CONSEQUENCES OF CROSSING OVER IN
INTERSTITIAL SEGMENTS OF BARLEY INTERCHANGE
HETEROZYGOTES

by
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DEPARTMENT OF AGRONOMY
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1964
STATEMENT BY AUTHOR

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APPROVAL BY THESIS DIRECTOR

This thesis has been approved on the date shown below:

R. T. RAMAGE
Professor of Agronomy
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INTRODUCTION

A chromosomal interchange is the result of an exchange of segments of nonhomologous chromosomes. An interchange heterozygote has two normal nonhomologous chromosomes and two interchanged chromosomes. The percent of pollen and ovule abortion in an interchange heterozygote depends on the frequencies of alternate and adjacent segregation of the quadripartite configuration, and the frequency of crossing over in the interstitial segments (the regions of the chromosomes between the centromeres and the interchange break-points.)

In the absence of crossing over in the interstitial segments, alternate segregation results in the formation of fertile gametes, while adjacent segregation produces aborted gametes. Burnham (1934) in corn and Garber (1948) in sorghum, found that alternate and adjacent segregations were about equal in frequency, resulting in 50 percent sterility. Cleland (1936) in Oenothera found almost all alternate configurations, accounting for the observed high fertility. Crossing over in the interstitial segments gives results different from those after crossing over in any of the other regions of the chromosomes in the interchange complex. The latter cross-overs merely transfer blocks of genes between the interchanged and the normal chromosomes. When crossing over in the interstitial segment is followed by alternate or adjacent segregation, half of the resulting products of that meiosis (spores in plants and gametes in animals) are normal and half carry a deficiency - duplication. In higher
plant species with directed segregation, these cross-overs are a source of spore abortion. One with completely directed disjunction should have normal pollen and seed set if there is no crossing over in the interstitial segments. These relations seem to have been recognized first by Samsome (1932) and were clearly stated by Lamm (1948), Hanson and Kramer (1949) and Burnham (1949).

An average of 28.8 percent pollen abortion was reported in various barley translocations by Burnham et al. (1954). Ramage and Suneson (1961) found that genetic recombination in a particular interstitial segment (long arm of chromosome 7 in T6-7b) was about 50 percent. Ramage (unpublished) found that even when genetic recombination was 50 percent the observed sterility was about 28 percent in T6-7b.

Barley interchange heterozygotes show an excess of alternate disjunction and should have close to 50 percent sterility when a long interstitial segment is involved.

This study is an attempt to understand why T6-7b shows a high degree of crossing over in the interstitial segment and a low degree of sterility. With this view in mind, the following studies were undertaken:

1. The percentage sterility of the ovules and pollen grains was determined in interchange heterozygotes T6-7a and T6-7b.

2. The frequency of different kinds of configurations at metaphase I and diakinesis of meiosis was determined in heterozygous T6-7a and T6-7b and these frequencies were compared with pollen and ovule sterility.
3. Percentages of centromere orientation, i.e., adjacent or alternate were determined and related to ovule and pollen sterility.

4. The effect of length of interchanged arm on sterility was noted.
REVIEW OF LITERATURE

Gates (1908) was the first to observe the attachment of more than 2 chromosomes to form a ring in Oenothera rubrinervis. Digby (1912) observed that two chromosome pairs were joined together in a ring in the amphidiploid Primula kewensis (2n = 36). Cleland (1922, 1929) observed that the chromosomes in a ring in any Oenothera race are associated in a specific and constant order. Sands (1925) saw the characteristic chain in Tradescantia virginia and also in Rhoeo, but interpreted them in terms of the synopsis theory current at that time.

Belling (1914 a, 1915 a.b.) gave the first accounts of the breeding behavior of a probable chromosomal interchange in plants. Belling reported 50 percent sterility in hybrids between Florida velvet bean (Stizolobium deeringianum) and Lyon (S. niveum), Yokohama (S. hassjo) and Chinese velvet bean. Since he observed 50 percent of aborted pollen and ovules, the condition was termed "semi-sterility". The progeny of these hybrids included semisterile and normal plants in 1:1 ratio. In the subsequent generations the semisteriles continued to segregate in this manner while the normals bred true.

Hammerlund (1923) reported in Pisum a close linkage between characters ordinarily independent. The association of 4 chromosomes in a ring in this material was reported by Hakansson in 1929.

McClintock (1930) made the first cytological demonstration of the location of an interchange between two non-homologous chromosomes in
plants for a case of semisterility in maize. The 4 armed figures at the pachytene stage of meiosis were drawn by camera lucida and measured to determine the points of exchange.

Burnham (1956) described clearly the general cytological and genetical behavior of interchange heterozygotes in diploids. He mentioned that in individuals heterozygous for one interchange, 2 pairs of chromosomes are usually associated in a ring or a string or a chain at meiosis. The quadripartile group of chromosomes may be orientated at metaphase I so that either alternate or adjacent chromosomes are directed towards the same pole, zigzag or open ring configurations, respectively. For adjacent segregation there are 2 types, adjacent I in which homologous centromeres go to the opposite poles and Adjacent II, in which homologous centromeres go to the same pole. If there is no crossing over in an interstitial segment all products of alternate segregation will be fertile and all products of adjacent segregation will abort.

Hagberg (1954) recognized another type of orientation in which two chromosomes on opposite sides of a ring are directed to opposite poles, but the ones adjacent to them are not co-oriented toward either pole. He reported that the tetravalents in a translocation heterozygote of barley appeared as zigzag rings in about 80 percent of the pollen mother cells while 20 percent had open rings. The zigzag tetravalents always separated 2-2, resulting in fertile gametes. The open ring occurred in 2 types: (1) All four chromosomes orientated in the metaphase I spindle, resulting in sterile deficiency - duplication gametes and (2) only 2 centromeres orientated with the spindle, the other two
remaining unattached on the metaphase plate. Although lagging chromosomes frequently resulted from the non-oriented open ring, the interchange complex occasionally separated 2-2 producing sterile gametes, or less frequently 3-1, possibly forming fertile n+1 gametes; viable eight chromosome gametes resulted in trisomic progeny. He found that some of the translocations produced a high frequency of trisomics. Such plants (with one of the interchanged chromosomes duplicated) formed pentavalent associations.

Burnham (1956) gave in detail the effect of crossing over in an interstitial segment in the formation of gametes. Crossing over in an interstitial segment would result in 50 percent of the alternate and adjacent segregation gametes being normal and the other 50 percent being aborted because of a deficiency - duplication.

McClintock (1945) gave evidence that chromosomes that cross over in the interstitial segment pass to opposite poles, hence adjacent 2 segregation does not occur.

Belling (1915 b) was the first to report linkage data between semisterility and other segregating characters. In maize, linkage of semisterility with factors in two linkage groups, was demonstrated by Brink and Cooper (1931). Rhodes (1931, 1933) was the first to report the expected linkage between factors in two linkage groups involved in a translocation in maize.

Burnham (1934) and Anderson (1934) stated that in general, crossing over is greatly reduced in regions near the break-points in maize. Burnham (1934b) observed that in the same region, crossing over
was much higher in the pollen than in the ovules, both greatly reduced as compared with that in normal plants.

Hanson and Kramer (1950) and Hanson (1952) reported that reduced crossing over in an interstitial segment probably results from a high frequency of alternate segregation in barley.

Burnham (1953) found that in maize, alternate and adjacent segregations are equally frequent after crossing over in an interstitial segment. Burnham (1956) further stated that reduction of crossing over due to the type of segregation is expected only if there is a much higher frequency of alternate than of adjacent I segregation.

Anderson, Kramer, and Longley (1955) reported that translocations in the proximal portion of the short arm of chromosome 4 in maize did not suppress recombinations, while those at the proximal portion of the long arm of that chromosome did. They suggested that the greatest suppression may be in the heterochromatic regions proximal to the centromere.

Kramer and Swamley (1961) pointed out that the extent of reduction of crossing over in interstitial segments impose an upper limit on the sterility that is expected. They presented formulae for calculating the degree of spore abortion and the recombination values with different assumed frequencies of alternate segregation. The frequencies may be different for crossover and non-crossover meiocytes. Burnham et al. (1962) pointed out that variable pairing near the break-points may account for part of the reduced recombination without any corresponding increase in sterility.
Smith (1941) reported 2 naturally occurring translocations in barley, one of which had 67.2 percent ring configuration, the other 57 percent.

White and Burnham (1948) and Burnham et al. (1954) found that the average sterility of interchange heterozygotes of barley is 25 percent.

Hanson and Kramer (1949) pointed out that this low percent of sterility in barley indicates a high proportion of alternate segregation of the chromosomes in ring.

Burnham et al. (1954) observed sterility in a group of 34 chromosomal interchanges produced by X-rays in barley. Pollen abortion in different translocation lines, each having a ring of 4 chromosomes, averaged 28.8 percent (with a range from 14 to 58 percent). In a number of lines ovule abortion was greater than that of the pollen.

Similarly, a number of plant species have been reported in which a ring of 4 has shown low spore abortion e.g., *Datura stromanium* (Blackslee, 1928) *Triticum monococcum* (Thompson and Hutcheson, 1942; Yamashita, 1947), *Lycopersicon esculentum* (Cleland, 1929 and others).

Burnham (1956) has reviewed the literature on interchanges and surveyed the plant species in which they have been reported.
Plants from two different stocks were made available by R. T. Ramage. The translocations T6-7a and T6-7b are in the barley variety Bonus and were obtained by A. Hagberg by X-radiation.

**T6-7a:** (Figure 1a) In this stock chromosome 6 is broken at the centromere or very close to it and chromosome 7 is also broken either in the centromere or very close to the centromere.

**T6-7b:** (Figure 1b) In this stock, chromosome 6 is broken in the long arm and chromosome 7 is also broken in the long arm.

As T6-7a is broken either in the centromere or very close to it, there is a very small, or no interstitial segment and crossing over should not affect formation of gametes as shown in Figure 2a. Only the type of disjunction at metaphase I would determine functional or non-functional gametes. Without crossing over, alternate disjunction will give functional gametes and adjacent disjunction will give nonfunctional gametes.

In T6-7b there is a long interstitial segment (Figure 2b) where crossing over can take place. Without crossing over in this segment, if there is alternate disjunction all gametes will be functional, whereas, if there is adjacent disjunction, all gametes will be non-functional. If there is a cross over in the interstitial segment, then alternate and adjacent disjunction, will both give 50 percent functional and 50 percent non-functional gametes. The translocation heterozygotes were obtained
Figure 1. Diagramatic representation of chromosome 6 and 7 indicating the break positions of T6-7a and T6-7b.

Figure 2. Diagramatic representation of Pachytene Pairing of T6-7a and T6-7b.
Figure 1

A. CHROMOSOME 6

B. CHROMOSOME 7

Figure 2

7 A

7

7 A

7

6

7

6

7
by crossing stock T6-7a and stock T6-7b with the normal nontranslocated stock and the progenies of these crosses were used in this investigation.

The $F_1$ interchange heterozygotes T6-7a and T6-7b were planted in the field in November, 1961. The spikes were collected in February, 1962. The immature spikes were collected in the morning when different stages of meiotic divisions can be obtained. The spikes were fixed in a fresh 3:1 solution of absolute alcohol and glacial acetic acid. After 48 hours of fixation, the anthers were used for acetocarmine smears for cytological studies. Different configurations and their frequencies were studied; laggards in first or second meiotic divisions, and any other abnormalities in meiosis were noted. The slides which had good figures were made permanent. The interchange configurations were classified at diakinesis and metaphase I as to whether they would result in alternate or adjacent separation. Pollen mother cells containing ring configurations such as \[ \infty , \infty \] and \[ \infty \] were considered to result in alternate disjunction and ring configurations such as \[ \infty , \infty \] and \[ \infty \] were considered to result in adjacent disjunction. The chain configurations, which were usually oriented like "\[ \infty \]", appeared to be closer to alternate disjunction than to adjacent disjunction and therefore were classified as alternate disjunction.

The ring configurations considered to result in adjacent disjunction were further subdivided on the basis of chromosome orientation on the metaphase plate. In one group, all of the 4 chromosomes were orientated and in the other group 2 were orientated on the metaphase plate and 2 were non-coorientated.
For pollen abortion studies, spikes were collected and fixed in fresh 3:1 absolute alcohol and glacial acetic acid solution. The pollen grains were then stained with aceto-carmine with the precaution that very little acetocarmine was used on the slide so that the aborted pollen could not move towards the periphery of the slide. This precaution was taken so that the aborted and normal pollen lay randomly on the slide. The aborted pollen grains can be identified as they are shrieveled and also do not absorb stain; whereas, the normal pollen is spherical in shape and stains well. The aborted and normal pollen grains were counted on 10 random spots from the slide and averaged, (each spot consisted of the area of the low power (100 X) field of view). The percent abortion was then calculated.

For ovule sterility, 10 spikes from each plant were collected and the seed formed in each spike were counted. This was deducted from the total expected seed to be formed in the spikes. The average of 10 ears was calculated and the percent ovule sterility was determined.

The different configurations observed were correlated with sterility. Also, their frequencies were compared with the ovule and pollen sterility. The percentage of centromere orientation was related to sterility. The effect of the length of arms and their effect on sterility was determined.

The same material was planted in December, 1961 (about a month later) to see the effect of late planting on ovule sterility.
RESULTS

The results of the metaphase I and late diakinesis analyses of the interchange complex (configuration frequencies) of heterozygous T6-7a are summarized in Table 1 and heterozygous T6-7b in Table 3. The configuration frequencies are classified either as alternate or adjacent disjunction. T6-7a shows 68.6 percent alternate disjunction and 31.4 percent adjacent disjunction; and in T6-7b the frequency of alternate disjunction is 60.7 percent and the adjacent disjunction was 39.3 percent.

The ovule and pollen fertility data for heterozygous T6-7a and T6-7b are summarized in Tables 2 and 4, respectively. In T6-7a, ovule fertility is 70.9 percent and pollen fertility is 74.4 percent in the interchange heterozygote under normal planting and ovule fertility in the late planting is 59.7 percent. Homozygous T6-7a under normal planting gives 97.1 percent fertility. In heterozygous T6-7b under normal planting, ovule fertility is 70.8 percent; whereas, in homozygous T6-7b the fertility is 97.4 percent. In the same stock under late planting, the interchange heterozygote gives 66.1 percent fertility and in the homozygous interchange the ovule fertility is 82.6 percent. The pollen fertility in T6-7b is 75.1 percent.

Ovule fertilities of less than 100 percent in the homozygous interchanges show the environmental influence on fertility. The differences in fertility in the late planting as compared to normal planting
indicate that the environment may be an important cause of ovule sterility. Anthers were collected only from interchange heterozygotes of the normal planting for pollen abortion studies. No data, therefore were obtained on the effect of late planting on pollen abortion.
Table 1. Frequencies of different types of configurations at metaphase I and late diakinesis in heterozygous T6-7a.

<table>
<thead>
<tr>
<th>Configurations</th>
<th>Alternate Disjunction</th>
<th>Adjacent Disjunction</th>
<th>% non co-orientation</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>∞∞ ∞ω ωω ωω</td>
<td>∞* ω+ ω+ ω+</td>
<td></td>
<td>4959</td>
</tr>
<tr>
<td>Observed frequency</td>
<td>1613  625  381  781</td>
<td>472  949  138</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Percentage</td>
<td>32.4  12.6  7.6  15.7</td>
<td>9.5  19.1  2.7</td>
<td></td>
<td>12.3</td>
</tr>
<tr>
<td>Total of the type of disjunction in %</td>
<td>68.6</td>
<td>31.4</td>
<td>100.0</td>
<td></td>
</tr>
</tbody>
</table>

+ indicates orientation of all 4 chromosomes on the Metaphase I Plate.

* indicates non-coorientation of 2 of the 4 chromosomes on the Metaphase I Plate.
Table 2. Ovule and pollen fertility and sterility in T6-7a.

<table>
<thead>
<tr>
<th></th>
<th>Normal Planting</th>
<th>Late Planting</th>
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<tbody>
<tr>
<td></td>
<td>Interchange</td>
<td></td>
</tr>
<tr>
<td></td>
<td>heterozygote</td>
<td>Interchange</td>
</tr>
<tr>
<td></td>
<td>homozygote</td>
<td>homozygote</td>
</tr>
<tr>
<td>A) Ovule</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Observed grains</td>
<td>2399</td>
<td>1541</td>
</tr>
<tr>
<td>No. of Florets</td>
<td>3384</td>
<td>2582</td>
</tr>
<tr>
<td>Percent ovule fertility</td>
<td>70.9</td>
<td>59.7</td>
</tr>
<tr>
<td>B) Pollen</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Observed Normal</td>
<td>1509</td>
<td></td>
</tr>
<tr>
<td>Observed Aborted</td>
<td>521</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>2030</td>
<td></td>
</tr>
<tr>
<td>Percentage Normal</td>
<td>74.4</td>
<td></td>
</tr>
</tbody>
</table>
Table 3. Frequency of different types of configurations at metaphase I and late diakinesis in heterozygous T6-7b.

<table>
<thead>
<tr>
<th>Configurations</th>
<th>Alternate Disjunction</th>
<th>Adjacent Disjunction</th>
<th>% non co-orientation</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>∞ ∞ ∞ ∞</td>
<td>∞ ∞ ∞ ∞</td>
<td>∞ ∞ ∞ ∞</td>
<td></td>
</tr>
<tr>
<td>Observed frequency</td>
<td>1769 922 171 504</td>
<td>1145 783 281</td>
<td></td>
<td>5576</td>
</tr>
<tr>
<td>Percentage</td>
<td>31.7 16.5 3.1 9.4</td>
<td>20.2 14.0 5.1</td>
<td>25.3</td>
<td>100</td>
</tr>
<tr>
<td>Total of the type of disjunction in %</td>
<td>60.7</td>
<td>39.3</td>
<td>100</td>
<td></td>
</tr>
</tbody>
</table>

+ indicates orientation of all 4 chromosomes on the Metaphase I Plate.

* indicates non-co-orientation of 2 of the 4 chromosomes on the Metaphase I Plate.
Table 4. Ovule and pollen fertility and sterility in T6-7b.

<table>
<thead>
<tr>
<th></th>
<th>Normal Planting</th>
<th>Late Planting</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>Interchange</td>
<td>Interchange</td>
</tr>
<tr>
<td></td>
<td>heterozygote</td>
<td>homozygote</td>
</tr>
<tr>
<td></td>
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<td></td>
</tr>
<tr>
<td>A) Ovule</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Observed grains</td>
<td>2419</td>
<td>302</td>
</tr>
<tr>
<td>No. of Florets</td>
<td>3450</td>
<td>310</td>
</tr>
<tr>
<td>Percent ovule</td>
<td>70.8</td>
<td>97.4</td>
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<tr>
<td>fertility</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>B) Pollen</td>
<td></td>
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</tr>
<tr>
<td>Observed Normal</td>
<td>1751</td>
<td></td>
</tr>
<tr>
<td>Observed Aborted</td>
<td>582</td>
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</tr>
<tr>
<td>Total</td>
<td>2333</td>
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<tr>
<td>Percent Normal</td>
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</table>
DISCUSSION

In T6-7a chromosome 6 is broken in the centromere or very close to it and chromosome 7 is also broken in the centromere or very close to it. In T6-7b chromosome 6 is broken in the long arm and chromosome 7 is also broken in the long arm.

The purpose of this study is to find why interchange heterozygote T6-7b shows a high degree of crossing over in the interstitial segment and a low degree of sterility. Therefore, the frequency of different configurations at late diakinesis and metaphase I of the meiotic division were studied and the pollen and ovule abortion were calculated for both T6-7a and T6-7b.

There is no interstitial segment in T6-7a. If present, it is so small that crossing over would be extremely unlikely in the region. Therefore, T6-7a is a standard (control) to compare with T6-7b where there is a long interstitial segment in which one or more crossovers are expected. The functional or non-functional gametes formed in heterozygous T6-7a will depend entirely on the type of anaphase I disjunction of the rings. All gametes formed by alternate disjunction will be normal and those formed by adjacent disjunction will abort.

In T6-7b, there is a long interstitial segment and one or more crossovers in this regions can take place as indicated in Fig. 3. Following single crossing over in the interstitial segment, either alternate or adjacent segregation will form 50 percent normal and 50
percent aborted gametes. The diagramatic representations of the expected pachytene pairings for T6-7a and T6-7b are shown in Figure 2A and 2B. The photographs, a and b, show late diplotene configurations in which a 4 arm configuration can still be seen. Also, the attachment of the nucleolus organiser to the short arm of chromosome 7 is quite visible. The pachytene configurations are hard to study as barley chromosomes are long and the size of the cell is comparatively small.

In the reports of Smith (1941), Burnham (1948), Burnham et al. (1954), Hanson and Kramer (1949) and Hagberg (1954), the low sterility, in barley interchange heterozygotes is considered to be the result of a high percent of alternate disjunction. They did not mention the length of the interstitial segment.

**Part I: Interchange heterozygote T6-7a**

The interchange heterozygote T6-7a gives an average of 29.1 percent ovule sterility in the normal planting and 40.3 in the late planting. The pollen sterility in T6-7a is 25.6 percent in normal planting. Burnham et al. (1954) observed that in different translocation lines (34 chromosomal interchanges produced by X-ray) each having a ring of 4 chromosomes, pollen abortion ranged from 14 to 58 percent (with an average of 28.8 percent). He also observed that the ovule abortion was greater than the pollen abortion. Interchange homozygote T6-7a shows 2.9 percent ovule abortion in normal planting and 25.7 percent ovule abortion in late planting. The chromosomal behavior of the interchange homozygote should be normal and no sterility is expected. Heterozygous T6-7a gives different sterility estimates in the
Photograph A T6-7b: 5 II + 1 IV, Diplotene showing quadrivalent configuration and the relative lengths of the arms.

Photograph B T6-7a: 5 II + 1 IV, Late diplotene. Nucleolus is attached to chromosome 7.
normal and the late planting. The sterility shown by homozygous T6-7a and also the different estimates of sterility in normal and late planting of the heterozygous T6-7a are due to environments influences. The ovule sterility calculated from the normal planting is treated as the environmental influence. Therefore, the ovule sterility after removing environmental influence in T6-7a is \((29.1 - 2.9)\) 26.2 percent and the ovule fertility is 73.8 percent.

The ovule fertility and sterility percentages can be related to the observed frequencies of the alternate and adjacent disjunction configurations given in Table 1. There is no interstitial segment in T6-7a and therefore, all alternate disjunction gametes should be normal and all adjacent disjunction gametes should abort. The quadrivalent configuration analysis shows that 68.6 percent of the gametes would result from alternate and 31.3 percent from adjacent. Thus the expected fertility and sterility is 68.3 percent and 31.4 percent respectively.

But the fertility after removing the environmental influence is 73.8 percent. The discrepancy between the expected fertility on the basis of the quadrivalent configurations and the observed (after removing the environmental influence) is 5.2 percent. This could be due to the following:

1. It could be due to chance only. The observed configurations may not be adequate and more configurations are needed.

2. The orientation of the chromosomes in the ring at metaphase I in adjacent segregation can be 292 or 3-1. The latter segregation behaves normally through the female as an \(n + 1\) gamete.
The percentage of non-orientation of the 2 out of 4 chromosomes in ring in T6-7a is 12.2 percent. In the 3-1 disjunction n + 1 gametes are functional in females which could increase the ovule fertility to some extent.

3. The observed 2.7 percent configurations classified as IXI, could have been figures eight types.

The results for interchange heterozygote T6-7a, discussed above are in agreement with the breeding and cytological behavior found by Smith (1941), White and Burnham (1948), Burnham et al. (1954) and Hanson and Kramer (1949).

Part II: Interchange heterozygote T6-7b.

The interchange heterozygote T6-7b has a long interstitial segment and one or more cross overs in this segment can take place. Functional or non-functional gametes formed, after crossing over in this region will depend on the following:

1. Number of cross overs and the type of cross over (single cross over, two strand double cross over, three strand double cross over, and four strand double cross over).

2. Type of disjunction.

The observed results of the ovule and the pollen fertility and sterility are summarised in Table 4. It can be seen from this table that in normal planting the interchange heterozygote T6-7b gives 29.2 percent sterility and the interchange homozygote should show normal chromosomal behavior and the sterility is only because of the environmental influences. Therefore, the ovule sterility in the heterozygous T6-7b is 20.0 percent.
after removing the environmental influence, from the observed sterility. Further evidence about the environmental influence on sterility can be seen from the observed data of normal and late plantings on the interchange homozygote and the heterozygote. In late planting the interchange heterozygote gives 33.9 percent ovule sterility and the interchange homozygote gives 17.4 percent sterility. The interchange heterozygote in the normal planting gives 29.2 percent ovule sterility and in the late planting gives 33.9 percent ovule sterility. The interchange homozygote in the normal planting gives 2.6 percent ovule sterility and in the late planting it gives 17.4 percent ovule sterility. This all indicates that the environment has some influence on the determination of ovule sterility.

The pollen abortion studies of interchange heterozygote T6-7b were from the normal planting. Therefore, the sterility shown by the interchange homozygote T6-7b in normal planting is treated as environmental influence. After removing the environmental influence from the observed sterility of interchange heterozygote T6-7b, the expected sterility is 26.6 percent.

The 26.6 percent sterility in T6-7b is now being related with the tetravalent configurations and the type of disjunction. From table 3 it can be seen that the frequency of the alternate disjunction configurations was 60.7 percent and the adjacent disjunction configurations was 39.3 percent. If there is no crossing over in the interstitial segment, all alternate disjunction gametes will be normal and all adjacent disjunction gametes will abort, giving rise to 60.7 percent fertility and 39.3 percent sterility. Among the adjacent disjunction configurations, 25.3 percent show non-coorientation of 2 out of the 4 chromosomes in the ring.
From these non-oriented tetravalents there could be 3-1 or 2-2 disjunction. The 2-2 disjunction will form gametes which will abort in male and female. The 3-1 disjunction will give n + 1 gametes which are functional in the female, non-functional--not aborted in male.

The observed pollen sterility is 24.9 percent. The ovule and pollen sterility are in a close agreement, but the ovule sterility is greater than the pollen sterility.

Assuming single crossing over in this interstitial segment 50 percent of the alternate configurations (i.e. 30.35 percent) will form normal gametes and 50 percent will form aborted gametes. In adjacent disjunction 50 percent of the gametes (i.e. 19.65 percent) will be normal and 50 percent will abort. Thus, overall fertility and sterility should be equal to 50 percent, if we assume single crossing over in the interstitial segment of each meiocyte.

The ovule sterility is 27.3 percent and the pollen sterility is 24.9 percent for which there is no evident explanation, but some of the possible explanations could be the following:

1. As shown in Fig. 3 there is a small interstitial segment in interchanged chromosome 6 and there is a long interstitial segment in interchanged chromosome 7. If there is a single cross over in each of the interstitial segments following the orientation of the chromosomes at metaphase II, as shown in Fig. 4C, higher percentages of normal gametes are expected. Thus, if the orientation of the chromosomes at metaphase II is directed, fertility will be increased.
2. If there is a 2-strand double cross over in the interstitial segment of chromosome 7, all gametes from alternate segregation will be normal and will give 60.7 percent fertility. There could be some n + 1 gametes formed from the non-oriented tetravalents which can function through the female. This will increase the percentage of fertility.

3. Any 4-strand double crossovers in the interstitial segment followed by adjacent I disjunction will produce normal gametes i.e. 39.3 percent. But if single crossing over in the interstitial segment takes place, 50 percent of the alternate gametes will be normal i.e. 30.4 percent. The total normal gametes therefore will be 69.7 percent. This would require further explanation to prove that 4 strand doubles form adjacent disjunction and single cross overs form alternate disjunction.

4. A three-strand double crossover will behave similar to a single crossover. Therefore a four strand double cross over, giving adjacent disjunction and a three strand double crossover giving alternate disjunction, will give similar results as explained in 3.

5. The observed ovule abortion is greater than the pollen abortion which could be due to the environmental influence.

6. The type of terminalisation of chiasmata from the interstitial segment, might be a factor affecting the type of disjunction.
Figure 3. Diagramatic representation of Pachytene in T6-7b illustrating double crossing over in the interstitial segment of chromosome 7.

Figure 4. Diagramatic representation of the formation of functional gametes by directed orientation of metaphase II following crossing over in both interstitial segments and either alternate or adjacent anaphase I disjunction.
Part III: Terminalisation of chiasmata as a factor affecting segregation from tetravalent configuration

Terminalisation of chiasmata from the interstitial segment is an unsolved problem. Various theories on the basis of observations have been forwarded all of which have some exceptions. In the interchange heterozygote T6-7b there is a long interstitial segment and one or more chiasmata may be formed in this region. It is not yet fully understood if chiasmata terminalise through the centromere which is median or almost median. The chiasma can either terminalise from the interchange segment or it has to pass through the centromere. If the chiasma does not terminalise either from the interchange segment or does not pass through the centromere, should result in a protuberance visible on the chromosomal segments of the ring configuration at metaphase I.

A late diplotene quadrivalent configuration can be seen in photograph C. A small protuberance of the chromosomal segments is quite obvious in this quadrivalent configuration which indicates that the chiasmata are stuck either at the interchange point or at the centromere. Non-terminalisation of chiasma should result in non-disjunction of the bivalent at anaphase I. This bivalent, if it is pulled to one of the poles will give rise to n + 1 gametes, the other dyad giving rise to n - 1 gametes. Evidence of 8-6 disjunction is given in photograph D. This bivalent, if not pulled to either pole, would remain on the equitorial plate at anaphase I as laggard. Bivalent laggards are shown in photographs E.

Univalent laggards should be visible if the terminalisation of chiasma from the interstitial segment takes place late as compared to
the chiasma in any other segment. The evidence for univalent laggards is shown in photograph F. All laggards eventually end up as micronuclei. And the chromosomes are lost.

Laggards were also observed in anaphase II as shown in photograph G. Anaphase II laggards in interchange heterozygotes are not reported in the literature. The diagramatic representation of a possible explanation for anaphase II laggards is given in Fig. 5. Assume that there are 2 interstitial chiasmata in a ring of 4 chromosomes as indicated in Fig. 5A. These chiasmata fail to terminalise, and therefore, hold the bivalents together as shown in Fig. 5B. The two chiasmata which are in the interstitial segments of the ring configuration do not terminalise completely at metaphase I. Thus the two bivalents of the ring are still held by chiasmata. These two bivalents go to opposite poles giving adjacent II disjunction at anaphase I as shown in Fig. 5c. The chiasmata are still holding each of the bivalents. Then these bivalents enter telophase I and interphase. During interphase, the bivalents are still held together by chiasmata or the two chromatids which possess chiasma may fuse leaving the other chromatids of each bivalents free.

At metaphase II, the two bivalents held either by chiasmata or fusion of the two chromatids line up at the equitorial plate in a twisted manner as shown in Fig. 5d. At anaphase II the centromere breaks, and the chromatids, not held by the chiasma, or fusion, move towards the pole. But, the chromatids held by chiasma or fusion still remain at the equitorial plate. So there could be 2 chromatids lagging in each of the dyad cells. If an independent relationship is established by the spindle
Photograph C T6-7b: 5 II + 1 IV, Diakinesis - quadrivalent configuration in a ring, shows a short protuberence. It appears that the chiasma did not terminalise.

Photograph D T6-7b: Anaphase I, showing 3-1 disjunction from a quadrivalent configuration.
Photograph E T6-7b: Telophase I, showing first division bivalent laggard.

Photograph F T6-7a: Anaphase I, showing first division univalent laggard.
Photograph G T6-7b: Telophase II, showing laggards of anaphase II.
with the chromatids, one or both chromatids, held by chiasma or fusion might move towards the pole. The spindle if joined earlier to one of the chromatids than to other, one chromatid might move to the pole leaving the other chromatid as a laggard. This could give rise to 2 chromatid laggards, one chromatid laggard or no laggards at anaphase II, depending on the spindles to which they are first picked up.

This is all hypothetical and detailed studies are necessary to elucidate more about it. The following studies are therefore suggested:

1. To confirm that chiasmata are formed in each of the interstitial segments.
2. Adjacent II disjunction only takes place.
3. During first meiotic division, the chiasma still holds the bivalent.
4. If two chromatids held by chiasma fuse during the interphase between first and second meiotic divisions.
5. Whether bridges are formed in anaphase I and anaphase II.
6. If the spindle establishes an independent relationship with the chromosomes in the ring configurations at metaphase I and with chromatids in bivalent condition at metaphase II.
7. Whether the laggards at anaphase II are univalents or chromatids.

A brief review of the work and speculations about the terminalisation of chiasmata, its effect on the formation of quadrivalent configurations, and the ultimate effects on sterility and fertility will be considered.

Burnham (1956) mentioned that the species which show a high frequency of alternate segregation of the chromosomes at meiosis have certain cytological features in common: (a) the chromosomes are relatively
Figure 5. Diagramatic representation of the possible origin of anaphase II laggards observed in T6-7b.
Figure 5

A: Pachytene

B: Diakine-SIS

C: Anaphase I

D: Metaphase II

E: Anaphase II
uniform in length (b) the centromeres are median or nearly so and (c) the chiasmata are located at the ends and terminalise completely.

All these features are true for the *Oenothera* rings which have survived in nature. Alternate segregation seems to be a characteristic not only of the larger rings but also of the $\Omega 8$, $\Omega 6$, and $\Omega 4$ which are found in certain species.

Alternate segregation for a ring of 4 in *Oenothera blandina* with very unequal interchanged segments (produced by X-rays) has been reported by Catcheside (1954). Hence equality in length of chromosomes in the interchange complex is not a necessary feature. Catcheside also mentioned that partial sterility was observed when an interstitial segment was long.

Gairdner and Darlington (1930) noted in *Campanula* that non-disjunction was more frequent in rings with non-terminal chiasmata at metaphase I. In this study, the barley interchange heterozygotes T6-7a and T6-7b showed laggards on the metaphase I plate. In T6-7b laggards at metaphase II were also observed. Sax and Anderson (1933) in *Tradescantia edwardsiana* reported a relationship between the kind of segregation from the ring and the relative frequencies of terminal and subterminal chiasmata in the interchange complex. For configurations with only terminal chiasmata there was a ratio of about 2 alternate: 1 adjacent; for those with one subterminal chiasmata it was 1:1 · 2, with two it was 1:1 · 8; and with three subterminal chiasmata all segregations were adjacent (12 figures). When considered as a whole it was about 1:1. Pollen counts showed 45.6 percent of imperfect pollen.
Levan (1939) reported an exceptional case with high terminalisation, yet an equal frequency of zigzag and open rings and about 50 percent pollen abortion in *Allium cernum*.

In 1937 Darlington and Gairdner stated that the absence of terminalisation prevents regular zigzag orientation of the chromosomes in a multiple ring.

In this study T6-7a has no interstitial segment and the terminalisation of chiasma could take place forming a high percent (68.3 percent) of zigzag configurations which supports the view of Darlington and Gairdner. But in the interchange heterozygote T6-7b there is a long interstitial segment and the chiasma terminalisation will be obstructed because of the non-homologous segments. Still 60.7 percent of the configurations show alternate disjunction which does not agree with the Darlington and Gairdner postulations.

Gairdner and Darlington (1931) suggested that the different segregations are the result of 2 assumed forces: (a) repulsion between the poles and the associated two chromosomes, (b) repulsion between the paired chromosomes, probably localized at the centromere. The latter was supposed to act more strongly along the axis of the spindle to repel adjacent chromosomes in the rings and orient them on opposite sides of the equator. Then the gradual movement onto the plate of the chromosomes along the ring would complete the alternate arrangement. Non-disjunction would be the result of different chromosomes in the ring establishing a relationship with the spindle independently. "Interstitial" chiasmata were believed to interfere with the gradual orientation along the ring.
Ostergren (1951), in explaining the arrangement and behavior in Oenothera rings stated that the chromosomes in chains or rings dance around during prometaphase, a mutual pulling one another resulting in orientation of adjacent chromosomes, to opposite poles. This works best when chiasmata are terminal and the centromeres are median. Kinetochores separated by the long arms or by arms having a sub-terminal chiasma were supposed to be in effect too far apart for mutual effect.

Darlington (1937) P. 152-153) has stated that "an association with interstitial chiasmata" is relatively rigid, its shape shows little variation, and is determined by the number and distribution of the chiasmata. An association of terminal chiasmata is extremely pliable. Forces of repulsion associated with the centromere are therefore able to effect regular disjunction of a ring with terminal chiasmata although they are powerless to distort a configuration held together by numerous "interstitial chiasmata". In 1939 he stated that "if chiasmata are terminalised a higher proportion of regular gametes are produced." Terminalisation of the chiasma in the interstitial segment of T6-7b is obstructed sometimes as shown in Photograph C, yet a high proportion of regular gametes are produced non affected as the fertility of the ovules is 70.8 percent and of the pollen is 75.1 percent. Darlington did not mention anything about the terminalisation of the chiasmata in an interstitial segment. If repulsion of the centromeres takes place, then a chiasma which cannot pass through the centromere will try to terminalise from the interchange segment and adjacent disjunction will take place forming aborted gametes. As regards the
mechanism of crossing over very little is known. Chiasma may be just an artifact and crossing over and chiasma might not be related. Therefore, to understand more about chiasma and its terminalisation, the mechanism of crossing over needs detailed understanding.

According to the one plane theory of crossing over given by Darlington (1937) the chiasma is supposed to be the result of crossing over. If single crossing over takes place in the interstitial segment, all types of disjunction will result in 50 percent fertility and 50 percent sterility in interchange heterozygotes. Then why in barley is a higher percentage of fertility observed? Thus, Darlington's hypothesis of crossing over needs some modification as it does not fit these results.

H. Lewis (1951) has suggested that some factors associated with the sequence of the chromosomes in translocation multivalents, not terminalisation of chiasmata, may determine or insure alternate segregation. This does fit observations of T6-7a. In T6-7a there is no interstitial segment and therefore chiasma will terminalise. Even then alternate segregation is 68.6 percent. In T6-7b there is a long interstitial segment and chiasma sometimes may not terminalise. This according to Lewis will give alternate segregation. The observed tetravalent configurations showing alternate segregation are 60.7 percent whereas in T6-7a where terminalisation of a chiasma is expected, the alternate segregation configurations are 68.6 percent. This discrepancy is unexplicable on Lewis's suggestion. Therefore, in barley interchange heterozygotes, terminalisation of chiasmata does not appear to be the only factor determining alternate segregation. There might be several other factors acting individually
and/or combined which determine the type of disjunction. Some of these factors are discussed in the earlier part of the discussion.

Hoar (1931) stated that the metaphase I chromosomes of Hypericum punctatum tended to show alternate segregation, yet about half of the pollen was aborted at maturity. This could be explained on the basis of single crossing over in the interstitial segment forming 50 percent non-functional and 50 percent normal gametes even if the disjunction was alternate.

Haga (1943) reported one extreme case of almost complete abortion and no seed set in Lilium hansonii with a ring of 4 + 10 II. His explanation was that orientation of the ring was always such as to result in adjacent segregation. Why a ring orients in that way no one knows, but it appears that there might be some genic or physiological constitution of the organism which makes a very definite type of orientation.

It appears that in barley, the segregation may basically be alternate and some conditions might be interfering to some extent giving still higher percent of fertility.

Thompson and Hutchinson (1942) in discussing translocation in wheat, also regarded the segregation as basically alternate, but thought that special conditions interfered and gave the appearance of randomness. They concluded that the forces which cause chromosome segregation involve whole chromosomes.

Price (1959), while working with an induced heterozygous reciprocal translocation in a Secale cereale L. X S. montanum Guss. hybrid, mentioned that the general validity of the idea that changes in homology stop
terminalisation must be rejected. He found that the above hybrid resulted in first metaphase configurations which show that the point of translocation is so located that chiasmata form both proximal and distal to it. He observed several of the configurations exhibiting terminal multiple chiasmata.

In this investigation there is evidence to show that in a few of the cases the interstitial chiasmata do not terminalise. The failure of the terminalisation of chiasmata from the interstitial segment should show some protuberence. In Photograph C, a small protuberence is visible supporting the hypothesis that in some cases non-homology interferes with the terminalisation of chiasmata. There appears to be no evident reason for the anaphase II laggards in heterozygote T6-7b if terminalisation of chiasmata takes place in metaphase I. The observed anaphase II laggards are, therefore, attempted to explain on the basis of the failure of terminalisation of chiasmata at metaphase I as shown in Fig. 5.

In maize, by using interchanges involving chromosome 6 (which has a nucleolus organiser as a marker), the frequencies of the different kinds of chromosome segregations have been determined by McClintock 1934, and Burnham, 1949, 1950b. It has been found that when both interstitial segments were short, alternate segregation was roughly 50 percent, the remainder being made up of adjacent 1, and adjacent-2 segregations in about equal proportions. When at least one interstitial segment was very long, there was little or no adjacent 2 segregation.

In one group of ring-forming interchanges with short interstitial segments there was an average of about 25 percent of adjacent 2 segregation. Therefore, the length of the interstitial segment is one factor
affecting the segregation (Burnham, 1949, 1950b). Relative length of the
two axes does not seem to be a factor. Burnham (1953) showed in other
experiments that both alternate and adjacent-I segregations occur follow­
ing crossing over in the interstitial segment, possibly with equal
frequency. Pollen abortion was close to 50 percent, regardless of
whether the interstitial segment was long or short (Burnham 1949, 1950b).
Therefore, in maize, when there is a ring configuration and interstitial
crossing over has not occurred, the ring may orient as an open configura­
tion in two ways, giving rise to adjacent 1 or to adjacent-2 segregations;
and that for each there is a corresponding zigzag or alternate configura­
tion (all of one type), resulting in 50 percent of alternate segregation.
When there is crossing over in an interstitial segment, the chromosomes
that cross over pass to opposite poles, but both alternate and adjacent-I
segregation occur with relatively high, if not equal frequencies (Burnham,
1953). In such figures, if the centromeres pass to either pole indepen­
dently, the two types of segregations would be in 1:1 ratio.

Adjacent-2 segregation occurs only in the ring configurations in
which no interstitial crossing over has occurred. This described the
behavior in maize, but does not explain the mechanism. If as Darlington
says, the configurations with interstitial crossovers are rigid, it is
difficult to explain why they have enough flexibility to produce alter­
nate, as well as adjacent segregations in maize. Also the configurations
without such cross overs should be free to react to the centromere effect,
which, according to Darlington, should bring about alternate segregations.
In maize interchanges with short interstitial segments, this would occur
in a large proportion of meiocytes, yet alternate and adjacent segregations are about equally frequent.

In general it appears that directed segregation in certain species may be accompanied by certain cytological features, but that these features may not be present in other species showing directed segregation. Detailed studies are needed in each species.
Photograph H T6-7b: 5 II + 1 IV, Diakinesis showing triangular configuration with nucleolar organising region.

Photograph I T6-7a: 5 II + 1 IV, Diakinesis representing "U" type quadrivalent configuration.
Photograph J  T6-7a:  5 II + 1 IV, Metaphase I. Quadrivalent configuration showing a figure eight.

Photograph K  T6-7b:  4 II + 1 IV, Metaphase I. Figure eight type configuration but one arm of the eight is free.
Photograph L T6-7b: Metaphase I showing 7 bivalents.

Photograph M T6-7b: 5 II + 1 IV, Metaphase I. Quadrivalent configuration in which 2 out of 4 chromosomes are oriented and the other 2 are not oriented. A small protuberance is seed showing non-terminalisation of chiasma.
Photograph N T6-7a: 5 II + 1 IV, Metaphase I, showing twisted chain of 4 chromosomes.

Photograph O T6-7a: 5 II + 1 IV, Metaphase I, showing folded ring configuration.
Photograph P T6-7b: 5 II + 1 IV, Early anaphase I indicating 2-2 disjunction from a ring of 4 chromosomes.

Photograph Q T6-7b: Telophase I, showing first division univalent laggard.
Photograph R  T6-7b:  Pollen grains showing differential staining and size.
   1. Small but heavily stained.
   2. Large but light stained.
   3. Medium size and intermediate stained normal pollen grain.

Photograph S  T6-7a:  Fertile and sterile pollen grains.
   1. Unstained, non-spherical--sterile.
   2. Well stained, spherical--fertile.
SUMMARY

(1) Barley interchange heterozygotes T6-7a and T6-7b obtained by A. Hagberg using X-radiation were selected for study. In T6-7a chromosome 6 is broken in the centromere or very close to it and chromosome 7 is also broken in the centromere or very close to it. In T6-7b chromosome 6 is broken in the long arm and chromosome 7 is also broken in the long arm. T6-7a is treated as a standard because there is no interstitial segment, or a very short interstitial segment where crossing over is not likely to take place. In T6-7b there is a long interstitial segment where crossing over is likely to take place. The object of this study was to find out why T6-7b shows a high degree of crossing over in the interstitial segment and a low degree of sterility. The following factors were studied: (1) The frequency of different types of configurations at diakinesis and metaphase I were determined for T6-7a and T6-7b; (2) The percentage of alternate and adjacent disjunction was calculated in both stocks; (3) Percentages of pollen and ovule sterility were calculated; (4) The percentage of nonorientation of chromosomes from the ring configuration was determined; (5) The environmental influence on ovule sterility was noted.

(2) The frequencies of different configuration at diakinesis and metaphase I for T6-7a and T6-7b are as follows:

49
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<th>Type of configuration</th>
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<th>T6-7b frequency in %</th>
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<td>15.7</td>
<td>9.4</td>
</tr>
<tr>
<td></td>
<td>9.5</td>
<td>20.2</td>
</tr>
<tr>
<td></td>
<td>19.1</td>
<td>14.0</td>
</tr>
<tr>
<td></td>
<td>2.7</td>
<td>5.1</td>
</tr>
</tbody>
</table>

(3) Percentages of alternate disjunction and adjacent disjunction in T6-7a and T6-7b were 68.6 and 31.4, and 60.7 and 39.3, respectively.

(4) Observed pollen and ovule sterility in the interchange heterozygote T6-7a is 25.6 percent and 29.1 percent, respectively, whereas in T6-7b pollen sterility is 24.9 percent and ovule sterility is 29.2 percent.

(5) The percent of non-coorientation of 2 of the 4 chromosomes in a ring for T6-7a is 12.3 percent and for T6-7b is 25.3 percent.

(6) The environmental influence on ovule sterility was determined by the observed ovule sterility in the interchange homozygotes in normal planting. T6-7a shows 2.9 percent and T6-7b shows 2.6 percent.

(7) The problem of terminalisation of chiasma from the interstitial segment and its bearing on the object of this research is discussed.
(8) The bearing of the above findings is discussed briefly as to why barley interchange heterozygote T6-7b shows a high degree of crossing-over in an interstitial segment and a low degree of sterility.
LITERATURE CITED


__________ 1934 (a) Chromosomal interchanges in maize: reduction of crossing over and the association of non-homologous parts. Amer. Nat. 68:81-82.

__________ 1934 (b) Cytology and genetics of interchange between chromosome 8 and 9 in maize. Genetics 19:430-447.


__________ 1950 (a) Chromosome segregations in chain forming translocations in maize. Genetics 35:99 (Abstr.).

__________ 1950 (b) Chromosome segregation in translocations involving chromosome 6 in maize. Genetics 35:446-480.


____ 1950. The determination of linkage intensities from F<sub>2</sub> and F<sub>3</sub> genetic data involving chromosomal interchanges in barley. Genetics 35:559-569.


ABSTRACT OF THESIS

CONSEQUENCES OF CROSSING OVER IN INTERSTITIAL SEGMENTS OF BARLEY INTERCHANGE HETEROZYGOTES

by

M. R. Wanjari

Barley interchange heterozygotes T6-7a and T6-7b obtained by A. Hagberg using X-radiation, were used in this study. The purpose of this study was to find out why T6-7b shows high degree of crossing over in the interstitial segment and low degree of sterility. In T6-7a there is no interstitial segment. If present, it is very small so that crossing over in this segment is unlikely. But in T6-7b there is a long interstitial segment and multiple crossovers in this region are expected. T6-7a was treated as standard to T6-7b. T6-7a and T6-7b were studied for pollen and ovule abortion and the frequency of different types of configurations at late diakinesis and metaphase I. The frequencies of different types of configurations for T6-7a and T6-7b respectively were as follows: $\mu 32.4, 31.7; \mu 12.6, 16.5; \mu 7.6, 3.1; \mu 15.7, 9.4; \mu 9.5, 20.2; \mu 19.1, 14.0; \mu 2.7, 5.7$. Percentages of alternate and adjacent disjunction in T6-7a and T6-7b were 68.6 and 31.4, 60.7 and 39.3 respectively. The observed pollen and ovule sterility in interchange heterozygote T6-7a was 25.6 and 29.1 percent respectively whereas in T6-7b pollen sterility was 24.9 and ovule sterility was 29.2 percent. From the results it appears that T6-7a and T6-7b do not show much differences in ovule and pollen sterility in spite
of presence of long interstitial segment in T6-7b and absence of interstitial segment in T6-7a. The effect of the environment on ovule abortion in the interchange homozygote of T6-7a and T6-7b was determined and the effect of late planting on the ovule abortion in the interchange heterozygote and homozygote was also determined. The ovule abortion in the interchange homozygote and in the late planting show that the environment affects ovule sterility to some extent.