

THE CYTOLOGY AND BREEDING BEHAVIOR OF A COMPLETE  
INTERCHANGE STOCK IN BARLEY  
(HORDEUM VULGARE L.)

by  
Sharon Lee Morgan

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SIGNED: Sharon Lee Morgan

APPROVAL BY THESIS DIRECTOR

This thesis has been approved on the date shown below:

R. T. Ramage  
R. T. RAMAGE  
Professor of Plant Sciences

February 17, 1978  
Date

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## ABSTRACT

Barley (Hordeum vulgare L.) plants containing a ring of fourteen chromosomes at metaphase I were obtained by crossing a complete interchange stock onto a large number of normal male sterile plants. These plants were observed cytologically to determine the extent of the chromosome association at metaphase I, numerical disjunction at anaphase I, and the per cent of stainable pollen.

The association of the chromosomes into one large ring was consistently observed in the  $F_1$ . At anaphase I, either 7-7 equal numerical disjunction or 8-6 disjunction types predominated. The frequency of equal numerical disjunction was found to have no correlation with the percentage of stainable pollen.

Application of normal pollen to  $F_1$  females was shown to produce more seed than self-pollination of the same plants. The amount by which seed set was increased varied from plant to plant.

Plants containing seven bivalents and plants containing a ring of fourteen chromosomes occurred in a 1:1 ratio in the  $F_2$ . Trisomics and double trisomics were also observed.

Several of the interchanged chromosomes could be distinguished from normal chromosomes morphologically in root tip preparations.



## INTRODUCTION

A complete interchange stock is one in which all of the chromosomes of the haploid set of an organism are involved in a series of interchanges. When such a stock is crossed with a line containing normal chromosomes, a large ring containing all of the chromosomes is observed at metaphase I of meiosis. Only two types of viable haploid gametes are expected from such an  $F_1$ . These gametes would contain either the centromere segments of the normal chromosomes or the centromere segments of the interchanged chromosomes. All other gametes would contain duplication-deficiencies and would be expected to abort.

Only three types of diploid individuals are expected in the  $F_2$  of such a cross since only two types of viable haploid gametes are produced by the  $F_1$ . The three types should include individuals homozygous for the complete interchange, individuals homozygous for the normal chromosomes, and individuals heterozygous for a large ring containing all of the chromosomes.

It may be possible to utilize complete interchange stocks in plant breeding programs once the behavior of such stocks is more clearly understood. Burnham (1946) has suggested that such stocks may be used to select favorable gametes. This would be an alternative to such expensive

practices as tissue culture and searching for quite infrequent spontaneous haploids.

Barley, which shows an excess of alternate segregation from a ring of four chromosomes, would appear to be a favorable organism for studying the behavior of a complete interchange stock. A high frequency of alternate segregation from a large ring of chromosomes would be necessary for obtaining viable gametes in sufficient numbers to make a plant breeding program worthwhile.

The purpose of this study is to examine the cytological behavior of a complete interchange stock in barley. The main area of interest is the meiotic behavior of the  $F_1$  of a cross of a complete interchange stock with a genotypically diverse array of plants containing normal chromosomes.

## LITERATURE REVIEW

Extensive research has been done on the genus Oenothera since the late 1880's. The unusual breeding and cytological aspects of this genus have been shown to have far reaching applications to many other organisms.

DeVries (1901-03) based much of his Mutation Theory on the behavior of Oenothera lamarckiana. This species was observed to breed true consistently. Very infrequently, however, off-types were obtained in the progeny. DeVries concluded that these off-types were gene mutations which represented observable steps in the evolutionary process. It is now known that the off-types were produced as a result of the unusual genetic system of Oenothera rather than as a result of gene mutation. The vast amount of work done by DeVries, however, laid the groundwork for much of the genetic research which followed.

Renner began a detailed study of the breeding behavior of Oenothera in 1913 (Sturtevant, 1926). He noted that when Oenothera lamarckiana was selfed, the progeny obtained were all O. lamarckiana. Half of the seed produced by selfing were inviable. If O. lamarckiana was crossed with either O. biennis or O. muricata there was no problem with seed viability but two types of progeny were obtained. These two types of progeny were termed laeta and

velutina. Renner proposed that seed inviability was the result of combinations of genomes in the homozygous condition.

A situation in Drosophila similar to the one in Oenothera was reported by Muller (1917). A race which bred true for the character Beaded was established. This race was shown to be homozygous for the locus involved. Further, any homozygous combinations which were obtained were lethal. Muller postulated that each allele was tightly linked with a lethal gene. This was referred to as a balanced lethal system.

The evidence presented during this time led Renner to formulate a theory of "complexes" (Sturtevant, 1926). He proposed that the genomes in Oenothera do not segregate at meiosis, but are transmitted as a whole to each gamete. A genome which behaved in this manner was termed a complex. A species which contained two different complexes was termed a complex-heterozygote. Such a system could be maintained by the presence of a balanced lethal system.

Very little work on the cytogenetics of Oenothera was done until the 1920's. The association of chromosomes into large rings at meiosis had been observed earlier in O. rubrinervis (Gates, 1908). Cleland (1922) found that in O. franciscana four of the chromosomes did not pair normally. Instead, a ring of four chromosomes was formed consistently at meiosis. In further studies, Cleland (1923,

1924) found that a given number of chromosomes were associated into large rings rather than pairs at meiosis. The number of chromosomes in the ring varied from species to species, but was very consistent within the species. The rings were observed to remain associated through the first metaphase. At metaphase, the rings arranged themselves in a zigzag fashion such that adjacent chromosomes passed to opposite poles.

The cause of ring formation was not at first obvious. The work of Belling and Blakeslee (1926) with a trisomic in Datura led to the understanding of the ring formation in the Oenothera's. This trisomic was found to contain a trivalent in which one of the chromosomes was smaller than the other two and obviously not a total homologue. In some cases a chain of five chromosomes was observed rather than a trivalent. They proposed that the smaller chromosome was the result of "segmental interchange." Two non-homologous chromosomes had broken and exchanged segments. This would mean that each end of the smaller chromosome was homologous with a different chromosome. Belling (1927) felt that the segmental interchange hypothesis could explain the formation of large rings in Oenothera.

Belling's suggestion led many workers to attempt to explain the behavior of Oenothera on the basis of segmental interchange. Emerson and Sturtevant (1931) and Cleland and

Blakeslee (1930, 1931) attempted to determine experimentally whether this hypothesis could be applied to Oenothera. In order for large rings to be formed as the result of segmental interchange, a considerable amount of exchange must have occurred. Theoretically, each of the "Renner complexes" should have a unique arrangement of end sequences. The analysis of a number of complexes in terms of the arrangement of their end sequences was begun. On the basis of this analysis, the investigators attempted to predict the types of chromosome configurations which should be observed when various complexes were combined. Such predictions were proved correct and served as proof of the validity of the application of the segmental interchange hypothesis to Oenothera.

The degree of ring formation in Oenothera follows a definite geographic pattern (Cleland, 1950). Oenothera hookeri, found in the western United States, has seven bivalents at metaphase I. The size of the ring of chromosomes at meiosis increases with species found eastward. Oenothera biennis, found on the east coast, has a ring containing all fourteen chromosomes at metaphase I.

The situation by which structural heterozygotes are maintained in a population has been found to occur naturally in plants other than Oenothera. James (1965) described a case in Isotoma petraea in which the evolution of a complex-heterozygote system can be observed. This

species is found in western Australia. In one area, all of the populations found are interchange heterozygotes. This area extends southwest from Lake Barlee. The population at Lake Barlee has normal bivalents at metaphase I. Rings of chromosomes are found in the remainder of the populations with the size of the ring increasing as one moves toward the southwest.

Isotoma has been found to have cytological characteristics very similar to those of Oenothera (James, 1965). The chromosomes are all fairly equal in size, the centromeres are median, and chiasmata tend to be terminalized. Burnham (1956) associated these morphological traits with species displaying an excess of alternate segregation from large rings. This would compensate for the sterility which usually results from adjacent segregation at prophase I in interchange heterozygotes. Both Isotoma and Oenothera possess cytological features which enable them to exist as a complex-heterozygote without appreciable loss in fertility.

Rhoeo discolor is a complex-heterozygote which sharply contrasts with the situation observed in Oenothera and Isotoma. Sax (1931) made a detailed study of ring formation in Rhoeo. The somatic chromosomes could easily be distinguished from each other. They were found to vary in length and position of the centromeres. Darlington (1929) had found that a ring containing all twelve

chromosomes was not found consistently in all pollen mother cells. A plant might contain some cells with a ring, some with a chain, or some with several chains.

In Rhoeo, between 80% and 90% of the pollen grains were found to be abnormal (Sax, 1931). This is assumed to be due to the high frequency of non-disjunction of the ring. The result is a very small amount of seed being produced on a given plant. A balanced lethal system is present in Rhoeo such that only the heterozygotes survive.

The presence of large rings in natural populations has led many workers to attempt to synthesize large rings in other organisms. Burnham (1930) built a ring of six chromosomes in maize. At the time, he was studying the segregation of marker genes in relation to the presence of several interchange heterozygotes. These heterozygotes had a ring of four chromosomes at metaphase I of meiosis. When he crossed two of the heterozygotes together, a ring of six chromosomes was observed in some of the  $F_1$  plants. Burnham concluded that the interchange heterozygotes involved one common chromosome pair. If the chromosomes are labelled A, B, and C, respectively, one member of the B pair has interchanged with an A chromosome while the other member of the B pair has interchanged with a C chromosome. When the two interchanges are brought together, a ring of six chromosomes is observed due to the pairing of all homologous end segments.



Sansome (1932) obtained a ring of six chromosomes in Pisum sativum in the same manner as demonstrated by Burnham (1930) in maize. Chains were occasionally seen at metaphase I of meiosis, but for the most part the six chromosomes were found in a complete ring. In earlier work (Pellew and Sansome, 1931), it was demonstrated that the points of attachment of the chromosomes in a ring were due to chiasmata. A "median chiasma" was observed in roughly 78% of the pollen mother cells which had a ring of six chromosomes (Sansome, 1932). This chiasmata occurred between chromosomes which were non-adjacent. Further, the position of this chiasma was consistent for the cells observed. It was postulated that the normal terminalization was stopped at the point where the chromosomes become non-homologous.

A complex-heterozygote resembling those seen in the Oenothera's has been constructed in the Einkorn wheats (Yamashita, 1950). Two species, Triticum aegilopoides and T. monococcum, were irradiated to induce reciprocal interchanges. A series of successive crosses was then made to combine all of the interchanges together. The large ring formed consisted of alternating T. aegilopoides and T. monococcum chromosomes. It was noted that viable gametes could only be produced by complete alternate segregation from the large ring. Einkorn wheats with a ring of four chromosomes display alternate segregation in roughly 82% of

the pollen mother cells.<sup>4</sup> As the size of the ring is increased, the amount of complete alternate segregation is decreased. A plant with a ring of fourteen chromosomes shows complete alternate segregation in only 13% of the pollen mother cells.

Inman (1957) suggested a method of constructing large rings which would minimize sterility problems. Crosses would be made between two single interchange homozygotes sharing a common chromosome. The breakpoints in the chromosome common to both interchanges should be in opposite arms and a fairly wide distance apart. The resulting  $F_1$  would contain a ring of six chromosomes at metaphase I of meiosis. The appropriate crossover in the interstitial segment of the common chromosome would combine the two interchanges, i.e., interchange A-B and interchange B-C would become interchange A-B-C. The appropriate crossover when crossed with a normal should contain a ring of six chromosomes. Once the desired crossover is identified it would be necessary to obtain the new interchange in the homozygous condition. Interchanges of this type would need to be established such that each chromosome is involved in two such interchanges, i.e., A-B-C, B-C-D, C-D-E, etc. When setting up this series of interchanges, the same single interchange is used to combine each chromosome into the appropriate interchanges. Once these lines are obtained, no crossing over in the interstitial segment is

required for future steps in the procedure. The next step would be to cross the homozygous interchange lines. A-B-C would be crossed with B-C-D, B-C-D would be crossed with C-D-E, etc. Two rings of four chromosomes should be observed in the  $F_1$ 's. Simply by segregation from the two rings, the  $F_2$  will contain plants which are composed of three interchanges. The plants containing three interchanges will be one of the possible fertile progeny obtained. These plants can be recognized by test crossing them with normals. A ring of eight chromosomes would be seen in the  $F_1$  of the desired plant from the test cross. This procedure can be continued to add further interchanges to the ring. If plants which contain three interchanges, two of which are common interchanges, are crossed together, the  $F_1$  should contain two rings of four chromosomes. Plants combining four interchanges can be selected in the  $F_2$  based on test cross progeny. The amount of sterility observed using this procedure should never be higher than that expected from two rings of four chromosomes.

Complete interchange stocks have been synthesized in barley, Hordeum vulgare L. Shih and Shebeski (1960) made crosses between various interchange lines to determine the effect of interchanges on the fertility of barley. The  $F_1$ 's of each cross were examined cytologically to confirm the metaphase configuration. They found that the sterility increased as the size of the ring of chromosomes was

increased. It was also noted that the size of the ring rather than the number of interchanges present was the greatest factor in determining the degree of sterility; i.e., a plant with a ring of eight chromosomes was more sterile than a plant with two rings of four chromosomes. Crosses which resulted in the same metaphase configuration often varied in the amount of sterility observed.

Nishimura (1961) reported a ring of fourteen chromosomes in a winter barley. Sisodia and Shebeski (1965) successfully synthesized a complete interchange stock in the variety Montcalm. They used two methods to obtain this stock with equal success. One method was by successive crosses of interchange lines involving common chromosomes. The other method was the use of irradiation to induce interchanges. Ten crosses were made of the homozygous complete interchange stock onto normal plants. The  $F_1$ 's obtained were completely sterile. They concluded that the method of gamete selection proposed by Burnham (1946) would not be feasible for use in barley. It was pointed out earlier, however, that a large population would be required to test the method in barley (Shih and Shebeski, 1960).

The fertility of a stock containing a large ring is dependent upon the amount of alternate segregation which occurs. If the type of segregation at metaphase I of meiosis is at random, 50% spore abortion would be expected from a ring containing four chromosomes. In reality, the

amount of spore abortion in interchange heterozygotes has been found to vary for different organisms. Sterility has been reported at 66% for Strelitzia augusta (Simmonds, 1954), 50% for Zea mays (McClintock, 1930), 29% for Hordeum vulgare L. (Burnham, White, and Livers, 1954), and none for Oenothera muricata (Cleland, 1926). The amount of sterility was observed to be quite constant for a given species. The constant observed differences suggest some underlying mechanism which controls segregation from a ring of chromosomes.

Thompson (1956) was able to show that genotypic control of segregation existed in rye. The progeny of a plant which was heterozygous for two separate interchanges was observed and subsequent generations studied. Three plants in the  $F_2$  heterozygous for both interchanges were selected. In the  $F_3$  there were significant differences as to the degree of alternate segregation from each of the three selected plants. Results in the  $F_4$  correlated closely with the observations in the  $F_3$ . The evidence suggests segregation in the  $F_1$  of a gene or genotype which favors alternate segregation.

It has been demonstrated that it is possible to select for genotypes favoring alternate segregation in rye (Lawrence, 1958). Lines which were heterozygous for two separate interchanges were used in this study. By selecting plants within lines which were heterozygous for both interchanges and which displayed the greatest fertility, the

amount of alternate segregation was increased 10% from the  $F_3$  to the  $F_6$ .

Dennhofer (1973) has described a single locus with multiple alleles which determines the type of segregation from a large ring in the mosquito, Culex pipiens L. She used interchange lines which were tightly linked with M, the male sex determinator. The amount of sterility had been found to be different in different males carrying this same interchange. Males which were heterozygous for the interchange and which produced 34% lethality were mated to normal females. The males obtained in the  $F_2$  were mated to normal females to determine the amount of sterility associated with the interchange. The males in the  $F_2$  produced either 50% lethality or 34% lethality. The ratio of the two types was 9:7. The presence of different alleles governing segregation is postulated as an explanation for the results obtained. It is stated that  $sg^1$  causes 66% lethality,  $sg^2$  causes 50% lethality,  $sg^3$  causes 34% lethality, and  $sg^4$  causes no lethality. The results of the crosses could be explained by this hypothesis. If the females were homozygous for  $sg^2$  and the males were heterozygous for  $sg^2sg^3$ , two types of progeny would be obtained in the  $F_1$ . Random mating would result in the ratio observed in the  $F_2$ .

The possibility of using a complete interchange stock to select genotypes which favor alternate segregation

has been suggested for use in barley (Ramage, 1976). The  $F_2$  obtained from crossing the complete interchange stock with a normal line should contain only three types of progeny: homozygous normal lines, homozygous interchange lines, and lines heterozygous for a ring of fourteen chromosomes. Homozygous lines of each type could be crossed to obtain an equivalent  $F_2$ . The heterozygous lines could simply be grown out. Such a method would contrast with the one used in rye by selecting a genotype for alternate segregation by the existence of a plant rather than by degrees of fertility.

The behavior of complete interchange stocks in essence reflects the cytological make-up of an organism. Oenothera (Cleland, 1972) and Isotoma (James, 1965) are examples in nature of organisms possessing a large ring whose cytological features enable them to maintain a high level of fertility. In both genera, the chromosomes comprising the large rings are all of almost equal length, the centromeres are median, and the chiasmata tend to be terminalized. These morphological features appear to preadapt an organism to an excess of alternate segregation from a large ring (Burnham, 1956). Other organisms exist as complex-heterozygotes in spite of a lack of complete alternate segregation. Rhoeo is one such organism (Sax, 1931). Its cytological features are quite different than those observed in Oenothera and Isotoma. Both the position

of the centromeres and the length of the chromosomes vary considerably in the large ring.

Sansome (1932) pointed out that it is possible to get certain viable gametes from non-disjunction in a large ring. Such gametes would contain the complete haploid genome plus an extra chromosome. If this gamete were united with a haploid gamete, the zygote formed would be trisomic; i.e., contain the normal diploid genome plus an extra chromosome.



## MATERIALS AND METHODS

A complete interchange stock in the variety Mars was used in this study. This stock is described in the coordinator's report in Barley Genetics Newsletter (Ramage, 1973). In the spring of 1976, this stock was crossed onto 450 male sterile  $F_2$  plants in breeding populations grown at Marana and Tucson, Arizona. Approximately 18,000 crossed seed were obtained. The crossed seed were grown in Bozeman, Montana, in the summer of 1976. One seed from each cross was thin planted in Montana to facilitate making cytological collections. The remainder of the seed were planted in bulk in order to obtain an  $F_2$  population.

It was observed in the  $F_1$  plants that the anthers appeared quite shriveled as compared to anthers of normal plants. The possibility that seed set would be reduced due to lack of anther development rather than ovule fertility was considered. Two to three heads of each of 31 of the  $F_1$  plants were pollinated by a source of normal pollen. A line, 6-3, was chosen as a pollinator due to the abundance of pollen produced by this line. A corresponding number of heads were bagged on each plant to insure that all seed set on these heads were the result of self-pollination. Seed set was determined for the self-pollinated heads versus the outcrossed heads on the basis

of number of seed per head. Since the heads were bagged at approximately the same stage of development, the number of florets per head was fairly consistent for a given plant.

All cytology conducted in this study was done following standard techniques. Microsporocytes were collected and immediately killed and fixed in a solution of three parts absolute ethanol to one part glacial acetic acid. After approximately 72 hours in the killing and fixing solution, the samples were transferred to 70% ethanol for storage. Microsporocytes were examined using the aceto-carminé squash technique.

In all microsporocytes examined, two meiotic characteristics were of special interest. Metaphase I was observed to determine the extent of chromosome association. In those plants which were observed to contain all chromosomes in one large ring, the numerical disjunction at anaphase I was counted in as many cells as possible. The cells were scored as to the number of chromosomes at each pole and the number of chromosomes remaining on the equatorial plate.

The percentage of aborted pollen was determined for many of the plants examined. Pollen was classified as aborted if it failed to stain dark blue in iodine potassium-iodide. At least 500 pollen grains per plant were scored in the determination of per cent aborted pollen.

The number of seed per head for each plant examined was determined to estimate relative differences in ovule fertility. Since an extremely small amount of seed was produced by each plant, it was decided that the number of seed per head would give a relatively good indication of any extreme differences between plants in terms of ovule fertility.

Cytological material examined was from  $F_1$  plants from the crosses of the complete interchange stock onto normal male steriles. Samples were collected in Montana in the summer of 1976. Additional  $F_1$  seed were grown in the greenhouse in Tucson, Arizona, in the fall of 1976 and cytological samples were collected from these plants.

It was of interest to determine the fate of the large ring in following generations. In the fall of 1976,  $F_2$  seed were grown in the greenhouse. In addition,  $F_1$  seed of the cross of the heterozygous ring of fourteen material with the line, 6-3, were grown in the greenhouse. Cytological samples were obtained from these plants.

The chromosomes of the complete interchange stock used have not been karyotyped. In order to get some indication of the morphology of the chromosomes, the root tips of several heterozygous ring of fourteen plants were examined. The root tips were placed in bromonapthalene for a period of three hours, 8:30 to 11:30 a.m. The root tips were then placed in 1 N hydrochloric acid at 55°C for a

period of two minutes, after which they were immediately rinsed in deionized water and placed in acetocarmine solution for staining. The cells were examined using the acetocarmine squash technique.

## RESULTS AND DISCUSSION

The complete interchange stock was crossed onto 450 male sterile plants. The male sterile plants used were  $F_2$  plants from segregating breeding populations. Every crossed seed could, therefore, contain a unique genotype. The  $F_1$  plants obtained should have had a ring of fourteen chromosomes at metaphase I of meiosis.

### Appearance of $F_1$ Plants

The  $F_1$  plants appeared normal in terms of vegetative growth and vigor. Wide variation was seen in plant type, time of heading, and time of maturity due to the diverse genotypes present. This variation was no more than would be expected in a normal segregating population.

Two plant characteristics were observed to be quite different in the  $F_1$  plants as compared to normal plants. The characters obviously affected were ovule fertility and anther development.

Seed set on the  $F_1$  plants was much lower than that on normal plants. Most of the  $F_1$  plants did not produce any seed. The plants that did set seed produced a maximum of ten seed per entire plant.

The anthers of the  $F_1$  plants appeared much less developed than anthers of normal plants. Normal anthers

and anthers from the heterozygous ring of fourteen plants are shown in Figure 1a. The normal anthers appear very plump and well filled as opposed to the anthers of the  $F_1$  plants which look quite shriveled. It was difficult to distinguish an  $F_1$  plant from a genetic male sterile plant on the basis of observable plant characteristics.

Comparison of Seed Set Between Selfed  
and Outcrossed Heads on  $F_1$  Plants

Pollen from the line 6-3 was placed on heads of 31 of the heterozygous ring of fourteen plants to determine if low seed set could be partially explained by poor development of the anthers. A corresponding number of heads on each of the 31 plants were bagged to insure that the seed set on these heads resulted from self-pollination. Each plant, therefore, constituted a replication since each of the two possible treatments were performed on each plant. The average number of seed per head was determined for each treatment type for each of the 31 plants. These data are shown in Table 1.

A student's t-test was conducted on the data obtained to see if the seed set varied significantly between the two treatments. The difference in the average number of seed per head obtained from both treatments was determined for each plant. An average difference of 2.88 seed per head between the two treatments was calculated for the 31 plants tested.

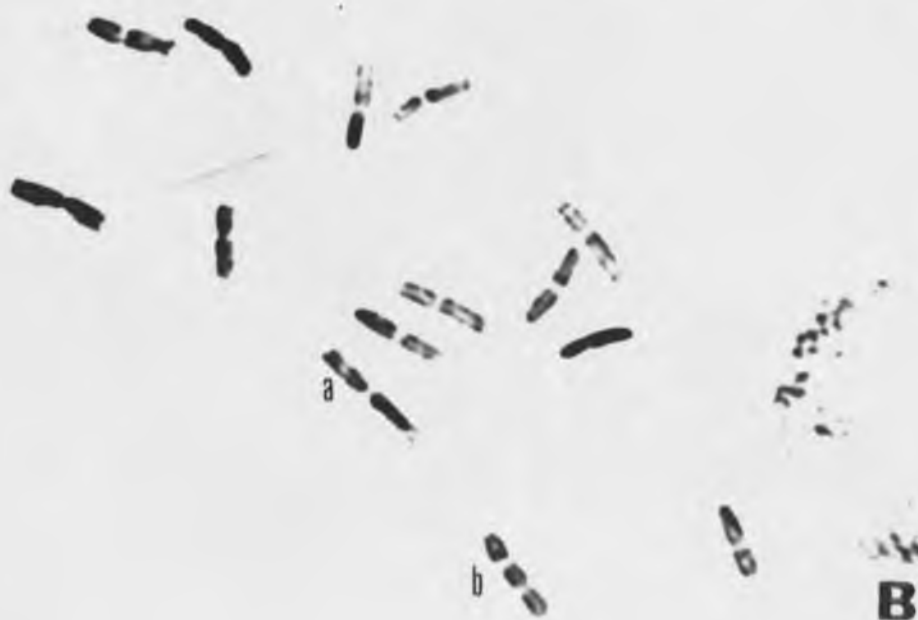


Figure 1. Anthers of normal plants and  $F_1$  plants containing a ring of fourteen chromosomes (A) and somatic chromosomes of an  $F_1$  plant heterozygous for the complete interchange (B).

Table 1. Relative seed set of self-pollinated versus artificially pollinated heads on heterozygous ring of fourteen plants.

Plant #	Seed per head		
	Self-Pollinated	Pollinated by 6-3	Difference
2a	0.50	4.25	3.75
2b	0.00	4.00	4.00
3a	0.00	2.00	2.00
3b	0.00	0.00	0.00
3c	0.33	2.00	1.67
6a	0.00	3.00	3.00
7a	2.00	1.66	-0.34
7b	0.25	1.50	1.25
8a	0.66	1.33	0.67
8b	1.00	3.00	2.00
9a	0.33	2.83	2.50
10a	0.00	1.25	1.25
10b	0.50	2.66	2.16
10c	0.00	2.00	2.00
12a	0.66	1.00	0.34
14a	1.00	4.00	3.00
15a	0.00	2.00	2.00
18a	0.00	2.00	2.00
19a	0.00	2.00	2.00
19b	0.00	0.50	0.50
23a	3.33	42.00	38.67
23b	0.20	1.60	1.40
25a	0.00	1.00	1.00
25b	0.00	3.00	3.00
29a	0.40	1.75	1.35
30a	1.00	1.00	0.00
31a	1.50	5.00	3.50
31b	0.40	2.80	2.40
34a	0.00	2.33	2.33
36a	0.50	0.50	0.00
36b	0.00	0.00	0.00



A standard deviation for the entire sample was obtained by comparing the differences observed for each plant with the average difference of the sample. The following formula was used:

$$S_D = \sqrt{\frac{\sum (D - \bar{D})^2}{N - 1}} .$$

A standard error was computed in the following manner:

$$S_D = \sqrt{\frac{S_D}{N}} .$$

The t-value for the data was obtained by dividing the average difference of the sample by the standard error. The statistical methods used were from Scheffler (1969).

A t-value of 2.3776 was calculated from the data obtained. The critical values with 30 degrees of freedom are 1.697 at the .05 significance level and 2.457 at the .01 significance level.

Plant #23a showed a much larger difference than any of the other plants tested. This may have influenced the t-value which was obtained. This plant, therefore, was disregarded and the remaining data analyzed. The average difference in the number of seed per head of the remaining 30 plants was 1.69. The calculated t-value for these plants was 7.764. The critical values with 29 degrees of freedom are 1.699 at the .05 significance level and 2.462 at the .01 significance level.

Significant t-values were obtained in both instances. The availability of sufficient pollen apparently increased seed set by a certain degree. The determination of per cent seed set from self-pollinated heads would not appear to be a valid estimate of ovule fertility in the heterozygous ring of fourteen material.

The production of viable megagametes was shown to be variable from plant to plant. Plant #23a showed an excessive increase in seed set as compared with the other plants tested. This was especially noticeable on heads that had been pollinated with pollen from the line 6-3. Such behavior would be expected from a male sterile contaminant. A ring of fourteen chromosomes, however, was observed at metaphase I in plant #23a. The amount of seed produced on the self-pollinated heads of this plant was slightly higher than that produced by self-pollination of the other plants observed. The major difference was the high degree of ovule fertility displayed by plant #23a as opposed to the low ovule fertility observed in the other thirty plants. Several possible explanations can be considered to explain the different behavior of plant #23a. A high degree of complete alternate segregation from the large ring at meiosis may have occurred in this female. If crossing over occurred in an interstitial segment, adjacent segregation of the chromosomes involved would also produce viable gametes. Still another possible explanation

might be that plant #23a had a high survival rate of megagametes containing an extra chromosome. Possibly all duplications were viable in the megagametes of this plant.

#### Metaphase I in F<sub>1</sub> Plants

The extent of chromosome association at metaphase I was determined for all plants examined cytologically. Normal barley contains seven bivalents at metaphase as shown in Figure 2a. One large ring was consistently observed in the F<sub>1</sub> plants from the cross of the complete interchange stock with male steriles. The formation of a chain or several chains was never seen in the material examined.

Figures 2c and 2d illustrate large rings in which it is possible to follow all chromosomes in the ring. Chromosome ends were found to be closely associated with each other in the ring. Certain points in the ring can be seen to be pulling toward the poles. In these rings, it appears that a combination of alternate and adjacent segregation will occur.

A ring in which a portion of the chromosomes appear to be arranged in a zigzag manner is shown in Figure 2b. The remainder of the chromosomes are arranged in a more irregular fashion. The chromosomes which are arranged in the zigzag manner would presumably undergo alternate segregation from the ring.

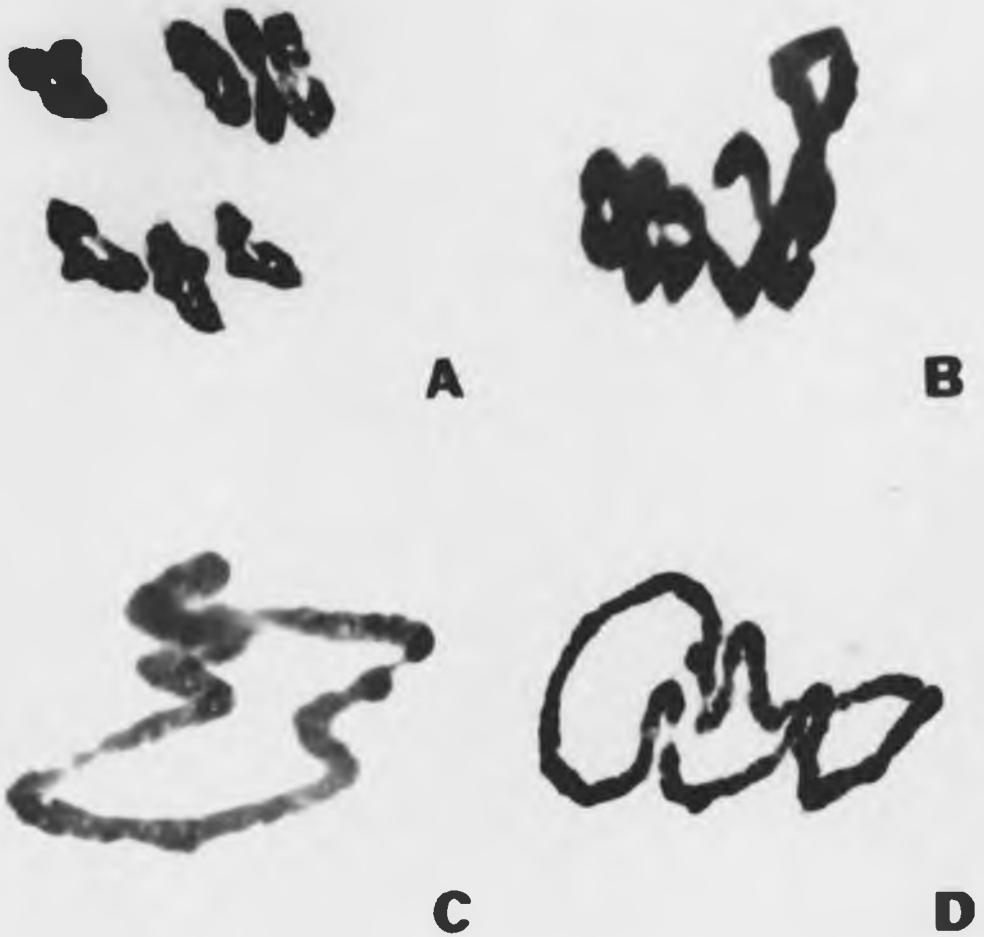


Figure 2. Metaphase I of normal and heterozygous ring of fourteen plants -- (A) exhibits seven bivalents and (B)-(D) exhibit rings of fourteen chromosomes.

Microsporocytes in which all chromosomes in the ring were arranged in a zigzag manner were extremely rare. The fact that some seed was obtained on self-pollinated heads would indicate that some viable pollen was being produced. The amount of selfed seed is much greater than would be predicted if it were assumed that the only viable gametes produced were the result of complete alternate segregation from the large ring, i.e., a complete zigzag arrangement in the metaphase I cells.

Crossing over in the interstitial segments followed by adjacent segregation will produce viable gametes. Crossing over, however, is normally expected to be greatly reduced in interstitial segments. Some of the abnormal metaphase configurations observed could produce viable gametes if crossing over had occurred in interstitial segments.

#### Numerical Disjunction at Anaphase I in F<sub>1</sub> Plants

The manner in which the chromosomes were numerically dividing at anaphase I of meiosis was analyzed. As many cells per plant as possible were scored as to the number of chromosomes at each pole and the number lagging on the equatorial plate. Table 2 shows the frequency of numerical disjunction types in some of the F<sub>1</sub> plants analyzed.

The analysis of numerical disjunction at anaphase I of meiosis revealed some interesting aspects about the

Table 2. Frequency of numerical disjunction types in some of the heterozygous ring of fourteen plants at anaphase I.

	7-7	8-6	7-1-6	9-5	8-1-5	6-2-6	7-2-5	Total observed
<u>Cytology #:</u>								
34	.470	.49	.020	.020				49
38	.490	.39	.050	.050	.020			41
41	.530	.38	.090					34
44	.590	.38		.030				29
49	.410	.53	.060					34
52	.450	.48	.035	.035				58
53	.460	.42	.070		.025	.025		43
57	.450	.26	.140	.030		.030	.09	42
62	.620	.29	.050	.040				61
64	.540	.42	.010	.020	.010			91
65	.460	.49	.020	.030				67
67	.550	.38	.035	.035				29
68	.440	.50	.060					48
71	.560	.39	.010	.030	.010			101
74	.510	.44	.010	.040				100
77	.440	.50		.040	.020			48
157	.500	.41	.050	.030			.01	64
163	.400	.38	.160			.040	.02	45
197	.460	.50		.040				52
<u>Pot #:</u>								
3	.525	.40	.050		.025			40
4	.480	.52						31
9	.530	.35	.090			.030		34
13	.520	.41	.070					46
14	.550	.36	.070	.020				42
60	.620	.32	.060					34
109	.460	.37	.120	.020	.030			93
147	.620	.34		.040				29
153	.630	.29	.060	.020				35

Note: Those plants designated by a cytology number were grown in Bozeman, Montana in the summer of 1976. The plants designated by a pot number were grown in the greenhouse in Tucson, Arizona in the fall of 1976.

behavior of large rings in barley. Approximately 90% of the cells scored segregated either seven chromosomes to each pole or eight to one pole and six to the other. These two classes tended to occur about equally. In the material examined, the fewest number of chromosomes observed passing to one pole was five.

Some mechanism may be present which enables the large ring to distribute chromosomal material fairly equally to all gametes. Such a mechanism would not insure the production of viable gametes, however, the daughter cells produced would contain roughly equal amounts of chromosomal material. Cells which have begun dividing are shown in Figure 3a and 3b. Both cells appear to be dividing equally as far as chromosomal material is concerned. It can be seen, however, that not all of the chromosomes in the ring are segregating in an alternate fashion.

Lagging chromosomes such as that shown in Figure 3c were frequently observed. Quite often the lagging chromosome could still be seen in very late anaphase as shown in Figure 3d. It would appear that none of the gametes obtained from such a division would receive this lagging chromosome. The resulting gametes would contain deficiencies and should not be viable.



Figure 3. Anaphase I of heterozygous ring of fourteen plants -- (A)-(B), early anaphase. (C)-(D), late anaphase with lagging chromosome.



### Pollen Stainability in F<sub>1</sub> Plants

Pollen was classified as to whether or not it stained with IKI, which detects the presence of starch in the pollen grains.

The percentage of stainable pollen was determined for 42 plants which contained a ring of fourteen chromosomes at metaphase I of meiosis. The average amount of unstained pollen was 91.6% in the plants examined. The range was from 81.12% to 100% unstained pollen. Table 3 indicates the distribution within this range.

As far as we know, viable gametes can be obtained from a large ring in only two ways. Complete alternate segregation from a large ring will produce viable gametes if no crossing over occurs in an interstitial segment. If crossing over were to occur in an interstitial segment, the chromosomes involved would have to segregate adjacently in order to produce viable gametes. If random chance alone determined which centromere from each pair of homologous centromeres present in the large ring that a gamete would receive with equal numerical disjunction,  $(\frac{1}{2})^7$  gametes would be expected to contain either all centromeres of the interchanged chromosomes or all centromeres of the normal chromosomes. Therefore,  $2 \cdot (\frac{1}{2})^7$  or 1.56% of the pollen grains resulting from equal numerical disjunction should be viable. It has been noted that equal numerical disjunction (Table 2) occurs in about 50% of the microsporocytes. Thus, only

Table 3. Distribution of per cent unstainable pollen in heterozygous ring of fourteen plants.

% Unstainable pollen	# of plants observed
81.01-82	2
82.01-83	2
83.01-84	0
84.01-85	3
85.01-86	1
86.01-87	2
87.01-88	2
88.01-89	2
89.01-90	2
90.01-91	2
91.01-92	1
92.01-93	5
93.01-94	5
94.01-95	2
95.01-96	1
96.01-97	2
97.01-98	0
98.01-99	0
99.01-100	8

0.78% of the pollen produced from a large ring would be expected to be viable.

Whether or not the frequency of equal numerical disjunction at anaphase I was associated with the amount of unstainable pollen was a question that was raised in connection with the variation observed in pollen stainability. Fifteen  $F_1$  plants which contained a ring of fourteen chromosomes at metaphase I were selected to use in an analysis of the correlation between the frequency of equal numerical disjunction and the amount of unstainable pollen. These plants were chosen because sufficient information was obtained regarding pollen unstainability and frequency of equal numerical disjunction at anaphase I. The data from these plants are shown in Table 4.

The regression analysis conducted was from Scheffler (1969). The first step in the procedures was to calculate a correlation coefficient,  $r$ . The following formula was used:

$$r = \frac{\Sigma XY - \Sigma X \Sigma Y / N}{\sqrt{(\Sigma X^2 - (\Sigma x)^2 / N) (\Sigma Y^2 - (\Sigma Y)^2 / N)}} .$$

The frequency of equal numerical disjunction was designated the X variable and the percentage of unstainable pollen was designated the Y variable. A value of 0.033 was computed for  $r$ .

A t-distribution was used to analyze the  $r$ -value which was obtained. The  $t$ -value was computed in the

Table 4. Comparison of per cent unstainable pollen and frequency of equal numerical disjunction at anaphase I in heterozygous of fourteen plants.

	Frequency of equal disjunction	% Unstainable pollen
<u>Cytology #:</u>		
38	.49	81.49
44	.59	100.00
49	.41	99.62
53	.47	86.73
62	.62	94.07
65	.46	92.94
67	.41	95.54
68	.44	99.42
71	.56	92.56
157	.51	99.00
163	.40	93.01
197	.46	85.21
<u>Pot #:</u>		
9	.53	96.51
14	.55	87.08
60	.62	99.37

Note: Those plants designated by a cytology number were grown in Bozeman, Montana in the summer of 1976. Those plants designated by a pot number were grown in the greenhouse in Tucson, Arizona in the fall of 1976.

following manner:

$$t = \frac{r - 0}{\sqrt{(1-r^2)/(N-2)}} .$$

This computation resulted in a t-value of 0.0091. The critical value with 13 degrees of freedom is 2.16 at the .05 significance level.

In the sample analyzed, there was no correlation between the frequency of equal numerical disjunction at anaphase I of meiosis and the percentage of unstainable pollen. The frequency of equal numerical disjunction does not appear to be as variable as does the amount of unstainable pollen.

It would appear that many factors may be involved in gamete viability from a large ring in barley. The data in Table 2 indicate that the frequency of 8-6 disjunctional types is roughly the same as the frequency of equal numerical disjunction. This would mean that a large number of gametes received an extra chromosome. Some of these gametes could be viable.

The ability to stain with IKI may not be an indication of pollen viability. It does demonstrate the development of the pollen to the point of the filling of the pollen grain with starch. This would not necessarily mean that the pollen grain would be able to germinate. Pollen stainability, however, should be a fair means of observing definite differences in the material examined.

Metaphase I in F<sub>2</sub> Plants

The metaphase configurations of some of the F<sub>2</sub> plants were analyzed. These observations are summarized in Table 5.

Table 5. Metaphase I configurations of F<sub>2</sub> plants from the cross of the complete interchange stock with male steriles.

Metaphase I Configuration	Observed # of Plants
7II	17
⊙14	17
5II+⊙4	2
2n+1	4
2n+1+1	1

The number of plants containing seven bivalents and the number containing a ring of fourteen chromosomes occurred in a 1:1 ratio. Viable gametes containing seven normal chromosomes and viable gametes containing the complete interchange chromosomes would be expected to be equally frequent. This supposition is supported by the data obtained. It would not appear that there is a competitive advantage for either of these two types of gametes.

Plants containing one extra chromosome and plants containing two extra chromosomes were observed. Duplications are being transmitted at least through the egg and

possibly through the pollen. The low number observed containing duplications might suggest that transmission through the pollen is not frequent. Pollen containing seven chromosomes is possibly more competitive.

Two plants were observed which contained five bivalents and ring of four chromosomes. This could be explained by the occurrence of two crossovers in the large ring in either the egg or the pollen. A single crossover in each of two separate interstitial segments would need to occur. The chromosomes involved in the crossover would have to be positioned in the ring such that each of the interchange chromosomes involved was attached to the normal chromosome which was homologous with the interstitial segment of the other interchange chromosome involved in one of the two crossovers. Alternate segregation of the chromosomes involved in the crossover would result in deficiencies which should not be viable. Adjacent segregation of the chromosomes involved would result in the production of a gamete in which two of the chromosomes are simply reciprocal interchanges. The reciprocal interchange chromosomes would be viable only in combination with the five remaining normal chromosomes. The combination of this gamete with one which contains seven normal chromosomes would result in a plant which contained five bivalents and a ring of four chromosomes.

Metaphase I in F<sub>1</sub> Plants from the Cross  
of the Heterozygous Ring of Fourteen  
Plants with a Normal Line

A few F<sub>1</sub> plants from the cross of heterozygous ring of fourteen plants with the normal line, 6-3, were analyzed at metaphase I of meiosis. These results are summarized in Table 6. Plants containing seven bivalents and plants containing a ring of fourteen chromosomes were observed. The small number of plants observed makes it difficult to determine if the two types occurred with equal frequency. Trisomic plants were also observed. This would indicate that transmission of an extra chromosome is occurring at least in the female.

Table 6. Metaphase I configurations of F<sub>1</sub> plants from the cross of heterozygous ring of fourteen plants with the normal line 6-3.

Metaphase I Configuration	Observed # of Plants
7II	6
@14	3
2n+1	4

Somatic Chromosomes in F<sub>1</sub> Plants

Very little is known about the complete interchange stock used in this study. The somatic chromosomes of some F<sub>1</sub> plants were examined to determine if there were any



gross differences in chromosome morphology. The somatic chromosomes of an  $F_1$  plant are shown in Figure 1b. Seven of the chromosomes are normal and seven are from the complete interchange stock.

Some of the interchange chromosomes can be quite easily distinguished from normal chromosomes. Chromosome a in Figure 1b is obviously longer than the other chromosomes present. This would indicate that the length of the interchanged segments was variable for the chromosomes involved in the production of the complete interchange stock.

Chromosome b in Figure 1b contains a satellite which is much longer than the satellite of a normal chromosome 6 or 7. The satellite on the interchanged chromosome appears as long as either arm on that chromosome.

The location of the breakpoints in the interchange chromosomes may be a factor in the low fertility observed in the plants containing a ring of fourteen chromosomes. Chromosomes of uniform length with median centromeres have usually been associated with plants displaying an excess of alternate segregation from large rings.

## SUMMARY

F<sub>1</sub> plants containing a ring of fourteen chromosomes were obtained by crossing a complete interchange stock onto male steriles. These F<sub>1</sub> plants were as vigorous as normal plants, however, anther development and ovule fertility were obviously reduced.

It was demonstrated that by supplying the F<sub>1</sub> plants with a sufficient amount of pollen, the amount of seed set was increased. The amount by which seed set was increased varied for different plants. Apparently ovule fertility was influenced by at least two factors. The first factor affecting ovule fertility was the availability of pollen. The second factor was differences in control of chromosomal segregation from the large ring.

Chromosome association at metaphase I of meiosis was examined in many of the F<sub>1</sub> plants. The formation of one large ring was consistently observed. The association of the chromosomes in the ring was very close.

Numerical disjunction of chromosomes from the large ring at anaphase I of meiosis was analyzed. The predominant types of segregation were either equal numerical disjunction or 8-6 disjunction. Lagging chromosomes could often be seen even at very late anaphase.

The ability of pollen to stain with IKI was determined in the  $F_1$  plants. Stainability was found to vary between none and around 20%. Less than 1% would be predicted by random segregation of chromosomes from the large ring. It was seen that there was no correlation between equal numerical disjunction and pollen stainability.

In the  $F_2$  plants, there was a 1:1 ratio of plants with seven bivalents and plants containing a ring of fourteen chromosomes. Trisomics and double trisomics were observed in the  $F_2$ . Plants containing five bivalents and a ring of four chromosomes were seen indicating that some crossing over in the interstitial segment was occurring.

In  $F_1$  plants from the cross of the complete interchange stock with the normal line 6-3, plants with seven bivalents and plants containing a ring of fourteen chromosomes were observed. It appeared that in the progeny of this cross, there was a higher recovery rate of trisomics than in the  $F_2$ .

The somatic chromosomes of an  $F_1$  plant from the cross of the complete interchange stock with male steriles were observed. Some of the interchange chromosomes could be distinguished from the normal chromosomes. One interchange chromosome was definitely longer than normal. Another interchange chromosome had a satellite which was much longer than the satellite of a normal chromosome 6 or 7.

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