# THE DEVELOPMENT OF DEFICIENT ANEUPLOIDS IN DURUM WHEAT

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by

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# INTRODUCTION

Deficient[1] aneuploids are proving of great value in the systematic genetic analysis of Triticum aestivum L. emend. Fiori et Paoletti (= T. vulgare) (2n = 42). More and more wheat breeders and geneticists are utilizing the monosomic (20" + 1') and nullisomic (20") lines developed by Dr. E.R. Sears in the variety Chinese Spring. These aneuploids, by providing a means of associating genes with certain chromosomes, have simplified inheritance studies in the hexaploid wheats. Moreover they make possible the controlled synthesis of new varieties since specific chromosomes from other varieties, species or even genera may be substituted for the deficient one.

The development of a monosomic or nullisomic series in the emmer wheats would aid greatly in the genetic study of that group. A program is underway at the University of Saskatchewan to develop such a series in T. durum Desf. var. Golden Ball (2n = 28). This program involves the use of deficient Chinese Spring lines as well as irradiation methods.

The material for the present paper was obtained from the aforementioned program. Information is presented showing

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[1] Monosomic and nullisomic plants having respectively one or both members of a single chromosome pair missing are referred to as deficient aneuploids. In contrast, plants having a certain chromosome represented more than twice (trisomes and tetrasomes) are referred to as duplicated aneuploids.

that it may be very difficult, if not impossible, to obtain deficient lines in Golden Ball.

## THE BACKCROSS PROGRAM

### Review of Literature

Genome Designations in Triticum

All the species of Triticum fall into one or the other of three groups; einkorn, emmer or vulgare. The chromosomes of these groups, which have seven, 14 and 21 pairs respectively, exhibit characteristic pairing relations in their various intra- and intergroup hybrids. Studies of this pairing behaviour led to the concept that the various Triticum species have from one to three distinct sets of seven chromosomes. These chromosomal sets or genomes have been designated A, B and D[2] by Kihara & Lilienfeld (1932) and Kihara (1940). From pairing relations in intergroup hybrids the einkorn wheats have been designated AA, emmer AABB and vulgare AABBDD (Kihara, 1924; Lilienfeld and Kihara, 1934).

This scheme of genomic individuality has, in general, borne up well under extensive testing. There is ample evidence however that the genomic designations are over simplifications. As Sears (1948) has pointed out "The concept of relationships by genomes tends to over look the fact that in polyploid

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[2] American and British workers commonly use C instead of D but D has priority since it was used by Kihara in 1924 while Gaines and Aase did not propose C until 1926.

species translocations may occur between chromosomes belonging to different genomes with a consequent tendency for loss of individuality of the genomes."

## Aneuploid Series in Plants

The einkorn, emmer and vulgare wheats form an euploid series having a basic chromosome number of seven. In contrast, aneuploids do not have chromosome numbers which are exact multiples of a basic haploid number.

Aneuploids in Datura. The first complete aneuploid series was obtained in the Jimson weed Datura Stramonium (2n = 24). Blakeslee, Belling and their co-workers (Blakeslee & Belling, 1924) found 12 different 25-chromosome mutants in this plant. Each of these 12 different trisomes (2n + 1), called by Blakeslee the "twelve apostles", was morphologically distinguishable (Blakeslee & Belling, 1924 and Blakeslee, 1930). Pollen tubes of unbalanced gametes grew more slowly than pollen tubes of normal gametes (Buckholz & Blakeslee, 1930) with the result that the extra chromosome was rarely transmitted by the male.

Aneuploids in Nicotiana. The first complete series of deficient aneuploids was obtained in the amphidiploid Nicotiana Tabacum (2n = 48). Clausen & Cameron (1944) reported obtaining the last of 24 possible monosomic (2n - 1) types and demonstrated their usefulness in associating genes with whole chromosomes. In both N. Tabacum (Olmo, 1935) and N. rustica (Lammerts, 1932) the monosomic condition was demon-

strated to be transmitted frequently by the egg but only rarely by the pollen. No nullisomes were obtained in Nicotiana. In this connection Lammerts (1932) has suggested that a species must be highly polyploid before n - 1 gametes and 2n - 2 zygotes will function.

Aneuploids in Triticum aestivum. Although various deficient aneuploids have been reported in Triticum species from time to time (Huskins, 1946; Kihara, 1939; Thompson, 1928; Uchikawa, 1938 and Love, 1940) few, until recently, had ever been obtained in any one variety. Sears (1939) reported obtaining five monosomic plants from a haploid of T. vulgare var. Chinese Spring pollinated with normal pollen. In the first of several papers relating his progress in the establishment of a nullisomic series, Sears (1939, 1941, 1944) postulated several ways in which the original aberrations could have originated. The development of the complete nullisomic series in Chinese Spring was aided by the fact that one of the plants obtained early in the program was asynaptic, giving rise to further deficient lines.

Based on their pairing relationships with T. durum, Sears designated his deficient lines I - XIV and XV - XXI depending on whether or not the missing chromosome was homologous to ones in the AB or D genomes. Within these two groups the lines were numbered in the order in which the nullisomic types were found (Sears, 1944).

Under favorable environmental conditions few of the monosomes differ appreciably from normal. Most of the nulli-

somes, however, are strikingly abnormal and all exhibit reduced vigor and fertility (Sears, 1944). Transmission of the deficiency by the egg is about 75% regardless of the chromosome concerned while pollen transmission varies from 1 - 15% depending on the line (Sears, 1944).

Sears' monosomic lines occasionally give rise to telocentric chromosomes (chromosomes deficient for one arm) and isochromosomes (chromosomes deficient for one and duplicated for the other arm) because of misdivision of the univalent's centromere (Sears, 1944). Morrison (1953) found that centromere misdivision in wheat monosomes takes place in 12% of the cells. The transmission frequencies of chromosome IX univalent isochromosomes and telocentrics have shown no striking difference from that for the normal monosome (Sears, 1946).

Larson (1949) has recently outlined the use of deficient aneuploid lines in the genetical study and breeding of common wheat. The method of monosomic analysis has already proved valuable in inheritance studies of numerous morphological and physiological characters (Sears, 1947; Sears & Rodenhiser, 1948; Unrau, 1950; Larson, 1953).

Aneuploids in Emmer Wheats. Sears (1939) early recognised that "By backcrossing these hybrids" (Chinese Spring lines I - XIV x emmer) "to the emmer parent, it should be possible to obtain monosomics in emmer, if they are viable ....". Although at least two programs (Sears, 1944, Unrau[3]) to obtain

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[3] Personal communication with Dr. B. C. Jenkins.

monosomes or nullisomes in an emmer wheat have been initiated, so far as the writer is aware no success has yet been reported.

The development of deficient aneuploids in emmer wheat by crossing with Chinese Spring lines is similar to earlier studies made on pentaploid hybrids. It is desirable, therefore, to briefly review this earlier work.

The cytogenetics of the so-called "pentaploid hybrids" between emmer and vulgare wheats has been extensively studied in the past. Kihara and his coworkers were especially active in this work. The formation of 14" + 7' in pentaploid hybrids was first observed by Kihara (1919). His results were soon confirmed by Sax (1921, 1922). This characteristic pairing behaviour led to symbolizing the emmer genomes AB and the vulgare genomes ABD. In the pentaploid hybrid it was considered that the AB of vulgare paired with the AB of emmer leaving the seven chromosomes of the D genome as univalents. Although this type of pairing behaviour has frequently been reported, Love (1941) has shown that hybrids of certain varieties of emmer and vulgare have striking irregularities.

At meiosis, the bivalents of pentaploid hybrids behave normally while the univalents divide equationally at first anaphase and pass at random to the poles at second anaphase (Kihara, 1919; Sax, 1922). Although it was realised that univalents were regularly lost due to lagging at second anaphase, the frequencies of various chromosome numbers in $F_2$ and subsequent generations were not what could be expected on random union of resulting gametes. Plants with the parental

numbers (14" and 21"), appeared far more frequently than those with intermediate numbers whereas the reverse would be expected if all types of gametes were functional.

Much controversy developed in trying to account for the missing types. Kihara (1924) attributed the results to failure of certain classes of zygotes. Other investigators, Watkins (1925), Sax (1923) suggested that pollen with either fourteen or twenty-one chromosomes was more likely to function than that with intermediate numbers. Kihara (1925) concluded from results of backcrossing pentaploid hybrids to the pure parents that the pollen grains which function are those which have a chromosome number similar to that of the female.

Kihara & Matsumura (1940) present their summarised results of fifteen years experience with pentaploid hybrids somewhat as follows (Sears, 1948).

1. Univalents tend to go to the poles in groups at second anaphase, thus increasing the proportion of 14- and 21-chromosome gametes.
2. Univalents tend to be lost.
3. Eggs and especially pollen with intermediate chromosome numbers tend to be eliminated. Pollen with 21 chromosomes is favored over that with 14.
4. Most zygotes formed by eggs with 14 chromosomes and pollen with around 21 chromosomes fail to survive.
5. Zygotes with from one to six D chromosomes or pairs are largely eliminated, except in certain crosses, notably (*T. polonicum* x *T. spelta*).
6. Germination and viability are lower for embryos with low chromosome numbers.

Thompson and Cameron (1928) reciprocally backcrossed

pentaploid hybrids to both parents in order to study univalent transmission by egg and pollen. They summarized their results as follows:

1. In nearly every case the gametes with numbers of extra vulgare chromosomes intermediate between 0 and 7 were in much smaller proportions than were expected, if all gametes are capable of functioning.

2. In most cases the gametes with no extra univalent chromosomes (14 in all) were much more numerous than those with 7 (21 in all).

3. There are marked differences between the male and female gametes of $F_1$, the latter showing less elimination of intermediate numbers than the former, though by no means all eggs are capable of functioning and a proportion of functioning pollen grains do have intermediate numbers.

4. There are marked differences between the different kinds of hybrids with respect to their functional gametes, and slight differences depending on whether a hybrid was backcrossed with the vulgare or with the emmer-type parent.

They concluded by stating, "It is dangerous to draw sweeping conclusions from a limited variety of material."

## Material and Methods

A program to develop a deficient aneuploid series in emmer wheat was initiated in 1950 by Dr. B. C. Jenkins of the Field Husbandry Department. In that year crosses were made between the durum wheat variety Golden Ball and those nullisomic or monosomic Chinese Spring lines then available which corresponded to the A & B genome chromosomes. Subsequently, as the remainder of the required Chinese Spring lines became available, all necessary crosses were made. Hybrids thus

obtained and found by cytological examination to be monosomic for a certain chromosome of the A or B genome (13" + 8') were backcrossed to Golden Ball. When the present investigation was begun most of the hybrids had undergone two backcrosses to the emmer parent.

The material for this study was chosen with a view to finding the fastest way of losing the seven D-genome chromosomes contributed by Chinese Spring while at the same time maintaining the monosomic condition in the A or B genome chromosome. For this purpose first backcross plants were grown of those lines in which reciprocal crosses had been made and for which seed was available. Likewise second backcross plants were grown of certain lines where reciprocal crosses had been made both in first and second backcross generations. Reference to Tables I and II will show the pedigree of the material used.

TABLE I

SUMMARY OF FIRST BACKCROSS MATERIAL EXAMINED

| Chromosome line | No. of plants examined | |
|---|---|---|
| | Direction of cross | |
| | F | M |
| IV | 9 | 6 |
| V | 10 | 9 |
| VI | 4 | 2 |
| VIII | 8 | 0 |
| XII | 7 | 5 |
| IX | 8 | 5 |
| XIV | 10 | 2 |
| Total | 56 | 29 |

TABLE II

SUMMARY OF SECOND BACKCROSS MATERIAL EXAMINED

| Chromosome line | Direction of first backcross | Plants examined | | | |
|---|---|---|---|---|---|
| | | Direction of second backcross | | | |
| | | F | | M | |
| | | Cytology of $BC_1$ | No. examined | Cytology of $BC_1$ | No. examined |
| IV | F | 14" + 4' | 8 | 14" + 4' | 9 |
| | M | 13" + 6' | 4 | 13" + 6' | 5 |
| | | 13" + 7' | 5 | 13" + 7' | 3 |
| V | F | 13" + 5' | 9 | 13" + 5' | 8 |
| | M | 14" + 1' | 2 | 13" + 3' | 7 |
| | | 13" + 3' | 3 | | |
| VI | F | 13" + 4' | 3 | 13" + 4' | 3 |
| VIII | F | 13" + 4' | 3 | 13" + 4' | 1 |
| Total | | | 37 | | 36 |

The chromosome line, that is the A or B chromosome in the monosomic condition, is indicated by a Roman numeral. The direction of the cross is indicated by the initial F or M. The designation VF thus means that the plant was monosomic for chromosome five and that in the first backcross the $F_1$

hybrid was used as female parent. Similarly IV MF indicates that an $F_1$ plant monosomic for chromosome four was used as male parent in the first backcross and that the resulting first backcross plant was used as female parent in the second backcross.

The material for this study was planted in pots on January 25, 1955 and grown in the Field Husbandry Department greenhouses. Specimens for cytological examination of PMC's were collected as they became available and fixed in Carnoys fluid 6:3:1 (95% ethyl alcohol:chloroform:glacial acetic acid). Counts were made from fresh acetocarmine smears at a magnification of x 475. The numbers of plants in the different lines for which counts were obtained are given in Tables I and II. Photomicrographs were mainly of fresh smears. Certain slides were made permanent by the use of Zirkle's mounting fluid[4].

## Results

### Direction of Cross and Transmission of Univalents

First Backcross. The effect of direction of cross on the number of univalent chromosomes transmitted in the first backcross is graphically presented in Fig. 1 and 2. There were just over half as many counts of first backcross plants in which the $F_1$ had been used as male parent as there were of first backcross plants in which the $F_1$ had been used

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[4] See Appendix A.

## EXPLANATION OF FIGURES 1 - 6

Fig. 1 and Fig. 2*

Frequency distributions of gametes carrying varying numbers of univalents in reciprocal backcrosses between $F_1$ plants (Chinese Spring (monosomic ) x Golden Ball) and Golden Ball.

Fig. 3 and Fig. 4**

Frequency distributions of gametes carrying varying numbers of univalents in reciprocal backcrosses between $F_1$ plants (Marquis x *T. durum* and *T. dicoccum*) and the 14 chromosome parent.

Fig. 5 and Fig. 6**

Frequency distributions of gametes carrying varying numbers of univalents in reciprocal backcrosses between $F_1$ plants (*T. aestivum* var. "of Chinese origin" x *T. durum* ) and *T. durum*.

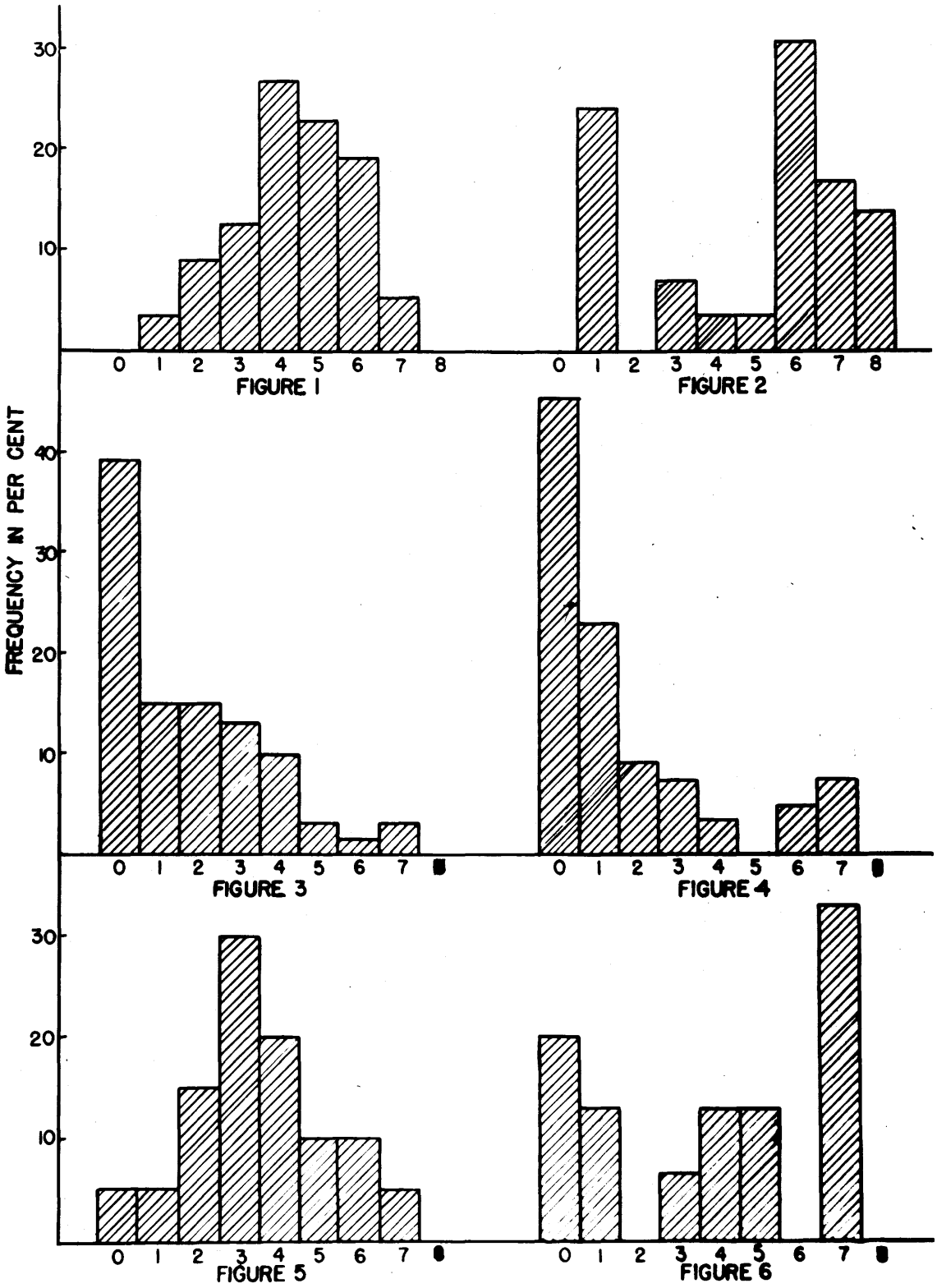
* The cytological records from which these histograms were prepared are presented in Appendix B.

** These histograms were prepared from the data of Thompson & Cameron (1928).

# UNIVALENTS CARRIED BY

EGGS POLLEN

(SEE TEXT FOR FURTHER EXPLANATION OF FIGURES)



## EXPLANATION OF FIGURES 7 - 10*

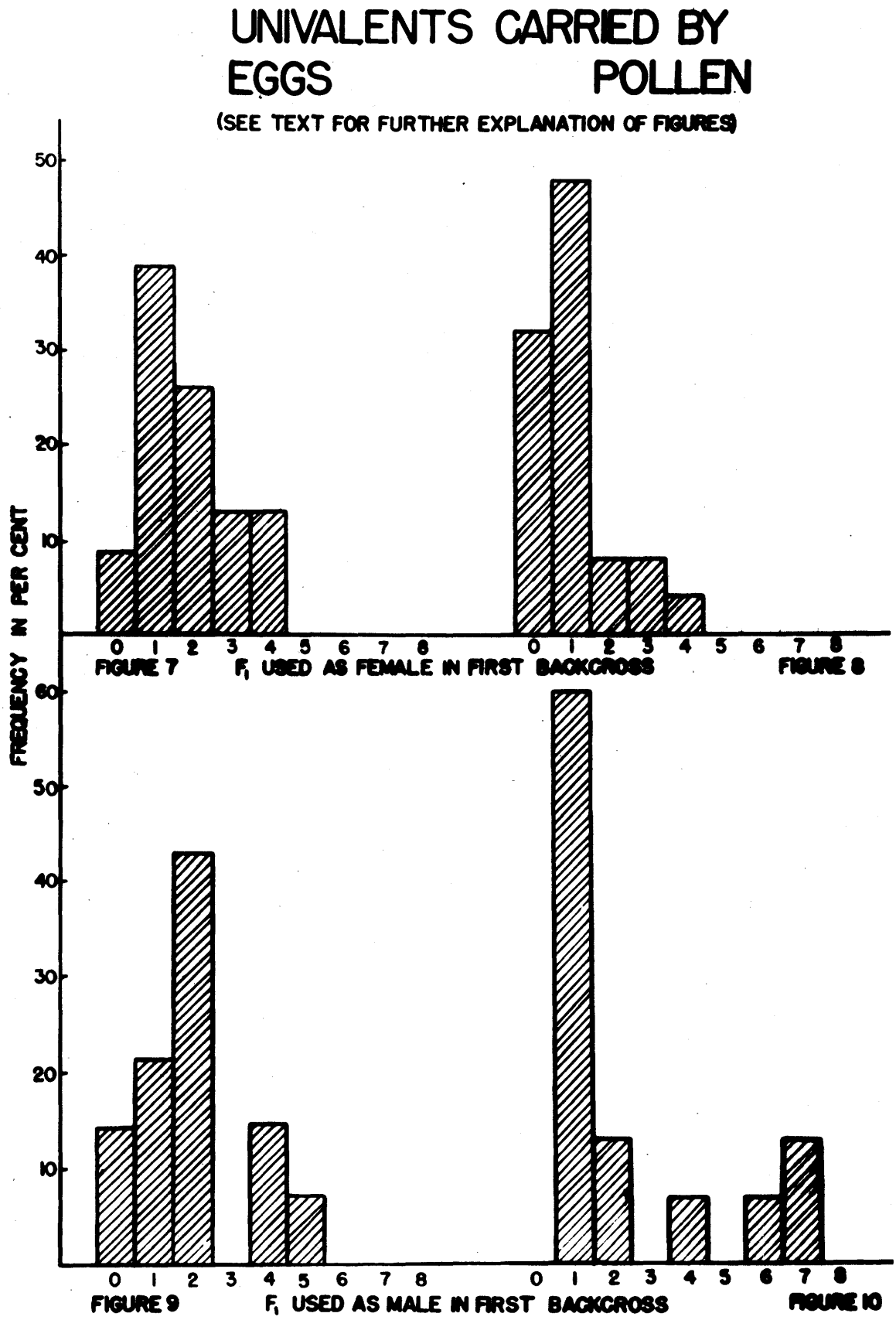
Fig. 7 and Fig. 8.

Frequency distributions of gametes carrying varying numbers of univalents in reciprocal second backcrosses between $BC_1$ plants and Golden Ball. The $BC_1$ plants used in these backcrosses were obtained from the cross ($F_1$ x Golden Ball).

Fig. 9 and Fig. 10.

Frequency distribution of gametes carrying varying numbers of univalents in reciprocal second backcrosses between $BC_1$ plants and Golden Ball. The $BC_1$ plants used in these backcrosses were obtained from the cross (Golden Ball x $F_1$)

* The cytological records from which these histograms were prepared are presented in Appendix B.


UNIVALENTS CARRIED BY
EGGS
POLLEN
(SEE TEXT FOR FURTHER EXPLANATION OF FIGURES)
FREQUENCY IN PER CENT
50
40
30
20
10
0 1 2 3 4 5 6 7 8
0 1 2 3 4 5 6 7 8
FIGURE 7
F1 USED AS FEMALE IN FIRST BACKCROSS
FIGURE 8
60
50
40
30
20
10
0 1 2 3 4 5 6 7 8
0 1 2 3 4 5 6 7 8
FIGURE 9
F1 USED AS MALE IN FIRST BACKCROSS
FIGURE 10

as female (89 and 56 respectively). To permit ready comparison of male and female transmission the frequencies of the various classes have been expressed in terms of per cent.

Second Backcross. The results of a check on univalent transmission by egg and pollen in the second backcross are presented in Fig. 7, 8, 9 and 10. In presenting these results several pertinent facts should be mentioned. First, it was found that the material designated IV FF and IV FM and two of the plants V MF had originated from first backcross plants which were not monosomic (see Table II). This fact was discovered when tardy examination was made of cytological material collected the year previously. Second, it should be pointed out that, unlike the $F_1$ plants used to obtain first backcross progenies, first backcross plants used to obtain second backcross progenies do not necessarily carry the same number of univalents. While both of these factors could complicate comparison of male and female transmission of univalents in second backcross generations, they are, in fact, not too serious here. Although the first backcross parent of IV FF and IV FM had the cytology 14" + 4', a comparison of univalent transmission is not interfered with since both directions of cross were equally affected. In the case of the V MF plants however, only one direction of cross was involved. Fortunately only two counts were influenced. The fact that the same first backcross plant, (or one having identical or very similar cytology - see Table II), gave rise to the reciprocal second backcross material used in these investigat-

ions largely eliminates the second possible complication in comparisons. One difficulty introduced into the comparisons arises from the fact that in certain of the reciprocal crosses, different numbers of plants were examined. The exact number of plants in the various reciprocal crosses that were cytologically examined are presented in Table II.

On the basis of the direction of the previous backcross, the second backcross data were divided into two parts, that where the $F_1$ hybrid was used as maternal parent in the first backcross (Fig. 7 and 8) and that where it had been used as paternal parent (Fig. 9 and 10). Within each of these two main groupings the transmission of univalents by egg and pollen may be compared.

Direction of Cross and Transmission of Deficiency

Twenty-nine first backcross plants which had been obtained by pollinating Golden Ball with $F_1$ pollen were cytologically examined. Of these nine or 31.0% were monosomic for the emmer chromosome, that is, they showed 13 bivalents plus varying numbers of univalents (see Fig. 11). Similarly, cytological examination of 56 plants from the reciprocal cross showed that 19 or 33.9% were monosomic.

Of the second backcross plants only nine or 16.7% of the total population were monosomic. The breakdown of this average figure for the various cross combinations was as follows:

| FF | FM |
|---|---|
| $\frac{2}{15}$ = 13.3% | $\frac{1}{12}$ = 8.3% |
| **MF** | **MM** |
| $\frac{3}{12}$ = 25% | $\frac{3}{15}$ = 20% |

Although a total of 37 plants was thus found to be monosomic for an emmer chromosome, none had lost all of the D-genome chromosomes contributed by Chinese Spring. Six plants had, however, metaphase or anaphase configurations showing 13" + 2'. They had lost all but one of the "extra" D-genome chromosomes. Fig. 16 shows a metaphase smear of such a plant.

In several of the plants carrying 13 bivalents plus two or more univalents it was noticed that there was a tendency for two of the univalents to be loosely associated at metaphase. Fig. 13, 14, 15 and 20 illustrate this tendency. It was noticed too that the number of univalents seen at anaphase I often varied in different cells of the same smear. Some anaphase stages showed all the univalents tardily undergoing equational division while other cells on the same slide showed two less univalents (cf. Fig. 17 and 21). Even at metaphase I a few instances were recorded where the number of bivalents varied from cell to cell. Some showed 13 regular and one loose pair (Fig. 22) while others showed only 13 bivalents plus two more univalents than in the previously

## Description of Plate I

Acetocarmine smears of plants obtained from the Backcross Program. Magnification x 1000 except where otherwise stated.

Fig. 11. Prometaphase of a plant having 13" + 5'.

Fig. 12. Diakinesis of previous plant. Note side by side association of two univalents.

Fig. 13. Metaphase of a plant having 13" + 5'. Note how two univalents are closely associated.

Fig. 14. Metaphase of previous plant showing a loose end-to-end association of two univalents. x 900.

Fig. 15. Prometaphase of a plant with 13" + 2'. Note univalents united by a thin chromatin strand.

Fig. 16. Metaphase of a plant with 13" + 2'.

Fig. 17. Anaphase showing three univalents tardily dividing. Note that one of the divided univalents appears to be broken.

Fig. 18. Metaphase figure of previous plant showing 13" + 3'.

Fig. 19. Anaphase figure showing one univalent, one telocentric and one fragment chromosome tardily dividing.

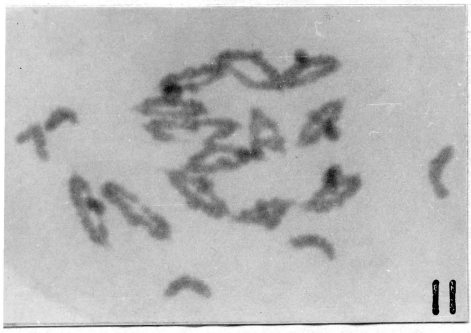
Fig. 20. Metaphase of same plant as shown in Fig. 16. Note close association of univalents. x 1035.

Fig. 21. Anaphase figure of same plant as shown in Fig. 17. Note here that 14 chromosomes have gone to the poles while only one univalent lags. x 1035.

Fig. 22. Metaphase figure of previous plant. Note one open bivalent and one univalent. x 1035.

Fig. 23. Metaphase of a plant having 15" + 2'.

Fig. 24. An aberrant microspore formed by the previous plant. x 640.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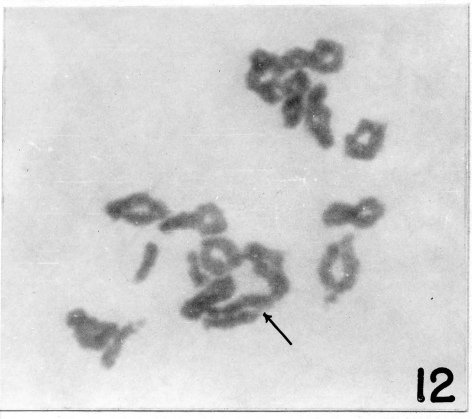
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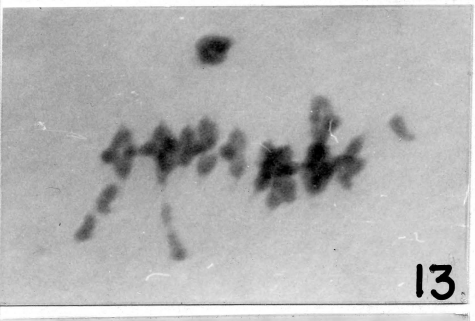
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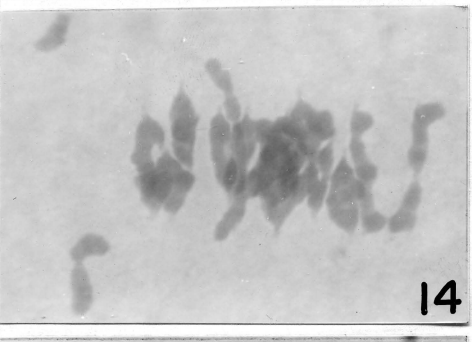
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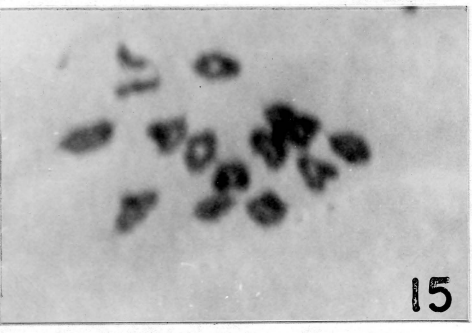
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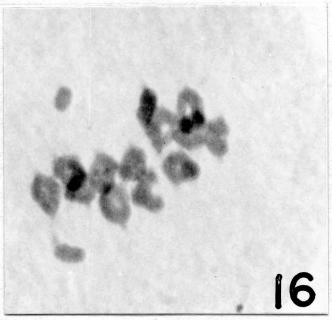
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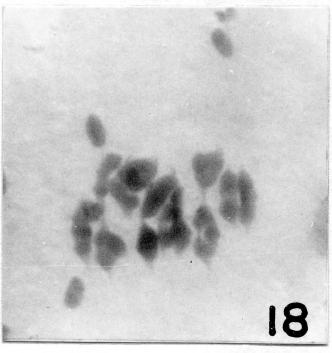
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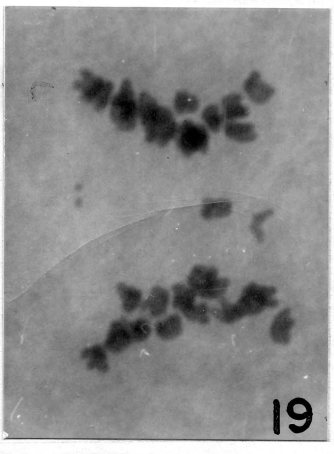
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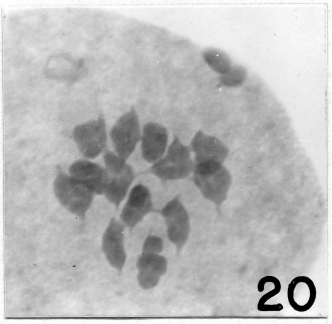
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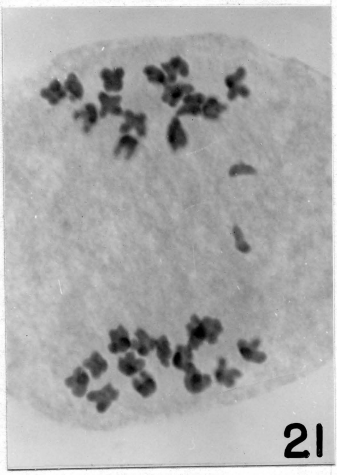
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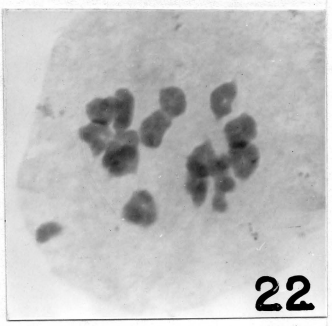
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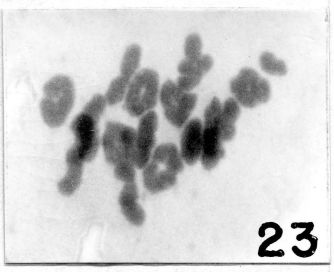
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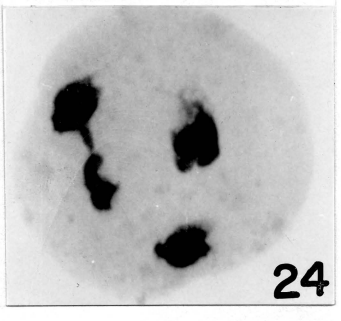
24

PLATE I

mentioned cells (Fig. 18).

Plants with aberrant cytology were also observed. Fig. 19 shows the anaphase I configuration of a plant carrying one univalent, one telocentric and one fragment chromosome in addition to 14 regular bivalents. One plant with apparently 15" + 2' (Fig. 23) showed irregular anaphase I stages which resulted in the formation of abnormal microspores (Fig. 24).

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## Discussion

### Direction of Cross and Univalent Transmission

First Backcross. The frequency of functional gametes having various numbers of univalents is shown by Fig. 1 and 2 to depend greatly on whether the egg or pollen is concerned. Whereas Fig. 1 indicates that eggs most frequently carry intermediate numbers of univalents, Fig. 2 shows that pollen with either high or low numbers of univalents is most often successful in fertilization. Pollen with intermediate numbers of univalents seems to be largely eliminated.

These results agree, in part at least, with those obtained by previous workers. As has already been mentioned in the Review of Literature, the many workers who studied chromosome numbers in $F_2$ and later generations of pentaploid hybrids found that their results did not agree with expectation. Instead of a majority of the $F_2$ plants having intermediate chromosome numbers (approximately 35 would be expected on the basis of random assortment of univalents), parental types

having 14 and 21 pairs occurred most frequently. The proportion of $F_2$ plants having approximately 35 chromosomes was very small in comparison with expectation. To explain these results various factors were postulated as operating. These have been presented in the Review of Literature. Suffice to say here that the random union of eggs and pollen shown by this investigation to be functional would not give rise to the $F_2$ results commonly described. The preponderance of functional pollen having either low or high numbers of univalents is in line with the reported $F_2$ results but the close to theoretical distribution shown by functional eggs is not. Of course, elimination of plants containing certain chromosome numbers at the zygote or seedling level, or some kind of selective fertilization could possibly operate.

As pointed out by Thompson & Cameron (1928) the chromosome numbers of functioning gametes can not be satisfactorily determined by studying $F_2$ results. To determine the chromosome numbers of those gametes which are capable of functioning these workers therefore utilized the backcross method. The results of their findings have been graphically presented in Fig. 3 - 6. The results presented in Fig. 3 and 4 are the grouped data obtained by Thompson and Cameron in first backcrosses to the emmer parent of the pentaploid hybrids (Marquis x T. dicoccum) and (Marquis x T. durum). Since the results of these two backcrosses were similar their grouping was considered permissible.

The preponderance of 14-chromosome gametes is very

evident in both directions of cross. Clearly gametes with intermediate numbers of extra vulgare chromosomes, although showing less elimination in the female than in the male, are in much smaller proportions than theoretically expected if all are capable of functioning. Although the difference is not pronounced there seems to be some tendency for gametes carrying all or nearly all the seven extra chromosomes to be more prevalent in the male than in the female.

The results obtained by Thompson and Cameron in reciprocal backcrosses of the pentaploid hybrid ("vulgare-2" x T. durum) to the emmer parent are presented in Fig. 5 and 6. "Vulgare-2" is described as "of Chinese origin" while the variety of T. durum used was Iumillo. In contrast to the results shown by Fig. 3 and 4, those in Fig. 5 and 6 are strikingly similar to the ones obtained in the present investigation (cf, Fig. 1 and 2). It is evident that the variety of T. vulgare and perhaps also of T. durum used in the production of a pentaploid hybrid influences the frequency with which various chromosome numbers appear in following generations. Perhaps certain combinations of chromosomes are better able to function as unbalanced eggs and zygotes than are others.

The possible significance of the absence of gametes carrying no univalents in our material will be discussed later. Gametes carrying eight univalents (see Fig. 2) would result if all univalents in our $F_1$ hybrid (13" + 8') went to one pole at anaphase II.

Second Backcross. The frequency of second backcross gametes carrying various numbers of univalents is shown in Fig. 7, 8, 9 and 10. The tendency noted in the results of the first backcross for functioning pollen to carry "all of none" of the extra seven vulgare chromosomes is continued in these results. (Fig. 8 and 10). Similarly the less severe elimination of eggs carrying intermediate numbers of univalents is again evident in the second backcross data (Fig. 7 and 9).

The first backcross parents of the plants represented in Fig. 7 and 8 were developed by using $F_1$ hybrids as female parent. According to the results presented (Fig. 1) the majority of such first backcross plants would have intermediate numbers of univalents. A check of the pedigrees showed these lines to carry either 4 or 5 univalents (see Table II). The consequence of this parentage is well illustrated by Fig. 8. Since functional pollen is most likely to have either "all or none" of the seven extra chromosomes, it may be expected that pollen from plants carrying intermediate numbers of chromosomes will carry none or very few univalents. This is the result illustrated by Fig. 8. In comparison, Fig. 7 shows that the female gametes experience a less severe selection. The slightly skewed frequency distribution is undoubtedly a result of the varied parentage of this group.

Referring now to Fig. 9 and 10 it will be noticed that the maximum number of univalents carried by both egg and pollen is greater than was found in the previous second backcross results. This undoubtedly is due to the fact that these plants

had a different pedigree. The first backcross parents of this group were obtained by using the $F_1$ hybrids as pollen parent. On the basis of the results shown in Fig. 2 such first backcross plants would most likely carry either small or large numbers of univalents. A check of the pedigrees showed that the plants in question carried either 1, 3, 6 or 7 univalents (see Table II).

The "all or none" selection of pollen could, in certain of these cases, result in large numbers of univalents being carried. A slight tendency for this to occur is shown by Fig. 10 which obviously comes closer to representing the situation shown in Fig. 1 than does Fig. 8. Even here there is a strong selection of gametes with 14 chromosomes. The transmission of univalents by the egg as shown by Fig. 9 is fairly well in accord with expectations based on first backcross results.

From these results of reciprocal backcrosses certain factors come to light as influencing the number of univalents carried from one generation to the next. Firstly, pollen with intermediate numbers of chromosomes is largely eliminated while eggs are not. Secondly, pollen carrying either few or nearly all of the seven vulgare chromosomes is functional. Finally, the direction of the first backcross influences slightly the number of univalents carried by the pollen in the second backcross. On examining the relative importance of these factors it appears that the most rapid method of losing unwanted D-genome chromosomes is by using hybrid plants as pollen parent. Plants still carrying high numbers of univalents in the second

backcross because both $F_1$ and first backcross plants had been used as pollen parent (Fig. 10) are not considered numerous enough to warrant the use of $F_1$ as maternal parent followed in second backcross by the use of first backcross plants as pollen parents.

In developing a monosomic or nullisomic series in emmer wheat, loss of the unwanted vulgare chromosomes is not the only consideration to be kept in mind. It is imperative that the deficient condition be maintained in the emmer chromosome throughout the backcross program. The results of univalent transmission must therefore be considered in this light.

Direction of Cross and Transmission of Deficiency

The first backcross data showed that the monosomic condition of the A or B genome chromosomes was transmitted with about the same frequency by egg and pollen. Approximately 38% of male and female gametes were shown to transmit the deficiency. This result is in striking contrast to those reported for other aneuploids. Unbalanced male gametes are usually non-functional while female gametes are less subject to genic unbalance. Aneuploid series are consequently perpetuated largely through the female. In Chinese Spring monosomes Sears found that the transmission of the deficiency by the egg was about 75% regardless of the chromosomes concerned, while pollen transmission varied from 1 - 15% depending on the line. Similarly in both N. Tabacum and N. rustica the monosomic condition has been demonstrated as being frequently transmitted by the egg but only rarely by the pollen. Even in Datura trisomes the

extra chromosome was rarely transmitted by the pollen.

The second backcross results showed that the transmission of the monosomic condition by the egg was approximately 19% while approximately 14% of the pollen was deficient. The breakdown of this average figure has already been given on page 16. If these second backcross results are considered as belonging to two main groups based on the direction of the first backcross, it will be seen that within each group the monosomic condition is transmitted only slightly more frequently by the egg than it is by the pollen (25 and 13.3% as compared to 20 and 8.3% respectively). The percentage transmission of the deficiency is considerably lower in the second backcross than it was in the first backcross. A possible explanation for this discrepency will be discussed later.

The tendency for both first and second backcross results to show male monosomic transmission frequencies approaching those of the female can perhaps be explained. Unlike the Nicotiana and Triticum aestivum aneuploids, this material carries, in addition to the deficiency, varying numbers of univalents. It is considered very likely that a D-genome chromosome homoeologous[5] to the missing emmer one could compensate, at least partially, for the deficiency. Compensating combinations would be equally frequent in male and female gametes. If the compensation was complete, normal pollen would

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5. Homoeology (Sears 1952) refers to similarities between chromosomes which can be detected by genetic or compensation studies but are not apparent cytologically because they are not sufficient to bring about pairing at meiosis.

have no advantage over compensated pollen but if the compensation was only partial, certation would result in fewer compensating combinations being effective in the male than in the female.

The first indication of homologies among the 21 chromosomes of T. aestivum were reports of bivalent formation in haploid plants. Kihara (1936) reported up to three chromosome pairs in haploid emmers. Hexaploid haploids have been studied at meiosis by several investigators (Gaines & Aase, 1926; Kostoff, 1939; Krishnaswamy, 1939; Raw, 1937, Yamasaki, 1936; McGinnis & Unrau, 1952). The number of open bivalents (closed bivalents are seldom observed) ranged from rarely more than one to as many as nine. In this latter instance it should be noted that the majority of cells had no bivalents while only 0.4% had more than four pairs. Chromosome pairing in haploids however cannot be expected to show the extent of duplicated segments amongst the 21 chromosomes. Some duplicated chromosome segments would undoubtedly be too small to cause pairing.

A more sensitive method of detecting genetic homologies between various chromosomes is by studying the compensating effect of tetrasomes when in the presence of nullisomes. Kihara (1939) was the first to report the occurrence of specific tetrasomes compensating partially or completely for each of the four nullisomes he was studying. Shortly afterwards Huskins (1941) and Sears (1944) reported similar occurrences. Eventually Sears (1952) was able on the basis of compensating effects of tetrasomes for nullisomes to arrange the 21 chromo-

somes of T. aestivum into seven genetically homoeologous groups.

O'Mara (1950) has presented limited but interesting data which indicate that even a chromosome from Secale cereale significantly compensates for the loss of a normal chromosome in T. aestivum pollen. This report stimulates the interesting speculation that perhaps there is more than one D-genome chromosome which can compensate for a particular A- or B-genome chromosome. Thus, there may be eight different functional 14-chromosome gametes in this material. One would be normal having all of the AB chromosomes, another would have, in addition to 13 of the AB chromosomes the D-genome chromosome shown by Sears to be homoeologous to the missing emmer one, while six others would contain 13 AB chromosomes plus different ones of the remaining six aestivum chromosomes. Sears (1952) believes that "the possibility that additional homologies exist", should not be excluded "since the compensation effect observed in any particular combination probably involves only a few genes" and thus "the chromosomes concerned may actually have only a small fraction of their genetic material in common".

Considering the amount of pairing reported in haploids of hexaploid wheats it is surprising that Sears (1944) reports that no tendency for homoeologous chromosomes to be associated at meiosis has been detected. That at least certain homoeologous chromosomes may occasionally associate at meiosis is considered evident from the results of this investigation. Undoubtedly homoeologous chromosomes were involved in the several instances

noted of very loose, end-to-end and side-by-side pairing (Fig. 13, 14, 15 - 20). The anaphase configurations which showed that in certain cells of the same plant the number of univalents may differ by two are considered good evidence for homoeologous pairing.

Östergren's theory of co-orientation (Östergren, 1951) is pertinent to the discussion at this point. Briefly, Östergren's theory concerns the mechanism whereby partner chromosomes are directed to opposite poles at first anaphase. According to this theory material association between chromosomes is the requisite for their co-orientation. Normally this association involves chiasmata formation between homologous pairs. Östergren maintains, however, that association by simple stickiness is sufficient to cause two chromosomes to behave as a normal bivalent. Such "quasi-bivalents" as he calls them have been observed in the hybrid (Lilium candidum x chalcedonicum) (Östergren & Vigfusson, 1953). They behaved as expected on the basis of this theory.

If homoeologous chromosomes could be considered to occasionally exhibit loose side-by-side prophase pairing (see Fig. 12), it is conceivable that under certain circumstances they would become attached by simple stickiness. Once materially associated the homoeologous chromosomes could, on the basis of Östergren's theory, co-orientate like normal bivalents. Prophase pairing followed by formation of "quasi-bivalents" would often be a matter of chance however since the attractions between homoeologous chromosomes would undoubtedly be small and stickiness possibly governed by environmental factors.

It is understandable then, why plants carrying compensating combinations would often show variance in the number of univalents at first anaphase. Understandable too is the variation in frequency of monosomic transmission in first and second backcrosses. If stickiness is influenced by environment, then the fact that the first backcrosses were made in the field whereas the second backcrosses were performed in the greenhouse would explain the discrepancy in our first and second backcross monosomic transmission frequencies. The more often "quasi-bivalents" formed the more often would a homoeologous chromosome be transmitted by a gamete rather than be lost by lagging. It would appear that field conditions favored "quasi-bivalent" formation.

The evidence to date seems to indicate that, unless the deficiency is compensated for, either (n - 1) gametes or (2n - 1) zygotes fail to function in the variety Golden Ball. Although six plants were found to have 15" + 2' none with only one univalent was observed. It may be argued that insufficient backcrosses have yet been made to get rid of all the extra chromosomes, but to the writer this explanation seems unlikely. If six plants have been reduced to carrying only two univalents, others would be expected to carry only one if such types are viable. The absence in this material of functioning gametes carrying no univalents seems to be strong evidence that even monosomic lines, at least for certain chromosomes, are going to be unattainable in Golden Ball. The presence of plants carrying 15" + 2' would seem to be supporting evidence

for this view. Apparently Sears has had a similar experience in his attempts to establish deficient aneuploids in emmer wheats. In a recent paper on another subject (Sears, 1953) he says, "Judging from results from monosomics in AABB wheats, most female gametes with less than the full AB complement would not be expected to function".

## THE RADIATION PROGRAM

### Review of Literature

Interest of geneticists in radiation methods was first stimulated by the reports of Muller (1927), and Stadler (1928a, b) that X-rays and radium radiations produced gene and chromosomal mutations in plants and animals. Since that time geneticists have used various radiations not only to produce useful genetic variants but also to study fundamental problems concerned with gene and chromosomal mutations, the time of chromosome doubling and estimations of gene size.

It was the purpose of the present investigation to explore the possibility of producing monosomes in T. durum by radiation methods. Other chromosomal abnormalities and aspects of the technique were of secondary importance. This review, therefore, is limited in its scope. For comprehensive reviews of radiation genetics the reader is referred to Lea (1947) and Catcheside (1948).

Structural rearrangements in the chromosomes arise as a result of breaks followed by recombination of breakage ends

in various ways.

> It has been established that the types of induced aberrations depend on whether the chromosomes are "effectively" monopartite or bipartite at the end of irradiation. If the chromosomes are "effectively" monopartite when "irradiated", the aberrations are of the chromosome type; if bipartite, they are primarily of the chromatid type. (Caldecott & Smith, 1952).

The types, mode of origin and frequencies of chromosomal aberrations resulting from irradiating somatic and meiotic nuclei have been discussed in the literature many times. Reference to these papers may be found in the review by Lea (1947) which also includes a diagrammatic representation of the types of chromosome and chromatid aberrations as they appear at mitosis.

Stadler (1931a), by pollinating normal recessive eggs of maize with irradiated dominant pollen, was able to show genetically that deletions were frequently induced. He found, however, that these deficiencies eliminated themselves in a single generation because deficient haploid gametophytes were inviable. He realised however (Stadler, 1931b) that deficiencies could probably be transmitted by gametophytes of polyploid species. It was also evident from Stadler's (1931a) work that "while deficiency appears to be lethal to the gametophyte, it is not necessarily lethal to the gamete when induced shortly before fertilisation".

Translocations were suggested by Stadler (1931a) as being the explanation of transmissible partial sterility. He cites Randolph's (1929) cytological detection of a ring-of-four

in semi-sterile plants as support for this view. Randolph (1929) was also cited as having detected a maize plant in which an entire chromosome was absent following the use of irradiated pollen.

Goodspeed and Avery (1933) obtained a monosome in Nicotiana by "using normal pollen on a flower which had been subjected to X-radiation when it was almost at anthesis". Their cytological evidence indicated that, "the X-ray dosage ..... produced an attachment between two homologous chromosomes". The behavior of the dicentric chromosome at second anaphase occasionally resulted in deficient gametes.

Smith (1943, 1946) using irradiated mature pollen reported "whole chromosome deficiencies particularly in the hexaploid but also in the tetraploid" (T. durum) wheat. Arnason et al (1952) reported radiation-induced monosomes in hexaploid wheat.

## Material and Methods

An irradiation program was initiated at the University of Saskatchewan during the summer of 1952 in an attempt to obtain monosomic plants in T. durum var. Golden Ball. Since the material used in the present investigation was obtained from this program, the irradiation methods used will be described.[6]

The source of the gamma ($\gamma$) rays and neutrons used

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[6] Information supplied by Dr. B.C. Jenkins who was in charge of the program. Dr. Jenkins acknowledges that Dr. J. G. O'Mara (State College of Agriculture & Mechanic Arts, Ames, Iowa) suggested the use of radiations to induce monosomes in emmer wheat.

was a cylinder of Radium-Beryllium. This source, enclosed in a wooden phantom, was placed in a short section of mailing tube and attached to an iron rod. In the field the rod was stuck upright in the earth and the wheat heads drawn about and fastened to the source. The treatments varied in duration and were estimated[7] to be equivalent to 250, 500 and 750 roentgens (r)[8]. The 750r treatment was not continuous but consisted of a 500r dose followed in 3/4 of an hour by another 250r dose. Heads estimated to be at a stage two days prior to pollination were given the three doses. In addition, heads still in the boot and heads just emerging from the boot were given the 500r treatment.

Thirty-nine rows of 10 seeds each were planted in the Field Husbandry Department greenhouses on December 21, 1952. Of these, two rows were untreated checks, 10 were treatment I (750r), 7 were treatment II (500r), 10 were treatment III (250r), five were treatment IVA (500r - heads still in boot), and five were treatment IVB (500r - heads just out of boot).

Cytological specimens were fixed, stained and examined as previously described.

$R_3$ progenies from this $R_2$ planting were grown during

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7 R. W. Hummel of the Chemistry Department supplied this information.

8 Dosages of $\gamma$ rays are usually measured in roentgen (r), this unit being the dose of radiation required to liberate one electrostatic unit of charge in 0.001293 g. of air (Catcheside, 1948).

the summer of 1953. The seed from $R_2$ plants showing good fertility were planted in the field while where little seed was formed on $R_2$ plants, the $R_3$ progenies were again grown in the greenhouse. No cytological specimens were collected from the $R_3$ generation.

## Results

Observation of the germination and growth of seed from treated plants soon indicated that the irradiation had induced mutations. Approximately seven percent of the seeds planted either did not germinate or the plants died before reaching maturity. Some morpholigcally abnormal plants were, however, able to grow to maturity. At the heading stage various abnormal spikes were evident and reduced fertility or complete sterility was quite common.

Chromosome counts were obtained of 288 $R_2$ plants. In addition, the cytology of 19 untreated plants was determined. All the untreated plants were normal showing 14 bivalents at metaphase. Among the treated plants the most commonly observed chromosomal mutations were deficiencies (heteromorphic bivalents) and translocation rings or chains. The deficiencies varied from slight to extreme. If most of a chromosome had been lost it often was not able to pair with its mate, at least it was not paired at metaphase (see Fig. 34). The translocation rings and chains usually involved four chromosomes (Figs. 25, 26, 27) although larger complexes were also noticed (Fig. 28). The hetermorphic bivalents and translocation complexes were present

## Description of Plate II

Acetocarmine smears of plants obtained in the Radiation Program. Magnification as indicated.

Fig. 25. Metaphase of a plant showing 12" + ring-of-four. x 1110.

Fig. 26. Prometaphase of a plant showing 12" + chain-of-four. x 1110.

Fig. 27. Metaphase of a plant showing 10" + two rings-of-four. x 1640.

Fig. 28. Metaphase of a plant showing 11" + chain-of-six. x 1350.

Fig. 29. Metaphase of a plant showing six normal bivalents, one heteromorphic bivalent, one chain-of-four, one ring-of-four, one chain-of-three and three univalents. x 1125.

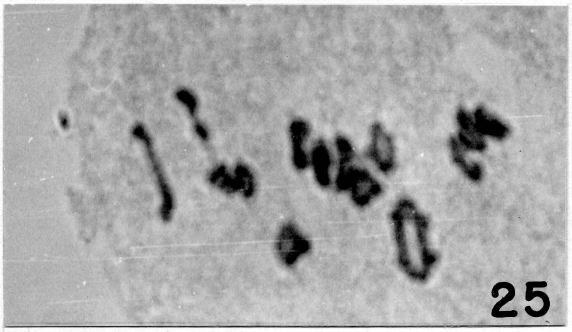
Fig. 30. Metaphase of a plant showing 11 normal bivalents, two heteromorphic bivalents and one univalent. X 855.

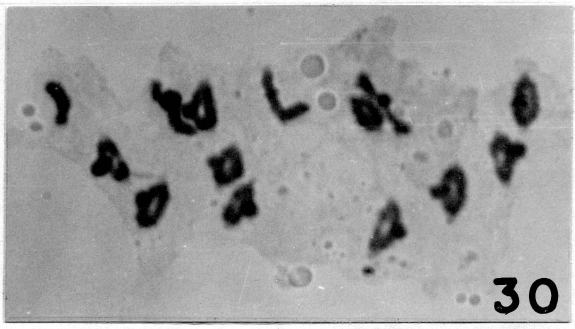
Fig. 31. Metaphase of the only plant found to have 13" + 1'. Unfortunately immersion oil leaking under the coverslip caused this photograph to be indistinct. x 1820.

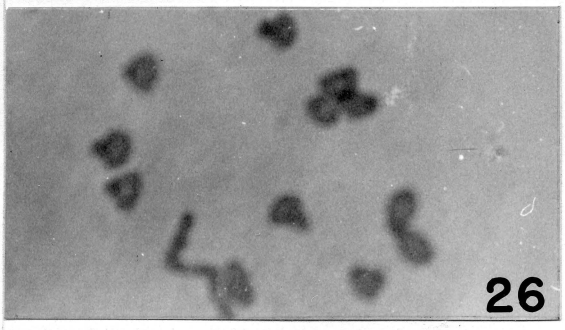
Fig. 32. Metaphase of a plant showing 13 normal bivalents plus one extremely heteromorphic bivalent. x 1395.

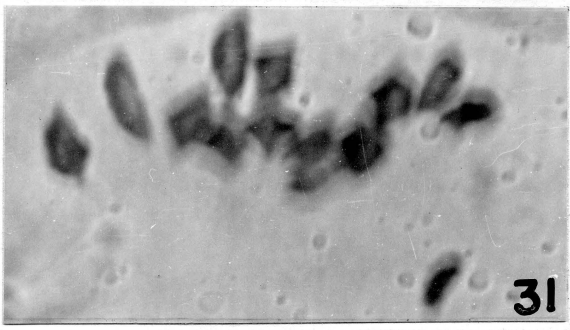
Fig. 33. Metaphase of a plant showing an isochromosome. x 1395.

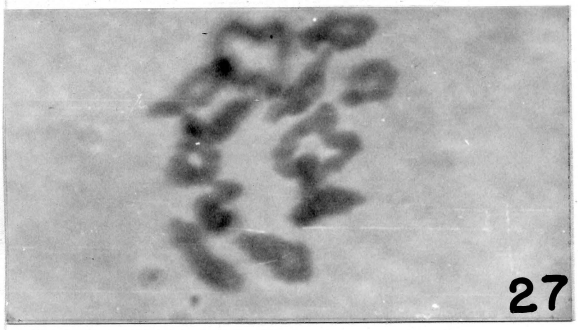
Fig. 34. Metaphase of a plant showing 12 normal bivalents, one heteromorphic bivalent and what is probably the two pieces of another heteromorphic bivalent. x 1338.

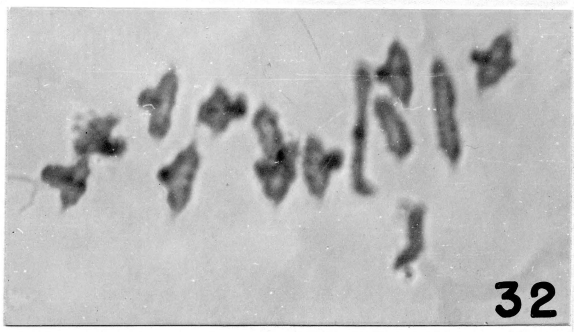


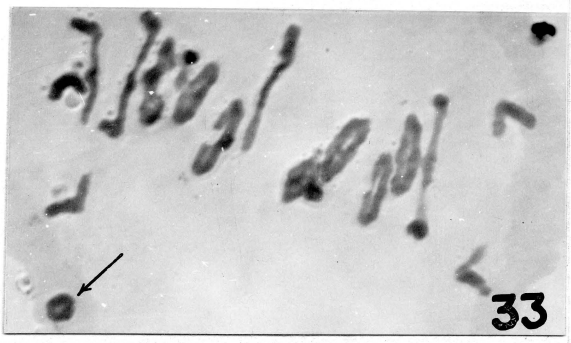



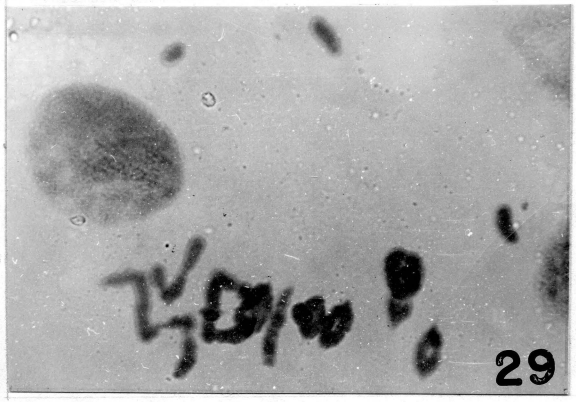





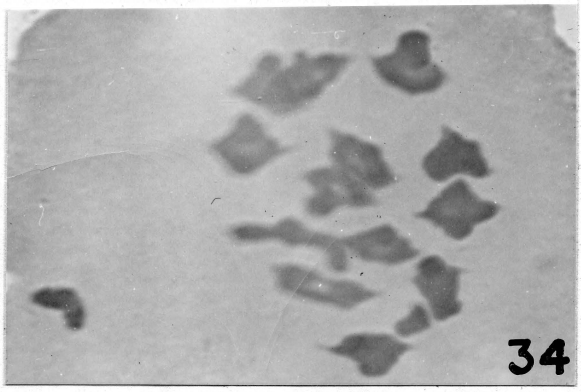




PLATE II

in numerous combinations (Fig. 29).

Only one plant was found to have the cytology 13" + 1' (Fig. 31) although whole chromosome deficiencies were noted in a few other plants having additional abnormalities (Fig. 30). A plant with an isochromosome was also noted (Fig. 33).

Rather than list the cytology of all plants examined, the data is presented (see Table III) in terms of per cent of broken chromosomes. In arranging the data for this table the following somewhat arbitrary procedure was followed. Heteromorphic bivalents and missing chromosomes were counted as one broken chromosome. A translocation ring or chain-of-four was counted as two broken chromosomes. Larger translocation complexes were scored in terms of rings-of-four. In calculating the percentage of broken chromosomes the 2n number (28) was used since both pollen and eggs were exposed to the $\gamma$ rays and neutrons.

Generally speaking, plants found to be carrying deficiencies were of abnormal morphology and fertility. Those having only rearrangement of essential genic material were apparently normal and usually quite fertile.

Although the plant found to be monosomic for one chromosome was more or less normal in morphology it was completely sterile. A plant having a very large portion of one chromosome missing (see Fig. 32) set only three seeds. These gave rise to plants of normal morphology and fertility.

TABLE III

SUMMARY OF RESULTS OBTAINED IN IRRADIATION PROGRAM

| Treatment | Stage when treated | No. of plants examined | Per cent of broken chromosomes |
|---|---|---|---|
| I 750r | Two days prior to anthesis | 68 | 5.3 |
| II 500r | " | 58 | 1.7 |
| III 250r | " | 85 | 1.2 |
| IVA 500r | Heads in boot | 37 | 3.4 |
| IVB 500r | Heads just out of boot | 40 | 2.0 |

## Discussion

Usually the relationship between dosage of ionising radiations used and number of aberrations induced is fairly direct. "In general 'one-hit' aberrations show a linear response to dosage, while the 'two-hit' aberrations vary as the square of the dosage, when the exposure time is held constant." (Sax, 1940). In our material there were several factors contributing to the lack of a consistent relationship between dosage intensity and per cent of chromosomes broken. First, the method of treatment would result in different heads and different parts of the same head being exposed to varying

intensities of radiation. In planting seed from different treatments, 10 seeds from different heads were planted in separate rows. The number of heads thus sampled was only a portion of the heads treated. Too small a sample of seeds within a head and heads within a treatment may have been partially responsible for the results obtained. Second, the chromosomal breaks which presumably were responsible for failure of seven per cent of the seed to germinate or at least to grow to the heading stage, were not considered. Third, the method used in counting chromosome breaks was arbitrary. It did not take into account the fact that certain abnormalities were probably due to two rather than one break. Finally application of the 750r treatment in two stages disturbs the dose-aberration relationship since restitution of breaks induced in the first part of the treatment could have occurred during the interval.

Two of the three 500r treatments (II and IVB) gave rise to approximately the same percentage of broken chromosomes (1.7 and 2.0% respectively) while the third (IVA) resulted in 3.4% broken chromosomes. This difference is probably due to the age of the plants in the various treatments. In Tradescantia Sax (1938) has clearly shown that the number of chromosome breaks induced by any given dose of X-rays depends greatly on the stage of the meiotic cycle at which it is given. Sax's results indicate that meiotic prophase is the stage at which chromosomes are most "susceptible" to breakage. He stated, "it seems probable that the chromosomes at meiosis are

at least 10 times as susceptible as chromosomes at the resting stage in the microspore." Although Treatments II, IVA and IVB were all 500r, Treatment IVA was given to spikes still in the boot while Treatments IVB and II were given to progressively older material. It would be expected that more gametes would be at meiotic prophase in Treatment IVA material than in the older plants of Treatments IVB and II. The wide range of meiotic stages present in the many florets of a wheat inflorescence makes comparison of the various treatments difficult.

The presence of many chromosomes showing deficiencies or translocations indicates that simple chromosome breaks occur frequently. Chromosome fragments lacking a centromere commonly lag at anaphase and fail to be included in the telophase nuclei. It is not surprising, therefore, that no acentric fragments were found in this material since the observations were made one complete life cycle after the irradiation.

The reduced fertility of plants carrying even small deficiencies and the complete sterility of most plants having large deficiencies is evidence that only gametes carrying the full AB complement of chromosomes are able to function. It emphasizes also the likelihood that the gametes which gave rise to such deficient plants or plants lacking a whole chromosome, carried all the essential genic material. The acentric fragments produced by the irradiation treatments must have been carried by the gametophytic generation and were subsequently lost during the first few somatic divisions of the new zygote. Similarly any structural rearrangements leading to loss of

whole chromosomes (see later) must not have been operative until after the gametophytic stage. In this regard McClintock (1938a) has reported that acentric fragments of meitoic chromosomes often are included in the daughter nuclei of Zea and that these acentric fragments act as normal chromosomes both in their cytological behaviour and in their genetic influence.

There are several ways in which plants deficient for whole chromosomes may have arisen. Fusion of broken ends of sister chromatids gives rise to a dicentric chromosome (McClintock, 1938a). When the two centromeres are close together lagging at anaphase is common with the result that the following cells are deficient for an entire chromosome (McClintock, 1938b). Another possible way in which monosomic plants may arise from irradiation treatment is by the inactivation of a centromere. Sax (1938) was of the opinion that "a centromere may be inactivated or prevented from dividing by X-ray treatment." A chromosome with such a nonfunctioning centromere would behave as an acentric fragment at anaphase and would soon be lost. Arnason et al (1952) were of the opinion that "monosomics may sometimes result from chromosome breakage and loss of both fragments."

Whatever the mode of origin, it appears evident from these results that n-1 gametes are largely incapable of functioning in T. durum var. Golden Ball. Plants having even small deficiencies were of poor fertility while those deficient for whole chromosomes or large portions of chromosomes were sterile.

## SUMMARY

An account is given of the backcross and radiation programs underway at the University of Saskatchewan to develop deficient aneuploids in the durum wheat variety Golden Ball.

Cytological examination of material obtained from the backcross program has shown that:-

1. Functional eggs most often carry intermediate numbers of the extra D-genome chromosomes while functional pollen carries either none or most of the univalents. These results are in close agreement with those obtained by Thompson & Cameron (1928) who used a vulgare parent "of Chinese origin".

2. The monosomic condition was found to be transmitted only slightly less frequently by the pollen than by the egg.

3. No monosomic plants were obtained although several plants having 13" + 2' were observed.

4. Evidence for loose association between compensating homoeologous chromosomes is presented.

5. Östergren's co-orientation theory is suggested as supplying a basis for explaining the high monosomic transmission frequency through the male.

Cytological examination of material obtained from the irradiation program has shown that:-

1. Heteromorphic bivalents and translocation complexes were the most frequent chromosome aberrations observed.

2. The number of chromosome breaks induced increased

with increasing dosage of radiation but no consistent relationship was observed.

3. Heads still in the boot were more susceptible to chromosome breakage than were heads at a stage two days prior to pollination.

4. Most plants having deficiencies showed marked reduction in fertility.

5. One monosomic plant was observed. It was completely sterile.

## CONCLUSION

On the basis of results obtained both in the backcross and irradiation programs it is evident that monosomic plants are going to be very difficult, if not impossible to obtain in the durum wheat variety Golden Ball. The failure of deficient gametes to function, as shown by the sterility of the one monosomic plant obtained, probably accounts for the absence of monosomic plants in this variety.

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# APPENDIX A

## Directions for making Zirkle's mounting fluid (as obtained from Dept. of Genetics, University of Wisconsin)

20 cc Venetian Turpentine
55 cc Phenol
35 cc Propionic acid
10 or 15 cc Glacial acetic acid
25 cc Water

Add the viscous venetian turpentine to propionic acid and mix thoroughly. Next, add phenol; either as loose crystals (crystals are easier to use) or a liquid. If liquid, phenol 88% is used; allow for the water it contains (use about 65 cc). Then add acetic acid, and lastly, water. DO NOT HEAT. May be filtered after 12 hours, but filtering is not necessary if solutions are mixed carefully.

Notes:

1. Special care should be taken to avoid contact with skin as phenol is poisonous.
2. A long time is required for the various ingredients to mix. Add chemicals in order given, and allow time for proper mixing.
3. It is easier to dissolve if propionic acid is measured first and the venetian turpentine added than the reverse. A long process of gentle shaking of the propionic acid and venetian turpentine mixture is needed in order to dissolve the turpentine. About now you wish you had not started this sticky, fragrant mess.
4. We have found that it is better to use 10 cc of glacial acetic acid than 15 cc.

5. Add water very slowly, and watch reaction. Stop if separation takes place. Sometimes all the water cannot be added.
6. Never make more than twice the quantity given above at one time.

Zirkle's fluid is used to make aceto-carmine preparations permanent when it is desirable not to remove the cover slip. It can not be used after aceto-orcein or Feulgen's stain.

# APPENDIX B

## BACKCROSS PROGRAM

### Cytological record of plants examined

I. First Backcross

| Chromosome Line | $F_1$ hybrid used as | | | |
|---|---|---|---|---|
| | Male | | Female | |
| | Cytology | Univalents | Cytology | Univalents |
| IV | 13" + 7' | 6 | 14" + 1' | 2 |
| | 14" | 1 | 13" + 5' | 4 |
| | 13" + 6' | 5 | 14" + 4' | 5 |
| | 14" + 6' | 7 | 14" | 1 |
| | 13" + 7' | 6 | 14" + 4' | 5 |
| | 14" | 1 | 14" + 5' | 6 |
| | | | 14" + 5' | 6 |
| | | | 14" | 1 |
| | | | 14" + 3' | 4 |
| V | 14" + 5' | 6 | 13" + 5' | 4 |
| | 14" + 7' | 8 | 14" + 4' | 5 |
| | 13" + 7' | 6 | 14" + 4' | 5 |
| | 13" + 2' | 1 | 13" + 8' | 7 |
| | 14" + 7' | 8 | 14" + 3' | 4 |
| | 13" + 7' | 6 | 14" + 4' | 5 |
| | 14" | 1 | 14" + 3' | 4 |
| | 14" | 1 | 14" + 4' | 5 |
| | 13" + 7' | 6 | 14" + 3' | 4 |
| | | | 13" + 6' + 1 fragment | 6 |
| VI | 14" + 2' | 3 | 13" + 4' | 3 |
| | 14" | 1 | 14" + 4' | 5 |
| | | | 14" + 5' | 6 |
| | | | 14" + 5' | 6 |
| VIII | | | 14" + 4' | 5 |
| | | | 14" + 2' | 3 |
| | | | 14" + 3' | 4 |
| | | | 14" + 1' | 2 |
| | | | 14" + 2' | 3 |
| | | | 13" + 8' | 7 |
| | | | 13" + 5' | 4 |
| | | | 13" + 3' | 2 |

APPENDIX B (continued)

| Chromosome Line | $F_1$ hybrid used as | | | |
|---|---|---|---|---|
| | Male | | Female | |
| | Cytology | Univalents | Cytology | Univalents |
| IX | 14" + 7' | 8 | 13" + 6' | 5 |
| | 14" + 5' | 6 | 13" + 4' | 3 |
| | 14" + 5' | 6 | 14" + 3 | 4 |
| | 14" + 7' | 8 | 14" + 4' | 5 |
| | 13" + 7' | 6 | 13" + 7' | 6 |
| | | | 14" + 3' | 4 |
| | | | 14" + 2' | 3 |
| | | | 14" + 3' | 4 |
| XII | 14" + 6' | 7 | 14" + 3' | 4 |
| | 14" | 1 | 13" + 6' | 5 |
| | 13" + 8' | 7 | 14" + 2' | 3 |
| | 14" + 6' | 7 | 14" + 5' | 6 |
| | 14" + 3' | 4 | 13" + 5' | 4 |
| | | | 14" + 3' | 4 |
| | | | 13" + 3' | 2 |
| XIV | 14" + 2' | 3 | 14" + 5' | 6 |
| | 14" + 6' | 7 | 14" +3' | 4 |
| | | | 14" + 6' | 7 |
| | | | 13" + 7' | 6 |
| | | | 13" + 6' | 5 |
| | | | 13" + 7' | 6 |
| | | | 14" + 3' | 4 |
| | | | 14" + 4' | 5 |
| | | | 13" + 4' | 3 |
| | | | 13" + 3' | 2 |

APPENDIX B (continued)

BACKCROSS PROGRAM

Cytological record of plants examined

II. Second Backcross

| Line | Cytology* 1 | | No. of Univ. | Cytology* 2 | | No. of Univ. | Cytology* 3 | | No. of Univ. | Cytology* 4 | | No. of Univ. |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | $BC_1$ | $BC_2$ | | $BC_1$ | $BC_2$ | | $BC_1$ | $BC_2$ | | $BC_1$ | $BC_2$ | |
| IV | | 14" + 2' | 2 | | 14" | 0 | | 14" + 3' | 4 | | 14" + 1' | 2 |
| | | 14" + 1' | 1 | | 14" | 0 | | 14" + 6' | 7 | | 13" + 3' | 2 |
| | | 14" + 1' | 1 | | 14" | 0 | 13" + 6' | 14" | 1 | 13" + 7' | 14" + 1' | 2 |
| | | 14" + 1' | 1 | | 14" | 0 | | 14" + 1' | 2 | | 14" | 1 |
| | 14" + 4' | 14" + 1 | 1 | 14" + 4' | 14" | 0 | | 14" | 1 | | 14" | 1 |
| | | 14" + 2' | 2 | | 14" | 0 | | 14" + 5' | 6 | | 14" + 3' | 4 |
| | | 14" | 0 | | 14" + 1' | 1 | 13" + 7' | 14" + 6' | 7 | 13" + 6' | 14" + 3' | 4 |
| | | 14" | 0 | | 14" | 0 | | 14" + 1' | 2 | | 13" + 3' | 2 |
| | | | | | 14" | 0 | | | | | 14" + 4' | 5 |
| V | | 14" + 1' | 2 | | 14" + 2' | 3 | | 14" | 1 | 14" + 1' | 14" | 1 |
| | | 14" + 2' | 3 | | 14" | 1 | | 13" + 2' | 1 | | 14" | 1 |
| | | 14" + 2' | 3 | | 14" | 1 | | 14" | 1 | | 14" + 1' | 2 |
| | | 14" + 1' | 2 | | 14" | 1 | | 14" | 1 | 13" + 3' | 13" + 2' | 1 |
| | 13" + 5' | 14" + 2' | 3 | 13" + 5' | 14" | 1 | 13" + 3' | 13" + 2' | 1 | | 14" + 1' | 2 |
| | | 14" | 1 | | 14" | 1 | | 13" + 2' | 1 | | | |
| | | 14" | 1 | | 14" + 1' + 1 telo + 1 frag. | 4 | | 14" | 1 | | | |
| | | 14" + 3' | 4 | | 13" + 3' | 2 | | | | | | |
| | | 14" + 3' | 4 | | | | | | | | | |
| VI | | 13" + 2' | 1 | | 14" | 1 | | | | | | |
| | 13" + 4' | 14" | 1 | 13" + 4' | 14" + 1' | 2 | | | | | | |
| | | 14" + 1' | 2 | | 14" + 1' | 2 | | | | | | |
| VIII | | 14" + 3' | 4 | | | | | | | | | |
| | 13" + 4' | 14" | 1 | 13" + 4' | 14" | 1 | | | | | | |
| | | 13" + 3' | 2 | | | | | | | | | |
| XII | | | | 13" + 6' | 14" + 1' | 2 | | | | | | |
| | | | | | 14" | 1 | | | | | | |
| | | | | | 14" | 1 | | | | | | |
| | | | | | 14" | 1 | | | | | | |

* Pedigree:
1 $F_1$ used as female; $BC_1$ used as female
2 $F_1$ used as female; $BC_1$ used as male
3 $F_1$ used as male; $BC_1$ used as male
4 $F_1$ used as male; $BC_1$ used as female

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