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CYTOGENETICS OF SYNTHETIC 6X AMPHIPLOIDS OF *AVENA*

by

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ABSTRACT

A_o, A_1, and A_3 generation plants of amphiploid Avena abyssinica x A. strigosa, autotetraploids of a diploid species A. strigosa, and two tetraploid lines derived from a synthetic 6x amphiploid were used as experimental material. A_o was the colchicine doubled tillers of the triploid hybrid.

Chromosome mosaicism in root-tips of A_1, A_3, and autotetraploid plants was observed. This observation suggested that in some root-tip cells of these plants one or more chromosomes had not undergone replication at interphase and therefore, at anaphase, these chromosomes had either moved to one of the poles at random or had misdivided. Only few cells with more than the modal chromosome number were observed.

Chromosome mosaicism in PMCs of A_o, A_3, and autotetraploid plants was observed. In autotetraploid plants, most of the PMCs with reduced chromosome complement had even number of chromosomes. This observation suggested that during premeiotic mitosis, homologous chromosomes, in some special way, were involved in the formation of these PMCs. Chromosome mosaicism in PMCs of A_o and A_3 plants may be due to the formation of germ line by mosaic cells, differential condensation of chromosomes in PMCs, PMCs with more than one nucleus, and disturbance in premeiotic mitosis. Chromosome mosaicism in PMCs and univalents as well as multivalent associations at metaphase I may lead to the formation of gametes with more or less than

the n number of chromosomes. Only few PMCs with more than the modal chromosome number were observed.

The observed difference in chromosome association in A_0 and A_3 plants suggested that the A genome of the parent species has differentiated, but to a lesser degree than that between the A genome chromosomes of abyssinica and sativa. In the later generation lines of the synthetic 6x amphiploids of Avena, higher fertility will be related to the shift toward higher quadrivalent formation. If the above interpretation is correct, fertile amphiploid lines can be selected after fewer generations of selfing from amphiploids of hybrids between derived tetraploids and their parental diploids than from amphiploids of hybrids between a tetraploid and a diploid species.

In A_3 plants, pollen grains with as few as 8 chromosomes and 3 fragments and with as many as about 28 chromosomes were observed.

Pachytene like feulgen positive threads were observed in pollen grains of two A_3 plants. Pachytene like threads stained with feulgen even after pollen grains were treated with Trichloroacetic acid. This observation suggested that the threads represent some abnormality in pollen grain wall.

Cytomixis, the process of extrusion of chromatin from the nucleus of one PMC into the cytoplasm of the adjacent PMC, seem to be affected by the fixative used as well as by the physiological state of the PMCs.

INTRODUCTION

Brown and Shands (1954) obtained amphiploid seeds of the triploid hybrid between Avena abyssinica Hochst. (2n=28) x A. strigosa Schreb. (2n=14) C.I. 3436 (= C.I. 7010 = Saia). They sent some of the A_2 or A_3 generation seeds to Dr. Frey who turned them over to Dr. Sadanaga[1]. In the A_5 or A_6 generation of this amphiploid material, Dr. Sadanaga picked up stable tetraploid (2n=28) derivatives in two lines.

In this study, an attempt was made to understand the cytogenetic mechanism(s) involved in the derivation of these tetraploids. Chromosome mosaicism in synthetic amphiploid and autoploid material is common in plants as well as in animals. Therefore, in this study, possible mechanism(s) of mosaicism was also explored.

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[1]Sadanaga, K., Dept. of Genetics, Iowa State University of Science and Technology, Ames, Iowa. Personal Communication. 1963.

REVIEW OF LITERATURE

Frankhauser (1945) observed a wide range of irregular chromosome numbers in some individuals of Triton palmatus (a newt) and named this phenomenon: "complex mosaics with irregular chromosome numbers." According to Frankhauser, this phenomenon was named "Poikiloploidy" by Levy in 1920 and "multiform aneuploidy" by Böök in 1944. Contemporary publications in English language simply describe this phenomenon as "chromosome mosaic" or "complex chromosome mosaic."

The literature on mosaicism and phenomenon which may be related to it is reviewed below and grouped in two sections, vegetative tissues and generative tissue.

Phenomena Which May be Related to Mosaicism in Vegetative Tissues

Chromosome variation and derivation of stable lower chromosome types

According to Huskins (1948), Sparrow in 1938 recovered 28 chromosome plants from a 42 chromosome hybrid wheat line. These recovered plants regularly formed 14 bivalents at metaphase I.

Vaarama (1949) found that in colchicine induced tetraploid Ribes nigrum and in their progeny raised from seed, chromosome numbers in the root-tips varied from 4 to 32. The cause of this variation was attributed to the presence of two separate spindles in the cells.

Menzel and Brown (1952) studied polygenomic hybrids of Gossypium. They produced two series of synthetic amphiploids, tetraploids produced by doubling F_1 hybrids between diploid species, and hexaploids produced by doubling F_1 hybrids between G. hirsutum (natural allotetraploid) and various diploid species. They intercrossed the synthetic amphiploids and also crossed them with various natural diploid species and with allotetraploid species G. hirsutum and obtained series of hybrids having complete chromosome sets from two, three or four different species having the diploid, triploid, tetraploid, pentaploid or hexaploid multiple of the basic chromosome number 13. In a number of hybrids, they observed striking color mosaics in leaves and petals. They concluded that the degree of mosaicism increased with an increase in chromosome number. At a given level of ploidy, the greater the number of species involved, the more exaggerated was the mosaicism. Moreover, among hybrids which had 52 chromosomes and combined three species, those having one genome present twice (e.g. A_2E_1/A_2B_1) showed more mosaicism than those in which each of the three genomes was present only once, (e.g. $A_2B_1/(AD)_1$ and $(AD)_1A_2/D_5$). In synthetic hexaploid material, the doubled generation itself had less mosaicism compared to its progeny. Hybrids produced by crossing synthetic amphiploids inter se, had more mosaicism than parent amphiploids. Mosaicism disappeared in hybrids produced by crossing synthetic amphiploids with a natural

species, either diploid or tetraploid. They concluded that mosaicism is not dependent upon the after-effect of colchicine treatment, upon particular combinations of species, either closely or distantly related, or upon the amount of segregation expected in the amphiploid parent. Occasionally, color mosaics in flowers was observed in two old colchicine-doubled plants of Asiatic species ($4A_2$ and $2A_2A_1$) and in leaves and flowers of a colchicine doubled (8x) plant of G. hirsutum. Moreover, they observed a case of somatic reduction in a mosaic hexaploid x hexaploid hybrid combining 3 species. Many, if not all cases of mosaicism suggested segregation rather than merely loss of chromatin. Therefore, they suggested: "some type of atypical segregational mitosis similar to these recently described by Huskins and his co-workers would explain the observed mosaicism and provide a common cytological basis for both mosaic phenotypes and somatic reduction." Moreover, they wrote: "mechanisms leading to mosaic phenotypes have been analyzed in detail only in aberrant lines within species. How any such known mechanisms might operate in hybrids composed of partially non-homologous genomes can only be conjectured." Out of 200 mitotic anaphases in root-tips of four different hexaploid combinations, they observed bridges, fragments or lagging chromosomes in nine cells, while among 250 root-tip anaphases of G. hirsutum, they observed only one cell with such irregularity. They did not make any conclusion from these data.

Sharma and Sharma (1956) studied a large number of vegetatively propagated plants of Aroideae, Liliaceae, Amaryllidaceae, and Dioscoreaceae. They observed mosaicism in root-tips of all the plants (more than a hundred) studied. No regularity in the sequence of their occurrence was noted and the numbers so far recorded suggest random behavior. In a later study (1957) they observed this chromosome mosaicism in stem-tips also. The percentage of mosaic cells in stem-tips was distinctly higher than in the root-tips. They thought that this was due to the fact that growing apices of stems differentiate more than root apices.

Pohlendt (1958) studied spontaneous as well as synthesized F_1 hybrids (2n=35) of Aegilops triuncialis x Triticum aestivum. A few F_2 plants from spontaneous F_1 hybrids were also studied. He observed chromosome mosaicism in root-tips as well as in epigeous (glume base, young anther, etc.) somatic tissue. In 12 root-tips of synthesized F_1 hybrids, the chromosome number varied from as high as 52 to as low as 10, while in spontaneous F_1 hybrid, the chromosome number varied from 3 to 53 ± 2 chromosomes. In F_2s produced from spontaneous F_1s, the chromosome number in root-tips varied from 3 to 70 ± 2. In epigeous somatic tissue of F_2s, the chromosome number varied from 8 to 63. Mosaicism was also reported in PMCs of two F_2 plants. One had PMCs with as many as 43 chromosomes and as few as 8, while the other varied from 3-43. He observed multipolar spindles

in mitosis as well as in meiosis. Fukumoto (1962) obtained somewhat similar type of mosaic cells in root-tips of high polyploid Solanum species and hybrids. He suggested that both high polyploidy and hybridity might be responsible for the mosaicism. From the study of various synthetic amphiploids and hybrids of Nicotiana, Yang (1965) concluded that ploidy, hybridity, as well as genotypes were responsible for the mosaicism.

Hegwood and Hough (1958) observed chromosome mosaicism in the root-tips of variety White Winter Pearmain apple and six of its seedlings. Cells with 2n chromosome number were observed in highest frequency. Cells with both higher and lower chromosome number than the 2n were observed. Cells with following abnormalities in mitosis were observed: chromosome excluded from the spindle, precocious movement of one or more chromosomes to the pole, lagging of one or more chromosomes at metaphase, rare occurrence of anaphase bridges, unequal division in anaphase, and cells with two metaphase plates.

Forsberg (1961) studied amphiploids of Avena abyssinica C.I. 2108 x A. strigosa C.I. 3436. In A_7 and A_8 generation plants, the mode chromosome number for each plant varied from 38 to 43. Chromosome mosaicism was observed in almost all the root-tips examined. Lowest chromosome number observed was 25 in a plant with mode of 42 chromosomes.

Krishnamurty and Satyanarayana (1962) studied F_1 plants of

the three species cross (Nicotiana glutinosa (n=12) x N. trigonophylla (n=12)) x N. megalosiphon (n=20). Somatic chromosome number of hybrids examined varied from 33 to 44. Based on variation in plant morphology, they assumed that the elimination of chromosomes was random. Variation in chromosome number was observed between plants but was not observed within plants. Therefore, they suggested that chromosome elimination took place during early stages of embryo development.

Rajhathy (1963) studied the cytology of colchicine doubled C_1 and C_2 generations of an allotetraploid species Hordeum murinum (2n=28). C_1 seeds were harvested only from partially sterile spikes (C_o) and plants (C_2) originating from only C_1 generation chromosome mosaics were studied. He observed chromosome mosaics in root-tips as well as in PMCs. In the C_1 generation, 30 out of 39 plants had the expected 2n=56 chromosomes. Chromosome number in the remaining 9 plants varied from 28 to 54. In the C_2 generation, 37 plants had 2n=56 chromosomes and in 13 plants, the chromosome number varied from 28 to 54. Rajhathy did not mention, but his data showed that only 2.5 and 15.4 percent of the plants in C_1 and C_2 generations, respectively, were without even numbers of chromosomes.

According to Yang (1965), Pfitzer in 1957 found a greater inconstancy of chromosome numbers in triploid hybrids of the genus Anthurium as compared to diploids, which was ascribed to the disturbance of the equilibrium of the genomes in triploids.

In most filamentous fungi, vegetative cells are usually haploid, and the zygote is the only diploid cell, which immediately goes through meiosis. Diploid vegetative cells were found in nature and were produced artificially (Pontecorvo, 1958) in many fungi. Pontecorvo (1958) observed haploids in the progeny of some diploids of Aspergillus nidulans. He suggested that haploidization might be the consequence of accidental and rare failure of regular mitotic separation of sister chromatids. He mentioned that most haploids could be shown to originate as aneuploids (2n-a, where a=1 at the onset of the process and a=n at the end). There carried at first some chromosomes in the disomic condition, which are rapidly reduced to the monosomic condition by accidental loss and selection in favor of the fully balanced haploid.

Käfer (1960) found haploid nuclei among diploid mycelia of Aspergillus nidulans. He attributed this process to chromosome non-disjunction, which would involve intermediate aneuploid steps. Lhoas (1961) found that in diploid Aspergillus niger, as a consequence of para-fluoro-phenylalanine treatment, haploidization occurs by successive losses of chromosomes.

Taylor et al. (1962) studied the effects of 5-fluorodeoxyuridine on breakage and reunion of anaphase chromosomes in the root-tip cells of Vicia faba. They reported that this chemical blocks DNA synthesis in the cell. Berger and Witkus (1962) studied cytological effects of 5-fluorouracid in the

root-tip cells of Allium cepa and concluded that this chemical blocks DNA synthesis almost immediately and, therefore, eroded regions of chromosomes were evident at anaphase. Moreover it prevented duplication of DNA in the resting stage following a normal mitotic division.

Genome separation

According to Stern (1958), in 1956 and in 1957, Glass observed genome segregation in rat liver cells. These cells were of varying ploidy level (1n to 6n) and cells with heteroploid chromosome numbers were either absent or rare. Among more than a thousand metaphase stages of diploid cells, 9-14 percent had the two haploid sets of 21 chromosomes grouped separately from each other. In triploid metaphases, 27-41 percent had separate genome groupings of the types 2n-1n and 1n-1n-1n, and similar high frequencies of nonrandom separations were counted in tetraploid and high polyploid cells. His designation of chromosome groups as genomic was not based simply on the number of chromosomes but was also based on the karyotype.

Many species of beetles are polyploid and parthenogenetic. According to Stern (1958), in 1940 and in 1947, Suomalainen reported that in beetles when the unpaired chromosomes arranged themselves for the first (and only) nuclear division of the maturing egg, they might appear on a single metaphase plate,

might arrange irregularly as if for a multipolar spindle or might arrange on different metaphase plates in groups that contain multiples of 1n=11 chromosomes. In triploid species, separation in 2n-1n was observed and in tetraploid species, distributions of the types 3n-1n, 2n-2n, and 2n-1n-1n occurred in 18 out of 40 eggs. These observations were based on sectioned material, and therefore the separation of groups of chromosomes on separate metaphase plates undoubtedly was not an artifact. According to Stern (1958), Glass in 1956 and in 1957 considered separation of chromosomes into genomes a prerequisite for the formation of cells with constitutions not belonging to the series 2^x (e.g. haploid and triploid cells from a tetraploid cell). According to Stern (1958), many authors assumed multipolar mitosis as the cause of somatic reduction. But this mechanism is inefficient in accomplishing segregations of whole genomes. He wrote: "Whatever the mechanism, the origin of haploid from diploid or lower degrees of ploidy from higher ones is an established source of somatic cell variation."

According to Shcherbakov (1960), Gottschalk in 1958 observed one diploid and one hexaploid PMC in an octoploid tomato plant. Moreover, in telophase I, the distribution of the genomes of the type 3x+x and 2x+x+x was observed.

Somatic pairing and related phenomena

In Oenothera, Gates (1912) observed somatic pairing of at least a few of the chromosomes in two cells of the nucellus.

He wrote: "On the whole, it seems probable that this pairing in the equatorial region of the spindle will be followed by the separation of the pairs, and hence by a reduction division. It is impossible not to be struck by the numerous differences between this figure and normal somatic metaphase. While the inference, that this intimate pairing is to be followed by a reduction or segregation division, is as yet unproven by actual observation, yet it seems more probable than any other explanation I can offer."

Watkins (1935) cited work of 33 workers who observed pairing of chromosomes during division of somatic nuclei of various plant species. He observed it in Yucca rupicola. According to Watkins (1935), Strasburger in 1907 reported that in plants attraction of chromosomes occurred only in pairs and not between higher multiples. This is not true of the Diptera (Metz, 1922). According to Berger (1941), polysomaty is the condition in which diploid and polyploid cells are present in the same tissue and the number of chromosomes in polyploid cells is the multiple of the diploid and not of the haploid chromosome number. Like Strasburger, he also reported that in spinach and other plants where polysomaty was observed, the chromosomes were either single or in pairs, but never in groups of four or eight as was the case in multiple complexes of mosquito.

Berger (1936) and Grell (1946 a,b) studied chromosome

changes during development in the mosquito, Culex pipens (2n=6). At the beginning of metamorphosis, the hind gut of the mosquito was formed of the ileum, colon, and rectum. During metamorphosis the colon disintegrated, while the cells of the ileum and rectum divided to form the ileo-colon of the adult. The dividing cells had the multiple complexes of chromosomes. These multiple complexes arose by repeated division of the chromosomes without the division of the cells during the long resting stage preceding metamorphosis. Throughout the nine to fourteen days of larval life and the first six or seven hours of pupal life, the cells remained in interphase. During interphase, there was progressive increase in size of ileum cells but not in their number. This increase in cell size was accompanied by increase in chromosome number from 6 to 12, 24, 48, 96, and rarely 192. All the cells did not reach the same degree of ploidy at the end of growth period when they began to divide. During metamorphosis the multiple complex cells underwent one, two, or more divisions. The divisions were found to be of two types--namely, first-division and later-divisions, respectively. A first-division was the initial division which any multiple complex cell underwent. Any division other than the first was considered a later-division. During both kinds of divisions the cells passed through the usual prophase, metaphase, anaphase and telophase stages, and two succeeding divisions were separated by short

interphases. At late prophase, chromosomes were in six separate groups each containing many similar groups. Later, the number of groups reduced to three in each of which the chromosomes appeared to be in pairs. Distinction of chromosomes into groups was lost at metaphase where chromosomes were fully condensed and were always in closely synapsed pairs. In first-division cells, chromatids of every chromosome went to opposite poles where each again synapsed with the chromatid of the previously synapsed chromosome. In general, at the end of the first-division, unlike every later-division, there was no somatic reduction. During every later-division of these autoploid cells, members of a metaphase pair separated at anaphase and went to opposite poles where each again synapsed with a homologous chromosome and thus somatic reduction occurred. The number of later-divisions depended on the degree of ploidy of the parent ileum cell at the beginning of metamorphosis. Newly formed ileo-colon cells did not divide. But it was estimated that these cells were probably tetraploid or octoploid.
First- and later-divisions were separated by a short interphase during which no reduplication of chromosomes occurred. Instead, chromosomes paired at anaphase were more closely associated (somatic synapsis) during subsequent interphase and prophase. The substitution of somatic synapsis for chromosome reduplication in the interphase was considered to constitute the essence for somatic reduction. Somatic pairing of homologues,

throughout the mitotic cycle, was also found in diploid as well as in spermatogonial cells. But according to Srinivasachar and Patau (1958), Wilson and Cheng (1949) did not find anything near regular segregation of homologous chromosomes.

In colchicine doubled apomictic Hieracium hoppeanum (2n=45), Christoff and Christoff (1948) found plants with reduced (2n=45) as well as with unreduced chromosome numbers. He concluded that embryos with the reduced chromosome number originated from integumental cells which had passed through meiosis.

According to Huskins (1948), Battaglia in 1947 showed that in Sambucus ebulus, the cells at the base of the style passed through an unusual heterotypic division with formation of bivalents and binucleate cells (two haploid nuclei). According to Huskins (1948), Hughes-Schrader in 1927 observed premeiotic reduction as a regular feature in a portion of the hermaphrodite gonad of coccid Icerya purchasi but preceding the appearance of haploid nuclei, no chromosome pairing or heterotype divisions were found.

Hiraoka (1958) studied somatic pairing of chromosomes in Daphne odora. He used basal region of young leaves, shoot- and root-tips, vascular bundle cells of young petals, shoot cortical and pith cells near the growing point, and tapetum cells. He studied all stages of mitosis and presented numerical data for the variations observed at each stage. He concluded that active pairing started at anaphase or telophase

and in a passive form, it might remain even at metaphase of succeeding division. He considered this conclusion as an evidence in favor of Darlington's (1937) hypothesis that chromosomes pair because they are single but a pair of chromosomes cannot pair with another pair.

Risler (1959) observed tetraploid mitosis in the third instar of Aedes aegypti (Culicidae). The number of tetraploid cells increased until the beginning of the fourth instar. During the fourth instar, somatic reduction took place and diploidy was reestablished. During reduction division chromosomes were not arranged as in equational division, but were superimposed along the length of the spindle.

Kitani (1963) studied orientation, arrangement and association of somatic chromosomes. He used root-tips of Crepis capillaris and endosperm tissue of immature seeds of Aegilops squarrosa as his main experimental materials. Tradescantia pardosa, T. reflexa, Vicia faba, Allium cepa, Zea mays, and Avena strigosa were also used, but to a lesser extent. All the tissues were tapped in a drop of aceto-orcein over a coverslip without prefixation. Only for the observations on the doubleness of anaphase chromatids, chromosome association in telophase, and the arrangement of interphase chromonemata, material was fixed in acetic-alcohol (1:3) and feulgen method was applied. He wrote: "For a particular photograph, pressing was applied before stain fixation to avoid the disarrangement

of chromosomes which is considered to occur when pre-fixed cells were pressed." In prophase, he consistently observed kinetochore regions of all chromosomes around one pole ("Kinetochore pole") of the nucleus and their free ends around the other pole ("free end pole"). This orientation was observed till chromosomes arranged themselves on an equatorial plate. At the beginning of prophase, distances between sister chromatids were wide and this distance was shortened at middle prophase. In Crepis, side-by-side arrangement of homologous chromosomes was observed from prophase till anaphase. In immature endosperm tissue of A. squarrosa, the side-by-side arrangement of homologous chromosomes at prophase was confirmed by studying the position of satellites in relation to each other. In 23 nuclei satellites of two of the three sat-chromosomes were side-by-side, while in only 2 nuclei, the satellite of the third sat-chromosome was close to the other two. No case was reported where satellites of all the three sat-chromosomes were located independently to each other. He presented pictures of two cells (one of interphase cell and another of prophase cell) in which 2 satellites were side-by-side while the third was separate. He did not clarify whether positions of satellites in relation to each other were observed at prophase, at interphase or at both of these stages. He wrote: "Since the satellite of this chromosome was clearly distinguished throughout interphase as a heteropycnotic body

attached on a nucleolus, the distribution of three satellites in a nucleus was analyzed." He studied 555 nuclei. Over 56% had two satellites on a larger nucleolus and the third one was on a smaller nucleolus and about 36% had all three satellites on a single large nucleolus. He interpreted this observation as indicating that the side-by-side arrangement was present at interphase as was the case at other stages of mitotic cycle. The homologous chromosomes which were situated side-by-side at anaphase, came closer to each other at telophase (chromosome association) and lost their visible individuality. In Crepis, three instead of six masses of homologous chromatids at a pole were observed in most of the cases. Three chromosome masses were also observed at the beginning of interphase. The definite sign of neither presence nor absence of chromosome association was observed in middle and later interphase. In general, homologous chromosomes were oriented side-by-side from prophase till telophase and were intimately associated before DNA replication.

Levan (1939) treated root-tips of Allium cepa and A. fistulosum with growth hormones. He observed polyploid mitosis with paired chromosomes at prophase in cells which were about 2 to 3 m.m. away from the tip. He concluded that the doubling of chromosomes took place during interphase. Such polyploid mitosis was called endoreproduction by Levan and Hauschka (1953) who also observed paired chromosomes at prophase and metaphase

in many cells of ascites tumours of the mouse. Mittwoch et al. (1965) observed paired chromosomes at metaphase in cultured human cells which went through two series of DNA replications at interphase and afterwards were undergoing mitosis.

Merriam and Ris (1954) observed that some diploid and polyploid somatic tissues of female honeybees had DNA content characteristic of haploid nucleus.

D'amato (1954) reviewed the literature on polysomaty and polyploidy in differentiated plant tissues. He concluded that mitosis with paired or grouped chromosomes is the result of interphase supernumerary chromosomal reproductions.

Chouinard (1955) obtained evidence that a gradient of endomitotic polyploidization exists in certain tissues along the longitudinal axis of the onion root. He also concluded that indoleacetic acid merely reveals the polyploid condition of differentiated tissues and does not cause polyploidization.

Colchicine induced somatic reduction and related phenomena

Ross and Holm (1960) treated tomato seedlings heterozygous (pale green color) for the aurea mutant (+A) with colchicine (apparently, chromosome number was not doubled). They observed increased frequency and size of spots indicating somatic segregation for homozygous tissue. The treated and untreated plants did not differ in the ratio of green (++) to yellow (AA) and twin (green and yellow) to single (green) spots. They suggested that these observations seem to support the hypothesis of

somatic reduction. It could however be concluded that at least the region of the chromosome containing the locus had undergone reduction division. Twin and single spots were observed more frequently in the tetraploid plants than in the diploid plants.

According to Chen and Ross (1963), during 1958-1960, Gottschalk studied tetraploid and octoploid tomato plants germinated on 0.5% colchicine in agar. He observed genome separations in certain PMCs as well as prior to meiosis, so that haploid gametes were formed in many instances.

Simantel and Ross (1963) studied somatic reduction in the variety Experimental 3 of Sorghum. They treated seedlings heterozygous for structural chromosome markers (F_1 seedlings produced by crossing plants homozygous for different reciprocal translocations and therefore had four pairs of structurally marked chromosomes) with 0.5% colchicine in lanolin. Out of 124 treated plants, 90 survived and 2 of them were homozygous for normal chromosome structure, dissimilar from either parent and therefore possibility of either selfing or androgenic origin was eliminated. It was proposed that cells with normal chromosome structure were formed by somatic reduction followed by doubling to restore the diploid number. Since four different chromosomes were involved, it was proposed that somatic reduction involved the whole chromosome complement. No explanation was proposed for the mutational effects that seem to be inherent in the somatic reduction phenomenon.

Sanders and Franzke (1964) proposed a hypothesis for the origin of colchicine-induced diploid mutants in Sorghum. Because of the confusion occassioned by the similarity of the words homologous and homoeologous, they discarded the word homoeologous in favor of the word analogous to designate phylogenetically similar chromosomes. The hypothesis stated that under the influence of colchicine, diploid mutants arose through substitution of chromosomes of similar phylogenetic origin. They wrote: "substitutions have not been detected cytologically because there is a tendency for bivalent rather than multivalent pairing to occur in sorghum with 2n=20, and because pairing may occur between analogous chromosomes. Information from the literature, as well as research on the mutants, lends support to this view."

Chromosome aberrations in root-tips

In Zea mays, Beadle (1932) observed that plants with sticky chromosome gene in homozygous condition caused alteration in chromosome behavior in somatic divisions and to a much greater degree, in meiotic divisions. Nine percent of the total 207 mitotic metaphase figures from the sticky chromosome plants were abnormal. Most frequently observed abnormality was loss of chromatin material from one of the chromosomes so that 19 chromosomes plus a fragment was observed at metaphase. At mitotic anaphase no stickiness of the chromosomes was observed. In meiosis, frequency of non-disjunction and

translocation increased considerably over normal.

Kato (1957) observed high polyploid cells in the central part of Allium endosperms. The cells with chromosome number higher than 6n, showed disorientation of chromosomes, and chromosome bridges at anaphase and fragments at metaphase. He wrote: "chromosome breakage seems to be associated with the polyploid condition."

Kato (1960) studied mitotic abnormalities in Clivia miniata (2n=22). The chief abnormality observed in root-tips was chromosome bridge(s). In shoot meristem, no abnormality was observed. These irregularities were more frequent among root-tips fixed in summer than those fixed in winter. It was suggested that both the external conditions (especially temperature) and cellular metabolic conditions were responsible for the aberrations.

From several studies of experimental mitotic stimulation and of spontaneously occurring mitosis in differentiated tissues, D'amato and Hoffmann-Ostenhof (1956) concluded that chromosome aberrations can be found almost exclusively in polyploid cells and practically never in diploid cells. They also reported that in 1948, D'amato studied several monocot species and observed spontaneous chromosome breakage and a pronounced increase in chromosome stickiness in the root-tips of plants approaching the rest period. They also reported that since Navashin's work with Crepis in 1933, it is known for many plant

species that the frequency of cells with chromosomal changes in root-tips of seedlings progressively decreases with increasing length of the root. They also mentioned the reports showing that in aged seeds of Triticum vulgare var. Baart and in T. durum var. Kubanka, the frequency of chromosomal changes in the shoot-tips and in the root-tips is in a ratio of 1:3, and in many plants root-tips have higher frequency of spontaneous chromosome aberrations than shoot-tips, which may be related to the known greater radiosensitivity of the root-tip meristem of dormant seeds compared with the shoot meristems.

Segregation of homologous chromosomes

Huskins (1948) presented many cytological figures of somatic reduction in Allium cepa and termed such natural and induced somatic reduction-divisions as "somatic-meiosis" without, however, implying that they necessarily involve all the essential features of normal meiosis. This separation might occur up to any degree and at any stage from early mitotic prophase to that of mitotic or even meiotic metaphase. His figure 1D showed that two chromosomes of different sizes are united by an almost terminal chiasma, and mentioned that in another cell, a median chiasma united two such chromosomes. Except for these two cases, none of his other figures or description implies that there were chromosomes which resembled meiotic chromosomes with chiasmata. He used sodium nucleate (sodium salt of ribose nucleic acid) and held the opinion that

it only enhanced the frequency of natural variations of nucleus and chromosome which because of the low frequency, was impossible to detect in natural material. He mentioned: "This is of course not surprising since nothing has been added to the cell that is not normally present." Evidence of crossing-over and segregation in somatic cell was given by Muller (1916) in a Drosophila male. Darlington (1937) reported: "somatic crossing-over is probably peculiar to the Diptera being conditioned by the exceptionally strong somatic pairing of the chromosomes which even leads to relational coiling. It seems that this will not of itself determine crossing-over, since the chromosomes do not divide while coiled, but if one of their chromatids is broken by X-rays at the earliest prophase the result should be the same as where a breakage occurs in meiosis, it should impose an increased strain on the partner chromosome exactly opposite and thus lead to double breakage and crossing-over. Rare natural crossing-over would be due to rare natural breakage, which should lead to the same regular result."

Kodani (1948) studied the effect of sodium ribose nucleate on mitosis of Allium cepa root-tips. He concluded that under the influence of this chemical, chromosomes did not duplicate and at anaphase, moved to one of the two poles at random. Few cases of chromosome bridges, fragments, etc. were observed. He did not present data showing either the combined or the separate frequencies of the abnormalities that he observed.

In sodium nucleate treated root-tips of Trillium sessile, like Huskins (1948), Wilson and Cheng (1949) also observed separation of homologues into two numerically equal groups. Moreover, they observed over twice as many equal (5:5) separations as expected on a completely random basis. These cells with 5:5 separations included many cases of genome separation. Wilson and Cheng (1949) concluded that in separation of chromosomes, not the nucleus as a whole but homologous chromosomes are acted upon in a unit which lead to separation of homologues. Such reductional groupings with equal or nearly equal distribution of chromosomes over all other groupings was also high in Allium cepa root-tips when bulbs were kept in water at 5-6°C. for 5 to 64 days (Huskins and Cheng, 1950). Huskins and Chouinard (1950) observed reductional groupings in two tetraploid plants of Rhoeo discolor. They reported that such genome separation increased in sodium nucleate treated roots.

Sachs (1952) treated root-tips of Allium cepa and Triticum monococcum with sodium salts of both ribose and deoxyribose nucleic acid in various combinations. Srinivasachar and Patau (1958) repeated the experiment of Huskins and Cheng (1950). Sachs (1952) as well as Srinivasachar and Patau (1958) failed to obtain any evidence of somatic reduction which achieved perfect segregation of homologous chromosomes. Sachs (1952) suggested that "somatic reduction" in Gossypium hybrids reported by Menzel and Brown (1952) could be explained if it is

assumed that hybrid genotypes produced multipolar spindles.

Effect of sodium nucleate on root-tips of Rhoeo was studied by Martinez-Pico in 1951 and by Owczarzak in 1953 and on root-tips of Tradescantia by Hershcope in 1951 (Srinivasachar and Patau, 1958). According to Srinivasachar and Patau, these workers, correctly interpreted that sodium nucleate treatment increased the frequency of at least metaphase reductional groupings in sections and in squash preparations in essentially the same manner. Srinivasachar and Patau wrote: "Up to now nothing has been established in plants that could be compared with the regular process of somatic reduction which has been reported by Berger (1938) for Culex. We have not observed any clear case of pairing nor any sign of chiasmata and we agree with Wilson, Hawthorne and Tsou (1951) and with Sachs (1952) that somatic reduction, as it occurs in onion, does not show any relation to meiotic processes."

Sharma and Sharma (1959) suggested that disturbance in the nucleic acid balance was the cause of reductional separation of chromosomes in somatic tissue and unequal separation of chromosomes resulted in cells with differing chromosome number. Sharma and Datta (1959) also suggested that increased synthesis of nucleic acid caused supersaturation in normal diploid nuclei and, therefore, these cells divided reductionally.

Chromosome Mosaicism in Generative Tissue

Variety Garnet of T. vulgare has Russian and Indian varieties as parents which have no known 28-chromosome parent in their ancestry. In a hybrid of Garnet with some unknown variety, Hollingshead (1932) observed an isolated PMC or a small group of PMCs with less than normal number of chromosomes. From this later observation he suggested that each group may have originated from one parental cell. He mentioned that at metaphase I, clear instances of PMCs with 1 II+7 I, 3 II+10 I, 5 II+8 I, and 8 II+8 I were recorded.

In a seventh generation H44 x Reward wheat plant (H44 was a vulgare selection from the cross Yarsolav Emmer x Marquis), Love (1936) observed two PMCs with less than the diploid chromosome number. At metaphase I, one of them had 7 II+7 I and the other had one heteromorphic trivalent, one heteromorphic bivalent, one open bivalent, five ring bivalents and six univalents (7 II+1 III+6 I). All the other (320) PMCs on the slide appeared to have either the normal 42 chromosomes, or there abouts.

Love (1938) studied F_5-F_7 generations of vulgare-like plants derived by crossing four varieties of Triticum vulgare with T. durum var. Iumillo. Total chromosome number of the plants studied varied from 39-42. In 16 out of 336 plants, PMCs with aberrant chromosome numbers were observed. These aberrant PMCs had 2n=15 to 30 chromosomes and one aberrant PMC

had 2n=61 chromosomes. It is interesting to note that adjoining the 61 chromosome PMC was a 23 chromosome PMC and total chromosomes in these two cells were 84. Mosaicism was attributed to upset in mitosis due to incompatible genes contributed by the different parents to some of their progeny or to incompatible cytoplasm-gene combinations. Chromosome number of mosaic cells varied from 16 to 61. Only one PMC (reported above) had a higher chromosome number (2n=61) than the euploid (2n=42) chromosome number of the plant.

Darlington and Thomas (1937) observed spindle abnormalities at meiosis in a 15 chromosome plant derived by backcrossing twice, the Lolium perenne (2n=14 x Festuca arundinacea (2n=6x=42) hybrid with Lolium perenne. In barley, Smith (1942) described spindle abnormalities governed by a single recessive gene which affects meiosis in such a manner that PMCs with 14, 21, 28, 56 and 112 pairs of chromosomes, instead of the normal 7, were observed. He suggested that this was apparently accomplished by assembling of chromosomes from adjoining PMCs rather than by any abnormality within the individual cells. Vaarama (1949) studied colchicine doubled plants of Ribes nigrum and attributed diminution in tetraploid number in mitotic cells to the formation of two separate and independent spindles in one cell.

It is interesting to note here that in autotetraploid rye, mosaicism at metaphase I was observed in one strain (O'Mara,

1942) and was not observed in four other strains (Müntzing, 1951). Moreover, O'Mara observed distinctly higher frequency of univalents at metaphase I as well as bridges at anaphase I than Müntzing. This difference in behavior of different strains may be due to their genetic difference(s). It may be appropriate to note here that rye is a cross-pollinated plant and the differences observed may be due to the dosage of a particular gene(s) and/or their ratio. This is important since only few plants of each strain were studied by Müntzing. In some instances O'Mara observed patches of deficient cells of approximately 30 cells while in other instances deficient cells were in pairs. Within some patches of deficient cells, smaller islands of cells with a more extreme deficiency were present. From these observations, he concluded that the loss of chromosomes usually occurs within five or six cell generations prior to meiosis and may be followed by additional loss within the islands of deficient cells. He assumed that gene products from contiguous normal cells diffused into the deficient cells and allowed normal development of these cells as proposed by Sax (1930).

Sachs (1952) studied 31 amphiploids involving Triticum, Aegilops, and Agropyron, three genera belonging to the subtribe Triticinae. Twenty-one of them were 8x (2n=54 to 56) amphiploids, nine were 6x (2n=41 or 42), and one was 10x (2n=70). He also studied hybrids involving two amphiploids and an

an amphiploid x T. aestivum var. April Bearded. First generation amphiploids were studied cytologically except that the 10x amphiploid was studied on a chromosome doubled part of the actual colchicine treated F_1 hybrid plants and two of the nine 6x amphiploids were studied in later generations. He found that chromosome number ranged from 9 to 48 in the 8x plants and 10 to 40 in the 6x plants. Chromosome mosaicism at metaphase I was dependent on the particular parent species involved in the production of the amphiploid. The amphiploids which did not show mosaicism included 10x amphiploid. He therefore, suggested that mosaicism was dependent upon genetic control of spindle abnormalities at mitosis as well as the magnitude of the chromosome number of the amphiploid. Variation in the percentage of cells with reduced chromosome number was present, both in different anthers of the same plant, and in different plants of the same amphiploid, and, therefore, he suggested that this variation was probably due to the environment. He compared 21 different amphiploids and showed that degree of chromosome pairing in PMCs with the complete chromosome complement was not related to the percentage of PMCs with reduced chromosome numbers. He did not observe any frequency peaks at particular chromosome numbers in the mosaic cells and these cells had high univalent frequency. Therefore, he concluded that these reduced numbers represent an almost random assortment of chromosomes which could have been produced by spindle abnormalities, such

as multipolar spindles, during pre-meiotic cell divisions. He observed mosaic PMCs either in small groups or singly in the same anther. Moreover, most of the cells had 2n chromosome number. He did not observe mosaicism in root-tips of any amphiploid. From these observations, he suggested that the mitotic irregularities must have occurred very late in the development of the plant, i.e., in the cell generations just preceding meiosis. He cited work of Darlington and Thomas (1937), Smith (1942), Vaarama (1949), O'Mara (1942) and Müntzing (1951) in support of his conclusion.

Precocity theory of meiosis (Darlington, 1937) states that, in meiosis, as compared with mitosis, the external changes outside the chromosomes are advanced (precocious) in relation to the changes inside the chromosomes. According to Sachs (1952), Oksala in 1944 concluded: "precocity in the dragonfly Aeschna juncea, is gradually developed and can be observed in at least two cell divisions preceding meiosis." From the studies of Darlington and Oksala, Sachs suggested that mitosis in cells with this developing precocity is different from mitosis in other cells and, therefore, mitotic abnormalities are more likely to occur at pre-meiotic cell divisions. Some other gene combinations such as those in tetraploid Ribes nigrum (Vaarama, 1949) may produce mosaicism quite early in development (i.e. in root-tips), while some others may produce mosaicism at some other stage in ontogeny.

Gerstel (1955) backcrossed the interspecific hybrid of Gossypium arboreum x G. herbaceum with G. arboreum and studied the backcrossed progeny cytologically. He found an intact PMC with only ten pairs of chromosomes at early metaphase I. All other PMCs had expected thirteen pairs of chromosomes. From this observation, he suggested: "the manouevers of meiosis are controlled from the outside of the cell undergoing them: but this idea is complicated by the fact that cells in various stages of meiosis may be found in the same anther locule." The author does not understand, how the presence of various stages of meiosis in the same anther locule contradicts the argument.

Snoad (1955) observed that chromosome number in root-tip cells of Hymenocallis calathinum ranged from 2n=23 to 83. But in PMCs, a smaller range (69 to 86) of variation was observed. It was concluded that level of tolerance to unbalanced chromosome numbers was different in anthers and roots.

Knott (1956) crossed Thatcher with a stable wheat-Agropyron derivative (Chinese Spring x Agropyron elongatum backcrossed to Chinese Spring) and backcrossed the F_1 with Thatcher. In an anther of a plant of the first backcrossed progeny he observed five PMCs with 22 and four PMCs with 20 chromosomes. He attributed their occurrence to somatic reduction in a premeiotic cell. From the distribution of chromosome numbers and from the constancy of the number of closed bivalents in each chromosome number group, he concluded that a

single initial reduction was responsible for the origin of these PMCs and that during somatic reduction, the chromosomes had separated at random into two groups. All of the remaining PMCs had 42 chromosomes and usually formed 21 bivalents.

Povilatis and Boyes (1956) studied the cytology of autotetraploid red clover. In one plant with autotetraploid chromosome number (2n=28), three PMCs had one or more extra chromosomes whereas 18 PMCs had the expected (2n=28) chromosome number. They did not mention the chromosome number of the three aneuploid cells. It was suggested that irregular chromosome distribution in a premeiotic mitosis was responsible for the occurrence of aneuploid PMCs.

Chopra (1960) studied a plant monosomic for chromosome 2D of the wheat variety Chinese Spring. At metaphase I, many PMCs had 20 II+1 I. In an anther, besides many PMCs with 20 II+1 I, he also observed PMCs with total chromosome number ranging from 16 to 21. A majority of these PMCs (9 out of 15) had 2n=20. The chromosome numbers 16, 18, 19, and 21 were observed in one, two, two and one PMCs, respectively. Fourteen PMCs showed both closed and open bivalents and in one PMC only one rod bivalent was present. The number of closed bivalents ranged from 1 to 3 per PMC, with a majority of PMCs having 3. From these observations he concluded that whole pairs of chromosomes were present in nulli-haploid PMCs and, therefore, in these PMCs chromosome distribution had occurred at random without pairing and

segregation of homologous chromosomes. Range of chromosome numbers observed in reduced cells did not fall into any regular pattern and, therefore, he did not draw any conclusion as to the number of premeiotic cells in which somatic reduction occurred.

Watanabe (1962) studied five plants (2n=42) of common wheat variety Shirahada. He observed mosaicism in all of these plants. Total chromosome number of five progeny plants from each of the above five plants was determined cytologically. Total of 42, 41, 40, and 39 chromosomes were detected in 13(52%), 9(36%), 2(8%), and 1(4%) plants, respectively. Chromosomal mosaicism at metaphase I was observed in these plants. Percentage of mosaic PMCs varied from 11.50 to 25.93. PMCs with less than the 2n number of chromosomes were observed more frequently and PMCs with more than the 2n number were observed quite less frequently. The 2n number of chromosomes in 120 PMCs was different than 42. Of these PMCs with chromosome number different than 42, 40 and 72 PMCs had even and uneven number of chromosomes, respectively. It was suggested that gene-controlled spindle abnormalities just before meiosis seem to be the probable cause of the origin of PMCs with hypo- and hyperchromosome numbers. He also mentioned that in previous studies he had observed hypo- as well as hyper- PMCs in amphiploids timopheevi-squarrosa (6x=2n=42, AAGGDD), timopheevi-monococcum (6x=2n=42, AAGGAA), timopheevi-longissima (6x=2n=42,

AAGGS'S'), timopheevi-comosa (6x=2n=42, AAGGMM), timopheevi-umbellulata (6x=2n=42, AAGGC^{u}C^{u}), and timopheevi-elongatum (6x=2n=42, AAGG??). According to Watanabe (1962), Mochizuki in 1943 reported the occurrence of hypo-PMCs in amphidiploid, Aegilops columnaris - Triticum timopheevi (8x=2n=56; AAGGCCMM).

Rajhathy (1963) as mentioned earlier, observed mosaicism in root-tips of colchicine doubled allotetraploid Hordeum murinum (4n=56). He also observed mosaicism in PMCs of this material. In C_1 generation, PMCs with reduced as well as normal chromosome number were observed in three plants. Out of 430 PMCs, 80(18.6%) cells had fewer than 56 chromosomes, and none with more than 56 chromosomes was observed. In mosaic cells, the chromosome number varied from 18 to 54. Rajhathy did not mention, but his data showed that only 15% of the PMCs with reduced chromosome complement were without even number of chromosomes. He indicated that the frequency of PMCs with euploid numbers (2n=28, 35, 42 and 49) was as high as 66% of the total number of reduced PMCs. The PMCs with original tetraploid chromosome number consisted of 51% of the total reduced PMCs and 77% of the total reduced euploid PMCs (2n=28, 35, 42, and 49). PMCs with 2n=28 chromosomes were of two types. One type had PMCs with univalents or multivalents or both. It was concluded that these PMCs were the products of independent assortment of chromosomes in somatic cells in which the reduction of chromosomes had taken place. The other type had

PMCs in which only bivalents were formed at metaphase I. Most likely, these cells had originated from a non-independent distribution of chromosomes as was suggested by Ross and his associates. Spindle abnormalities were not observed. Reduced PMCs were usually located together in patches and were not located in any particular part of the anther. From these facts, he indicated a very recent origin of the reduced PMCs, i.e., shortly prior to meiosis. Among C_2 generation plants, he found 4.1% with 2n=28 chromosomes and all these plants formed only bivalents at metaphase I. Mosaicism in the C_2 generation was similar to that observed in C_1 generation. He concluded that although genetic effect cannot be ruled out, dosage and high ploidy *per se* may have some effect. He thought that by doubling the chromosome number of the allotetraploid *H. murinum*, the cytogenetic balance was disturbed.

Yang (1964) studied *Nicotiana* amphiploids derived by doubling F_1 hybrids produced by crossing the species *tabacum* (n=24) with the diploid (n=12) species *sylvestris*, *otophora* and *tomentosiformis*. Chromosome numbers in microspores of these amphiploids varied from 33 to 39 with modes at the expected number of 36. Since modes were as expected, i.e., 36 chromosomes in microspores and 60 in progenies (amphiploids x *N. tabacum*), she excluded abnormal somatic divisions before meiosis as a major cause leading to the frequent appearance of deviants. She did not study meiosis. However she observed

cytomixis at early stages of sporogenesis and micronuclei in sporads. She wrote: "It seems clear that numerical unbalance found in spores and progenies must have arisen in the main as a result of disturbances during sporogenesis." Hyperploid spores and progeny plants were relatively few. It was concluded that this may be due to elimination of chromosomes during meiosis in amphiploids or to lower viability of hyperploid products of meiosis to reach first microspore division, or to both factors. The author thinks that the later possibility may also be responsible for not observing more extreme hypo- and hyperploid microspores.

MATERIALS AND METHODS

Materials used in this study were:

(1) A_3 generation seeds of the synthetic 6x amphiploid of the triploid hybrid of Avena abyssinica Hochst. (2n=28) x A. strigosa Schreb. (2n=14) C.I. 2630 produced by Dr. Sadanaga. Amphiploid seeds produced on colchicine doubled sectors of triploid plants are considered as A_1 generation seeds.

(2) Two tetraploid lines (designated as 57-127 and 57-133) derived from synthetic 6x amphiploid (Their source and details of their derivation are mentioned in the introduction).

(3) Panicles (A_o generation) produced on the colchicine doubled tillers of the triploid hybrid of Avena abyssinica Hochst. (2n=28) C.I. 2108 x A. strigosa Schreb. (2n=14) C.I. 7010.

(4) A_1 generation plants grown from seeds produced on colchicine doubled tillers of the triploid mentioned above.

(5) Autotetraploid (4x) of the diploid species Avena strigosa C.I. 2630 (2n=14), obtained from Dr. Sadanaga.

(6) Autotetraploid (4x) of the diploid species Avena strigosa C.I. 7010 (2n=14), obtained from Dr. Sadanaga.

Planting of Seeds

All seeds were treated in 2.0% solution of calcium hypochlorite (clorox) for approximately 3 minutes and then were germinated on moist filter paper in petri dishes kept at room

temperature. Seedlings were planted in 2-1/2" pots when the seminal roots were 1/2 to 3/4" long, or when the coleoptile was about 1/4 to 1/2" long. For studying mitosis, seminal root-tips were collected before the seedlings were planted in the pots. Plants were transferred to 4" pots after about 15 days and then to 6" pots after another 10-15 days. Most of the plants were grown in the green house. During the winter, 300-watt incandescent bulbs were used to compensate for the short day-length, and during the summer, some plants were grown in a plant growth chamber and others in the area between the botany and genetics green houses.

Treating Seeds with Colchicine

Four triploid hybrid plants, derived from the cross C.I. 2108 x C.I. 7010, were treated with 0.05% colchicine for about 24 hours (Bell, 1950). One of the plants was successfully doubled and many panicles with seeds were obtained. Some of the colchicine doubled panicles were studied cytologically.

Cytological Techniques

For mitotic studies, seminal root-tips as well as root-tips collected from pots were used. For metaphase study, root-tips were pretreated in 0.002 mol. oxyquinoline for 5-6 hours at about 15°C. (Tjio and Levan, 1950), fixed in acetic alcohol (1:3), hydrolyzed in 1N HCl at 60°C. for 9-10 minutes, stained in Feulgen, and squashed in a drop of lacto-propionic orcein (Dyer, 1963). For studying chromosomal aberrations at metaphase

and anaphase, root-tips were fixed in acetic-alcohol (1:3), hydrolysed in 1N HCl at 60°C. for 9-10 minutes, stained in Feulgen and squashed in a drop of either 45% acetic acid or Fe aceto-carmine.

For studying various meiotic stages, panicles were collected when the tip of the panicle enclosed within the sheath reached a position coinciding with the dewlap of the second leaf. The panicles were fixed in Carnoy's fluid (6 parts anhydrous ethyl alcohol : 3 parts chloroform : 1 part glacial acetic acid) and were stored in a refrigerator for at least 24 hours before PMCs were smeared and stained in Fe aceto-carmine. After lowering the cover slip on the slide, slide was warmed on the alcohol flame. Whenever the objective was to study cytomixis, no pressure was applied on the cover glass. To intensify the staining of chromosomes with carmine, a drop or two of ferric chloride solution was added to every 10 ml. of Carnoy's fluid before panicles were fixed.

Slides were made permanent according to the quick-freezing method described by Bowen (1956).

Photomicrographs were taken before the slides were made permanent.

Other materials and cytological techniques used are mentioned in the section on experimental results and discussion.

EXPERIMENTAL RESULTS AND DISCUSSION

In this report, mode number of root-tip cells with a particular chromosome count is considered and is used to designate the chromosome number of the plant.

Two Tetraploids Derived from Synthetic 6x Amphiploids of the Triploid Hybrid of *Avena abyssinica* C.I. 2108 x *A. strigosa* C.I. 7010

Two tetraploid derivatives are designated as 57-127 and 57-133. Dr. Sadanaga mentioned that the former derivative is more fertile than the latter. At mitotic metaphase, total of 28 chromosomes were counted in seven plants of each of the derivatives.

One plant of 57-127 and four plants of 57-133 were studied at diakinesis and at metaphase I. Results are presented in Table 1. Most of the PMCs had 14II (Fig. 1) as expected in a stable tetraploid. Among few anomalous PMCs observed, most frequently observed association was 12II + 1IV (Fig. 2). It should be noted here that a PMC with 29 chromosomes with 12II + 1IV + 1I was observed (Table 1).

Chromosome Numbers in Root-tip Cells of A_1 and A_3 Generation Plants

The chromosome number of the A_3 plants varied from 39 to 44 (Table 2). Moreover, chromosome mosaicism was observed wherever the number of root-tip cells examined was large (Table 3, Figs. 3 and 4).

Griffiths et al. (1959) in their study of nine A_2 and A_3 plants of synthetic 6x amphiploids found little variation in the chromosome number of the plants. Chromosome mosaicism in root-tips was not reported.

Forsberg (1961) reported that chromosome number of synthetic amphiploid lines developed at Wisconsin varied from 38 to 44. He observed chromosome mosaicism in the root-tips.

Thomas and Peregrine (1964) studied A_1 and A_2 generation plants of the synthetic 6x amphiploids of the triploid hybrids produced by crossing tetraploid species abyssinica with diploid species strigosa and brevis, and synthetic 6x amphiploids of the triploid hybrids produced by crossing A. barbata with diploid species longiglumis, strigosa spp. hirtula and F_3 selection of strigosa spp. hirtula x brevis. Chromosome number of these plants varied from 38 to 44. This variation in chromosome number is very similar to the one reported here (Table 2). They reported that no evidence for chromosome mosaicism in root-tips was found while in this study, chromosome mosaicism was observed (Table 3).

All the root-tip cells examined by Forsberg (1961) and almost all the root-tip cells examined in this study had either the chromosome number of the plant or fewer than this number (Table 3).

In this study a few cells with more than the chromosome number of the plant were also observed (Table 3).

Chromosome Numbers and Chromosome Associations Observed at Meiotic Metaphase in PMCs of A_3 Generation Plants and A_o Generation Panicles

Most of the PMCs had the chromosome number of the plant (Figs. 5 and 6) but some PMCs with less than the chromosome number of the plant were also observed (Table 4, Figs. 7-10).

Griffiths et al. (1959) indicated that chromosome mosaicism at metaphase I was present in the synthetic 6x amphiploids of Avena that they studied and mentioned that a detailed account of this phenomena will be presented elsewhere.

Forsberg (1961) did not study cytology of PMCs of the synthetic 6x amphiploids of Avena that he used.

Thomas and Peregrine (1964) observed chromosome mosaicism in the PMCs at metaphase I in A_1 and A_2 plants of various synthetic 6x amphiploids (for parents, see above) that they studied. They gave detailed account of the mosaic cells observed in one plant of the amphiploid Cc 4435 (A. abyssinica x A. strigosa) and one plant of the amphiploid Cc 4387 (A. abyssinica C.D. 4550 x A. strigosa C.D. 3220). The amphiploid generation of these plants was not given. These amphiploid plants had a higher percentage of mosaic cells than their sib plants and other A_1 and A_2 generation amphiploids that they studied. Chromosome number in PMCs of the two above mentioned amphiploid plants varied from 18 to 42. The author observed a PMC with as low as 6 chromosomes (Table 4, Fig. 7). Thomas and Peregrine (1964) did not mention that one or more PMCs with

more than the chromosome number of the plant were observed while the author observed such PMCs (Table 4).

Chromosome Associations in 42 Chromosome PMCs at First Meiotic Metaphase in Various Synthetic 6x Amphiploids

Thomas (1963) and Thomas and Jones (1964) reported that nearly all the bivalents observed in 12 different pentaploid hybrids of tetraploid x hexaploid species crosses were of the rod type and their synthetic decaploids showed near diploid-like pairing. From these observations, they concluded that the A genome of the tetraploid and the hexaploid species involved in the pentaploid hybrids has differentiated. On the basis of a relatively high frequency of ring bivalents in the triploid hybrid and the presence of multivalents in the synthetic 6x amphiploids (Griffiths et al., 1959), Thomas and Jones (1964) concluded that the A genome of A. strigosa and A. abyssinica does not show any differentiation.

Contrary to the conclusion of Thomas and Jones (1964), data of Tables 5 and 6 suggest that the A genome of A. strigosa and A. abyssinica do show differentiation. Mean number of bivalents and other associations observed (Table 5) in A_0 and A_3 generations were compared (Table 6). High chi-square value showed that the number of bivalents observed in the A_0 generation was significantly higher than the number observed in the A_3. Data in Table 5 show that the shift was from bivalent associations to univalents and trivalent associations and to

quadrivalent associations. These results suggest that the A genome of abyssinica and strigosa has differentiated. Since ring bivalents are observed in triploid hybrids, it is also suggested that this differentiation is not as much as between the A genome of abyssinica and sativa.

The suggestion that the A genome chromosomes have differentiated is supported by following observations:

The data of Nishiyama (1929) reproduced in Table 7 was interpreted by Rajhathy and Morrison (1960) as indicating no homology between the A genome and the other genome of tetraploid species. That same data, along with some other data (Nishiyama, 1934) can be used to support the view that the A genome is partially homologous with the genome other than A of the tetraploid (Table 7) as concluded by Nishiyama (1934). Furthermore, the presence of quadrivalents and trivalents in various derived tetraploids (Table 1 and Rajhathy and Morrison, 1960) and their complete absence in tetraploid species (Rajhathy and Morrison, 1960) suggest that the A genome of the diploid is partially homologous with the genome other than the A of the tetraploid, and this homology is higher than that between the two genomes of the tetraploid. Since the A genome of diploid is partially homologous with the genome other than the A of the tetraploid and multivalents are not observed in tetraploid species, the A genome of diploid can not be completely homologous with the A genome of tetraploid.

The advanced generation synthetic 6x amphiploid line, 68-31, used by Rajhathy and Morrison (1960) seems to be different than those used by other workers (Table 5). Dr. Zillinsky[1] wrote: "68-31 was the most productive of the (synthetic) hexaploids and under favorable greenhouse conditions almost complete seed set was obtained." Such high fertility is exceptional for synthetic 6x amphiploids of Avena (Table 5). Moreover, chromosome associations observed in line 68-31 by Rajhathy and Morrison (1960) are more similar to those reported in autotetraploid of strigosa than those reported in synthetic 6x amphiploids by other workers (Table 5). Therefore, itis suggested that in the development of the line 68-31, there was selection in favor of accumulating completely homologous chromosomes (a set of four homologous chromosomes) of A genome. If we designate the A genome chromosomes of diploid species as A^D and of tetraploid species as A^T, then a set of four homologous chromosomes of A genome in line 68-31 can be designated as A^{D-T} A^{D-T} A^{D-T} A^{D-T}. Instead of using A_o or A_1 plants, Rajhathy and Morrison (1960) used later generation, highly fertile line (68-31) to study homology of chromosomes. Therefore, the quadrivalents observed by Rajhathy and Morrison in line 68-31 (Table 5) could represent the association of four

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[1]Zillinsky, F. J., Canada Department of Agriculture, Ottawa, Ontario, Canada. Fertility of line 68-31. Personal Communication. 1966.

chromosomes of a set rather than the true affinity between chromosomes of diploid with A genome chromosomes of tetraploid.

In synthetic 6x amphiploids, due to some homology between A genomes of diploid and tetraploid species, early generation plants showed more trivalents and univalents than observed in line 68-31 (Table 5). It seems that this higher frequency of trivalents and univalents is, at least, partly responsible for lower fertility of lines other than 68-31. In such synthetic material, instead of a quadrivalent, a trivalent and a univalent or two bivalents are frequently formed, and higher fertility will be related to the shift toward higher quadrivalent formation. This suggestion is consistent with the data reported in Table 5.

If the interpretation of the data presented in Table 5 is correct, selection of fertile amphiploid lines from crosses of derived tetraploids and their parental diploids can be accomplished within fewer generations compared to the one developed by crossing tetraploid with diploid species.

The genome formula for some of the 28 chromosome plants obtained in the later generations of the triploid hybrid of A. barbata x A. strigosa was designated as AAAA (Nishiyama, 1934). Even though pairing associations observed in these plants are comparable to those observed in autotetraploids ($A^DA^DA^DA^D$) of the diploid species of Avena (Morrison and Rajhathy, 1960), author feels that, because of its hybrid

origin, the genome formula for these plants should be designated as $A^{D-T}A^{D-T}A^{D-T}A^{D-T}$ rather than $A^{D}A^{D}A^{D}A^{D}$ or $A^{T}A^{T}A^{T}A^{T}$. For similar reasons, genome formula for the A genome of derived tetraploids should be designated as $A^{D-T}A^{D-T}$ rather than $A^{T}A^{T}$. Nishiyama (1936) studied F_1 hybrid obtained by crossing tetraploid species with derived autotetraploid ($A^{D-T}A^{D-T}A^{D-T}A^{D-T}$) and Rajhathy and Morrison (1960) studied F_1 hybrid obtained by crossing derived tetraploid with autotetraploid of strigosa. It seems that, neither procedure followed by Nishiyama (1936) who used derived autotetraploid instead of doubled A. strigosa nor procedure followed by Rajhathy and Morrison (1960) who used derived tetraploids instead of tetraploid species were appropriate to study genome homology.

Metaphase of First Pollen Grain Mitosis in A_3 Generation Plants of Synthetic 6x Amphiploid of Avena abyssinica C.I. 2108 x A. strigosa C.I. 2630

In order to develop a method to study metaphase of first pollen grain mitosis, the various pretreatments tried to shorten the chromosomes were: oxyquinoline (0.002, 0.004, and 0.006 Mol.), colchicine (0.1, 0.2, 0.4, 0.5, and 1.0%), paradichlorobenzene (saturated solution), monobromonaphthalene (saturated solution), chloral hydrate (0.6, 1.0, and 1.4%) and cold treatment. Pretreatments were tried for 1, 2, 3, 4, 6, 7, 8, 12, and 24 hours (all pretreatments were not tried at all these different number of hours). The details of procedure followed

was as under:

(1) kept panicle in the pretreatment solution (at the beginning of the pretreatment, used aspirator to remove air from the panicle) for appropriate number of hours,

(2) fixed panicle in Carnoy's fluid and kept it in the fixative for at least 24 hours,

(3) squashed anthers in aceto-carmine, or using one of the anthers of a spikelet, detected metaphase of first pollen grain mitosis and used standard feulgen technique to stain remaining two anthers. In the latter procedure, anthers were rinsed thoroughly with distilled water before hydrolysis.

No statistical record was kept of the different procedures tried. But, none of the treatments tried was superior over others and all gave poor results. Good metaphase plates were observed in few preparations of a given treatment and the results were not consistent. It seems that not the pretreatment but physiological and anatomical nature of anthers was responsible for the poor results.

The compiled data, showing variation in chromosome number between pollen grains, are presented in Table 8. Pollen grains with 21 chromosomes were found in highest frequency. In general, the frequency of cells with various chromosome numbers was observed in descending order as the chromosome number increased as well as decreased from 21. Observed frequency of cells with 14 chromosomes (Fig. 11) was higher than those with

15, 16 or 17 chromosomes but was lower than those with 18, 19 (Fig. 12) or 20 chromosomes. A pollen grain with as low as 8 chromosomes and 3 fragments was observed. Total number of pollen grains with lower than 21 chromosomes was quite high (110) compared to those with higher than 21 chromosomes (36). PMCs with univalents and/or multivalents (Table 4) will lead to the formation of gametes with lower as well as higher number of chromosomes than 21 and PMCs with less than the chromosome number of the plant (Table 4) will form gametes with lower number of chromosomes than 21. PMCs with multivalents, univalents and with less than the chromosome number of the plant and the fact that the higher the chromosome number, the more difficult is the task of determining the chromosome number of the cell, seems to be the reasons for determining more cells with lower than 21 chromosomes and fewer cells with higher than 21 chromosomes.

A Probable Case of Abnormality in Pollen Grain Wall

To determine the time of the day when maximum number of pollen grains will be in the division stages of first pollen grain mitosis, panicles were fixed in Carnoy's fluid at 1-1/2 hour interval (8:30 A.M., 10:00 A.M., 11:30 A.M., 1:00 P.M. and 2:30 P.M.). Plants were outside the greenhouse and panicles were collected on May 24 and 25.

Considering maturity, spikelets studied were those which had anthers with either all or some cells with 1 nucleus, 2 nuclei or both as well as cells with various stages of first mitosis of pollen grains. In general, 8:30 A.M. and 10:00 A.M. samplings had more number of cells at metaphase of first pollen grain mitosis.

A panicle of a plant (64-10-26) with 41 chromosomes was collected at 1:00 P.M. on May 24. Besides one or two nuclei, many anthers showed pachytene like threads (Table 9 and Fig. 13). Some anthers, whose sister anthers showed these pachytene like threads in aceto-carmine smear preparations, were used to test whether the threads were Feulgen positive or not. These anthers were washed thoroughly in distilled water (to remove chloroform which is known to obstruct bright staining of chromosomes with Feulgen), hydrolysed in 1N HCl at 60°C. for about 10 minutes, stained with Feulgen and were smeared in 45% acetic acid. In this test, the threads showed brilliant staining with Feulgen. The data for aceto-carmine and Feulgen stained anthers are combined (Table 9). This abnormality, to a lesser extent, was also observed at comparable stages in pollen grains of a sister plant (64-10-30) with 42 chromosomes. The panicle of this plant was fixed at 2:30 P.M. on May 24.

Five percent solution of trichloroacetic acid (TCA) will destroy DNA as well as RNA if material is heated at 90°C. for 15-20 minutes (Schneider, 1945). Therefore, one anther of the

plant 64-10-26 (Table 9, X-3) and some anthers of the plant 64-10-30 were treated in the following manner: Anthers were treated with 5 percent solution of TCA at about 90°C. for 20 minutes, washed immediately in 70 percent alcohol for 30 minutes (3 changes, 10 minutes each), rinsed thoroughly in distilled water, hydrolysed in 1N HCl for about 10 minutes, stained with Feulgen and then were squashed in 45 percent acetic acid. The pollen grains still showed Feulgen positive threads (Fig. 14).

Mild hydrolysis causes liberation of the aldehyde groups of the nucleic acid. This liberated aldehydes react with leuco-basic fuchsin, giving rise to a violet coloration of the chromosomes. In the cell, three types of substances are capable of giving the aldehyde reaction (Darlington and La Cour, 1962): (1) free aldehydes in lignified cell walls, (2) DNA after hydrolysis, and (3) polysaccharides after oxidation with chromic acid.

The Feulgen positive thread like material may be some kind of abnormality in the pollen grain wall. It is appropriate to note here that McClymont and Larson (1964) concluded that intine of 7 families of moss contains carbohydrate material including some cellulose and this layer stained brilliantly with aqueous basic fuchsin.

This abnormality might be in some way related to the division of the nucleus. The abnormality was observed in cells

whose sister anthers were near metaphase or in anthers which were ontogenically close to active division of the chromosomes (Table 9).

Cytological Considerations for Probable Causes of Mosaicism in Root-tips of A_1 and A_3 Generation Plants

Chromosome mosaicism was observed in A_1 and A_3 plants (Table 3). Therefore, causes of mosaicism were explored.

To study whether the presence of four sets of A genome in 6x amphiploids is responsible for mosaicism or not, chromosomes were counted in synthetic autotetraploid (AAAA) plants of the diploid species A. strigosa. These plants showed mosaicism (Fig. 15, Table 10). Unlike A_1 and A_3 generation plants (Table 3), cells with extremely few chromosomes were rare.

Root-tips of control material fixed without pretreatment had no abnormality and chief abnormality in autotetraploid and A_1 and A_3 plants was one or more bridges at anaphase and telophase (Fig. 16 and Table 11) which may be responsible for the observed fragments and telocentrics at metaphase (Tables 3 and 10). The autotetraploids (Table 10), unlike A_1 and A_3 generation plants (Table 3), did not show any dicentric chromosomes. Therefore, it is possible that the bridges observed may not lead to the formation of dicentric chromosomes. On the other hand, the dicentric chromosome may separate at anaphase in such a way that the two chromatids "fall-free" (no bridge is formed) or a bridge is formed but ruptures before observation at

anaphase. In these cases a bridge will not be detected, but in certain cases a bridge will be observed before it will rupture (Conger, 1965). Among A_1 and A_3 plants, only one plant (63-10-32) had many cells with one dicentric chromosome (Fig. 17) and all other plants had either no dicentric chromosome or had only occassionally one dicentric chromosome (Table 3). Among the latter plants, chromosome number of most of the cells with a dicentric chromosome was less than the chromosome number of the plant. This may be related to the fact that a slightly higher frequency of anaphase and telophase bridges was found in A_3 plants (except plant 65-10-10) than in autotetraploid plants (Table 11). Only plant 63-10-32 showed some PMCs which indicated that the dicentric chromosome was also present in PMCs (Fig. 18), though, it should be admitted that the other A_1 and A_3 generation plants were not studied extensively at anaphase I. A PMC with a dicentric chromatid at anaphase I was observed (Fig. 19). High frequency of chromosome bridges in plant 65-10-10 suggested that this plant had many cells with dicentric chromosomes, though, no suggestion is offered for the difference in frequency of bridges between root-tips of this plant (Table 11).

The origin and fate of dicentric chromosomes in A_1 and A_3 plants are puzzling since anaphase bridges in high frequency was observed only in plant 65-10-10 and was not observed in any other A_3 plants (Table 11).

The chromosome number of the plant 64-10-85 was 41 (Table 3). In this plant, two adjacent cells had 19 and 22 chromosomes (Table 3, Fig. 4). The total chromosome number (19 + 22 = 41) of these two cells suggested that during anaphase of the preceding cell division, the movement of all chromosomes was similar to the movement of a single chromatid (formed from the univalent divided at anaphase I) at anaphase II. Fig. 16 and the observed chromosome fragments at metaphase (Tables 3 and 10) suggested that in some cells the chromosomes concerned had not undergone replication in interphase and therefore, had moved at random to either of the two poles at anaphase or had ruptured at metaphase (a telocentric is difficult to differentiate from a fragment). Bridges at anaphase may cause rupture of the chromosomes and two such ruptured chromosomes may unite to form a dicentric chromosome. A cell with 28 chromosomes had almost all chromosomes in pairs of two (Fig. 20).

Cytological Considerations for Probable Causes of Mosaicism in PMCs of A_0 Panicles and A_3 Generation Plants

As mentioned previously, PMCs with less than the chromosome number of the plant were observed (Table 4). Therefore, causes of mosaicism were explored.

Observed chromosomal mosaicism in root-tips

Previously it was reported that root-tip cells with less than the chromosome number of the plant were observed.

Therefore, it is possible that some of the PMCs were formed from such mosaic premeiotic cells.

Observed abnormalities in PMCs between the stages interphase and metaphase I

Since chromosome mosaicism in PMCs was observed at metaphase I, it was decided to study PMCs at interphase and at various stages of prophase I. During leptotene, zygotene, and pachytene, the nucleus of many PMCs seemed to have moved to the side of the cell and then extruded chromatin material into the cytoplasm of the adjoining PMC through the pit (or pits) in the cell wall. Gates (1911) observed such chromatin extrusions in Oenothera and for this process coined the word "cytomixis." According to Gates (1911), this phenomenon was first reported by Koernicke in 1901. In this study, many times cytomixis was observed in a row of PMCs (Fig. 21) as first reported by Gates. When looked for, cytomixis was observed in this study in almost all the plants. These included A_0 and A_3 plants, autotetraploid (4x) plants of the diploid species Avena strigosa C.I. 2630, and A. sativa variety Garry and Cherokee. In general, plants which were outside the greenhouse during May-June 1964 showed more pronounced extrusions than those which were in a temperature controlled chamber during August-September 1964 and those which were in the greenhouse during October and later in the season. According to Kattermann (1933) and Kihara and Lilienfeld (1934), temperature

fluctuations seem to be important for cytomixis.

At leptotene stage, many PMCs had oblong nuclei and at zygotene, extrusion in the neighbouring cells was observed in somewhat higher frequency than at other stages. In some PMCs, small chromatin balls near the periphery of the cells seemed to be the broken up chromatin (due to mechanical tearing while smearing PMCs on the slide) which was extruded in the cell from the neighbouring cell.

The literature on cytomixis was reviewed by Kattermann (1933) and Kihara and Lilienfeld (1934). Cytomixis is observed in many varietal materials and is not always observed in the same plant material. Therefore, it is usually considered to be an artifact. West and Lechmere (1915) studied chromatin extrusion in PMCs of Lilium candidum. In some loculi, they observed PMCs at either side, discharging extrusions toward the centre, whilst PMCs occupying a position near the centre of the loculus retained the typical condition of synapsis. Sinotô (1922) suggested that this observation seemed to indicate some relation between the fixing fluid and the direction of extrusion. It seems that this relation may be due to the speed and direction of penetration of the fixing fluid. It is important to note here that Sinotô (1922) observed more frequent extrusions in Oenothera lamarckiana when Bouin's fluid and stronger Flemming's solution were used as fixatives than when chrom-acetic solution and medium chrom-acetic solution

were used. In other words, both the fixative used and the physiological state of the PMCs seem to be important for cytomixis.

In recent years, cytomixis was studied by Sarvella (1958), Bopp-Hassenkamp (1959), Kamara (1960), Tarkowska (1960), Maréchal (1963), Bell (1964) and Nettancourt and Grant (1964). Maréchal (1963) studied cultivated species of Gossypium. He wrote: "There appears to be a relationship between cytomixis and the external conditions responsible for the disturbance in the normal thickening of the cell membrane." It may be appropriate here to note that, previously in this study, a probable case of abnormality in the pollen grain wall is reported. Maréchal (1963) concluded that cytomixis could be responsible for the decrease in pollen fertilization in certain plants when cultivated outside their natural milieu. In this study, occasionally, cytomixis was observed at metaphase I.

In some plants, PMCs with differences in condensation of chromosomes and PMCs with more than one nucleus were observed (Fig. 22). Such differences in condensation of chromosomes were observed by Jain (1957) in Lolium when plants were kept at high temperature (35°C.) and by Rees (1958) in Scilla when plants were presumably at high temperature. Prior to microsporogenesis, Rana (1965) kept plants of Triticum monococcum at 37°C. for 6 to 96 hours. Besides some other abnormalities, he observed the extrusion of the chromatin material from the

main nucleus. He wrote: "Extrusion took the form of loss of stainable (Feulgen positive) material from the chromosomes and could be associated with the degeneration process. One or more Feulgen positive bodies could be seen lying in the cytoplasm of the cell." The abnormalities observed in this study were more pronounced in three plants. Because one of these plants was in the temperature controlled chamber and another was in the greenhouse during the month of November, the author feels that the abnormality observed in these plants may not be due to high temperature. Chromosome number of two of these plants was 39 and the chromosome number of the third plant was not determined. A few PMCs with degenerating chromatin material were observed at metaphase I (Fig. 23). Such PMCs may lead to the formation of pollen grains with unusually small number of chromosomes.

Univalents as well as multivalents observed in A_0 generation panicles and A_3 plants (Table 3) will contribute toward the formation of gametes with higher as well as lower than the n number of chromosomes.

Chromosome mosaicism in autotetraploid Avena

Rajhathy and Morrison (1962) concluded that H. murinum is an allotetraploid (2n=28) and forms only bivalents at metaphase I. Rajhathy (1963) observed chromosome mosaicism in three C_1 generation colchicine doubled plants (autoallotetraploid) of H. murinum (2n=56). He did not mention, but his data showed

that only 15 percent of the PMCs with reduced chromosome complement had odd number of chromosomes.

After reading Rajhathy's (1963) paper, it was decided to count total chromosomes in PMCs of the synthetic autotetraploids of the diploid species strigosa. The observations are presented in Tables 12 and 13. In the plant 4x-2630-2, panicle II had most of the PMCs with 26 chromosomes while panicle V had most of the PMCs with 27 chromosomes. Somewhat similar type of variation was observed for different spikelets of panicle IV of the plant 4x-2630-3 as well as for some panicles of the plant 4x-2630-3. Therefore, it is suggested that in these two plants some early generation (not premeiotic) cells had misdivided (Table 10) and formed the germ line. The data of these two plants are not included in the interpretation presented below for the mosaic PMCs observed in autotetraploids.

Most of the PMCs of the plant 4x-2630-1 had 28 chromosomes (Table 12). Out of 22 PMCs with less than 28 chromosomes, 16 (72.72%), 5 (22.72%) and 1 (4.54%) PMCs had 26 (Fig. 24), 27, and 18 chromosomes, respectively (Table 11). In other words, 77.26 percent of the PMCs had an even number of chromosomes and only 22.72 percent of the PMCs had an odd number of chromosomes. In the plant 4x-Saia-2, most of the PMCs had 30 chromosomes and all cells with less than 30 chromosomes had an even number of chromosomes (Figs. 25 and 26) except 3 PMCs which had exactly one-half the expected chromosome number (Table 13).

Formation of diploid plants from autotetraploids has been reported previously (cf. Rajhathy, 1963). But, as far as author knows, this is the first report showing mosaicism in generative tissue of autotetraploid of a diploid species of Avena.

Most of the PMCs in autotetraploids (4x-2630-1 and 4x-Saia-2) had the expected chromosome number of the plant and fragments or telocentrics were not observed in PMCs. From these observations it is suggested that cells which gave rise to mosaic PMCs in these plants had fairly recent origin. Among mosaic cells of autotetraploids, comparatively high frequency of cells with even number of chromosomes suggested that homclogous chromosomes in some special way, were involved in the production of mosaic PMCs in autotetraploids and the A genome chromosomes may be involved in similar way in the production of mosaic PMCs in synthetic 6x amphiploids.

Table 1. Chromosome configurations observed at diakinesis and at metaphase I in PMCs of two tetraploids derived from the amphiploid of C.I. 2108 x C.I. 7010

Chromosome configuration	Plant Number 57-127-1	57-133-1	57-133-2	57-133-3	57-133-4	Total
14II	24	33	10	22	21	110
13II + 2I	1	2	1	-	1	5
12II + 1IV	4	3	1	-	-	8
12II + 1III + 1I	-	1	-	-	-	1
10II + 2IV	1	-	-	-	-	1
12II + 1IV + 1I	-	1	-	-	-	1

Table 2. Modal chromosome number in root-tips of A_3 generation plants of the amphiploid of C.I. 2108 x C.I. 2630

Year	Chromosome Number 39	40	41	42	43	44	Total
1963-64	1	1	2	22	4	1	31
1964-65	1	6	9	26	2	3	47
Total	2	7	11	48	6	4	78

Table 3. Chromosome mosaicism observed in root-tips of A_3 generation plants of the amphiploid of C.I. 2108 x C.I. 2630 and A_1 generation plants of the amphiploid of C.I. 2108 x C.I. 7010

Amphiploid Plant Generation Number	Chromosome Number Mode	2n Chromosome Number of Other Intact (Unless Otherwise Mentioned) Cells
A_3 63-10-32 (16 out of 25 cells have one dicentric chromosome)	42	16, 18 + 2f*, 37 (one dicentric), 39 + 1f, 39 (one dicentric), 40 (one dicentric), 40 + 2f, 41, 41 (one dicentric), 43 Following cells have one dicentric chromosome with a satellite. These cells may not be intact: 16, 24, 35, 36, 37, 38, 41
A_3 63-10-33	42	41 (one dicentric, cell may be broken)
A_3 63-10-35	42	30, 36, 37, 37, 38, 38, 40, 41
A_3 63-10-37	41	30, 38, 41 (one dicentric, cell may be broken), 42, 42, 42
A_3 63-10-38	42	26 + 3f, 30, 33, 37 + 1f, 40, 41
A_3 63-10-41	44	24, 32, 34
A_3 63-10-42	42	36, 41, 41 + 2f
A_3 63-10-47 (A cell has one dicentric chromosome)	42	-
A_3 64-10-11	43	41
A_3 64-10-12	42	14, 29, 38, 40
A_3 64-10-21	42	41
A_3 64-10-25	42	38, 39 (one may be dicentric)

*f = fragment (may include telocentric chromosome).

Table 3. (Continued)

Amphiploid Plant Generation Number	Chromosome Number Mode	2n Chromosome Number of Other Intact (Unless Otherwise Mentioned) Cells
A_3 64-10-26	41	17, 28 (Most of the chromosomes are in pairs of two)
A_3 64-10-27	40	19, 39 (one dicentric)
A_3 64-10-29	42	43
A_3 64-10-37	?	Seminal root-tip number 1: 41 or 42, 11 cells with 43 (One cell - one dicentric + a small chromosome. Another cell - one small chromosome) Seminal root-tip number 2: 39, 39 + 3f (One long), 41 (one long), 14 cells with 42 (5 cells may have one long, dicentric) Seminal root-tip number 3: 26, 43, 43, 43 + 3f
A_3 64-10-38	42	36 + 5 or 6f
A_3 64-10-39	41	37 (one dicentric, cell may be broken), 40 (one dicentric, cell may be broken)
A_3 64-10-40 (A cell has one dicentric chromosome)	41	36, 42, 42, 42, 42, 43
A_3 64-10-47	42	40
A_3 64-10-48	42	36
A_3 64-10-57	42	17
A_3 64-10-58	42	43
A_3 64-10-84	41	A cell has 26 chromosomes on one side and 15 on the other side
A_3 64-10-85	41	18, 19, 22, 23

Table 3. (Continued)

Amphiploid Plant Generation Number	Chromosome Number Mode	2n Chromosome Number of Other Intact (Unless Otherwise Mentioned) Cells
A_3 64-10-88	40	29
A_1 64-1-1	42	41
A_1 64-1-2	42	36 (one dicentric, cell may be broken), 43 + 3 or 4f
A_1 64-1-3	40	12, 35, 35, 36, 36 + 1f, 36 + 1f, 36 + 1f, 36 + 1f, 37, 37, 37, 38, 39 -
A_1 64-1-6	40	39, 41
A_1 64-1-12	41	38

Table 4. Chromosome numbers and associations observed at metaphase I in PMCs of some A_3 generation plants of the amphiploid of C.I. 2108 x C.I. 2630 and one A_o generation plant of the amphiploid of C.I. 2108 x C.I. 7010

Amphiploid generation	Plant number (chromosome No. of the plant)	Total chromosome number of the PMC	Observed chromosome association
A_3	63-10-32 (42 - one chromosome with 2 centromeres)	42	12II + 2IV + 1III + 7I
		42	14II + 1IV + 2III + 4I
		42	15II + 1IV + 1III + 5I
A_3	63-10-35 (42)	18	7II + 1IV
		42	16II + 1IV + 2III
		42	17II + 2IV (2 PMCs)
		42	20II + 2I
A_3	63-10-37 (41)	14	4II + 1IV + 2I
		38	10II + 1V + 2IV + 5I
		41	6II + 3IV + 5III + 2I
		41	10II + 1V + 1IV + 2III + 6I
		41	12II + 2IV + 2III + 3I
		41	13II + 1II (end to end) + 1IV + 2III + 3I
		41	14II + 1VII + 1III + 3I
		41	16II + 1III + 6I
		41	17II + 1III + 4I
		43	13II + 1V + 2IV + 1III + 1I
A_3	63-10-38 (42)	18	6II + 1IV + 2I
		26	10II + 1III + 3I
		32	10II + 1II (end to end)+ 1IV + 1III
		40	8II + 2IV + 3III + 7I
		42	9II + 1V + 2IV + 2III + 5I
		42	11III + 1V + 4III + 3I

Table 4. (Continued)

Amphiploid generation	Plant number (chromosome No. of the plant)	Total chromosome number of the PMC	Observed chromosome association
A_3	63-10-38 (42)	42	12II + 1IV + 3III + 5I
		42	12II + 1IV + 1III + 11I
		42	14II + 4III + 2I
		42	16II + 2II (end to end) + 1IV + 2I
		42	17II + 1III + 5I
		42	17II + 1V + 3I
		42	17II + 1VI + 2I
		42	18II + 1III + 3I
		45	15II + 1IV + 2III + 5I
A_3	63-10-47 (42)	21	4II + 3III + 4I
		13	6II + 1I
		32	11II + 3III + 1I
		40	12II + 4III + 4I
		42	9II + 1V + 2IV + 2III + 5I
		42	12II + 2IV + 3III + 1I
		42	13II + 2IV + 1III + 5I
		42	13II + 3IV + 1III + 1I
		42	15II + 2III + 6I
		42	16II + 2IV + 2I
		42	20II + 2I
		43	14II + 1IV + 3III + 2I
A_3	64-10-55 (41)	21	4II + 1V + 1III + 5I
		29	10II + 1III + 1V + 1I
A_3	64-10-56 (42)	42	10II + 1IV + 2III + 12I

Table 4. (Continued)

Amphiploid generation	Plant number (chromosome No. of the plant)	Total chromosome number of the PMC	Observed chromosome association
A_3	64-10-57 (41)	16 + 4 fragments	1II + 14I + 4I
		41	18II + 1III + 2I
		41	41I
A_3	64-10-60 (42)	14	7II
		42	10II + 4IV + 1III + 3I
		42	17II + 1IV + 1III + 1I
A_3	64-10-61 (39)	36	9II + 1IV + 1III + 11I
		39	5II + 4II (end to end) + 1IV + 2III + 1II
		39	11II + 2III + 11I
		39	14II + 1III + 8I
		39	14II + 2III + 5I
A_3	64-10-70 (42)	41	8II + 1II (+ 1 chromosome) + 4IV + 1III + 4I
		42	13II + 3IV + 1III + 1I
	64-10-80	6	2II + 2I
A_o	63-1-14 (Colchicine doubled panicles of a triploid plant)	9	1II + 1II (+ an isochromosome) + 1III + 2I
		20 or 21	4II + 1IV + 1III + 2I + (1III or 2II)
		42	15II + 1V + 1IV + 1III
		42	16II + 2IV + 2I

Table 4. (Continued)

Amphiploid generation	Plant number (chromosome No. of the plant)	Total chromosome number of the PMC	Observed chromosome association
A_o	63-1-14 (Colchicine doubled panicles of a triploid plant)	42	17II + 2IV
		42	17II + 1IV + 1III + 1I
		42	19II + 1III + 1I
		42	21II (2 PMCs)

Table 5. Chromosome associations observed at first meiotic metaphase in PMCs with 2n = 42 ch and of synthetic autotetraploid of a diploid species **A.** strigosa

Source	Desig-nation	Parents of the Amphiploid	Amphiploid Generation	Number of PMCs Examined	VI Mean	VI Range	V Mean	V Range	Obser IV Mean	IV Range
Table 4 of this report	-	abyssinica C.I. 2108 x strigosa C.I. 7010	A_0	7	0.00	-	0.14	0-1	0.85	0-2
Table 4 of this report	-	abyssinica C.I. 2108 x strigosa C.I. 2630	A_3	28	0.03	0-1	0.14	0-1	1.14	0-4
Griffiths et al. (1959)[2]	-	barbata x hirtula	A_2	At least 160	Not calculated				1.39	-
Griffiths et al. (1959)	240 Cx	barbata x longiglumis	A_2	At least 20	Not calculated				1.85	1-4
Griffiths et al. (1959)	Cc 4387/2	abyssinica C.D. 4550 x strigosa C.D. 3820[3]	At least A_4 or A_5[3]	At least 20	Not calculated				2.35	-
Thomas and Peregrine (1964)	Cc 4387/1	abyssinica C.D. 4550 x strigosa C.D. 3820[3] and abyssinica x strigosa	At least A_4 or A_5[3]	Not mentioned	0.12	0-1	0.18	0-1	1.88	0-3
	Cc 4435/1									
Rajhathy and Morrison	68-31	abyssinica C.D. 4550 x	Selection for fertility made for	50	0.00	-	0.00	-	5.20	0-7

2n = 42 chromosomes of various synthetic 6x amphiploids

Observed Associations								
IV		III		II		I		Fertility
Mean	Range	Mean	Range	Mean	Range	Mean	Range	(%)
0.85	0-2	0.42	0-1	18.00	15-21	0.57	0-2	-
1.14	0-4	1.32	0-4	14.42	9-20	3.71[1]	0-12	-
1.39	-	1.50	-	14.34	-	2.17	-	40.37 (19.77 - 70.77)
1.85	1-4	2.20	0-4	11.85	7-16	2.30	0-5	26.40
2.35	-	1.05	-	13.90	-	1.55	-	30-70[3]
1.88	0-3	1.41	0-4	13.10	8-19	2.35	0-9	2-5
.20	0-7	0.10	0-1	Closed 8.00 5-12 Open		0.40	0-4	Almost complete seed set under

Thomas and Peregrine (1964)	Cc 4387/1	[illegible] x strigosa C.D. 3820[3]	At least A_4 or A_5[3]	Not mentioned	0.12	0-1	0.18	0-1	
	Cc 4435/1	abyssinica x strigosa							
Rajhathy and Morrison (1960)	68-31	abyssinica C.D. 4550 x strigosa C.D. 3820[4]	Selection for fertility made for several generations[4]	50	0.00	-	0.00	-	
Morrison and Rajhathy (1960)	Ottawa	Auto-tetraploid of strigosa	Selected for fertility	125	-	-	-	-	

[1]In plant 63-10-38 (Table 4) a PMC is reported as having 16II + 2II (end to end 16II + 1IV + 6I.

[2]Data of 8 plants are combined.

[3]Griffiths, D. J., Welsh Plant Breeding Station, near Aberystwyth, Great Britai used. Personal Communication. 1966.

[4]Zillinsky, F. J., Canada Department of Agriculture, Ottawa, Ontario, Canada. 1966.

0.18	0-1	1.88	0-3	1.41	0-4	13.10	8-19	2.35	0-9	2-5
0.00	-	5.20	0-7	0.10	0-1	Closed 8.00 5-12 Open 2.30 0-8		0.40	0-4	Almost complete seed set under favorable greenhouse conditions[4]
-	-	4.40	-	0.10	-	5.00	-	0.10	-	-

II (end to end) + 1IV + 2I. Here, this PMC is considered as having

, Great Britain. Parents, amphiploid generation, and fertility of lines

rio, Canada. Parents and fertility of line 68-31. Personal Communication.

Table 6. Chi-square goodness of fit for the number of bivalents and other associations observed at metaphase in A_o generation of the amphiploid of C.I. 2108 x C.I. 7010 and in A_3 generation of the amphiploid C.I. 2108 x C.I. 2630

Amphiploid generation	No. of PMCs	Total No. of bivalents		Total No. of associations other than bivalents		
		Observed	Calculated	Observed	Calculated	Total
A_o	7[1]	126	102.77	14	37.23	140
A_3	28	404	427.23	178	154.77	582
		530		192		722

Chi-square = 24.49 d.f. = 1

Chi-square = 6.63 (Table value at 0.01 level)

[1]Since the unit of observation is a bivalent, this sample is not considered to be small.

Table 7. Author's interpretation of chromosome associations observed in triploid hybrids of *Avena* by Nishiyama

Chromosome associations	Frequency in different hybrids: *A. barbata* x *A. strigosa* (Nishiyama, 1929)		*A. barbata* x *A. strigosa* (Nishiyama, 1934)		*A. barbata* x *A. wiestii* (Nishiyama, 1934)		Author's interpretation
7II + 7I		38		11		24	Shows homology of A genome chromosomes.
6II + 1III + 6I	24		20		26		
5II + 2III + 5I	9	35	28	70	28	63	Shows partial homology between A genome and genome other than A of tetraploid.
4II + 3III + 4I	2		19		6		
3II + 4III + 3I	-		2		2		
2II + 5III + 2I	-		1		1		
8II + 5I	17	19	4	4	2	2	Shows autosyndetic pairing of chromosomes of genome other than A of tetraploid.
9II + 3I	2		-		-		
7II + 1III + 4I	6		6		3		Very likely, shows partial homology between A genome and genome other than A rather than autosyndetic pairing.
6II + 2III + 3I	2	8	5	15	7	11	
5II + 3III + 2I	-		4		1		

Table 8. Frequency of microspores (few anaphase II groups counted) with various chromosome numbers observed at metaphase of first pollen grain mitosis in 13 A_3 generation plants (2n=42) of the amphiploid of C.I. 2108 x C.I. 2630

Subtotal	No. of microspores	Chromosome number in microspore
	1	8+3 fragments
	1	12+3 fragments
	1	"12"[1]
	5	"13"
	2	14
	1	14+1 telocentric
12	1	14+2 fragments
	1	14+3 fragments
	6	"14"
	1	"14"+3 fragments
	2	"15"
	1	16
	2	"16"
12	4	17
	1	17+2 fragments
	1	17+3 fragments + 3 micronuclei
	1	"17"
	2	18
	3	18+1 micronucleus
14	1	18+2 micronuclei
	8	"18"
	15	19
	4	19+1 micronucleus
30	1	19+2 micronuclei
	2	19+1 fragment
	8	"19"
	15	20
34	6	20+1 micronucleus
	2	20+2 micronuclei
	11	"20"

[1]" " is used to show the approximate number of chromosomes with ± 1 chromosome, e.g. "15" = 15±1 chromosomes.

Table 8. (Continued)

Subtotal	No. of microspores	Chromosome number in microspore
	36	21
	2	21+1 micronucleus
	2	21+1 fragment
58	1	21+2 fragments
	1	21+3 fragments
	1	21+1 fragment + 1 micronucleus
	14	"21"
	1	"21"+1 micronucleus
	8	22
16	3	22+1 micronucleus
	2	22+1 fragment
	3	"22"
	5	23
10	1	23+1 fragment
	4	"23"
	2	24
	4	"24"
	2	"26"
—	1	"28"

Table 9. Number of abnormal pollen grains at various developmental stages in A_3 generation plant 64-10-26 (2n=41) of the amphiploid of C.I. 2108 x C.I. 2630

Relative maturity of the spikelets (based on the relative position of the spikelets on the panicle)	Developmental stages of the pollen grains		
	2 nuclei	Near metaphase	1 nucleus
x-2[1] 3 Anthers	236[2](0)[3]	7(0)	57(0)
x-3 Anther I	27(2)	18(8)	198(153)
Anther II	37(9)	36(10)	222(81)
x-4 Anther I	0	0	365(185)
Anther II	0	0	250(150)
Anther III	0	0	200(110)
x-5 3 Anthers	0	0	300(0)

[1]x-2 is the most mature spikelet and x-5 is the youngest spikelet.

[2]Total number of pollen grains.

[3]( ) = Number of abnormal pollen grains.

Table 10. Chromosome mosaicism observed at mitotic metaphase in root-tips of autotetraploids of the diploid species Avena strigosa, varieties C.I. 2630 and Saia

Chromosome number	4x-2630 4	5	6	7	4x-Saia 7	8	9	Total
5	-	-	-	-	-	-	1	1
6	-	-	-	-	-	-	1	1
6+1f*	-	-	-	-	1	-	-	1
7	-	-	-	-	-	-	1	1
16+1f	-	-	-	1	-	-	-	1
23+3f	1	1	-	-	-	-	-	2
26	-	2	-	-	-	-	-	2
26+1f	-	1	1	-	-	-	-	2
26+4f	1	-	-	-	-	-	-	1
27	1	1	-	1	1	1	-	5
27+1 small chromosome	1	-	-	-	-	-	-	1
27+2f	-	-	-	-	1	-	-	1
28	18	12	9	16	25	4	11	95
28+1f	2	2	2	2	1	-	-	9
28+2f	1	-	-	-	-	-	-	1
28+1 telo.	-	-	-	-	3	-	-	3

*f = fragment (may include telocentric chromosome).

Table 11. Abnormalities during mitosis in root-tips of A_3 generation plants of the amphiploid C.I. 2108 x C.I. 2630 and in autotetraploid (4x) plants of C.I. 2630 and Saia

Material	Plant Number	Root-tip Number	No. of normal looking anaphases and telophases	One or more bridges at anaphase and telophase	Other abnormalities
Amphiploid plants:	64-10-82	1	8	-	-
	64-10-85	1	17	2	-
		2	4	1	1 (metaphase with fragments)
		3	-	1	-
	64-10-86	1	8	-	-
	64-10-88	1	7	-	-
	65-10-1	1	-	1	-
		2-5	46	-	-
	65-10-2	1 and 3	-	-	-
		2,5,7-10	47	-	-
		4	12	1	-
		6	6	1	1 (tripolar anaphase?)
	65-10-4	1 and 3	-	-	-
		2,4,5, and 7	19	-	-
		6	-	-	1 (metaphase with fragments)
	65-10-5	1	-	-	-
		2	-	1	-
		3	-	2	-
		4	6	1	-
		5,7,8, and 11	38	-	-
		6	10	1	-
		9	4	2	-
		10	17	4	-

Table 11. (Continued)

Material	Plant Number	Root-tip Number	No. of normal looking anaphases and telophases	One or more bridges at anaphase and telophase	Other abnormalities
Amphiploid plants:	65-10-6	1	8	1	-
		2-5,7 and 8	40	-	-
		6	2	1	-
	65-10-7	1-3,5	24	-	-
		4	17	1	-
		6	6	1	-
	65-10-8	1 and 4-7	59	-	-
		2	11	1	-
		3	4	1	-
		8	20	2	-
		9	20	3	-
	65-10-10	1	3	-	-
		2	2	4	-
		3	9	12	-
		4	1	1	-
		5	13	1	-
		6	11	5	-
	65-10-11	1-6	28	-	-
		7	5	3	-
Autotetraploid plants:	4x-Saia-1	1-7	26	1	2 (metaphase with fragments)
	4x-Saia-2	1-2	11	-	-
	4x-Saia-3	1-4	48	1	-
	4x-Saia-4	1-6	16	-	-
	4x-Saia-5	1-3	25	-	-

Table 11. (Continued)

Material	Plant Number	Root-tip Number	No. of normal looking anaphases and telophases	One or more bridges at anaphase and telophase	Other abnormalities
Autotetraploid plants:	4x-Saia-6	1-3	16	-	-
	4x-2630-1	1-9	88	1	-
	4x-2630-2	1-5	78	-	-
	4x-2630-3	1-7	35	-	1 (anaphase with fragment) 1 (about 35 chromosomes with 3 or 4 fragments)
Control:	C.I. 2108 (7 plants)	1-7	121	-	-
	C.I. 7010 (3 plants)	1-3	60	-	-
	C.I. 2630 (3 plants)	1-3	42	-	-

Table 12. Chromosome number and chromosome distribution at anaphase I in PMCs of autotetraploids (4x) Avena strigosa C.I. 2630

Plant No.	Chromosome distribution 2n=28		2n=27		2n=26		Other 2n Nos.	
4x-2630-1	14+14	48	14+13	4	13+13	10	2n=18	1
	15+13	11	13+1*+13	1	14+12	4		
	13+2*+13	3			13+1*+12	2		
	14+1*+13	2						
	16+12	1						
	Total	65	Total	5	Total	16		
4x-2630-2								
Panicle II	1		1		11		-	
Panicle III	-		-		5 (includes 3 cells at metaphase I with 13II, 9II+1IV+1III+ 1I, and 6II+2IV+2III)		-	
Panicle V	1		8		2		-	
4x-2630-3								
Panicle IV Spikelet N[1] and N-1	47		-		-		2n=24	1

*Divided lagging chromosomes at equatorial plate.

[1]Considering position of florets on the panicle, spikelet N is more mature than spikelet N-1 and is less mature than spikelet N+1.

Table 12. (Continued)

Plant No.	Chromosome distribution 2n=28	2n=27	2n=26	Other 2n Nos.
Spikelet N+1	2	35	1	2n+27+fragment 1 2n=10 1 cell at metaphase I with 3II+2III+2I
Panicle V	11 (1 cell at metaphase I with 14II)	-	-	-
Panicle VI	2	7	-	2n=25 1
Panicle VIII	14	-	-	-

Table 13. Chromosome number and chromosome distribution at anaphase I and chromosome number at metaphase I in PMCs of an autotetraploid (4x) of Avena strigosa C.I. 7010 (Saia)

Plant No. 4x-Saia-2[1]

I. Frequency of different anaphase I groupings

2n=30		2n=28	
16+14	7	14+14	2
15+15	5	15+13	1
15+1*+14	4		
15+2*+13	4		
17+13	2		
17+1*+12	1		
Total	23	Total	3

*Divided lagging chromosomes at equatorial plate

II. Observed chromosome associations at metaphase I

Total chromosome number of the PMC	Observed chromosome associations
6	1II+3I+1 (telocentric?)
6 (2 cells)	1II+4I
14	7II
15	3II+1V+4I
15	3II+2III+3I
15	4II+1III+4I
26	10II+1III+3I
30	8II+2IV+1III+3I
30	9II+1IV+2III+2I
30	10II+2IV+2I
30	11II+3II (end to end) + 2I
30	12II+6I
30	13II+4I
30	15II

[1]Two siblings of this plant had 2n=28 chromosomes in most of the PMCs at anaphase I. Data for these plants were not large enough and, therefore, are not presented here.

Fig. 1. Diakinesis in a PMC of the derived tetraploid 57-127 with 2n=28 chromosomes. The cell shows 14II.

Fig. 2. Metaphase I in a PMC of the derived tetraploid 57-127 with 2n=28 chromosomes. The cell shows 12II+1IV.

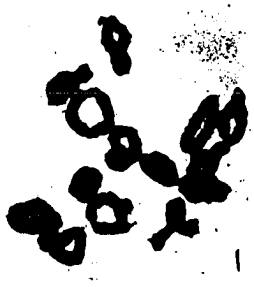
Fig. 3. Mitotic metaphase in a root-tip cell of the A_3 plant 64-10-85 with 2n=18 chromosomes.

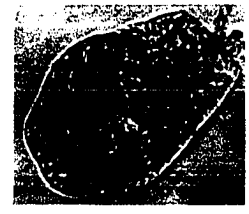
Fig. 4. Mitotic metaphases in two adjoining root-tip cells of the A_3 plant 64-10-85 with 2n=19 and 22 chromosomes.

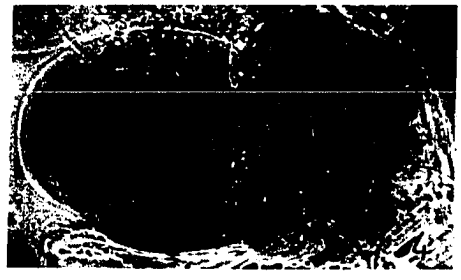
Fig. 5. Metaphase I in a PMC of the A_3 plant 63-10-37 with 2n=41 chromosomes. The cell shows 13II+1II (end to end) +1IV+3I.

Fig. 6. Metaphase I in a PMC of the A_8 plant 63-1-14 with 2n=42 chromosomes. The cell shows 21II.

Fig. 7. Metaphase I in a PMC of the A_3 plant 64-10-80 with 2n=6 chromosomes. The cell shows 2II+2I.







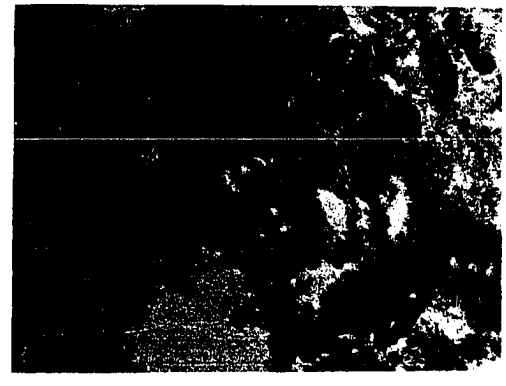


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Fig. 8. Metaphase I in two PMCs of the A_3 plant 63-10-47 with 2n-13 and 32 chromosomes. The cells show 6II+1I and 11II+3III+1I, respectively.

Fig. 9. Metaphase I in a PMC of the A_3 plant 63-10-38 with 2n=18 chromosomes. The cell shows 6II+1IV+2I.

Fig. 10. Metaphase I in a PMC of the A_3 plant 63-10-47 with 2n=21 chromosomes. The cell shows 4II+3III+4I.

Fig. 11. Metaphase of first pollen grain mitosis in the A_3 plant 63-10-32 with 2n=14 chromosomes and 2 fragments.

Fig. 12. Metaphase of first pollen grain mitosis in the A_3 plant 63-10-26 with 2n=19 chromosomes.

Fig. 13. Aceto-carmine stained pollen grain of the A3 plant 64-10-26. Pollen grain shows pachytene-like threads.

Fig. 14. Pollen grains of the A_3 plant 64-10-30. Pollen grains were treated with Trichloroacetic acid before staining with Feulgen. Therefore, it is concluded that the threads represent some abnormality in the pollen grain wall.

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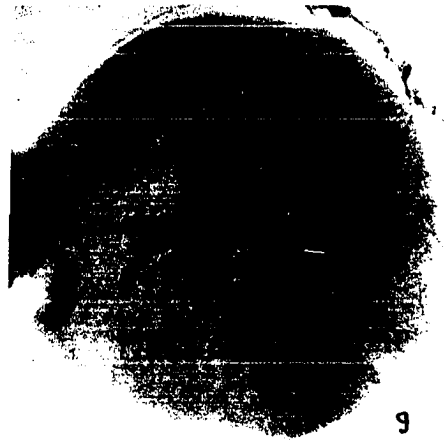

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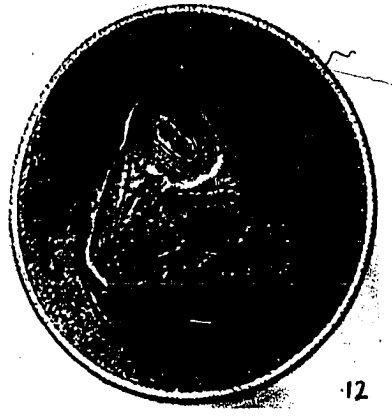

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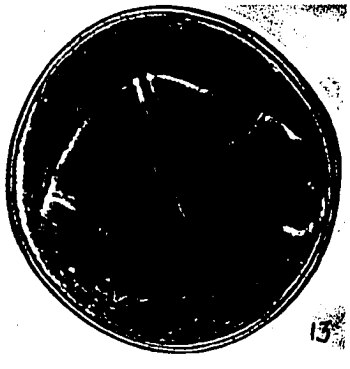

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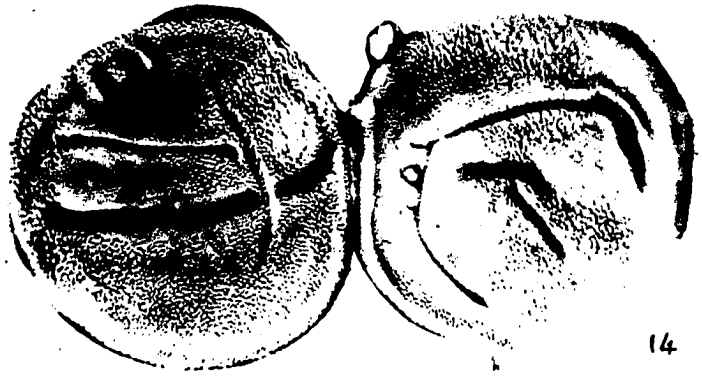

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Fig. 15. Mitotic metaphase in a root-tip cell of the autotetraploid plant 4x-2630-4 with 2n=26 chromosomes and 4 fragments.

Fig. 16. Telophase in a root-tip cell of the A_3 plant 65-10-10 with two chromosome bridges.

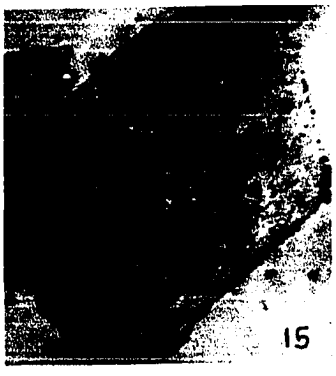
Fig. 17. Mitotic metaphase in a root-tip cell of the A_3 plant 63-10-32 with 2n=42 chromosomes. The cell has one dicentric chromosome.

Fig. 18. Anaphase I in a PMC of the A_3 plant 63-10-32. The cell shows a bridge involving a dicentric chromosome.

Fig. 19. Anaphase I in a PMC of the A_3 plant 64-10-70. The cell shows a dicentric chromatid at one of the poles.

Fig. 20. Mitotic metaphase in a root-tip cell of the A_3 plant 64-10-26 with 2n=28 chromosomes. The cell shows most of the chromosomes in pairs of two.

Fig. 21. Zygotene in PMCs of the A_3 plant 64-10-72. The cells show cytomixis.



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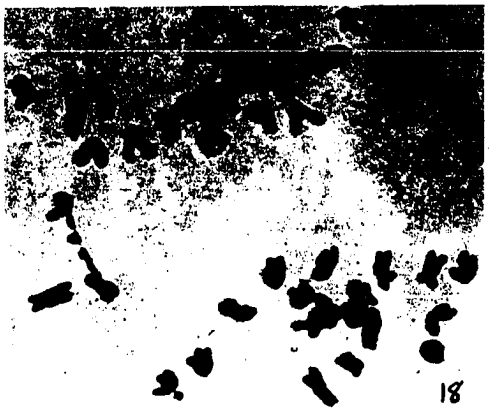

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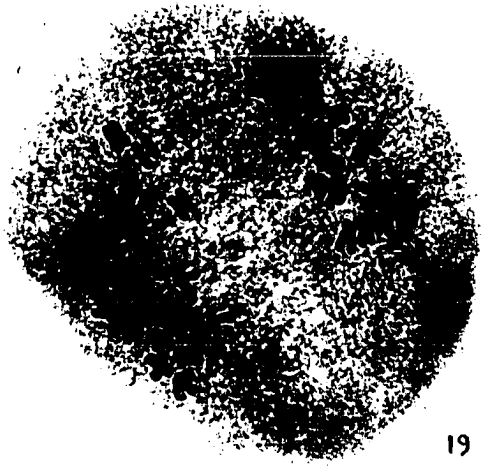

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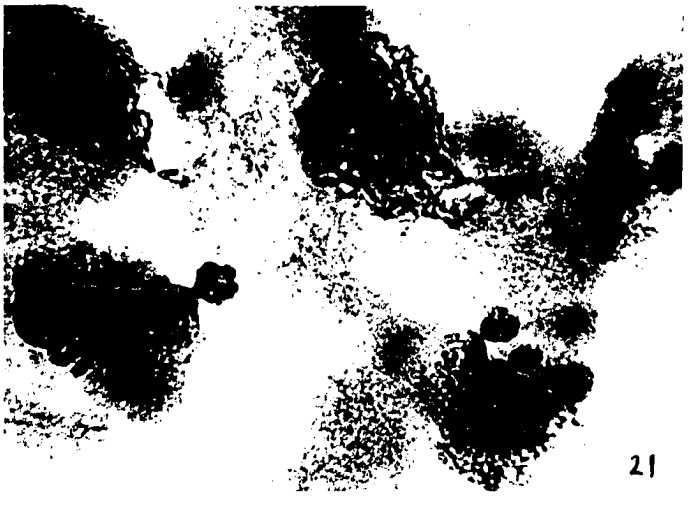

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Fig. 22. A PMC of the A_3 plant 64-10-61. The cell has more than one nucleus and shows differential condensation of chromosomes.

Fig. 23. A PMC of the A_3 plant 64-10-61. The cell has more than one nucleus and shows differential condensation of chromosomes.

Fig. 24. Anaphase I in a PMC of the autotetraploid plant 4x-2630-1 of the diploid species A. strigosa. The cell shows 2n=26 chromosomes.

Fig. 25. Metaphase I in two PMCs of the autotetraploid plant 4x-Saia-2 of the diploid species A. strigosa with 2n=6 chromosomes. The cells show $\overline{1}\overline{1}\overline{1}+4\overline{1}$.

Fig. 26. Metaphase I in a PMC of the autotetraploid plant 4x-Saia-2 of the diploid species A. strigosa with 2n=14 chromosomes. The cell shows $7\overline{1}\overline{1}$.

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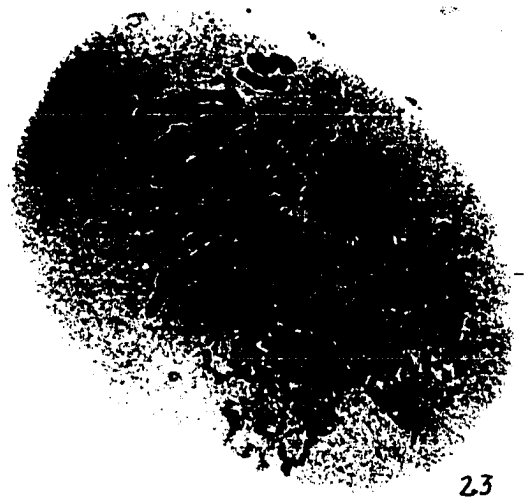

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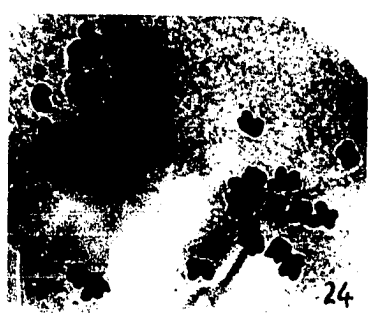

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SUMMARY AND CONCLUSIONS

(1) The A_0, A_1, and A_3 generation plants of the amphiploid of *Avena abyssinica* x *A. strigosa*, autotetraploid plants of the diploid species of *A. strigosa*, varieties C.I. 2630 and C.I. 7010, two tetraploid lines derived from a synthetic 6x amphiploid, and *A. sativa* varieties, Garry and Cherokee, were used as experimental materials.

(2) Chromosome mosaicism in root-tips of the A_1 and A_3 amphiploids and in the autotetraploid plants was observed. Chromosome numbers in the root-tip cells varied from 12 to 43 in the A_1 and A_3 amphiploids and varied from 5 to 28+1 telocentric in the autotetraploid plants. Cells with less than the modal chromosome number were observed more often than cells with more than the modal chromosome number. This observation suggested that in some root-tip cells of these plants, one or more chromosomes had not undergone replication at interphase and, therefore, at anaphase, these chromosomes had either moved to one of the poles at random or had misdivided. Among A_1 and A_3 generation plants, only one plant had many root-tip cells with one dicentric chromosome and all other plants had few or no cells with one dicentric chromosome. No explanation is suggested for the origin and fate of the dicentric chromosome in these plants.

(3) Chromosome mosaicism in the PMCs of the A_0, A_3, and autotetraploid plants was observed. Chromosome numbers in the PMCs of the A_0 and A_3 plants varied from 6 to 45, and in the

PMCs of autotetraploid plants, varied from 6 to 30. Few PMCs with more than the modal chromosome number were observed in the A_3 plants. In the autotetraploid plants, most of the PMCs with the reduced chromosome number had an even number of chromosomes. This observation suggested that in the autotetraploid plants, and probably in the synthetic 6x amphiploid plants, homologous chromosomes, during premeiotic mitosis, in some special way, were involved in the formation of the PMCs with less than the 2n chromosome number of the plant. Chromosome mosaicism in root-tips suggested that mosaic premeiotic cells may be involved in the formation of the germ line.

(4) In the A_3 plants, pollen grains with as few as 8 chromosomes and 3 fragments and with as many as about 28 chromosomes were observed. This observation suggested that chromosome mosaicism in the root-tips and the anomaly in premeiotic mitosis contributed to the formation of gametes with less than the n number of chromosomes in the A_0 and A_3 plants. Moreover, the PMCs with differential condensation of chromosomes, with more than one nucleus, as well as multivalents at metaphase I, also contributed to the formation of such gametes. Univalents and multivalents can also result in the formation of gametes with more than the n number of chromosomes.

(5) The chromosome associations in the 42 chromosome PMCs of the A_0 and A_3 plants were compared. The average number of bivalents per cell was higher, and the average number of

multivalents and univalents per cell was lower in the A_0 plant than in the A_3 plants. This difference in number of bivalents and other associations in the A_0 and A_3 plants suggested that the A genome chromosomes of A. abyssinica and A. strigosa paired less often in the A_0 plant, and the association within sets of four presumably homologous chromosomes of the A genome was more frequent in the A_3 plants. This means that the A genome chromosomes of A. abyssinica and A. strigosa have differentiated and that this differentiation is small enough to permit occasional pairing between them. Such pairing and crossing over will lead to the formation of four completely homologous chromosomes in a later generation amphiploid. The A_3 plants were in a transition stage and, therefore, multivalents were more frequent in the A_3 plants than in the A_0 plant. Some PMCs of the derived tetraploid lines showed quadrivalents, trivalents, and univalents, whereas natural tetraploid species showed only bivalents. This observation supports the suggestion that the A genome chromosomes of A. abyssinica and A. strigosa have differentiated. In the pentaploid hybrids of A. abyssinica x A. sativa only rod bivalents were observed and in the synthetic 10x amphiploids near diploid-like pairing was observed (Thomas and Jones, 1964). Therefore, the differentiation between the A genome chromosomes of A. abyssinica and A. strigosa is not considered as advanced as that between the A genome chromosomes of A. abyssinica and A. sativa.

(6) Pachytene-like feulgen positive threads were observed in pollen grains of two A_3 plants. The fact that the pachytene-like threads stained with feulgen even after the pollen grains were treated with Trichloroacetic acid indicated that the threads represent some abnormality in the pollen grain wall.

(7) Cytomixis, the process of extrusion of chromatin from the nucleus of one PMC into the cytoplasm of an adjacent PMC, was observed more frequently in the plants grown at higher than normal temperature. Cytomixis was observed in the A_0 and A_3 plants, the autotetraploid of the diploid species, A. strigosa, C.I. 2630, and in the A. sativa varieties, Garry and Cherokee. The varieties Garry and Cherokee have a very low frequency of abnormality at metaphase I. Therefore, in this study, cytomixis is considered to be an artifact.

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