. The author is also indebted to Ellen Dempsey and D. L. Shafer for constructive criticisms.

The work was supported in part by a Floyd Fellowship held at Indiana University.

LITERATURE CITED


