# A STUDY OF AUTOTRIPLOIDS AND TRISOMICS OF COMMON BARLEY, HORDEUM VULGARE L.

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# A STUDY OF AUTOTRIPLOIDS AND TRISOMICS OF COMMON BARLEY, HORDEUM VULGARE L.

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ABSTRACT

Triploid plants of Hordeum vulgare L. were found in an $F_2$ intervarietal hybrid population derived from colchicine treated $F_1$ plants and in a large nursery of the variety Gateway. Triploids occurred spontaneously in Gateway with an estimated frequency of one in 6000 plants. An attempt to produce triploids by crossing tetraploid with diploid Gateway was unsuccessful.

At meiosis in the Gateway triploids univalents lying off the equatorial plate during metaphase I were found to be distributed on opposite sides of the plate at random. Furthermore, only univalents located on the plate at the completion of metaphase I lagged and divided equationally at anaphase I. At anaphase I the distribution of the extra set of seven chromosomes to the poles was found to be in a binomial frequency. Microcytes formed by diads located on the periphery of the cells at anaphase I behaved as independent minute cells in succeeding meiotic stages.

Among the progeny of the triploids no aneuploid plants occurred with more than three extra chromosomes; the majority were either diploid or were primary trisomics. Data were given on the fertility and on the transmission of the extra chromosome through the gametes of a number of trisomics. Four morphologically distinct primary trisomic types were described in Gateway. One of these was cytogenetically demonstrated to be associated with Linkage Group II and independent of the remaining known groups.

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TABLE OF CONTENTS

TABLE OF CONTENTS (continued)

# A STUDY OF AUTOTRIPLOIDS AND TRISOMICS OF COMMON BARLEY, *HORDEUM VULGARE* L.

## INTRODUCTION

Autotriploid plants possess three basic sets of homologous chromosomes, whereas diploids have two. Triploids[1] have been reported and studied in numerous genera. The investigations have dealt with their natural occurrence and their experimental production, the pairing relationships of the chromosomes at prophase, the behavior of trivalent complexes and univalents at metaphase and anaphase of meiosis, and with the chromosome numbers of functional gametes. Although these studies have made valuable contributions to the elucidation of chromosome behavior in general as well as to the cytogenetics of the species concerned, the results have been varied and often inconclusive, particularly with regard to the behavior of the extra set of chromosomes at meiosis.

Common barley, *Hordeum vulgare* L., is a diploid having 14 chromosomes. Reports on triploids of this species are relatively rare, and the behavior of their chromosomes at meiosis has been described in only one report. The usual experimental methods of obtaining triploids in other species have been found generally unsuccessful in barley.

Among the aneuploid progeny produced by triploids, trisomics (plants with one chromosome in triplicate) have been of particular value in cytogenetic investigations. Since the genetic ratios for genes carried by the extra chromosome are modified, trisomics are useful for associating chromosomes with their respective linkage groups. This altered ratio

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[1] Hereafter the term 'triploid' refers to autotriploid.

technique has been successfully applied to Datura, tomato, corn and tobacco, to mention a few plants. It is only recently that trisomics of barley have been obtained and utilized in this manner.

According to published reports, four and possibly five of the barley linkage groups have been identified with their respective chromosomes through the use of chromosome translocations and trisomics. To date, however, a complete series of the seven possible primary barley trisomics has not, apparently, been developed and made available to barley cytogeneticists.

The discovery of a number of triploid plants in two field populations of common barley prompted the present study, the first part of which deals with the triploids and the second part with their trisomic progeny.

The objectives in the first part of the study were 1) to determine the frequency and origin of triploids that occurred spontaneously; 2) to attempt to produce triploids of barley experimentally; 3) to contribute further to the knowledge of the behavior of chromosomes in triploids, particularly of the extra set; and 4) to determine the frequency of the various chromosomal types among the progeny of the triploids.

The objectives in the second part of the study were 1) to describe the morphological characteristics of a number of primary trisomics; 2) to determine the fertility of trisomics and the transmission of gametes with an extra chromosome; and 3) to provide evidence for the association of one trisomic type with its corresponding linkage group, which heretofore had not been definitely shown to be independent of the remaining groups.

# PART I: TRIPLOIDS

## REVIEW OF LITERATURE

### Occurrence and Origin of Triploids

Triploid plants have been obtained from suitable experimental crosses and from natural populations in which they occurred spontaneously.

Triploids have been produced experimentally by crossing an autotetraploid with its related diploid in Datura (10, 12), Lolium (38, 39), Lycopersicum (16), Petunia (21), Primula (17, 19), Secale (25), and Zea (15, 47). In his extensive review Smith (56) cited no reports on triploids of common barley obtained by this method. Tsuchiya (63), however, produced a hypotriploid plant, $2n$[1] = 20, by crossing an artificially induced autotetraploid with its related diploid of the same variety. He further reported (64) a hybrid triploid obtained by crossing the same autotetraploid with Hordeum spontaneum nigrum (2n = 14). The latter is a wild species closely related to common cultivated barley.

The success with which triploids are obtained by intercrossing autotetraploids with diploids depends on the species and the direction in which the cross is made. Blakeslee et al. (10), and Buchholz and Blakeslee (12) observed that in Datura, triploids were produced, from the small proportion of viable seeds obtained, only when the tetraploid was used as the female parent. The reciprocal cross was completely incompatible due to pollen tube growth failure. A similar relationship was found in Primula sinensis by Darlington (19).

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[1] Throughout this study the symbols '2n' and 'n' refer to the zygotic and gametic chromosome complements, respectively.

When the autotetraploid of this species was pollinated by the diploid, pollen tube growth appeared quite normal. In another study involving the same cross Watkins (66) stated that fertilization failed to occur. However, apparently fertilization must have occurred rarely since Darlington obtained triploids from this cross. In the reciprocal cross 2x[1] pollen tubes were not functional in the style of 4x plants.

Randolph (48) found that in the cross 2x X 4x of Zea mays about 98 per cent of the seed was abortive and less than 0.5 per cent of the relatively well-filled seeds were viable, while the reciprocal produced seed with a viability of less than five per cent. Cooper (15) studied the development of the caryopsis of these reciprocal crosses and concluded that the high degree of incompatibility was not due to non-fertilization but to failure of the caryopsis to reach a germinable stage; endosperm development was abnormal and the embryo suffered from a lack of nutrients. More normal development was noted in the 4x X 2x cross than in the reciprocal. Triploid plants were obtained from seed of both combinations.

According to Chin (14), when autotetraploid rye was pollinated with the diploid, pollen tube growth was normal, and approximately 38 per cent of the florets set seed. The incompatibility of the reciprocal cross was attributed to growth failure of the pollen tube. On the other hand, Hakansson and Ellerstrom (25) found that in their stocks of rye, fertilization occurred regularly in both combinations of the tetraploid and diploid. However, only four triploid plants were obtained from 783

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[1] The symbol 'x' refers to the basic haploid chromosome number of a species.

tetraploid florets pollinated with the diploid and the same number from 1275 diploid florets pollinated with the tetraploid. The almost complete incompatibility in these matings was attributed to irregular development and disintegration of the endosperm. Endosperm development was somewhat more normal in the 4x X 2x cross, which probably accounted for the slightly greater success of this combination.

Seed collapse following crosses between diploid and autotetraploid races of Lycopersicum pimpinellifolium was studied by Cooper and Brink (16). They concluded that incompatibility of 2x X 4x and 4x X 2x crosses was not due to triploidy as such but to conditions surrounding the triploid embryo within the seed. The occasional triploid obtained from the 4x X 2x combination exhibited normal vegetative growth.

The spontaneous occurrence of triploids in diploid populations has been noted in Canna (66), Lycopersicum (30, 50), Nicotiana (22), Tulipa (42), Zea (33, 49), and in several genera of Gramineae, including Avena, Hordeum, Secale and Triticum (28, 29, 36, 37). The relative frequency in which they appeared has been determined in a few cases. Lesley (30) found from one to 0.4 per cent triploids in two different varieties of tomato. In the same species but different varieties Rick (50) calculated a frequency of one triploid in about 1200 plants, or less than 2.08 per cent, and observed variation in frequency from season to season within the same variety.

Triploids have occasionally occurred as members of twin seedlings. Muntzing (37) found that of 2201 twin seedlings from 14 genera, including Avena, Hordeum, Secale and Triticum, 2106 were diploid, while 95 had deviating chromosome numbers of which 77 were triploid. From the data of all species investigated there was noted a distinct tendency for one

member of a heteroploid twin to be triploid. The frequency of twins in eight varieties of Hordeum vulgare was found to be about one in 5900 seedlings, and one twin member of 93 examined was triploid. Aase (2) and Kostoff (28) have suggested that the frequency of twinning is genetically controlled since it varies between species and between varieties.

Several methods of origin have been proposed for the spontaneous occurrence of triploids:

1. They have occurred through natural intercrossing of tetraploids and diploids found within the species. The various aspects of this method have been discussed above.

2. A commonly supposed origin is the union of an unreduced with a reduced gamete of an otherwise normally behaving diploid plant. Unreduced gametes may be the result of failure of one of the meiotic divisions. That such unreduced gametes are formed has been rather definitely demonstrated cytologically (2, 8, 9, 35, 42, 66). Syndiploidy has been suggested as a further source of gametes with the diploid chromosome number (20). This is the failure of separation of daughter nuclei in divisions immediately preceding meiosis. It is thought that fusion takes place after the pachytene stage of meiosis, since usually no quadrivalents are produced. Aase (2) noted that pollen mother cells with multiple euploidy were not infrequently found in routine studies of anther material. It is logical to assume that similar phenomena occur to form doubled egg cells. Rick (50) noted considerable variation from season to season in the frequency of spontaneous occurrence of triploids in the same variety of tomato and suggested that high temperatures during the growing

season possibly influenced the production of diploid gametes. The fusion of a reduced with an unreduced gamete has been inferred as the method of origin of triploidy in Canna (5), Lycopersicum (30, 50), Tulipa (42), and Zea (33, 48).

3. A third possible method suggested for the origin of triploids is the fertilization of an egg cell by two male nuclei (10, 22).

4. The mode of origin of triploids from twin seedlings has been attributed to embryonal development of a fertilized endosperm cell or to a fertilized unreduced nucleus of a supernumary embryo sac. (2). According to Muntzing (36), a supernumary macrospore mother cell could give rise to an extra embryo sac having an unreduced chromosome number, which when fertilized by haploid pollen would result in a triploid zygote.

## Morphology of Triploids

The morphological appearance of triploids varies from species to species. Lesley (30) noted that the stems, leaves, and flowers of triploid tomato were more or less gigantic, but the fruits were undersized and few in number. Although triploid maize was observed to be more vigorous than diploid, there was no striking morphological difference (33). Lamm (29) found difficulty in distinguishing a triploid rye plant from normal diploids, the former being only slightly more vigorous. The triploid derived from crossing tetraploid common barley with the wild species Hordeum spontaneum appeared to exhibit heterosis (64).

## Meiosis in Triploids

### First Division

According to the generally accepted hypothesis of pachytene

pairing, homologous chromosomes synapse at random as pairing segments; that is, only two chromosomes pair at one point (7, 20, 42, 58). Since triploids possess three homologous chromosomes of each kind, equal lengths of paired and unpaired chromosome segments should occur. The variation in number of chiasmata formed in these paired lengths determines the type of association observed at metaphase. If no chiasmata are formed between two particular chromosomes in the paired sectors, univalents occur. Studies of triploids of Hyacinthus (18, 20) and Tulipa (42) have indicated that the frequency of chiasmata formation is in proportion to the length of the chromosomes. Consequently, longer chromosomes are more likely to form trivalents and, for the same reason, more complex associations, while a relatively greater frequency of shorter chromosomes will occur as univalents.

Myers (38) observed that at prophase of triploid Loleum perenne there was an excess of paired and a deficiency of single chromosome strands. However, never more than two chromosomes were associated at one point. The excess of paired strands appeared to result from pairing of normally nonhomologous segments. This form of illegitimate pairing evidently was not accompanied by chiasma formation, since only trivalents, bivalents, and univalents occurred at metaphase in frequencies expected from normal pairing.

Earlier papers on triploids of Canna (5), Datura (7), and Hyacinthus (6) reported that only trivalents were regularly observed at metaphase. However, more detailed later studies on these and other species indicated that bivalents and univalents also occurred (13, 17, 21, 22, 29, 31, 33, 38, 39, 47, 63, 64, 65). Usually various combinations of

trivalents, bivalents, and univalents were found at metaphase; the frequency of each varied from species to species and from cell to cell within the same anther. The combined total of trivalents and bivalents equalled the haploid chromosome number in true autotriploids; pairing rarely occurred between chromosomes of the haploid or extra set.

The five types of trivalent configurations that are theoretically possible from normal synapais of three homologues (20) are diagrammatically illustrated in Fig. 1. The tandem-chain and tandem - V types require a minimum of two chiasmata, one in each arm, while the triradial configuration also requires two chiasmata, both in

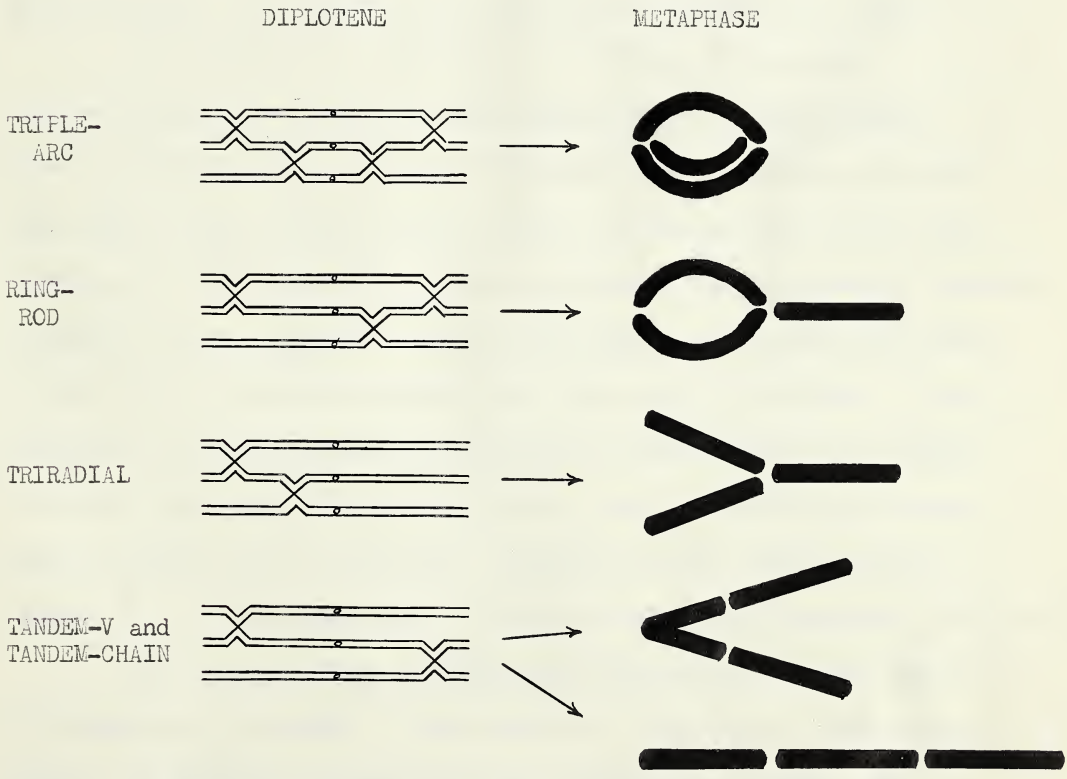


Fig. 1. Pairing arrangements of three homologous chromosomes at diplotene and resulting trivalent configurations at metaphase I.

the same arm; the ring-rod requires a minimum of three chiasmata, two involving one arm and one the other, while two chiasmata in each arm produce a triple-arc association.

In triploids of Datura and hyacinth Belling (7) found that short chromosomes with median centromeres formed any one of the trivalent configurations. Short chromosomes with subterminal centromeres, such as occurred in hyacinth, formed more complicated configurations because of interstial chiasmata. Long chromosomes with median centromeres tended to form ring-rod and both types of tandem configurations. The ring-rod and tandem types were most frequently observed in Canna (7), Datura (7, 9), Hordeum (63, 64), Lolium (38), Primula (17), and Secale (29), while triradial and triple-arc associations were rare or not found.

The behavior of trivalents and bivalents at metaphase I has been found to be similar in most triploids and in other plants, such as autotetraploids, interspecific hybrids and aneuploids, in which they occur (1, 13, 18, 22, 24, 32, 33, 38, 41, 42, 43, 45, 47, 53, 65). Normally, at late diakenesis and early metaphase, when the nuclear membrane disappears, these associations move to the center of the cell and become oriented into an equatorial plate within the spindle mechanism. At late metaphase and early anaphase the trivalents usually disjoin two members to one pole and the third to the opposite, while the bivalent daughter-members separate one to each pole. According to some investigations, members of a trivalent may lag and divide equationally at anaphase (20, 38, 64).

On the other hand, considerable variability has been found in the behavior of univalents. Since there are similarities of univalent behavior in triploids, interspecific and intergeneric hybrids, and

aneuploids, pertinent information from these sources will be discussed.

The following general description of univalent behavior has been given by Darlington (20): "Unpaired chromosomes usually lie at random on the spindle at metaphase. They do not move towards the equator as early as the paired chromosomes. It is sometimes stated that unpaired chromosomes lying to one side of the plate are moving to the pole in advance of the bivalents at anaphase, but this conclusion is unjustifiable. Their position is due to their never having reached the plate, and they actually do not move until after the bivalents have divided. When the paired chromosomes begin to separate at anaphase unpaired chromosomes follow one of two courses: (1) those lying far away from the equator are included with the group of daughter bivalents passing to the nearest pole; (2) those lying near the equator move on to the plate, orientate themselves axially, and divide after a short interval into their two chromatids, which then pass to opposite poles as in mitosis," Based on Kihara's study of Triticum - Aegilops hybrids (Kihara, H. Genomanalyse bei Triticum und Aegilops. I and II. Cytologia, 2. 1931.), Darlington concluded that univalent behavior was chiefly of the second type and that "variations commonly observed in univalent behavior are probably due to various degrees of delay in the movement of univalents relative to those of the bivalents."

The behavior of univalents in wheat monosomics as described by Smith et al. (57) and Sears (55) was similar to Darlington's second type. However, numerous descriptions of their behavior in various triploids and interspecific and intergeneric hybrids have indicated that both types may occur in the same stock.

In a series of papers on interspecific Triticum hybrids Melburn

and Thompson (34), and Thompson (59, 60) reported that univalent behavior in these hybrids followed a generally consistent pattern. At metaphase the univalents were more or less scattered throughout the cell, a few being observed near or on the plate together with the bivalents. After division of the bivalents the majority of univalents moved to the equator and divided equationally, the two halves separating to opposite poles. The remaining univalents did not move to the plate but joined the bivalent-halves at the nearest polar group. Thus, late anaphase polar groups consisted of bivalent-and univalent-halves and undivided univalents. Similar behavior of univalents was observed by Nishiyama (43) in an interspecific triploid hybrid of Avena and by Sax (53) in a pentaploid emmer - vulgare wheat cross. The latter paper also included a study of the hybrid Triticum monococcum X T. turgidum in which it was found that the univalents usually lay at or near either pole. In a few cases they moved to the plate and divided after the bivalents. This description is in contrast to that which has been given by Thompson (59) for the same hybrid, as already discussed, but involving different varieties. Sax and Sax (54) observed that in the intergeneric cross Aegilops cylindrica X Triticum vulgare the univalents remained at or passed to the poles without dividing at first division.

Myers (40) and Myers et al. (41) concluded from observations of meiosis in diploids and autotetraploids of Lolium perenne, and several other grasses, that some univalents were oriented on the plate with the bivalents and multivalents before anaphase and that others were scattered throughout the cell during metaphase but became oriented some time before completion of anaphase, after which they divided equationally. The few

unoriented univalents that were left intact in the cytoplasm formed micronuclei later. The descriptions of univalent behavior in autotetraploid Secale cereale that have been given by Chin (14) and O'Mara (45) agree closely with that for Lolium.

Gajewski (24) developed a series of Geum interspecific hybrids with increasing numbers of univalents and studied their behavior at metaphase - anaphase. Considerable irregularity was found in their behavior. In two hybrids with the fewest number of univalents, two to seven univalents were rarely found on the plate at metaphase, and most passed undivided to the poles at anaphase. The remainder were left at the plate at late anaphase where they either divided or passed whole to one of the poles. In two hybrids with 14 and 21 univalents, respectively, the univalents were scattered over the whole spindle. Later they congressed at the equator to form a more or less regular ring about the plate. At anaphase, after separation of the bivalents, all of the univalents rested on the plate. Their division and movement was very irregular; some divided; others did not; and many were omitted from the daughter nuclei. In the fifth hybrid, possessing 42 univalents, three groups of chromosomes tended to be formed, one at each polar end and one at the plate composed of a few bivalents and univalents. After division of the bivalents at anaphase the behavior of the univalents depended on their position on the spindle. Those on it moved without change to the nearest pole, while those on the equator appeared to stretch but moved as whole bodies to the poles. Gajewski attributed the differences of univalent behavior in the different hybrids to 1) differences in genotypical constitution (an important factor in meiotic pairing) and 2) to different numerical relationships between univalents and bivalents - the more

bivalents on the metaphase plate, the greater the proportion of univalents that became oriented at metaphase - anaphase.

A discrepancy between the relative proportions of metaphase univalents and anaphase laggards can be calculated from the data given by Boyle and Holmgren (11) who studied the $F_1$ hybrid between Agropyron trachycaulum (2n = 28) and Hordeum jubatum (2n = 28). An average of 13.8 univalents occurred at metaphase, which was approximately one half of the entire chromosome complement, while at anaphase only 3.51 laggards were observed (this value is calculated from data given in Table IA of their report). In the amphiploid of this hybrid an average of 1.3 univalents were observed at metaphase and 1.4 laggards at anaphase (3). The data from this hybrid and its amphiploid tend to substantiate Gajewski's second conclusion.

Most reports of univalent behavior in triploids, particularly those from early investigations, are largely descriptive. In triploid asters all of the univalents divided at first division (4), while in Solanum tuberosum they lagged but did not divide (35). The absence of lagging at second division further indicated that in the latter species univalents seldom, if ever, divided at first division. Although lagging univalents occurred occasionally in triploid tomato, they rarely divided at anaphase (31). In triploid Lilium Chandler et al. (13) found that univalents were usually together with the trivalents and bivalents at the plate. Most frequently they lagged and divided equationally at anaphase after the trivalents and bivalents had separated. McClintock (33) stated that in triploid corn univalents oriented on the plate probably divided at the same time as the trivalents and bivalents, while lagging univalents separated later. More precise and conclusive information on univalent

behavior in triploids has been obtained in more recent studies.

Approximately 43 per cent of the lagging univalents of triploid rye divided at anaphase (29). In triploid Phleum pratense (44) an average of 4.68 univalents occurred at metaphase, but an average of only 1.81 univalents lagged and divided at anaphase. According to the data given by Punjasingh (47) on triploid corn, approximately 49 per cent of the microsporocytes had one or more univalents at metaphase, while only about 25 per cent had one or more laggards at anaphase. The writer's calculations of Punjasingh's data indicate that about 55 per cent of all metaphase univalents lagged and divided equationally at anaphase. Tsuchiya (64) found an average of 2.33 univalents at metaphase of a hybrid triploid barley and an average of 1.46 laggards at anaphase. Of the latter, about 36 per cent were calculated to be derived from "improper" disjunction of trivalents and the rest from univalents oriented on the equatorial plate at metaphase. The results indicate that in these triploids a relatively large proportion of metaphase univalents did not divide at anaphase. In contrast to this behavior, Myers (38) calculated that in triploid Lolium perenne an average of 1.33 laggards occurred at anaphase as compared with 0.93 univalents at metaphase. The excess of anaphase laggards was attributed to "improper" disjunction of trivalents. In triploid hyacinths Darlington (18) also attributed an excess of anaphase laggards when compared with the number of metaphase univalents to imperfect disjunction of trivalent associations, although no supporting data were given.

In normal diploids the equational split at anaphase occurs simultaneously in all daughter-members of disjoined bivalents. They are then termed diads. Aase (1), Darlington (20), Gajewski (24), and Love (32),

stated that the equational split occurred simultaneously in all unpaired chromosomes and disjoined members of bivalents. In contrast to this behavior Myers (38) noted that many univalents oriented on the metaphase plate showed the 'split' before initiation of anaphase, while those off the plate did not. Aase (1) concluded from her studies on the cytology of numerous cereal hybrids that "The behavior of the univalents depends largely on their location on the spindle at the time of the equational split. The equational split may, however, overtake them at any location on the spindle, and consequently, if many univalents are at the equator at this critical time many univalents will divide."

It is generally assumed that univalents of aneuploids and interspecific hybrids and the extra set of chromosomes in triploids, whether they occur in associations as trivalents or unassociated, are distributed at random to the poles during meiosis (20). Some authors have claimed or assumed random distribution of univalents in certain interspecific gramineous hybrids without giving statistical data (1, 27, 43, 53, 54, 60). O'Mara (45) also assumed randomness of univalents in tetraploid rye. Anaphase distribution of chromosomes in triploid corn "appeared" random (45). Although Tsuchiya (64) presented data on the observed distribution of the chromosomes at anaphase of triploid barley no statistical comparison with a binomial distribution was given. Visual inspection of his data indicates that several distribution classes were not in accordance with randomness, perhaps because of the relatively small numbers of cells recorded. Chromosome distribution at anaphase has been described as approaching or resembling randomness by comparing, without statistical tests of significance, observed frequencies with frequencies

calculated according to the binomial in triploids of Canna (5), Datura (8), Lycopersicum (31), and Zea (47).

To date the hypothesis of random distribution of the third set of chromosomes in triploids has been adequately tested in only two species. Satina and Blakeslee (51) recorded the distribution of chromosomes at first division in 1000 microsporocytes of triploid Datura stramonium (3x = 36). They found an excess of the more extreme anaphase groupings, 12-24, 13-23, 14-22, 15-21, and a deficiency of the intermediate groupings, 17-19 and 18-18. The discrepancies were statistically significant. The authors concluded that "Despite the lack of direct evidence from other forms than Datura, it seems probable that the divergence of the assortments at the I division in P.M.C. from calculated values is of general occurrence and is to be attributed to the nature of chromosomes and the mechanisms involved in their movements at division." Myers (39) tested the randomness of chromosome distribution at anaphase of triploid Lolium perenne (3x = 21). The statistical treatment of the data obtained from 2,494 metaphase and 1636 anaphase microsporocytes indicated that 1) at metaphase "unoriented univalents lie in the microsporocyte at random relative to one another and to the equatorial plate", and 2) "The distributions at anaphase I also were consistent with the hypothesis of chance position of the unoriented metaphase I univalents and random assortment of the extra chromosomes of the trivalents." Thus, the behavior found in triploid Lolium differed from that in triploid Datura.

Descriptions of univalent behavior during the stage from late anaphase to early interphase were similar in several papers reviewed. In

Triticum (53, 60) and Avena (43) interspecific hybrids, autotetraploids of Secale (45) and Lolium (41), as well as triploid Lilium (13), the univalents that were oriented at the plate divided after the bivalents and other associations. The resulting univalent-halves usually moved to opposite poles in time to be included in the polar groups at telophase; if not, they were excluded to form micronuclei in the cytoplasm. Aase (1) and Melburn and Thompson (34) occasionally observed that after division of a univalent lying off the plate both daughter-halves moved to the same pole. Chromosome fragmentation has been attributed to misdivision of lagging univalents at telophase (32, 55) and to lagging univalent-halves being cut into two by cell wall formation at early interphase (18, 42, 63). Univalents beyond the influence of the spindle have been observed to form microcytes on the periphery of the cell (7, 13, 14, 18, 23, 33, 34, 64). The univalent within such microcytes has been found to carry on division and pass through stages comparable with the two main daughter cells (18).

Restitution nuclei formed at the conclusion of first division have been observed in triploids of Datura (8, 9) and Tulipa (42).

Second Division

Myers (40) and Smith et al. (57) observed that at second division all diads usually aligned to form an equatorial plate in each daughter cell and then divided equationally. The univalent-halves, derived from the previous division of univalents, lagged at the equatorial region during anaphase - telophase and either moved to the poles or were excluded to form micronuclei. In Avena hybrids (43) and in several other grasses that possessed univalents (41) some of the diads and univalent-halves remained at the poles to be included in the nuclei at telophase. Lagging

univalent-halves have been observed to misdivide at telophase (55). Without providing data Melburn and Thompson (34), Nishiyama (43), O'Mara (45), Sax and Sax (54), and Thompson (60) assumed that univalent-halves at anaphase - telophase passed at random to either pole. Muntzing (35) and Thompson (61) noted that restitution nuclei occurred following second division.

As a result of lagging chromosomes and their fragmentation at both divisions of meiosis a large proportion of the microspores of triploids have been observed to contain one or more micronuclei (13, 29, 38, 63, 64). The proportion of 'good' pollen has been found to vary among different triploids. From eight to nine per cent of triploid Lilium pollen germinated on artificial media (13), while from five to 15 per cent of triploid Datura pollen germinated on 3x stigmas (12). Approximately 92 per cent of the pollen of triploid corn (47) and 54 per cent of the pollen of triploid barley (64) was found to be well filled with starch. Similarly, the fertility has been noted to vary. Triploids of Lycopersicum esculentum (31) and Secale cereale (29) were completely self-sterile. Selfed triploid Lilium (13) had 20 per cent of the fertility of the diploid. Tsuchiya (64) found a seed set of 19 per cent on open-pollinated triploid barley; the fertility was increased by hand-selfing and by crossing with pollen from diploids. Punjasingh (47) determined that 11 per cent of the florets of triploid corn set seed, presumably when open-pollinated.

## Functional Gametes of Triploids

In all reports reviewed (8, 12, 21, 22, 26, 30, 33, 38, 47, 51,

52, 63) a marked discrepancy was noted in the frequency of the chromosome numbers in functional pollen and eggs of triploids when compared with a binomial distribution. These included triploids in which distribution of the chromosomes to the poles at meiosis had been found to be random. The number of extra chromosomes in functioning gametes has been found to vary among species. It has also been noted that extra chromosomes were transmitted with a higher frequency through female than male gametes (12, 22, 33). In triploids of Zea (33, 47) and Petunia (21) pollen with the haploid number or with one extra chromosome functioned exclusively, except for the occasional one with the diploid or near diploid complement. Pollen of triploid Secale (29) and triploid Lycopersicum (31) was nonfunctional on both 3x and 2x stigmas. Functional eggs of triploids of Datura (8, 12), Lolium (38), and Lycopersicum (30) were found to possess up to two or three extra chromosomes. In addition to these extra chromosome types, a relatively low proportion of the functional female gametes of triploid Nicotiana (22) and triploid Zea (33, 47) had intermediate numbers ranging between the haploid and diploid complement. Some triploids, therefore, tended to have progeny with chromosome numbers approaching almost exclusively the diploid number, while others produced additional types having intermediate numbers of the entire possible range but with a much lower frequency than theoretically expected.

## MATERIALS AND METHODS

The triploids described in this study were obtained from two sources. Three triploid plants were found in a large $F_2$ field population derived from intervarietal $F_1$ hybrids, which as germinating seeds had been treated with colchicine. Another group of 13 were found among 4500 progeny head-rows of a highly homozygous line of the variety Gateway which had been treated with various antibiotics, fungicides and insecticides in the previous generation. The original objective of this material was to test the mutagenic properties of these compounds. All but three of the triploids were discovered during the flowering period because of their high sterility, exhibited at this stage by the open florets. The three triploids of hybrid origin, and 10 of the Gateway triploids were cytologically identified by chromosome counts of pollen mother cells or of mitotic divisions in ovary tissue. The remaining three, detected at harvest time because of their very low seed set, were assumed to be triploid since they produced aneuploid offspring similar to those obtained from the cytologically proven triploids.

Secondary tillers were collected from the Gateway triploids for cytological study of microsporocytes. They were fixed in Carnoy's 6:3:1 at room temperature for two to three days and then stored under refrigeration in the same solution for periods up to 15 months before being examined.

Temporary aceto-carmine preparations were made for studying chromosome behavior. Mature pollen grains were stained with an iodine solution to determine the proportion of 'good' pollen.

Cytological data were recorded for three or four of the Gateway triploid plants. Although data for each were recorded separately, no attempt was made to analyze them individually because of insufficient material available from any one plant. Only those metaphase I and anaphase I cells in which all chromosomes and their associations could be clearly distinguished were recorded. Similarly, only microspore tetrads with the surrounding wall intact were recorded.

Seeds harvested from the triploid plants were sown either in pots in the greenhouse or in field plots. Chromosome numbers of the resulting progeny were determined by counts in pollen mother cells or in somatic ovary tissue. The latter method was used because some of the plants tillered poorly and were highly sterile. To obtain a maximum number of seeds from these, a few florets only were removed for cytological examination from a head as it emerged from the leaf sheath.

Microscopic observations and photomicrographs were made using a Ziess Opton microscope fitted with apochromatic lenses and a reflex plate camera attachment.

## OBSERVATIONS AND RESULTS

### Occurrence of Triploids

Three triploids were found among approximately 3000 progeny head-rows of an $F_2$ hybrid nursery derived from $F_1$ plants treated with colchicine. One of the triploids was found among the progeny of a plant which also produced diploids and tetraploids, while the other two occurred in the progeny of different plants in which no tetraploids were observed.

Three of the 13 triploids found in the Gateway material occurred among check progeny rows. This would indicate that some of these triploids originated spontaneously, without treatment effect. Except in one case where two triploids came from the same mother plant, the sibs of all triploids were fully fertile, and therefore, presumably diploid. No attempt was made to obtain a reliable estimate of the frequency of occurrence of triploids among treated rows. However, an estimate was calculated for check rows. Four hundred and thirty-three check rows, which constituted approximately one tenth of the entire population, were closely observed plant by plant for sterility, as an indication of possible triploidy, several times during the flowering period and again at harvest time when low set of seed could be detected. Under this careful scrutiny three cytologically identified triploids were found at flowering time. The average number of plants per check row was estimated from an exact count of 82 rows. The total number of plants in the 433 check rows was then estimated to be approximately 18,500. From this value the frequency of spontaneous triploids was determined to be about one in 6000 plants.

## Experimental Production of Triploids

When it was observed that triploids occurred spontaneously in check rows, an attempt was made to produce them experimentally by hand-pollinating Gateway autotetraploids with diploids of this variety. One poorly developed, inviable seed was obtained from 242 pollinated florets.

## Morphology of Triploids

The triploids were indistinguishable morphologically from diploid

sibs. As stated previously, they were noted only because of the high degree of sterility characteristically evident at flowering time when the florets remained open for several days beyond that normally observed in diploids. Fig. 2 shows representative plants of diploid, triploid, and tetraploid Gateway.

## Meiosis in Triploids

Observations were made on chromosome behavior at meiosis in microsporocytes at metaphase I and subsequent stages of the Gateway triploids. Data from several plants were combined for analysis.

### Metaphase I

At metaphase I the pollen mother cells contained various combinations of trivalents, bivalents, and univalents, as shown in Table I. The most common combinations were $6_{III} + 1_{II} + 1_{I}$ and $5_{III} + 2_{II} + 2_{I}$, each occurring with a frequency of about 30 per cent. No cells with $9_{III} + 7_{II} + 7_{I}$ were noted.

All types of trivalents that are possible from normal pairing of three homologous chromosomes were observed - tandem-V (Figs. 4, 5, 7), tandem-chain (Fig. 7), ring-rod (Figs. 3, 4, 5, 6, 7, 8), triple-arc (Figs. 3, 4), and triradial (Fig. 5). Table II shows that slightly more than 50 per cent of the trivalents were the ring-rod type. The triradial type was least frequent, only 14 being observed in 865 cells. The bivalents were either closed or open, with 5.71 per cent being of the latter type. An average of 5.22 trivalents and 1.78 bivalents per cell occurred.

In addition to the above expected associations of homologous chromosomes and their various combinations, the following unusual sorts

TABLE I

Frequencies of combinations of various chromosome associations at metaphase I

| Combination of associations | Frequency | |
|---|---|---|
| | No. of cells | % of total cells |
| $7_{III}$ | 159 | 14.96 |
| $6_{III} + 1_{II} + 1_{I}$ | 328 | 30.85 |
| $5_{III} + 2_{II} + 2_{I}$ | 317 | 28.82 |
| $4_{III} + 3_{II} + 3_{I}$ | 171 | 16.09 |
| $3_{III} + 4_{II} + 4_{I}$ | 69 | 6.49 |
| $2_{III} + 5_{II} + 5_{I}$ | 15 | 1.41 |
| $1_{III} + 6_{II} + 6_{I}$ | 4 | 0.38 |
| $7_{II} + 7_{I}$ | 0 | 0.00 |
| | 1063 | 100.00 |

were observed among 1091 cells examined:

1. A quadrivalent in each of three cells (Fig. 8).

2. Three hexaploid cells (2n = 42) having only trivalents, bivalents, and univalents.

3. One cell with an extra bivalent (2n = 23).

4. Seven trivalents and a fragment in a cell.

5. One microsporocyte deficient for three chromosomes (2n = 18).

6. One cell with five trivalents, one bivalent, and four univalents (Fig. 7).

7. Seven cells with a 'side-by-side', and seven cells with an 'end-to-end' association of two univalents. These have been also termed pseudobivalents and secondary associations (46). These associations were observed to lie off the plate in all but one cell.

TABLE II

Frequencies of various types of trivalents and bivalents observed in 865 metaphase I cells

| Configuration | Frequency | | |
|---|---|---|---|
| | Total no. | % of total | Average per cell |
| Trivalents | | | |
| Tandem-V | 1337 | 29.63 | 1.54 |
| Tandem-chain | 632 | 14.01 | 0.73 |
| Ring-rod | 2342 | 51.89 | 2.71 |
| Triradial | 14 | 0.30 | 0.02 |
| Triple-arc | 188 | 4.17 | 0.22 |
| | 4513 | 100.00 | 5.22 |
| Bivalents | | | |
| Closed | 1454 | 94.29 | 1.68 |
| Open | 88 | 5.71 | .10 |
| | 1542 | 100.00 | 1.78 |

Behavior of Trivalents and Bivalents.- At metaphase I the trivalents and bivalents usually were observed to form an equatorial plate. Occasionally one or two of these associations were seen to lie off the plate. Bivalent daughter-halves were oriented with the spindle fibre attachment regions toward opposite poles, while orientation of trivalent members depended on the type of configuration. Tandem-V, triple-arc, and triradial types were oriented two and one to opposite poles (Figs. 4, 5, 6, 7). Tandem-chain configurations were found to lie with a member oriented to each pole and the third member interposed between, unoriented (Fig. 7). The ring-rod type was most frequently found to be oriented with two members toward one pole and the third to the other




Fig. 2. Diploid, triploid and tetraploid Gateway.
Fig. 3. Diakenesis with $3_{III} + 4_{II} + 4_{I}$ and showing triarc configuration (arrow).
Fig. 4. Metaphase I cell with $7_{III}$; one tandem-V, four ring-rod and two triple-arc (arrows) configurations.

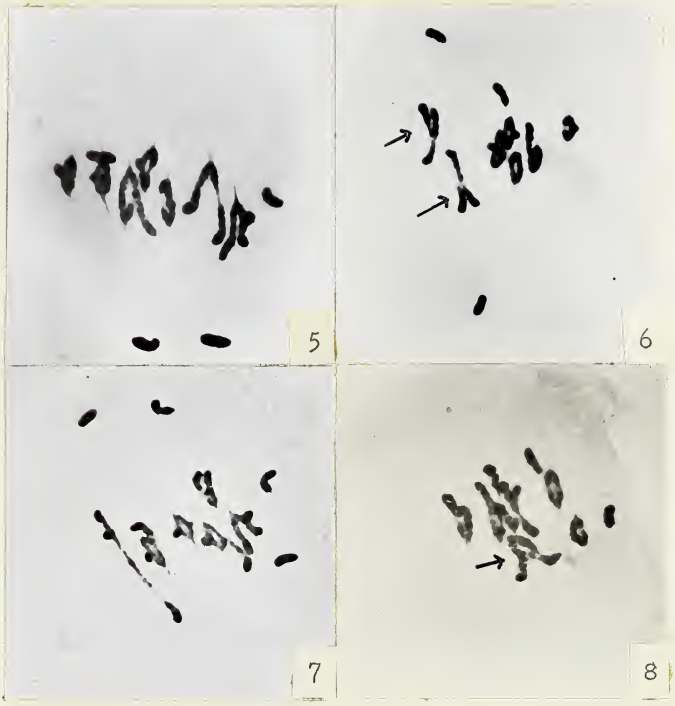


Fig. 5. Metaphase I with $3_{III} + 4_{II} + 4_{I}$; two univalents on plate, one being oriented; univalents off plate distributed 0 - 2.

Fig. 6. Metaphase I showing two triradial trivalents (arrows) and 1 - 1 distribution of univalents.

Fig. 7. Metaphase I with $5_{III} + 1_{II} + 4_{I}$; one tandem-V, and two ring-rod trivalents; one unoriented univalent on plate and three distributed 1-2 off plate.

Fig. 8. Metaphase I showing a quadrivalent (arrow).

(Figs. 4, 5, 6, 7). Occasionally, however, the rod member of this type lay more or less parallel to the plate without orientation of the attachment region to either pole.

Behavior of Univalents.- The position of the univalents in the cell varied considerably. They were observed to lie at the polar regions, in the vicinity of, or on the equatorial plate (Figs. 5, 6, 7). Those at the poles appeared to be oriented haphazardly, while those at the plate often were noted to lie parallel to it. Univalents not on the plate or at the polar regions were observed in various positions, extending from near the main group of equatorial chromosomes to the poles, randomly oriented.

In order to determine the significance of the position of univalents in the cell at metaphase in relation to their subsequent behavior, the following information was obtained on univalents: 1) their position, on or off the plate: 2) orientation of those on the plate; and 3) the distribution to opposite sides of the plate of those not located on it. A univalent was regarded as being on the plate when observed to lie in the equatorial region. This region was considered to extend from one side of the cell to the other along the equatorial axis and approximately two thirds the length of a tandem-chain trivalent along the polar axis. As an example, in Fig. 5 two univalents are on and two are off the plate. The position of some univalents was not clearly defined. These were more or less arbitrarily recorded into either group on the assumption that univalents so observed would be entered with equal frequency into both groups. For example, in Fig. 7 three univalents were recorded as off and one as on the plate. Univalents on the plate were further classed as oriented if lying more or less parallel to the

equatorial axis and as unoriented if in any other plane (Figs. 5, 7).

The proportion of univalents on the metaphase plate for each of the seven combinations of chromosome associations is given in Table III. The per cent of univalents on the plate in each class varied from 42.38 to 25.00. The latter value cannot be regarded as reliable, since only four cells were recorded for this class of $1_{III} + 6_{II} + 6_{I}$. The proportion of univalents on the plate in the remaining six classes ranged from 42.38 to 32.25 per cent. From the data in the table the average number of univalents per cell was calculated to be 1.74, and the average number of these on the plate was 0.66, or 37.73 per cent. Expressed in another way every 100 cells contained 174 univalents of which 66 were on the plate.

TABLE III

Proportion of univalents on the plate at metaphase I

| Combination of chromosome associations | Total no. of cells examined | Total no. of univalents | Univalents on plate | |
|---|---|---|---|---|
| | | | No. | % of total |
| $7_{III} + 0_{II} + 0_{I}$ | 159 | -- | -- | -- |
| $6_{III} + 1_{II} + 1_{I}$ | 328 | 328 | 139 | 42.38 |
| $5_{III} + 2_{II} + 2_{I}$ | 317 | 634 | 234 | 36.91 |
| $4_{III} + 3_{II} + 3_{I}$ | 171 | 513 | 196 | 38.21 |
| $3_{III} + 4_{II} + 4_{I}$ | 69 | 276 | 89 | 32.25 |
| $2_{III} + 5_{II} + 5_{I}$ | 15 | 75 | 34 | 45.33 |
| $1_{III} + 6_{II} + 6_{I}$ | 4 | 24 | 6 | 25.00 |
| Total | 1063 | 1850 | 698 | Av.37.73 |

For each combination of chromosome associations, univalents on the plate were further classified as oriented or unoriented, as indicated in Table IV. The per cent of total univalents oriented on the plate ranged from 25.10 to 16.67, with an average of 20.52. It was calculated that an average of 0.37 oriented univalents per cell occurred or, stated in another way, 37 univalents in every 100 cells. Approximately 54 per cent of all univalents on the plate were oriented. A summary of the data on the frequencies of the various classes of univalents is given in Table V.

TABLE IV

Proportion of univalents oriented on the plate at metaphase I

| Combination of chromosome associations | No. of cells | Total no. of univalents | Univalents on plate | | Oriented univalents | |
|---|---|---|---|---|---|---|
| | | | Total no. | No. oriented | % of total | % of total on plate |
| $7_{III} + 0_{II} + 0_{I}$ | 117 | -- | -- | -- | -- | -- |
| $6_{III} + 1_{II} + 1_{I}$ | 243 | 243 | 100 | 61 | 25.10 | 61.00 |
| $5_{III} + 2_{II} + 2_{I}$ | 246 | 492 | 187 | 100 | 20.33 | 53.48 |
| $4_{III} + 3_{II} + 3_{I}$ | 135 | 405 | 155 | 83 | 20.49 | 53.55 |
| $3_{III} + 4_{II} + 4_{I}$ | 57 | 228 | 80 | 38 | 16.67 | 47.50 |
| $2_{III} + 5_{II} + 5_{I}$ | 13 | 65 | 25 | 13 | 20.00 | 52.00 |
| $1_{III} + 6_{II} + 6_{I}$ | 4 | 24 | 6 | 4 | 16.67 | 66.67 |
| Total | 815 | 1457 | 553 | 299 | Av.20.52 | 54.07 |

TABLE V

Summary of univalent classes at metaphase I

| Class | Frequency | |
|---|---|---|
| | Per 100 cells | % of total univalents |
| From Table III | | |
| Univalents on plate | 66 | 37.73 |
| From Table IV | | |
| Oriented univalents on plate | 37 | 20.52 |

The proportions of cells having various numbers of univalents on the metaphase plate was also recorded (Table VI), since, as will be shown later, these may influence the proportion of anaphase cells with various numbers of lagging univalents.

TABLE VI

Frequency of metaphase I cells with various numbers of univalents on the plate

| No. of univalents on plate | No. of cells | % of total cells |
|---|---|---|
| 0 | 557 | 52.40 |
| 1 | 354 | 33.30 |
| 2 | 119 | 11.20 |
| 3 | 27 | 2.54 |
| 4 | 5 | 0.47 |
| 5 | 1 | 0.09 |
| 6 | 0 | 0.00 |
| 7 | 0 | 0.00 |
| | 1063 | 100.00 |

To test the assumption that univalents off the equatorial plate occurred on opposite sides of the plate at random, the observed distribution frequencies for each class of univalents off the plate can be compared with the calculated. For example, where two univalents occurred off the plate, they would be expected to lie on the same side (0-2 distribution) and one on each side (1-1 distribution) in equal frequencies. Similarly, with three univalents off the plate the 1-2 and 0-3 distributions would be expected in a 3:1 ratio. In cells with four univalents off the plate distributions of 0-4, 1-3 and 2-2 should be expected in a ratio of 1:3:4. The $X^2$ analysis for observed and calculated distribution frequencies for all classes of univalents off the plate is given in Table VII. In the table the data are arranged in classes according to the total number of univalents per cell, each of which is subdivided according to the number of this total that were off the plate. In all classes except one the fit of observed to calculated frequencies is satisfactory. The exception is for the class in which two univalents of a total of three occurred off the plate. A probability level of less than 0.01 indicates that the distribution of the 0-2 and 1-1 classes deviated significantly from the expected 1:1 ratio.

The data from all identical distribution classes in Table VII were combined so as to obtain a single test of fit of observed to calculated frequencies of the various distributions. For two univalents off the plate the fit to a 1:1 ratio of 0-2 and 1-1 distributions was poor ($P = 0.02 - 0.01$). This is due to the discrepancy, noted above in Table VII, for the class in which two of a total of three univalents occurred off the plate. The combined data for the distributions of three univalents off the plate (0-3 and 1-2) gave a good fit to a 1:3

TABLE VII

$X^2$ analysis of observed and calculated frequencies of distributions of metaphase I univalents to opposite sides of the plate

| Total no. of univalents per cell | Univalents off plate: Total no. | Univalents off plate: Distribution | No. of cells: Observed (O) | No. of cells: Calculated (C) | | $\frac{(O-C)^2}{C}$ | P |
|---|---|---|---|---|---|---|---|
| 2 | 2 | 0 - 2 | 76 | 67.00 | | 1.21 | |
| | | 1 - 1 | 58 | 67.00 | | 1.21 | |
| | | | 134 | 134.00 | $X^2=$ | 2.42 | 0.20-0.10 |
| 3 | 3 | 0 - 3 | 10 | 12.00 | | 0.33 | |
| | | 1 - 2 | 38 | 36.00 | | 1.11 | |
| | | | 48 | 48.00 | $X^2=$ | 1.44 | 0.30-0.20 |
| | 2 | 0 - 2 | 44 | 33.00 | | 3.67 | |
| | | 1 - 1 | 22 | 33.00 | | 3.67 | |
| | | | 66 | 66.00 | $X^2=$ | 7.34 | $<0.01$ |
| 4 | 4 | 0 - 4 | 3 | 2.38 | | 0.16 | |
| | | 1 - 3 | 10 | 9.50 | | 0.03 | |
| | | 2 - 2 | 6 | 7.12 | | 0.18 | |
| | | | 19 | 19.00 | $X^2=$ | 0.37 | 0.70-0.50 |
| | 3 | 0 - 3 | 7 | 5.25 | | 0.58 | |
| | | 1 - 2 | 14 | 15.75 | | 0.19 | |
| | | | 21 | 21.00 | $X^2=$ | 0.77 | 0.50-0.30 |
| | 2 | 0 - 2 | 8 | 10.00 | | 0.40 | |
| | | 1 - 1 | 12 | 10.00 | | 0.40 | |
| | | | 20 | 20.00 | $X^2=$ | 0.80 | 0.50-0.30 |

ratio (P = 0.95 - 0.50).

The data in Table VII were combined in a second manner to test the randomness of the distribution of univalents to opposite sides of the plate. Similar groupings of univalents, ie., 0, 1, 2, 3, 4, from each

of the distributions, ie., 0-2, 1-1, 0-3, 1-2, etc., were combined for all observed and calculated values, respectively, and then subjected to a $X^2$ analysis. A probability level of 0.10 - 0.05 for the combined data in Table VIII further substantiates the assumption of random distribution of univalents to opposite sides of the plate.

TABLE VIII

$X^2$ analysis of total frequencies of univalent groupings located off the plate at metaphase I

| Univalent grouping | Frequency: Observed frequency (O) | Frequency: Calculated frequency (C) | $\frac{(O-C)^2}{C}$ |
|---|---|---|---|
| 0 | 148 | 129.63 | 2.60 |
| 1 | 246 | 281.25 | 4.42 |
| 2 | 192 | 175.99 | 1.46 |
| 3 | 27 | 26.75 | .02 |
| 4 | 3 | 2.38 | .16 |
| | 616 | 616.00 | $X^2 = 8.66$ |

P = 0.10 - 0.05

The overall results of the statistical analysis of the data on univalents occurring off the equatorial plate indicate that in general they were distributed to opposite sides of the plate at random during metaphase I.

Anaphase I

At anaphase I all polar groups contained not less than seven diads nor more than 14. Presumably, therefore, one chromosome from each bivalent and trivalent invariably moved to each pole. From zero to four univalents were observed to lag at the equatorial region after separation

and movement to the poles of the daughter chromosomes of the bivalents and trivalents. At this time all diads appeared equationally 'split', whether found at the poles or lagging at the plate (Figs. 9, 12). Lagging univalents usually divided equationally at late anaphase, and the daughter-halves then moved to the poles (Fig. 9, 13). In a small proportion of cells lagging univalents were observed to misdivide (Figs. 10, 11). Also, in a few cells one and occasionally two univalents were noted to lie on the extreme periphery of the plate or polar group (Fig. 12), apparently beyond the influence of the spindle mechanism. These did not divide nor were they included in the main polar groups at anaphase or telophase (Figs. 12, 14).

It was calculated above that for every 100 cells at metaphase I there were a total of 174 univalents. Of these 66 were on the plate, 37 being oriented (Table V). From the data in Table IX it was further calculated that an average of 80 lagging univalents were present in every 100 cells at anaphase I, which is 45.97 per cent of the total number of univalents at metaphase I. This indicates that less than one half of the metaphase I univalents lagged and divided at anaphase I. Although this value is greater than the proportion of univalents on the plate at metaphase, that is 37.73 per cent (Table V), it does approach the latter value rather than 20.52 per cent (Table V), the proportion of oriented univalents on the metaphase plate. The evidence supports the assumption that all univalents on the plate at metaphase I, including those recorded as unoriented, lagged and divided at anaphase I. Presumably, univalents classed as unoriented became oriented by the time anaphase was initiated. The higher percentage of univalents found to lag at anaphase could be explained as the result of the recording system used. Conceivably, some

of the univalents lying close to the plate, and recorded as off, later moved on to it and lagged at anaphase, after division of the bivalents and trivalents.

TABLE IX

Frequency of anaphase I cells containing various numbers of lagging univalents

| No. of lagging univalents per cell | No. of cells | % of cells | Total no. of laggards |
|---|---|---|---|
| 0 | 191 | 48.23 | 0 |
| 1 | 131 | 33.08 | 131 |
| 2 | 51 | 12.88 | 102 |
| 3 | 10 | 2.53 | 30 |
| 4 | 11 | 2.78 | 44 |
| 5 | 2 | 0.50 | 10 |
| 6 | 0 | 0.00 | 0 |
| 7 | 0 | 0.00 | 0 |
| | 396 | 100.00 | 317 |

Visual comparison of Tables VI and IX indicates that the proportions of cells with various numbers of univalents on the plate at metaphase I approach the proportions of cells at anaphase I with corresponding numbers of lagging univalents. On the basis of the assumption stated previously that only those univalents on the metaphase plate lagged and divided at anaphase and the evidence already presented, the observed frequencies of anaphase I cells with zero to five laggards (Table IX) were compared with frequencies calculated from the proportion of metaphase I cells having the corresponding numbers of univalents on the

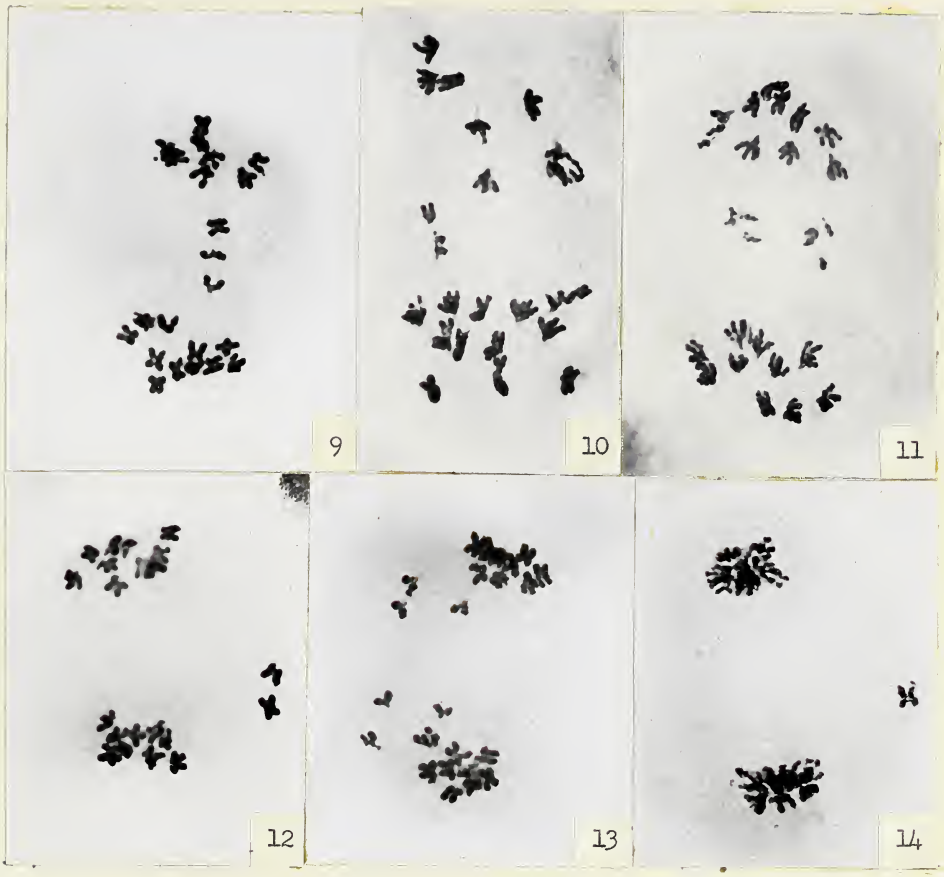


Fig. 9. Anaphase I showing 8 - 11 distribution, one lagging diad, and two lagging daughter-univalents.
Fig.10. Anaphase I with lagging univalent misdividing transversely.
Fig.11. Anaphase I with two lagging univalents misdividing.
Fig.12. Anaphase I cell with two peripheral lagging diads.
Fig.13. Early telophase I with lagging daughter-univalents.
Fig.14. Telophase I cell showing appearance and location of peripheral diad.

plate (Table VI). The $X^2$ analysis of the data in Table X indicates a discrepancy between the observed and calculated values. The poor fit ($P =< 0.001$) is due almost entirely to the excess of cells observed in the classes with four and five laggards, as indicated by a probability level of 0.70 - 0.50 when the remaining classes were subjected to a separate $X^2$ test. The discrepancy may be due to the relatively small number of cells observed in the two classes and also, as previously stated, to error in the system used for recording the position of univalents as either off or on the plate at metaphase I, which could have contributed to an inaccurate estimate of calculated values.

TABLE X

$X^2$ analysis of observed and calculated frequencies of lagging univalents at anaphase I

| No. of lagging univalents | Observed (O) | Calculated (C) | $\frac{(O-C)^2}{C}$ |
|---|---|---|---|
| 0 | 191 | 207.50 | 1.31 |
| 1 | 131 | 131.87 | 0.01 |
| 2 | 51 | 44.35 | 1.00 |
| 3 | 10 | 10.06 | 0.00 |
| 4 | 11 | 1.86 | 44.91 |
| 5 | 2 | 0.36 | 7.47 |
| | 396 | 396.00 | $X^2 = 54.80$ |

$P =< 0.001$

The results nevertheless indicate that in the majority of cells univalents located on the plate at metaphase I lagged at anaphase I, after division of the bivalents and trivalents.

On the evidence already presented to indicate that the distribution of univalents on opposite sides of the metaphase I plate tended to be random (Tables VII, VIII) and on the assumption that the third member of a trivalent passed to either pole also at random, it appears valid to compare the observed frequencies of chromosome distributions to the poles at anaphase I with that expected for none and various numbers of lagging univalents, according to a binomial frequency. In cells with no laggards at anaphase the distribution of the extra seven chromosomes can be calculated from the expansion $(\frac{1}{2} + \frac{1}{2})^7$. For cells with one laggard the distribution of the remaining six should be according to the expansion $(\frac{1}{2} + \frac{1}{2})^6$, for cells with two laggards $(\frac{1}{2} + \frac{1}{2})^5$, for those with three laggards $(\frac{1}{2} + \frac{1}{2})^4$, for cells with four lagging univalents $(\frac{1}{2} + \frac{1}{2})^3$, and for those with five laggards $(\frac{1}{2} + \frac{1}{2})^2$. No cells with more than five lagging univalents were noted in 369 that were examined. The observed and calculated frequencies of anaphase distributions and analysis by the $X^2$ method is presented in Table XI. No analysis is given for cells with five laggards, since there were only two in each category, one for each of the two possible types of distributions, 7 - 9 and 8 - 8, which are expected in a 1:1 ratio. The results of the analysis indicate that the chromosomes were distributed to the poles in a binomial frequency, except for the category with two laggards. The discrepancy here is due to the 7 - 12 and 8 - 11 distributions. When these were combined and tested with the remaining class a good fit of observed to calculated was obtained ($P = 0.30 - 0.20$).

To test further the randomness of chromosome distribution at anaphase I, the observed data on the one hand and the calculated on the other from all distributions in Table XI were combined to form eight

TABLE XI

$X^2$ analysis of observed and calculated frequencies of chromosome distribution classes at anaphase I

| No. of laggards per cell | | Distribution classes and frequency of cells | | | | |
|---|---|---|---|---|---|---|
| 0 | | 7 - 14 | 8 - 13 | 9 - 12 | 10 - 11 | Total |
| | Observed (O) | 4 | 23 | 64 | 100 | 191 |
| | Calculated (C) | 2.99 | 20.89 | 62.67 | 104.45 | 191 |
| | $\frac{(O - C)^2}{C}$ | 0.34 | 0.21 | 0.03 | 0.19 | 0.77 |
| | | P = 0.90 - 0.80 | | | | |
| 1 | | 7 - 13 | 8 - 12 | 9 - 11 | 10 - 10 | Total |
| | Observed (O) | 2 | 24 | 57 | 48 | 131 |
| | Calculated (C) | 4.09 | 24.56 | 61.41 | 40.94 | 131 |
| | $\frac{(O - C)^2}{C}$ | 1.07 | 0.01 | 0.32 | 1.22 | 2.62 |
| | | P = 0.50 - 0.30 | | | | |
| 2 | | 7 - 12 | 8 - 11 | 9 - 10 | Total | |
| | Observed (O) | 6 | 9 | 36 | 51 | |
| | Calculated (C) | 3.19 | 15.95 | 31.87 | 51 | |
| | $\frac{(O - C)^2}{C}$ | 2.47 | 3.02 | 0.54 | 6.03 | |
| | | P = 0.05 - 0.02 | | | | |
| 3 | | 7 - 11 | 8 - 10 | 9 - 9 | Total | |
| | Observed (O) | 1 | 8 | 1 | 10 | |
| | Calculated (C) | 1.25 | 5.00 | 3.75 | 10 | |
| | $\frac{(O - C)^2}{C}$ | 0.50 | 1.80 | 2.02 | 4.32 | |
| | | P = 0.20 - 0.10 | | | | |
| 4 | | 7 - 10 | 8 - 9 | Total | | |
| | Observed (O) | 4 | 7 | 11 | | |
| | Calculated (C) | 2.75 | 8.25 | 11 | | |
| | $\frac{(O - C)^2}{C}$ | 0.56 | 0.19 | 0.75 | | |
| | | P = 0.50 - 0.30 | | | | |

possible polar chromosome groups. The number of chromosomes per group ranged in consecutive order from seven to 14. A probability level of 0.80 - 0.70 obtained from the $X^2$ analysis of the combined data in Table XII indicates that the proportions of the various groupings occurred in frequencies expected from random assortment of chromosomes to the poles.

TABLE XII

Total frequencies of anaphase I polar chromosome groupings

| No. of chromosomes in group | Frequency | | $\frac{(O-C)^2}{C}$ |
|---|---|---|---|
| | Observed (O) | Calculated (C) | |
| 7 | 18 | 15.27 | 0.49 |
| 8 | 73 | 76.64 | 0.17 |
| 9 | 167 | 172.70 | 0.13 |
| 10 | 244 | 225.95 | 1.44 |
| 11 | 167 | 183.05 | 1.41 |
| 12 | 94 | 90.42 | 0.14 |
| 13 | 25 | 24.98 | 0.00 |
| 14 | 4 | 2.99 | 0.34 |
| | 792 | 792.00 | $X^2$ = 4.12 |

P = 0.80 - 0.70

The overall results of the analysis of chromosome behavior at anaphase I indicate that the extra set of seven chromosomes were distributed at random to the poles, whether they occurred at metaphase I as members of trivalent complexes or as univalents lying off the plate.

As mentioned previously in the general description of anaphase, a small proportion of cells contained univalents that behaved abnormally. In 396 cells examined, 13 were observed to have a diad lying on the periphery of the plate or pole. Apparently, these neither divided nor were included with the main group of polar chromosomes. Another four cells each had two diads similarly positioned. Altogether, 17 cells or 4.29 per cent had one or two peripheral diads. Misdivision of lagging univalents was noted in two of 396 cells. In one (Fig. 10) a univalent was observed to misdivide transversely at the centromere to produce two equal-armed daughter-univalents. In the second cell two lagging univalents appeared to misdivide in a manner that would produce single-armed fragments or telocentrics. In another two cells the daughter-halves of an equationally split univalent were seen to move to the same pole.

Telophase I

At telophase I the microsporocytes contained two polar groups of chromosomes with from zero to eight lagging daughter-univalents located at various positions between (Fig. 13). In some cells one or two peripheral diads were again observed (Fig. 14). Also, in a few cells misdivision could be inferred from the centric and acentric fragments that were observed.

The proportions of cells with various numbers of lagging daughter-univalents are given in Table XIII. The class with one lagging daughter-univalent was combined with that for two, since presumably one of two univalent-halves had already joined a polar group at the time of observation. The 'undetermined' class includes cells with irregular numbers of lagging univalent-halves and fragments that could not be definitely placed in the other classes. A legitimate comparison of observed frequencies of cells having various numbers of lagging daughter-

univalents with expected frequencies calculated from anaphase I data cannot be made because of the relatively large number of undetermined cells. However, the proportions of cells with and without laggards can be validly compared. At anaphase I 48.23 per cent (Table IX) and at telophase I 46.92 per cent of the cells showed no lagging. These values are in close agreement.

TABLE XIII

Frequencies of cells with various numbers of lagging daughter-univalents at telophase I

| | No. of lagging daughter-univalents | | | | | | |
|---|---|---|---|---|---|---|---|
| | 0 | 2 | 4 | 6 | 8 | Unde-termined | Total |
| No. of cells | 198 | 119 | 31 | 7 | 1 | 66 | 422 |
| % of cells | 46.92 | 28.20 | 7.34 | 1.66 | 0.24 | 15.64 | 100.00 |

Of the 422 cells examined 34, or 8.06 per cent, had one or two diads located at the periphery. Presumably these had remained in this position from anaphase through to the end of telophase. Misdivision of a univalent was observed or was inferred from fragments in eight cells, or in 1.90 per cent of the total. This value probably is somewhat less than the actual one because misdivision probably occurred in some of the cells classed as 'undetermined'.

Interphase

At interphase two large nuclei were usually observed, one in each of the two daughter cells that resulted from first division. One and rarely two microcytes were associated with 51, or 9.17 per cent, of 556 pairs of interphase daughter cells that were recorded (Fig. 15).

Each of two microcytes, associated with different interphase cells, had two distinct minute nuclei. The remainder had one. Presumably, the microcytes were formed by diads previously observed on the periphery of anaphase and telophase cells. Approximately 57 per cent of the interphase microsporocytes contained no micronuclei, while one to six were observed in the remainder. An average of 0.67 micronuclei occurred in each pair of daughter cells.

Second Division

The precise behavior of the chromosomes at second division could not be clearly observed in the material available. However, a general description can be outlined from the data obtained.

At metaphase II diad chromosomes were aligned on the plate, often simultaneously in both daughter cells (Fig. 16). Univalents derived from equational division of lagging diads at anaphase I - telophase I were observed lying throughout the cell, off as well as on the plate. The few fragments that occurred usually were scattered in the cytoplasm. No lagging univalents or fragments were observed in 45.59 per cent of the pairs of daughter cells examined. A single microcyte was associated with 8.05 per cent of the pairs of cells. Four of these 21 microcytes had two diads and the remainder had one. All of the above metaphase II observations are based on data recorded from 261 pairs of daughter cells.

At anaphase II, diads that were aligned on the equatorial plate separated and moved to the poles as in normal mitotic division (Fig. 17). Univalents were found lagging at the equatorial region in three of 27 pairs of daughter cells recorded. In each pair one laggard occurred in both daughter cells. Misdivision of lagging uni-

valents was also noted in a few cells.

During telophase II, daughter cells were observed to have a group of chromosomes at each pole and from zero to four univalents lagging in the equatorial region (Fig. 18). No laggards of any sort occurred in 54.80 per cent of 177 cells examined. Lagging univalents either 1) misdivided at the plate (Fig. 18), 2) remained intact in the cytoplasm but were excluded from the main polar group (Fig. 19), probably to form micronuclei later, or 3) were included with the polar groups. Misdivision of a lagging univalent was observed in six pairs of daughter cells and inferred in another seven from pairs of fragments that were noted (Fig. 19). This indicates that misdivision occurred in a total of 7.35 per cent of telophase II pairs of daughter cells. Misdivision at anaphase I - telophase I was inferred from a fragment noted in each cell of seven (3.95 per cent) of cells. Five pairs were recorded with one to three fragments. In all of these observations, made on 177 pairs of daughter cells, the fragments were the size of univalent chromosome arms; hence, they probably originated from misdivision of lagging univalents at first and second division.

At telophase II a microcyte was seen to be associated with 5.09 per cent of the pairs of daughter cells (Fig. 20). The diad within the microcyte divided equationally and the two halves moved to opposite ends of the minute cell, apparently following the same procedure as diads in normal cells. It was noted, however, that throughout second division the process lagged behind that in the main cells in passing through the stages of division. For example, in Fig. 20 the two main daughter cells are at late telophase while the microcyte is at the anaphase stage.

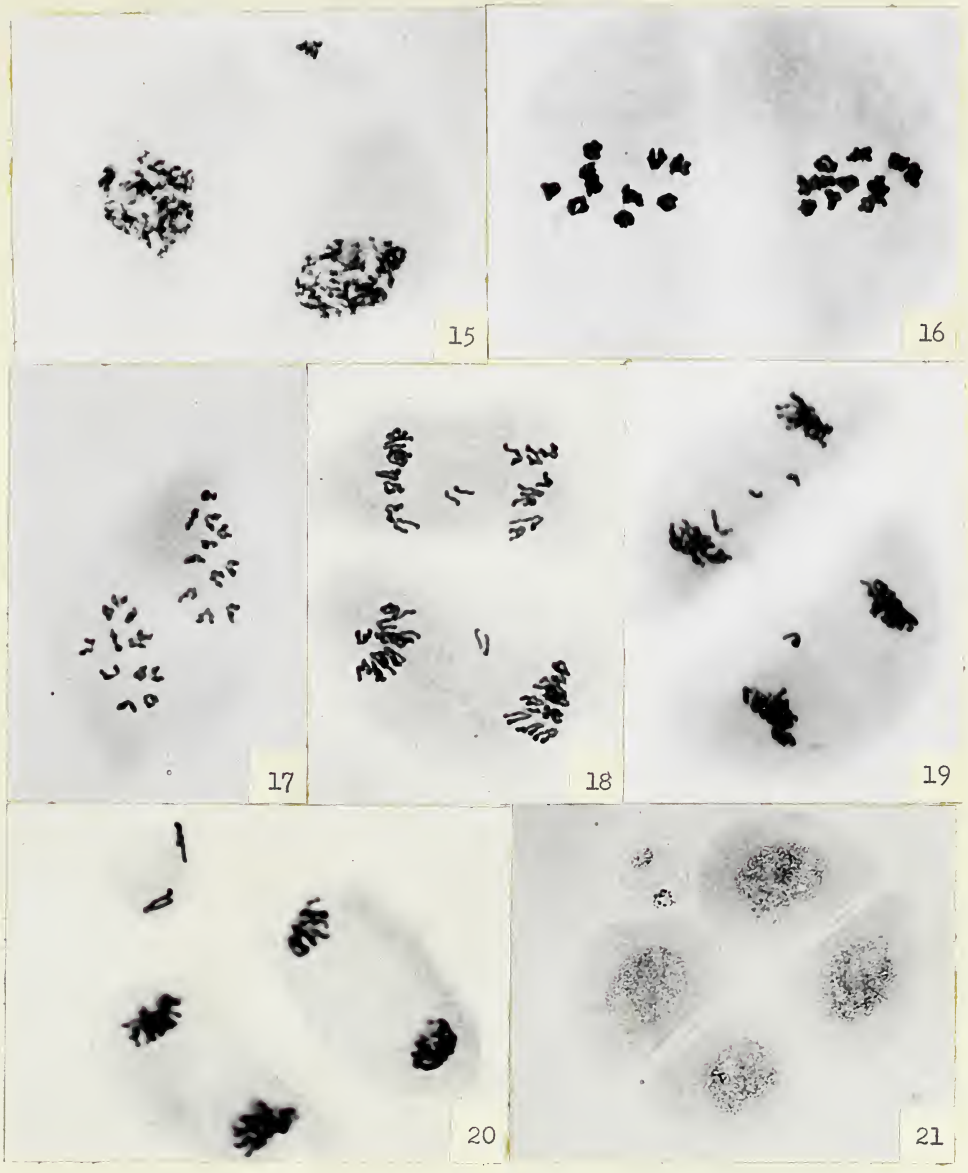


Fig.15. Interphase cell illustrating attached microcyte with nucleus formed from single diad.
Fig.16. Metaphase II with 10 diads in one and 11 in the other daughter cell.
Fig.17. Anaphase II daughter cell with 11 - 12 distribution.
Fig.18. Anaphase II - telophase II with misdividing daughter-univalent in each daughter cell.
Fig.19 Telophase II with lagging fragments and daughter-univalents.
Fig.20 Telophase II with divided diad in microcyte.
Fig.21. Tetrad with associated twin microcytes.

Tetrads were found to have from zero to nine micronuclei and an average of 1.81 per tetrad in 1346 that were recorded. A minute microspore containing one micronucleus was attached to 2.30 per cent of the tetrads. Presumably these were formed from univalents and fragments that had been isolated in the cytoplasm at second division.

At telophase II 54.80 per cent of the pairs of daughter cells had no lagging univalents or fragments. Assuming that laggards at this stage were the source of micronuclei in the tetrads, a similar or perhaps even greater proportion (because of laggards eventually included in the main nuclei) of tetrads should have occurred without micronuclei. Unexpectedly, however, only 29.72 per cent of the tetrads had no micronuclei.

Microcytes initially formed from anaphase I peripheral diads were associated with 2.16 per cent of the tetrads. They now appeared as single minute cells, each with two nuclei, or as twin microspores, each with a micronucleus. These microcytes were enveloped together with the main group of four microspores by a common sheath (Fig. 21). The lower proportion of these microcytes observed at the tetrad stage (2.16 per cent) when compared with their proportions at telophase II (5.09 per cent) and anaphase I (8.06 per cent) can be explained by their separation and loss from the tetrad envelope during preparation of the slide, in spite of the care taken to avoid this.

Restitution at second division was indicated in four cases by the association of one large microspore with two of normal size.

## Viability of Pollen from Triploids

To obtain an estimate of the proportion of good pollen, nearly

mature anthers from secondary tillers of one triploid plant were treated with an iodine solution. Grains were classed as good if they appeared large, well filled with starch, unshrunken, and comparable to those observed in a diploid plant (Fig. 22). The data in Table XIV show that there was considerable variability between anthers in the proportion of good pollen. The range was from zero to 21.3 per cent, the average being 5.5 per cent. Diploid plants grown under comparable conditions produced 98.7 per cent good pollen. Figs. 23 and 24 give an indication of the types of grains that were formed in two anthers from triploids.

TABLE XIV

Percentage of good pollen from triploid and diploid plants of Gateway

| Source | Total no. of grains | No. of good grains | % good grains |
|---|---|---|---|
| Diploid | 5200 | 5132 | 98.7 |
| Triploid | | | |
| Anther 1 | 1013 | 0 | 0.0 |
| " 2 | 672 | 1 | 0.2 |
| " 3 | 1007 | 22 | 4.6 |
| " 4 | 1010 | 25 | 4.0 |
| " 5 | 561 | 16 | 2.9 |
| " 6 | 1003 | 49 | 4.9 |
| " 7 | 1108 | 236 | 21.3 |
| Total | 6374 | 349 | Av. 5.5 |

## Fertility of Triploids

The fertility of all well-developed heads of open-pollinated


Fig.22. Pollen 98 per cent good from diploid plant.
Fig.23. Pollen 2.54 per cent good from anther of triploid.
Fig.24. Pollen zero per cent good from anther of triploid.

triploid plants was calculated by expressing the number of seeds as a percentage of the total number of florets. Data on the fertility of cytologically identified Gateway and hybrid triploids, Gateway triploids pollinated with diploid Gateway, and Gateway control plants are presented in Table XV. All plants were grown in field plots.

TABLE XV

Fertility of triploids and diploids

| Source | No. of florets | No. of seeds | % fertility |
|---|---|---|---|
| Open-pollinated Gateway triploids | 1418 | 60 | 4.2 |
| Gateway 3x x 2x | 142 | 9 | 6.3 |
| Open-pollinated hybrid triploids | 1024 | 119 | 11.6 |
| Open-pollinated Gateway diploids | 1531 | 1470 | 96.0 |

The average fertility of 10 Gateway triploid plants was 4.2 per cent and of three hybrid triploids 11.6 per cent. Although these values are not directly comparable, since the triploids from the two sources were grown in different years, the hybrid plants on the average were more fertile. Ten diploid Gateway plants taken at random from the same plots as the triploids had an average fertility of 96.0 per cent. The data in Table XV indicate little difference in seed set between Gateway triploids that were open-pollinated and those hand-pollinated with normal diploid Gateway.

Data on the viability of seeds from triploid plants are presented in Table XVI. Approximately 54 per cent of the seeds from

the Gateway and 45 per cent of those from the hybrid triploids germinated. Although the germinability of the seed from the Gateway triploids was higher, the ultimate survival of the hybrid seedlings was about 50 per cent greater.

TABLE XVI

Viability of seed from triploids

| Source | No. of seeds planted | Germination | | Adult plants | |
|---|---|---|---|---|---|
| | | No. | % | No. | % of germinated seeds |
| Gateway triploids | 59 | 32 | 54.2 | 20 | 62.5 |
| Hybrid triploids | 119 | 54 | 45.4 | 50 | 92.6 |

Progeny of Triploids

The number of the various chromosome types recovered among the progeny of Gateway and hybrid triploids is given in Table XVII.

TABLE XVII

Chromosome constitution of progeny of triploids

| Source of seed | No. of seeds sown | No. of adult plants | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | Total | 2n | 2n+1 | 2n+2 | 2n+3 | 3n | Other | Unknown |
| Gateway triploids | 81 | 25 | 5 | 12 | 5 | | 1 | | 2 |
| Hybrid triploids | 119 | 50 | 13 | 26 | 4 | 2 | | 2n+f(2)<br>2n+1+f<br>2n+2+f | 1 |
| Total | 200 | 75 | 18 | 38 | 9 | 2 | 1 | 4 | 3 |

Approximately 38 per cent of the seeds that were sown produced mature plants. Of these, 24 per cent were normal diploids, and approximately 51 per cent were primary trisomics. Three plants had three extra chromosomes; this was the maximum number of extra chromosomes found among the aneuploids. Four had a fragment in addition to one or two extra chromosomes. One plant was identified as triploid. In general, aneuploid plants with more than one extra chromosome were dwarfed, lacked vigor, had few tillers, and all but three of 12 plants in this catagory were completely self-sterile. Characteristics of 2n + 1 plants are described under the second part of this study.

## DISCUSSION

The apparent difficulty in producing triploids of common barley experimentally is indicated by the paucity of reported attempts and the failure noted in the present study. Undoubtedly, the method of producing triploids of barley by intercrossing tetraploids and diploids has been unsuccessfully attempted numerous times and, consequently, has not been reported. Although Tsuchiya (64) was successful in obtaining a triploid by this method, his triploid cannot be considered a true autotriploid of common barley since the diploid involved was a closely related wild species, Hordeum spontaneum. His success with this particular cross suggests that combinations between widely divergent, unrelated stocks of tetraploids and diploids of common barley are more likely to be fruitful as a source of triploids. Based on the few known attempts the conclusion can be drawn that crosses between tetraploids

and diploids of common barley are highly incompatible.

Barley triploids have been reported to occur spontaneously as members of twin seedlings (37). The present study has provided evidence that they occur spontaneously in another manner. Of three triploids found in a hybrid $F_2$ population derived from colchicine treated $F_1$ plants, one was noted among the progeny of a mother plant that also produced diploids and tetraploids. Presumably, it originated from the union of a haploid and a diploid gamete produced by the treated mother plant. The other two triploids were descendents of treated $F_1$ plants which otherwise produced only normal diploid offspring, as indicated by their normal fertility. Consequently, the mother plants of these presumably had produced only haploid gametes. Apparently, therefore, either 2x pollen from other nearby treated plants participated in fertilization or the two triploids were of spontaneous origin; that is, they were not the result of colchicine treatment. The latter supposition is more likely since it has been shown that 2x pollen usually does not function in 2x X 4x crosses (10, 12, 19, 14, 16). Further evidence to support the hypothesis of the spontaneous occurrence of triploids in this manner was provided by an additional 13 that were found in a nursery of approximately 4500 progeny head-rows of treated and untreated Gateway barley. Since three of these were found among the progeny of untreated mother plants, it is assumed that these and probably a portion of those from treated plants were of spontaneous origin. The sibs of all triploid plants but one were fully fertile and, therefore, presumably diploid. In the one exception two triploids were found in the same family.

It is likely that none of these spontaneous triploids were derived from twin embryo seeds, since according to Müntzing (37) when

one member is triploid the other is diploid and, consequently, completely fertile. All spikes produced by the triploid plants described in this study were highly sterile; hence, none could have been members of diploid-triploid twins. It is probable that they resulted from the fertilization of an unreduced by a reduced gamete produced by otherwise normal diploid plants. Cytological studies of several diploid species have shown that unreduced gametes are occasionally formed (2, 8, 9, 35, 42, 66). The writer has observed such gametes also in cytological studies of diploid barley. The evidence from the present and other studies further indicates that the triploid plants, described as being of spontaneous origin in this study, probably resulted from the fertilization of diploid eggs with haploid pollen produced by normal diploid plants; diploid pollen most probably was not involved since, as stated above, studies have shown that it rarely functions in the fertilization of diploid plants.

Undoubtedly, triploid barley plants of spontaneous origin have been rarely observed in natural populations because of their close resemblance to normal diploids and the consequent difficulty in detecting them. The triploids described in this study were noted in field plots only because of their sterility which at the time of flowering was indicated by florets which remained open for an abnormally long period and by a very low set of seed on mature spikes.

A higher proportion of trivalents and, consequently, lower proportion of univalents was found in the Gateway triploids than Tsuchiya noted in his triploid (64). These differences can be attributed to incomplete homology between the chromosome sets of the parents of

Tsuchiya's triploid, common barley and Hordeum spontaneum.

Only three quadrivalents were noted in 1092 metaphase I cells of the Gateway triploids. This substantiates evidence obtained from a haploid (62) and a hypotriploid (63) to indicate that very little reduplication of chromatin occurs within the basic set of chromosomes of common barley.

Univalents observed at metaphase I in the Gateway barley triploids were divided into two groups: 1) those located off the equatorial plate and 2) those found on the plate. By means of suitable statistical treatment of the data it was shown that the univalents positioned off the plate were distributed on opposite sides of it at random. These results agree with those reported by Myers (39) who studied univalent behavior in triploid Lolium perenne. As might be expected the relative proportions of these two classes of univalents has been found to vary among different triploids (38, 39, 64). However, of more significance is the behavior of each group at late metaphase I and at anaphase I. In his study Myers (38) noted that an average of 1.33 laggards per cell occurred at anaphase I, as compared with a total average of only 0.93 univalents per cell at metaphase I. He attributed this excess at anaphase to "improper" disjunction of trivalents. Although Tsuchiya (64) noted a much lower average proportion of laggards at anaphase I than of average total univalents at metaphase I (1.46 per cell as compared with 2.33), he found that there was a greater proportion of anaphase I laggards than could be accounted for by the proportion of univalents located on the plate at metaphase I. He assumed that only univalents located on the metaphase I plate lagged and divided equationally at anaphase I and concluded that the excess of anaphase I

laggards originated from "improper" disjunction of trivalents. The results from the present study indicate that trivalents were not a source of lagging univalents at anaphase I in Gateway barley triploids. An average of 0.66 univalents per cell were found on the plate at metaphase I and 0.80 laggards per cell at anaphase I. The difference between these values is considerably less than between the corresponding values for Tsuchiya's triploid. A $X^2$ test of observed frequencies of anaphase I cells with zero to five laggards and the expected frequencies based on the proportion of metaphase I cells with corresponding numbers of univalents on the plate indicated that there was close agreement for all but the two classes with four and five laggards (Table X). The number of cells observed in these two classes was too small (four per cent of the total) to permit a valid test. The average for the remaining four classes, having from zero to three univalents on the metaphase plate, and that for the corresponding classes of anaphase laggards was 0.64 univalents and 0.69 laggards per cell, respectively. Since these two values are in close agreement, the conclusions can be drawn that only those univalents located on the plate at metaphase I lagged and divided equationally at anaphase I and that members of trivalent associations were not an additional source of anaphase laggards.

The assumption that the extra set of chromosomes in triploids is distributed to the poles at random during meiosis has been inferred in several studies of triploids but has been adequately tested in only two (37, 51, 52). In the present study the statistical analysis of the data obtained from a relatively large number of cells indicated that at

anaphase I the chromosomes were distributed to the poles in a binomial frequency in all classes of cells, with and without lagging univalents. (Tables XI and XII). These results agree with Myers' (39) conclusions, based on a study of triploid Lolium perenne, that "the distributions at anaphase I also were consistent with the hypothesis of chance position of unoriented metaphase I univalents and random assortment of the extra chromosomes of the trivalents." That these findings do not have general application to all triploids has been shown by Satina and Blakeslee (51) in their detailed study of triploid Datura stramonium. They noted a statistically significant divergence of certain distribution classes from that expected according to random assortment at anaphase I. They concluded that "it seems probable that the divergence of the assortments at the I division in P.M.C. from calculated values is of general occurrence and is to be attributed to the nature of the chromosomes and the mechanisms involved in their movements at division."

The results from the present and from previous investigations of triploids emphasize the caution to be taken in forming broad, general conclusions from a single study and also indicate the need for further intensive, detailed statistical analysis of chromosome behavior in triploids of other species.

Although the precise behavior of the chromosomes was difficult to trace at second division in the material available for this study, a general pattern could be perceived. At metaphase II - anaphase II the diads formed an equatorial plate and divided equationally. Univalents derived from division of anaphase I laggards were either positioned on the plate or lagged in the cytoplasm at metaphase II. Subsequently

their behavior at anaphase II - telophase II followed one of three courses: 1) they were included with the polar groups; 2) they remained lagging intact in the cytoplasm, probably to form micronuclei later; or 3) they misdivided at the equatorial region in a small proportion of cells. As a result of the division of lagging chromosomes at anaphase I - telophase I and the subsequent lagging of daughter-univalents and fragments at second division, tetrads with an average of 1.81 micronuclei were formed. No micronuclei occurred in approximately 30 per cent of the tetrads. This is an unexpectedly low frequency when compared with 55 per cent of the pairs of telophase II daughter cells that contained no micronuclei.

Microcytes possessing one or two chromosomes have been observed associated with normal cells at meiosis in a variety of plants, including triploids of Datura (7), Lilium (13), hyacinths (18), Zea (33) and Hordeum (64). In the present study it was possible to follow the behavior of microcytes through all stages of both meiotic divisions. They originated at anaphase of first division from one or, less often, two univalents that were positioned on the periphery of the equatorial region or of a polar group of chromosomes. These univalents showed the typical anaphase 'split' but the halves did not separate. At telophase - interphase each peripheral diad formed a minute cell, although in rare instances two were seen together in a single microcyte. Each minute cell was attached to the microspore mother cell (Fig. 15). At second division the chromosome(s) in each microcyte divided equationally, and the resulting univalent-halves moved to opposite ends of the microcyte. At the completion of second division each microcyte had developed into twin minute

microspores that were attached to the main group of four (Fig. 21). These observations are similar to those reported in hyacinths (18), Lilium (13) and Triticum (23). They indicate that a minute cell with a single chromosome is capable of independently undergoing division, exhibiting spindle activity and polarity during the process, and they add to the evidence for the autonomous nature of individual chromosomes during meiosis.

Although the distribution of the extra set of chromosomes has been shown to be random or to approach randomness in a number of different triploids, in none has the frequency of functional eggs and pollen been found to correspond to the expected. Usually gametes with chromosome numbers approaching the haploid complement of the species were found to function most frequently. Those with intermediate numbers and numbers near the diploid complement functioned infrequently, rarely, or not at all. Furthermore, it has also been noted that there is usually a more pronounced selection against aneuploid gametes on the male than on the female side. The results of the study on triploid Gateway barley agree with these general observations in other species. On the basis of random assortment of the chromosomes at anaphase I, plants with 18 to 24 chromosomes would be expected most frequently. It was found, however, that approximately 75 per cent of the progeny from open-pollinated triploid plants were diploids (14 chromosomes) and primary trisomics (15 chromosomes). Apparently, gametes with more than one extra chromosome functioned infrequently, and most probably these were female. The low frequency of progeny with more than one extra chromosome may be attributed to the following factors, assuming that chromosome distribution at

macrosporogenesis was also random: 1) Gametes with more than one extra chromosome were probably less viable than those with the haploid number of seven or with eight. 2) Male gametes with more than one extra chromosome were less viable than female gametes with the same number of chromosomes. 3) Male gametes with extra chromosomes probably failed to function in fertilization because of certation. 4) Aneuploid embryos with more than 15 chromosomes were probably less viable than diploid and trisomic embryos, as suggested by the low fertility of the triploids. 5) Reduced seed germination and seedling lethality probably were further effects of 4).

A comparison of the fertility among the triploids from the two sources reported in this study and Tsuchiya's triploid would seem to indicate that fertility of triploids is influenced by the degree of heterozygosity. The average fertility of the triploid plants obtained from a highly homozygous stock of the variety Gateway was four per cent, of those derived from highly heterozygous, intervarietal hybrids about 12 per cent and of Tsuchiya's interspecific hybrid triploid, produced from the cross between tetraploid common barley and the closely related wild diploid species, Hordeum spontaneum, 19 per cent. Thus, the triploid plants from the most homozygous stock were lowest in fertility, while the triploid from the interspecific hybrid was highest, despite the higher proportion of univalents found at meiosis in the latter.

## SUMMARY

1. Three triploid plants were found in an $F_2$ population of common barley derived from colchicine treated $F_1$ intervarietal hybrids. An additional 13 were found in a large population of the variety Gateway. Triploids occurred spontaneously in Gateway with a frequency of one in approximately 6000 plants; the origin of these was attributed to the fertilization of unreduced female gametes with reduced pollen produced by diploid plants.

2. Morphologically, adult triploid plants were indistinguishable from diploids.

3. An attempt to produce triploids by pollinating tetraploid Gateway with the related diploid was unsuccessful.

4. At meiosis in the Gateway triploids an average of 5.22 trivalents and 1.78 bivalents occurred. All types of trivalents that are possible from pairing between three homologous chromosomes were observed. In addition, three quadravalents were noted in 1091 cells examined.

5. It was statistically determined that univalents lying off the equatorial plate at metaphase I were distributed on opposite sides of the plate at random.

6. $X^2$ analysis of the data indicated that univalents located on the plate at the completion of metaphase I lagged and divided equationally at anaphase I. Univalents off the plate did not divide but were included with the nearest polar group at anaphase I.

7. The distribution of the chromosomes to the poles at anaphase I was in a binomial frequency and, therefore, in accordance

with the hypothesis of random assortment.

8. Misdivision of lagging univalents occurred in 1.90 per cent of telophase I cells.

9. At second division diads divided equationally. Univalents derived from equational division of lagging chromosomes at anaphase I either were included with the main anaphase II polar groupings or lagged in the cytoplasm. Misdivision of univalents at telophase II was inferred in 3.95 per cent of the pairs of daughter cells.

10. As a result of chromosome lagging and misdivision at first and second meiotic divisions, about 70 per cent of the tetrads had one or more micronuclei.

11. Minute twin microcytes were associated with 2.16 per cent of the tetrads (Fig. 21). Each set originated from a lagging univalent located on the periphery of the cell at anaphase I where it formed a microcyte at telophase I - interphase I (Fig. 15). During second division the microcyte behaved as an independent cell; the enclosed univalent divided equationally to form daughter cells, each with a chromatid. (Figs. 20, 21).

12. Pollen from one Gateway triploid plant averaged 5.5 per cent good.

13. The fertility of open-pollinated Gateway and hybrid triploids was 4.2 and 11.6 per cent, respectively (Table XV).

14. Approximately 54 per cent of the seed from the Gateway triploids and 45 per cent from the hybrid triploids germinated.

15. Of a total of 75 offspring obtained from the triploids, 24 per cent were diploid, 51 per cent were trisomic, and one plant was triploid. None of the aneuploid plants had more than three extra chromosomes.

# PART II: TRISOMICS

## REVIEW OF LITERATURE

The first trisomic plants were described in Datura in 1919 by Blakeslee and Avery (2). In 1920 Blakeslee et al. (5) reported that 12 morphologically distinct trisomic types were found, corresponding to the haploid chromosome number of Datura. By means of the modified ratio technique Blakeslee and Farnham (4) identified the gene 'white' with the trisomic type 'Poinsettia'. Subsequently, genes were identified with each of the 12 trisomic types. Since these early reports on Datura, trisomics have been obtained and studied in Nicotiana (12, 16), tomato (17, 18, 19, 27), corn (21, 22), rye (30), and more recently in common barley (25, 28, 31).

McClintock and Hill (22) used trisomics to associate the gene r with the smallest chromosome pair of corn and also reported the identification of trisomics with five additional linkage groups. Lesley (17, 18, 19, 20) first obtained 11 of the 12 primary trisomics in tomato and identified certain of these with specific linkage groups. More recently Rick and Barton (27), using different material, obtained the complete series of 12 primary tomato trisomics and cytogenetically identified six with the corresponding linkage groups.

Trisomic plants have been obtained from several sources. Occasionally they have occurred in the progeny of normal diploids and presumed to have resulted from nonconjunction or nondisjunction at meiosis (7, 12, 30). A further source has been from the progeny of plants heterozygous for an interchange. Burnham et al. (11), and Ramage (25) attributed the origin of these to a 3:1 disjunction from a translocation

complex of four chromosomes and subsequent formation of gametes with one extra chromosome. The most prolific source, however, has been from the progeny of triploids (6, 8, 13, 14, 16, 18, 21, 23, 24, 27, 31).

In most species the presence of an extra chromosome changes the phenotype of the plant. Usually a group of characters specific for each chromosome is altered. Thus, in Datura the 12 primary trisomic types were phenotypically distinct (1), as were those of Nicotiana sylvestris (16) and tomato (27). On the other hand, none of the chromosomes in corn produced distinctive morphological changes when in triplicate other than decreased size and vigor (21). Characters that have been observed to be modified by the presence of an additional chromosome include growth habit; plant height; shape, size, color and position of the leaf; thickness and stiffness of the stems; and enlargment or decrease in size of the floral parts and fruits. In addition, trisomic plants generally have been noted to be less vigorous than diploids and completely to partially sterile, particularly on the male side (16, 18, 21, 25, 27, 31).

On the basis of random chromosome distribution to opposite poles at meiosis, gametes with the haploid number and with an extra chromosome should be produced by trisomics in equal frequencies. Furthermore, if both male and female gametes are fully functional, equal proportions of diploid and trisomic progeny should be produced. Actual transmission of the additional chromosome is, however, considerably less than expected and varies with the trisome involved. In Datura (3) transmission through the ovules varied from about three to 33 per cent; in tomato (27) from less than one per cent to about 25 per cent, and in corn from 22 to 52 per cent (15). The frequency of transmission through

the pollen has been found to be considerably less than through the eggs. Under favorable conditions only five of the 12 primary trisomics of Datura were transmitted through male gametes (9). In Nicotiana sylvestris transmission through pollen varied from zero to 34 per cent, and in eight of 11 trisomics it was less than 10 per cent (16). In corn McClintock and Hill (22) found that approximately 1.4 per cent of the progeny of one trisome were trisomic from the cross 2n X 2n + 1. Rhoades (26) found no trisomics in a population of 1845 plants of a similar cross involving a different trisome of corn.

In common barley Smith (28) found trisomics in a diploid stock, the origin of which he attributed to a 6:8 chromosome segregation at meiosis. Later, Ramage (25) noted that an interchange was carried by the same stock and concluded that Smith's trisomics likely were the result of a 3:1 separation from a ring of four chromosomes. Although the trisomics from this material were shorter than normal, they were vigorous and had no distinguishing characteristics. In 1952 Tsuchiya (31) recovered six trisomic plants among the progeny of a hypotriploid plant. They differed from each other and from diploids in several characteristics, including plant height; stem thickness; number of tillers; length, width and color of leaves; shape of heads; habit of growth; time of maturity; fertility; and cytological behavior. The most striking characteristic among the trisomics was their fertility, which varied from zero to 92 per cent under self-fertilization.

In 1955 Ramage (25) was the first to report on the morphological and cytogenetical identification of barley trisomics. He isolated primary and secondary trisomics from interchanges involving each of the seven

chromosomes of the variety Mars. The morphological descriptions of the trisomic types found to be genetically associated with specific chromosomes were given as follows:

> Trisome of Chromosome a[1].- This type was weak, dwarf and highly sterile.
>
> Trisome of Chromosome b.- This type had narrow, dark-green leaves.
>
> Trisomes from Interchange c - d.- One type was dwarf with slender stems and short, narrow leaves; it was completely self-sterile; most spikes did not reach the heading stage. The second type from this interchange was later in maturity than normal but otherwise was indistinguishable from diploid sibs.
>
> Trisomes from Interchange e - f.- One type was dwarf with short, wide leaves, particularly the flag leaves. The other type was readily distinguished by the long, narrow, light-green, drooping leaves.
>
> Trisome of Chromosome g.- This type was not readily distinguished from diploid sibs, although it produced fewer tillers.

Burnham and Hagberg (10) have summarized the results obtained by Ramage and by several other workers who have utilized trisomics and translocation stocks to determine the association of barley linkage groups with their respective chromosomes. The evidence establishes the independence of all linkage groups except III, VII, and V, on the one hand, and II and V on the other. No genes have been located on chromosome g; Burnham and Hagberg suggested the possibility that a and d also have no known genetic markers. According to their summerization, the following associations appear probable at the present time:

| Chromosome | f | b | c | e | a | g | d |
|---|---|---|---|---|---|---|---|
| Linkage Group | I | III VII | VI | IV | II? | - | V? |

[1] The seven barley chromosomes have been designated temporarily by the small letters a to g by Burnham et al. (11).

## MATERIALS AND METHODS

Primary trisomics described in this part of the study were obtained from the progenies of the hybrid and Gateway triploids discussed in the first part. Each original trisomic plant was cytologically identified as such and affixed with a 'T' number. All trisomic descendents of each original trisomic plant retained this number. The morphological descriptions are based on data and notes taken on the original trisomics and their trisomic progenies grown in the field in two different years. Measurements on maximum leaf width and length were taken on the three uppermost leaves of the original trisomic plants and diploid sibs. Data on spike density were taken on a minimum of 19 spikes collected from six or more plants of each trisomic type and from the diploid. Data on the number of days required to head are based on a minimum of six plants, while the average values for plant height were determined from not less than five plants. All measurements were made on plants grown in the field in 1955, except of leaf width and length, which were obtained from plants grown in the greenhouse.

The varieties Nigrinudum I and Colsess V were used as tester stocks to determine the association of Gateway trisomic type T39 with its corresponding linkage group. These two tester stocks together possess at least one contrasting marker gene for the corresponding locus in Gateway for each of the linkage groups. The characters used, their symbols, the linkage group to which each has been assigned (29) and the genotype of Gateway are summarized in Table I. The two genetic stocks were used as pollen parents in crosses with trisomic plants. Seeds from

$F_1$ trisomic plants were space-planted to facilitate classification for seedling characters. Individual $F_2$ plants were classified for each segregating character. Tests for association were made by $X^2$ analysis of $F_2$ populations for disomic and trisomic ratios.

TABLE I

Linkage groups and marker genes involved in tests for association with Gateway trisomic T39

| Linkage group | Character | Gene symbol | Genotype of Gateway |
|---|---|---|---|
| I | Two-row vs. six-row spike | V, v | vv |
| II | Black vs. white pericarp and lemma | B, b | bb |
| III | Covered vs. naked caryopsis | N, n | NN |
| IV | Hooded vs. awned spike | K, k | kk |
| IV | Blue vs. white aleurone | Bl, bl | blbl |
| V | Rough vs. smooth awns | R, r | rr |
| VI | Normal vs. albino seedlings | $A_n$, $a_n$ | $A_nA_n$ |
| VII | Normal vs. chlorina seedlings | $F_c$, $f_c$ | $F_cF_c$ |

## OBSERVATIONS AND RESULTS

### Morphological Characteristics of Primary Trisomics

Primary trisomic plants obtained from the progeny of the triploids differed from one another and from diploid sibs in characteristics such as rate of growth; relative vigor; height; length, width and

color of leaves; degree of tillering; and length and density of the spike. Although a few of the trisomic types derived from the hybrid triploids appeared to have certain distinct characteristics, it was difficult or impossible to distinguish these trisomics morphologically from one another and in some instances from the diploids because of the high degree of heterozygosity present in the original stocks. One type, however, differed conspicuously from all others and from the diploids by having extremely long, narrow, drooping leaves with enlarged auricles and ligules. This also was the only type that could be distinguished in the seedling stage. A similar type occurred among the trisomic progeny of the Gateway triploids. Since the Gateway trisomics were derived from a pure line stock, morphological differences among them could be attributed to the effects of specific chromosomes when present in triplicate.

On the basis of the data on some measurable characteristics given in Table II and on additional visual differentiating characteristics, four Gateway trisomic types could be readily distinguished from one another and from diploids. Compared with diploids, all trisomic types were shorter, later in heading, had fewer tillers and were considerably lower in fertility. Specific differences between the four types and between these and the diploids were as follows:

Type T31(Fig. 1).- Leaves darker green, broader and more erect than diploid; neck distinctly coiled (Fig. 2); head relatively short and dense when compared with diploid and other trisomics; awns appressed rather than spreading; tallest of the four trisomics.

Type T39(Fig. 3).-Spike shorter but more lax than diploid and tended to be tapered from base toward apex, rather than oblong in shape; leaves shorter and narrower than normal and rolled under at the margins, particularly toward the apex; compared with other trisomics, this type had the

TABLE II

Comparative data on morphological characteristics of Gateway trisomic types

| Primary trisomic type | Av.width of leaves, mm. | Av.length of leaves mm. | Length-width ratio of leaves | Av.length of rachis, mm. | Av.rachis internode length, mm. | Av.no. of days to head | Av.height in inches | Av. no. of tillers |
|---|---|---|---|---|---|---|---|---|
| T31 | 15.0 | 157.2 | 10.7 | 42.0 | 3.02 | 55.4 | 20.3 | 8.8 |
| T39 | 11.2 | 147.5 | 13.4 | 56.7 | 3.29 | 49.3 | 17.5 | 10.7 |
| T46 | 10.0 | 215.0 | 21.4 | -- | -- | -- | -- | -- |
| T51 | 10.9 | 126.7 | 11.7 | 42.0 | 3.21 | 51.2 | 15.7 | 7.0 |
| Diploid | 11.7 | 191.1 | 17.0 | 67.9 | 3.08 | 46.5 | 28.0 | 17.2 |

longest spike, was most prolific in stooling and had the greatest fertility.

Type T46.- Leaves extremely long, narrow and drooping with relatively large auricles and ligules; head emerged from the side of flag leaf.

Type T51 (Fig. 4).- Leaves very broad relative to length, particularly the flag leaf, dark blue-green in color, erect, and base tended to clasp the thick stem; florets were small and flaccid at the heading stage with supernumery organs on the upper ones; at emergence from sheath the awns projected in all directions giving spike a ragged appearance.

The original trisomic type T46 from the Gateway stock was lost because of high sterility. However, a very similar type (previously referred to), probably trisomic for the same chromosome, was recovered from the hybrid material; it reproduced readily under open-pollination.

When all of the adult characteristics of each Gateway trisomic type were taken into consideration, it was relatively easy to distinguish them from each other and from the diploid under field conditions. In the greenhouse certain of the differentiating features tended to be modified or absent. For example, T51 did not show the extremely broad, dark-green leaves and thick stems in the greenhouse, and the development of the head and florets was more normal, facilitating emasculation and hand-pollination.

## Fertility of Trisomics

The fertility among 14 original trisomic plants obtained from the progeny of the hybrid triploids ranged from about 20 to 67 per cent and averaged 46 per cent under open-pollination in the greenhouse. The average fertility of nine diploid sibs was about 92 per cent. Twelve additional trisomics obtained from the same source but grown in the field had a fertility from zero to approximately 92 per cent, with an average of about 52 when open-pollinated. Two of the 26 trisomic plants were


Fig.1. Trisomic type T31 and diploid Gateway.
Fig.2. Trisomic type T31 (left)showing coiled neck and diploid Gateway (right).
Fig.3. Trisomic type T39 and diploid Gateway.
Fig.4. Trisomic type T51 diploid Gateway.

completely self-sterile. The self-fertility of three morphologically distinct Gateway trisomic types and the diploid when grown in the field is given in Table III. Type T31 had the lowest fertility with a seed set of about 17 per cent, while Type T39 was highest with about 50 per cent.

TABLE III

Fertility of open-pollinated Gateway trisomics and the diploid

| Trisomic type | No. of plants | No. of florets | No. of seeds | % fertility |
|---|---|---|---|---|
| T31 | 6 | 1014 | 170 | 16.8 |
| T39 | 9 | 1575 | 789 | 50.1 |
| T51 | 4 | 246 | 91 | 37.0 |
| | | 2835 | 1050 | Av. 37.0 |
| Diploid | 6 | 663 | 637 | 96.1 |

## Transmission of Trisomics

Data on the frequency of trisomic plants among progenies of open-pollinated trisomics are shown in Table IV. Trisomic plants were distinguished from diploids in the hybrid progenies by lack of vigor, short growth, lateness, sterility and certain morphological characteristics that were known from previous experience to differentiate them. In a few instances trisomic plants were cytologically verified. The Gateway trisomics were readily identified from diploid sibs by the morphological characteristics already described. The frequency of trisomic plants

TABLE IV

Frequencies of transmission of trisomic plants in progenies of open-pollinated trisomics

| Trisomic type | Total no. of 2n + 1 and 2n | No. of 2n + 1 | Per cent 2n + 1 |
|---|---|---|---|
| Hybrids | | | |
| T4-2 | 35 | 8 | 22.9 |
| T5-1 | 56 | 10 | 17.9 |
| T6-2 | 51 | 9 | 17.6 |
| T10-2 | 19 | 4 | 21.1 |
| T12-1 | 98 | 25 | 25.5 |
| T15-1 | 56 | 14 | 25.0 |
| T16-1 | 18 | 4 | 22.2 |
| T21 | 45 | 14 | 31.1 |
| T22 | 24 | 6 | 25.0 |
| T23 | 12 | 4 | 33.3 |
| T26 | 49 | 18 | 36.7 |
| T27 | 20 | 4 | 20.0 |
| T28 | 17 | 4 | 23.5 |
| T29 | 36 | 6 | 16.7 |
| T30 | 26 | 7 | 26.9 |
| | 562 | 137 | Av. 24.4 |
| Gateway | | | |
| T31 | 61 | 15 | 24.6 |
| T39 | 72 | 19 | 26.4 |
| T51 | 42 | 9 | 21.4 |
| | 175 | 43 | Av. 24.6 |

among the progenies of open-pollinated hybrid types ranged from 16.7 to 36.7 per cent, averaging 24.4. The frequencies of transmission among the progenies of the three morphologically identified Gateway types T31, T39 and T51 were 24.6, 26.4 and 21.4 per cent, respectively.

Limited data were obtained on the frequency of transmission of the extra chromosome through the pollen. Of 237 plants from the cross 2n X 2n + 1, involving five hybrid trisomic types and four diploid varieties, only one trisomic plant was cytologically identified (Table V).

TABLE V

Frequencies of transmission of trisomics in progenies of 2n X 2n + 1

| Trisomic type | Total no. of 2n + 1 and 2n | No. of 2n + 1 | % of 2n + 1 |
|---|---|---|---|
| T12-1 | 51 | 0 | 0.0 |
| T15-1 | 67 | 1 | 1.5 |
| T21 | 68 | 0 | 0.0 |
| T22 | 32 | 0 | 0.0 |
| T27 | 19 | 0 | 0.0 |
| | 237 | 1 | Av. 0.4 |

## Cytogenetic Identification of Gateway Trisomic T39

Tests were completed for the cytogenetic identification of Gateway trisomic type T39. The observed $F_2$ segregations for each of the marker genes were tested for deviations from a 3:1 ratio. The $X^2$ analysis of the data in Table VI indicates disomic inheritance for

TABLE VI

$X^2$ analysis of
$F_2$ populations of crosses between trisomic
T39 and tester stocks for 3:1 disomic segregations

| Linkage group | Marker gene | Frequency Observed | Frequency Calculated | $X^2$ | P |
|---|---|---|---|---|---|
| I | V | 281.00 | 277.50 | 0.04 | |
| | v | 89.00 | 92.50 | 0.13 | |
| | | 370.00 | 370.00 | 0.17 | 0.95-0.50 |
| II | B | 226.00 | 274.50 | 8.57 | |
| | b | 140.00 | 91.50 | 25.71 | |
| | | 366.00 | 366.00 | 34.28 | < 0.001 |
| III | N | 272.00 | 275.25 | 0.04 | |
| | n | 95.00 | 91.75 | 0.12 | |
| | | 367.00 | 367.00 | 0.16 | 0.95-0.50 |
| IV | K | 233.00 | 237.75 | 0.09 | |
| | k | 84.00 | 79.25 | 0.29 | |
| | | 317.00 | 317.00 | 0.38 | 0.95-0.50 |
| IV | Bl | 98.00 | 103.50 | 0.29 | |
| | bl | 40.00 | 34.50 | 0.88 | |
| | | 138.00 | 138.00 | 1.17 | 0.30-0.20 |
| V | R | 351.00 | 340.50 | 0.32 | |
| | r | 103.00 | 113.50 | 0.97 | |
| | | 454.00 | 454.00 | 1.29 | 0.30-0.20 |
| VI | $A_n$ | 297.00 | 290.25 | 1.18 | |
| | $a_n$ | 90.00 | 96.75 | 0.54 | |
| | | 387.00 | 387.00 | 0.72 | 0.50-0.30 |
| VII | $F_c$ | 267.00 | 261.75 | 0.11 | |
| | $f_c$ | 82.00 | 87.25 | 0.32 | |
| | | 349.00 | 349.00 | 0.43 | 0.95-0.50 |

all markers except B, b, located in Linkage Group II. The observed segregation for this factor was then tested for goodness of fit to a trisomic ratio based on the assumption of nontransmission of n + 1 pollen, 25 per cent transmission of n + 1 female gametes, and chromosome segregation. On this basis 7/18 of the $F_2$ are expected to be homozygous recessive. The $X^2$ analysis in Table VII shows a good fit of observed to calculated values, indicating trisomic inheritance for B, b. The analysis of the data in Tables VI and VII, therefore, establishes the association of trisomic type T39 with Linkage Group II and its independence of the other known groups.

TABLE VII

$X^2$ analysis of $F_2$ population for 11:7 trisomic segregation of B, b in Linkage Group II

| Marker gene | Frequency | | $X^2$ |
|---|---|---|---|
| | Observed | Calculated | |
| B | 226.00 | 223.70 | 0.02 |
| b | 140.00 | 142.30 | 0.04 |
| | 366.00 | 366.30 | 0.06 |

P = 0.95 - 0.50

## DISCUSSION

On the basis of the descriptions of the trisomics of the variety Mars and their associations with certain chromosomes, as given by Ramage (25), it is possible to indicate which of the four Gateway trisomic types likely correspond with specific chromosomes. Trisomic type T39 of the variety Gateway was genetically identified with Linkage

Group II and shown to be independent of the remaining known groups. Therefore, according to the probable associations between the chromosomes and linkage groups indicated by Burnham and Hagberg (10), type T39 should be trisomic for chromosome a. However, the morphological description given by Ramage for this trisomic does not agree with that for T39. Possibly the morphological characteristics expressed by a certain chromosome when in triplicate vary from variety to variety. For example, Ramage observed no trisomic type in Mars with a coiled neck (personal communication), a characteristic that was very distinct and invariable for type T31 of Gateway. Otherwise, the latter type appears to correspond to Ramage's trisome of either chromosome c or d, on the one hand, or g on the other; more likely it is the trisome of g, since this type produced fewer tillers, which was also a characteristic of T31. Ramage's two trisomic types involving chromosomes e and f (the two types were not specifically identified as to which of these two chromosomes was associated with each) probably correspond to Gateway trisomic types T46, which had long, drooping leaves, and to T51, which approached dwarfness under field conditions and had short, broad leaves, particularly the flag leaves.

In accordance with the findings on trisomics of other species (3, 15, 16, 27), the frequency of trisomic plants among the progenies of open-pollinated barley trisomic types was considerably less than the theoretical 50 per cent, approaching an average of 25 per cent in the present study. Limited results also indicated that the extra chromosome was transmitted through the pollen with a frequency of less than one per cent; therefore, it is probable that transmission of trisomics in barley is

largely, if not entirely, through female gametes, at least for certain chromosomes. This is also in agreement with the results reported in Datura (9), Nicotiana (16), and corn (22, 26).

Both Tsuchiya (31) and Ramage (25) found that certain simple trisomic types obtained from pure varieties of barley were completely self-sterile. In the present study two of 26 original hybrid and two of 12 Gateway primary trisomic plants were completely self-sterile. Two additional trisomics of Gateway were lost because of almost complete self-sterility. This evidence indicates that certain of the primary trisomes of common barley, particularly if established in a pure variety, are completely self-sterile, or nearly so. They would have to be maintained by hand-pollination with pollen from diploids of the same variety or, as suggested by Ramage (25), maintained as heterozygous stocks, since trisomics from intervarietal crosses are more highly fertile.

## SUMMARY

1. Four morphologically distinct primary trisomic types of the variety Gateway were identified.

2. The fertility of each of three Gateway trisomic types under open-pollination was approximately 17, 37 and 50 per cent, respectively, averaging approximately 37 per cent.

3. The frequency of trisomic plants among progenies of 15 unidentified open-pollinated hybrid trisomic types varied from about 17 to 37 per cent and averaged 24 per cent. In progenies of three open-pollinated, morphologically distinct Gateway trisomic types approximately 21, 25 and 26 per cent of the plants were trisomic, respectively.

4. Transmission of the extra chromosome through the pollen of five unidentified hybrid trisomics averaged 0.4 per cent in a total population of 237 plants.

5. One Gateway trisomic type was found to be associated with Linkage Group II and independent of the other known groups.

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