



Morphological and cytogenetic investigations in the genus *Agropyron* Gaertn
by Penelope Witte Allerdice

A thesis submitted to the Graduate Faculty in partial fulfillment of the requirements for the degree of
DOCTOR OF PHILOSOPHY in Genetics

Montana State University

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Abstract:

Forty-one plants representing 30 strains of 22 *Agropyron* species were analysed in meiosis. The chromosome number was determined for each plant and a total of 4354 cells were interpreted. Irregularities of meiotic behavior were calculated as the percentage of cells per stage with a specific irregularity. Sixty-four percent of the 41 plants studied showed irregularities in the meiotic stages observed. Three plants showed an increase in univalent frequency from diakinesis to metaphase I.

The present study was concerned with genome distribution within sections *Goulardia* and *Holopyron* of the genus *Agropyron*. Meiotic chromosome behavior was analysed for ten plants from strains of eight allopolyploid *Agropyron* parent species and for six new synthetic interspecific *Agropyron* hybrids.

The four tetraploid hybrids within section *Goulardia* were: *A. subsecundum* x *A. latiglume*, *A. caninum* x *A. latiglume*, *A. arizonicum* x *A. caninum* and *A. semicostatum* x *A. trachycaulum*. The two synthetic intersectional hybrids between sections *Goulardia* and *Holopyron* were *A. caninum* x *A. riparium* and *A. brachyphyllum* x *A. riparium*. *A. caninum* x *A. latiglume* produced fertile seeds. Each of the other hybrids was completely sterile.

Cytogenetic study of these hybrids has given further evidence of the world-wide and intersectional distribution of genomes derived from the diploid *A. spicatum* genome. Derivatives from the S genome were shown to be present in hexaploid *A. brachyphyllum* from Iran, in the North American tetraploids *A. arizonicum*, *A. latiglume*, *A. subsecundum*, *A. trachycaulum* and *A. riparium*, and in tetraploid *A. caninum* from Hungary.

Derivatives of a genome of similar origin to the B genome present in tetraploid *A. trachycaulum* were also present in *A. brachyphyllum*, *A. arizonicum*, *A. latiglume*, *A. subsecundum*, *A. riparium*, and *A. caninum*.

Tetraploid *A. semicostatum* from Asia possessed one genome of undetermined origin or distribution. The second *A. semicostatum* genome was most likely of S, but possibly of B derivation.

Hexaploid *A. brachyphyllum* possessed derivatives of the S and B genomes and a third genome of undetermined origin and distribution.

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ABSTRACT

Forty-one plants representing 30 strains of 22 Agropyron species were analysed in meiosis. The chromosome number was determined for each plant and a total of 4354 cells were interpreted. Irregularities of meiotic behavior were calculated as the percentage of cells per stage with a specific irregularity. Sixty-four percent of the 41 plants studied showed irregularities in the meiotic stages observed. Three plants showed an increase in univalent frequency from diakinesis to metaphase I.

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Tetraploid A. semicostatum from Asia possessed one genome of undetermined origin or distribution. The second A. semicostatum genome was most likely of S, but possibly of B derivation.

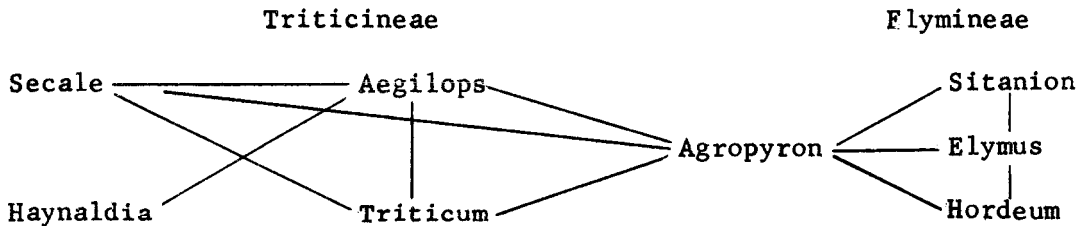
Hexaploid A. brachyphyllum possessed derivatives of the S and B genomes and a third genome of undetermined origin and distribution.

INTRODUCTION

The genus Agropyron Gaertn. is the largest and the most heterogeneous of the genera within the tribe Triticeae Dumort. of the family Gramineae. Some of the characteristics of the tribe Triticeae are: a compound spike, 2 glumes (sometimes strongly reduced), simple starch grains, and rather large chromosomes of the Festucoid type in multiples of 7. Within the tribe there are transitional forms between genera and many natural hybrids, making generic descriptions difficult.

Of the some 150 described species of Agropyron, some 100 are native to Eurasia, about 30 to North America, and the remainder to South America, Australia, and Africa. In the United States, many introductions have been made, particularly for use in seeding abused rangelands. When it was realized that some Agropyron species can be hybridized with wheat and rye, it was hoped that some characteristics of Agropyron species such as their perennial nature and disease resistance, might be transferred through repeated backcrosses to the economically important grain varieties.

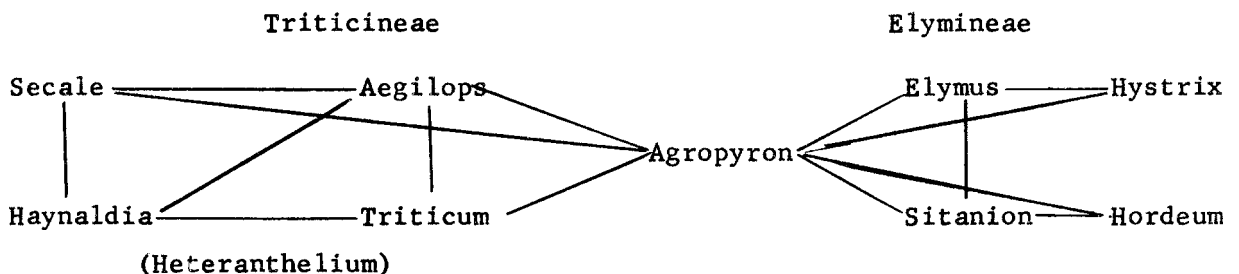
Though morphologically closer to the subtribe Triticineae than to Elymineae, intergeneric hybridizations have indicated that the genus Agropyron biosystematically occupies an intermediate position between the two subtribes. The relationship of the genera within the tribe was diagrammed in the following manner by Godley (1951):



The lines indicated the genera which had been connected either directly or indirectly with each other by various natural and synthetic intergeneric hybrids. Godley concluded that Agropyron serves as an evolutionary link between Triticineae and Elymineae and that no intersubtribal hybrids may be produced, except through species of the genus Agropyron.

Stebbins (1956) suggested that the genera Hystrix and Heteranthelium also belong in this comparium. Disregarding the little known genus Heteranthelium, he stated that 20 of the possible 36 intergeneric combinations have been synthesized or are known to exist as natural hybrids.

Godley's diagram amended with data on intergeneric hybrids listed by Stebbins (1956) and Carnahan and Hill (1961) follows:



The first important work on the cytology of grasses was that of Avdulov (1931). He found that his classification of grasses, based on the numbers and sizes of chromosomes, was quite similar to that earlier proposed

by anatomists and different from that of the classical taxonomists.

Stebbins (1956) remarked that specialists who recognize most grass genera at a glance, still find it difficult to identify the species. A source of this difficulty has been demonstrated through cytogenetic studies, which show that possible distinct ancestral boundaries among species have been eliminated through hybridization and polyploidy. As a result, evolutionary relations of most grass genera can be represented only by highly complex networks.

Seeking the common ground of agreement between the different species definitions by Clausen et al. (1939), Huxley (1940), Dobzhansky (1941), and Mayr (1942), Stebbins (1950) proposed they would agree that "in sexually reproducing organisms a species is a system consisting of one or more genetically, morphologically, and physiologically different kinds of organisms which possess an essential continuity maintained by the similarity of genes or the more or less free exchange of genes between its members. Species are separated from each other by gaps of genetic discontinuity in morphological and physiological characteristics which are maintained by the absence or rarity of gene interchange between members of different species." Mayr (1963) commented that most of the recently proposed species definitions have avoided all reference to morphological distinctness between individual organisms. He stated that species are defined better by their relation to non-conspecific populations than by the relation of the conspecific individuals to each other. He felt the decisive criterion was the reproductive isolation of populations, not the fertility of individuals.

The biosystematic approach to the study of differences within and between species utilizes data obtained through genetic, cytological, ecological, morphological, physiological and biochemical methods. This approach has been described in essays on "The New Systematics" (Huxley 1940), "Alpha and Omega Taxonomy" (Turrill 1938b), and has been proposed as the basis for new disciplines; e.g., "experimental taxonomy" (Clausen et al. 1940), "biosystematy" (Camp and Gilly 1943) and "genonomy" (Epling 1943).

Keck (1957) wrote that the biosystematic approach results in an understanding of the biological and evolutionary status of the taxon under consideration, and produces an objective and dependable classification.

A long range research program on the biosystematics of the genus Agropyron Gaertn. is in progress at Montana State University, directed by Dr. J. Schulz-Schaeffer. The sectional division of the genus by Holmberg (1936) was chosen as a system of taxonomic classification against which to integrate and evaluate data to be obtained through cytological, chromatographic and serological methods. The morphological observations by Nevski (1934) and by Hitchcock and Chase (1950), were chosen to provide the basis for the biosystematic survey.

Schulz-Schaeffer and Jurasits (1962) compared the karyotypes of 33 species of the genus, in a search for genome indicator chromosomes (Schulz-Schaeffer 1960). Schulz-Schaeffer et al. (1963a) studied the nature of polyploidy in tetraploid and hexaploid species of section Agropyron. Two dimensional paper chromatographic analysis was made of polyphenolic compounds from Agropyron species spikelets (Lorenz and Schulz-Schaeffer 1964). They found parallels to Holmberg's sectional classification of the genus.

Extracted leaf proteins from different species have been compared through serodiagnostic techniques (Creel 1964, Creel et al. 1965, Collins 1965). Creel found species of sections Holopyron and Goulardia to be quite closely related, while those of Agropyron formed a distinct group. Collins (1965) studied the morphology and cytology of 14 native Montana Agropyron species, from sections Holopyron and Goulardia. He also studied the cytotaxonomy of a sterile, natural hybrid, concluding that suspected parents were A. latiglume, and A. scribneri belonging to section Goulardia.

Stebbins (1950) divided interspecific hybrids into two groups. The first included those hybrids capable of producing some viable pollen and seed through selfing, F_1 intercrossing, or back crossing to the parent species. The second group included those which are completely sterile, except for possible production of allopolyploid derivatives. Clausen et al. (1939) identified the first group as hybrids between ecospecies, and the second, as those between cenospecies.

The present study is concerned with the determination of genome distribution within the genus Agropyron. Meiotic chromosome behavior was analyzed for ten strains of eight Agropyron species used for parents and for six new synthetic interspecific Agropyron hybrids. The results were compared with previous genome analyses of closely related interspecific and intergeneric Agropyron hybrids reported by Matsumura (1942), Stebbins et al. (1946a, 1946b), Stebbins and Pun (1953), Boyle and Holmgren (1955), Stebbins and Snyder (1956), Witte (1956), Boyle (1963), Dewey (1964), Sakamoto (1964), Collins (1965).

The four synthetic tetraploid hybrids within section Goulardia were:

A. subsecundum x A. latiglume, A. caninum x A. latiglume, A. arizonicum x A. caninum, and A. semicostatum x A. trachycaulum. The two synthetic intersectional hybrids were: A. caninum of section Goulardia x A. riparium of section Holopyron, and A. brachphyllum of section Goulardia x A. riparium. Each of these hybrids except A. caninum x A. latiglume appeared to be completely sterile. A. caninum x A. latiglume produced fertile pollen grains and viable seed.

REVIEW OF LITERATURE

Nevski (1934) reorganized the tribe Triticeae, and recognized some twenty genera. He established three series of genera, each with two subtribes: 1) Clinelymineae series: Clinelyminae and Roegneriinae 2) Hordeinae series: Hordeinae and Aegilopinae 3) Elyminae series Elyminae and Agropyrinae. In each series one subtribe contained spikelets in groups at the nodes, and the other had one spikelet per node. Nevski split the genera Agropyron, Elymus, and Hordeum and arranged the species into several new genera. Gould (1947) proposed that the genera Sitanion, Hystrix, and most of Agropyron should be placed in the genus Elymus. However, Melderis (1950) and Stebbins (1956) agree that Gould did not provide sufficient evidence for uniting, nor for defining the proposed genus.

Stebbins et al. (1946a,b) showed that some of the Agropyron species may have evolved from crosses with Elymus species; but meiotic behavior in additional intergeneric hybrids indicated that genomes from other genera have contributed during the process of evolution of the now existing Agropyron species. Stebbins and Walters (1949) concluded that the established species names should be used until accumulating biosystematic data might provide a basis for a natural classification system with the comparium.

Holmberg (1926) described the following taxonomic sections within the genus Agropyron: Goulardia (Husnot) Holmberg, Holopyron Holmberg, Agropyron Gaertn. and Eremopyron (Ledeb.) Jaub. et Spach. Nevski (1934) moved some of these section names to generic status; however, in the study at Montana State University they have been used for sectional comparison of species within the genus Agropyron.

Peto (1930) published chromosome numbers of Agropyron species. He found a polyploid series, with 7 being the basic genome number.

Meiotic irregularities are common in species hybrids and generally are indicative of the hybrid origin of the organism. Cytogenetic studies have been made of intergeneric hybrids between Agropyron species and Secale, Triticum, Hordeum, Elymus, and Sitanion species, as well as of various interspecific and intraspecific Agropyron hybrids. As a result of studies on meiotic chromosome pairing in species and species hybrids, genome formulae have been published for at least eighteen Agropyron species as well as for Elymus glaucus, Sitanion hystrix, and Sitanion jubatum (Matsumura 1942, Godley 1947, Stebbins and Singh 1950, Stebbins and Pun 1953, Stebbins and Vaarama 1954, Boyle and Holmgren 1955, Cauderon 1958, Dewey 1961b, 1963a, 1963c, Schulz-Schaeffer et al. 1963a).

In the literature genomes are usually identified by letters, and variations within a basic genome are indicated by subscripts of different numbers and letters. Matsumura (1942) used abbreviations of the species names as subscripts, in place of numbers.

Following study of the meiotic chromosome associations in the parent species and their interspecific hybrid, Godley (1947) proposed the gametic genome formulae of A_1A_2B for A. pungens and A_1A_2aC for A. repens. A. pungens and A. repens are both in section Holopyron. Creel (1964) found a low serological difference index between these two species, lending support to the similarity of the formulae.

Stebbins and Singh (1950) studied interspecific and intergeneric hybrids of the allotetraploids A. parishii and Elymus glaucus, and the

diploid A. spicatum and A. inerme. Based on chromosome associations in the hybrids, the following gametic genome formulae were postulated: diploid A. inerme and A. spicatum, A_1 ; A. parishii A_1E_1 ; Elymus glaucus A_2E_1 . The genome A_1 was identified as carrying genes for slender growth, narrow glaucous leaves, elongate spikes with the spikelets single at the nodes, glumes shorter than lemmas and large anthers. The genome E_1 carried genes for tall growth, narrow glaucous leaves, elongate spikes, spikelets double at the nodes, relatively long glumes, and probably large or medium sized anthers. The diploid species carrying this genome was not known. It was also proposed that the genome A_2 of Elymus glaucus carried genes for relatively short, stocky growth; broad, possibly green leaves, short dense spikes, and narrow glumes and long awns.

It was suggested by Stebbins and Pun (1953) that some species of Agropyron contain a genome more or less homologous to that of diploid A. spicatum of section Holopyron. They found bivalent pairing in hybrids within the diploid complex A. spicatum, A. inerme, and A. caespitosum. The hybrid A. spicatum x A. inerme had nearly normal meiosis and was fertile. The hybrid A. spicatum x A. caespitosum often formed seven bivalents in metaphase I, but sometimes had two to four univalents which lagged in later stages. The nearly normal pairing between the species from North America, and A. caespitosum from Iran led them to suggest that the genome in this complex may have contributed to the evolution of polyploid Agropyron species on a world wide basis.

Artificial hybridizations between nine different strains of Elymus glaucus, six strains of Sitanion jubatum and two strains of Sitanion hystrix

were reported and interpreted by Stebbins and Vaarama (1954). The ability to cross varied widely with the combination used, ranging from failure of the cross, to weak, to vigorous hybrids. The F_1 hybrids were intermediate between their parents in morphology and resembled natural hybrids called Sitanion hansenii. The gametic genome formula given Sitanion hystrix and Sitanion jubatum was A_2E_s . Elymus glaucus had been given the formula A_2E_1 by Stebbins and Singh (1950). E_s was thought to be the genome carrying the distinctive morphological characteristics of Sitanion. Sterility in the F_1 hybrids was postulated to be due to cryptic structural hybridity, with genic sterility also present to a varying degree.

Hunziker (1955) studied the morphology and cytogenetics of some North and South American species of Agropyron and Elymus, and four of their interspecific hybrids. These species were: A. spicatum var. inerme, Elymus glaucus from North America, A. agroelymoides, A. sp. "Calmuco", and Elymus patagonicus from South America. The two South American Agropyron species had morphological characters intermediate between those of Agropyron and Elymus, and Agropyron and Hordeum. Hunziker postulated that A. agroelymoides arose as a result of hybridization between species of Agropyron and Elymus. Its meiotic behavior suggested that it was a segmental allohexaploid. He postulated that A. sp. "Calmuco" and Elymus glaucus each appeared to possess a genome partly homologous to that of the North American, diploid A. spicatum.

The intergeneric hybrid A. scabrifolium x Hordeum hexaploidum was studied by Hunziker and Covas (1955). The morphology of the hybrid was

intermediate between the parents and very close to that of the unidentified species A. sp. "Calmuco". They postulated that the latter may have arisen through allopolyploidy, between a tetraploid species of Agropyron and a diploid Hordeum. The most frequent chromosome association in the hybrid meiosis was 1^{III} , 5^{II} and 22^I suggesting that the parental species had one genome in common.

Knowles (1955) studied 29 of the 30 possible F_1 interspecific hybrids among diploid A. cristatum and the tetraploids A. desertorum, A. fragile, A. imbricatum, A. michnoi, and A. sibiricum. Trivalents were present in all the triploid hybrids, and quadrivalents in meiosis of all the tetraploid hybrids in addition to bivalents indicating a degree of relationship among the genomes concerned. A. michnoi x A. desertorum had fewer quadrivalent and more bivalent associations than either parent. These species all belong to section Agropyron.

Conner (1956) studied the New Zealand Agropyron species A. enysii, A. kirkii, A. scabrum, and A. tenue, and four of their possible interspecific hybrids. He related the results of the study to the taxonomic treatment of the genus in New Zealand. He found agreement between the conclusions reached on the biosystematic status of the four native species with those from morphological study of the plants. He reported that the four indigenous species are members of one comparium and consist of three distinct cenospecies A. enysii, A. tenue, and A. scabrum-kirkii.

A hybrid of the Asian species A. tsukushiense x A. mayebaratum was studied by Sakamoto (1957). He found normal bivalent pairing and stated that the hybrid sterility must have been due to cryptic structural

hybridity. Greel (1964) compared the species serologically, and found the very low difference index of 0.04 between the parent species.

In France, Cauderon (1958) studied the meiotic chromosome associations in the hybrids: A. campestre x A. repens ($2n=49$), A. junceum var. mediterraneum x A. campestre ($2n=49$), A. junceum var. mediterraneum x A. intermedium ($2n=42$), A. intermedium x A. littorale ($2n=42$), and A. junceum var. mediterraneum x A. littorale ($2n=42$). Taking under consideration the conclusions of Stebbins (1953) on A. intermedium, and Stebbins (1956) on A. caninum, Cauderon postulated the following genome formulae: A. elongatum, $n=7$, E; A. caninum $n=14$, SX (S originating from A. spicatum, and X from an unknown diploid species); A. junceum var. boreo-atlanticum $n=14$, 2 close genomes J_1J_2 (Ostergren 1940); J. originating from an unknown diploid species. A. junceum var. mediterraneum $n=21$, $J_1J_2E_3$; A. intermedium $n=21$, $E_1E_2N_1$ (Stebbins 1953); A. campestre $n=28$, $K_1K_2E_4E_5$ (K could be close to N); A. littorale $n=21$, $N_2N_3Y_1$ (Y could be close to J); A. repens $n=21$, $R_1R_2Z_1$ (the origin of R and Z is unknown, one, probably R, must be close to K). A. caninum belongs to section Goulardia. The other species with which Cauderon worked belong to section Holopyron. Cauderon considered that the E genomes are modifications of the A. elongatum genome. When Greel (1964) compared A. elongatum and A. intermedium he found a close serological relationship.

Dewey (1961b) studied hybrids of A. repens x A. desertorum. He concluded that A. repens (belonging to section Holopyron) and A. desertorum (belonging to section Agropyron) share no common genomes, and gave the gametic genome formulae of B_1B_2C to A. repens, and $A A$ to A. desertorum.

Serologically Creel (1964) found a high difference index between these two species. Dewey concluded that A. trichophorum and A. intermedium share the same genomes. Creel (1964) found a low difference index in his serological study of the two species. Dewey (1963a) investigated meiosis in the synthetic hybrid A. trichophorum x A. desertorum and assigned the gametic formulae $A_1B_1B_2$ to A. trichophorum and AA to A. desertorum. Creel (1964) found a low serological difference index between these two species, indicating that they may share a genome. From the frequency of meiotic chromosome associations in the hybrid A. trichophorum x A. cristatum, Dewey (1963b) concluded that one of the A. trichophorum genomes was partially homologous with the genomes of hexaploid A. cristatum. He proposed the following gametic genome formulae: A. cristatum AAA, A. trichophorum $A_1B_1B_2$ and for the hexaploid hybrid, the formula A A A $A_1B_1B_2$. Creel (1964) found a low difference index between the two parent species.

A. trichophorum belongs to section Holopyron, and A. desertorum and A. cristatum belong to section Agropyron. The two diploids studied by Dewey (1963a, 1963b) provided evidence that the diploid A. cristatum (= A. cristatiforme) genome A, is present in some species in both sections.

Synthesizing evidence from cytological and chromatographic studies, Schulz-Schaeffer et al. (1963a) proposed the following gametic formulae: A. cristatiforme A_1 , A. desertorum A_1A_2 , and A. cristatum $A_1A_1A_2$. Creel (1964) found very close serological relationship between A. desertorum and hexaploid A. cristatum.

Dewey (1964b) studied a synthetic triploid hybrid between autotetraploid A. spicatum and diploid A. cristatum. At metaphase I 90.5

per cent of the cells examined showed autosyndesis of the spicatum chromosomes, which resulted in seven bivalents, with the cristatum chromosomes present as seven univalents. The gametic genome formulae assigned were: A. spicatum B B, and A. cristatum A. A. spicatum has morphological characteristics of species of section Holopyron (Stebbins 1961) and A. cristatum belongs to section Agropyron. The A. spicatum genome has not been found in species belonging to section Agropyron (Matsumura 1942).

Proposed genome formulae for Agropyron, Sitanion, and Elymus species are listed in chronological order in Table I (Godley 1947, Stebbins and Singh 1950, Stebbins and Vaarama 1954, Cauderon 1958, Dewey 1961b, 1963a, 1963c, Schulz-Schaeffer et al. 1963a, Dewey 1964).

Most of the interspecific Agropyron hybrids studied have been between species indigenous to a common geographic region, however, a few hybrids have been synthesized between species native to different continents. (Stebbins and Pun 1953, Stebbins and Snyder 1956, Hunziker 1955, Dewey 1964). Evidently there are some genomes shared by species in more than one section of the genus, and there are some genomes with world-wide distribution.

Table 1. Published genome formulae for Agropyron, Sitanion, and Elymus species studied through intergeneric and interspecific hybrids.

Species	Gametic formula	Author
<u>A. pungens</u>	$A_1 A_2 B$	Godley (1947)
<u>A. repens</u>	$A_1 A_2 a C$	Godley (1947)
<u>A. inerme</u> <u>A. spicatum</u>	A_1	Stebbins & Singh (1950)
<u>A. parishii</u>	$A_1 E_1$	Stebbins & Singh (1950)
<u>Elymus glaucus</u>	$A_2 E_1$	Stebbins & Singh (1950)
<u>Sitanion hystix</u> <u>Sitanion jubatum</u>	$A_2 E_s$	Stebbins & Vaarama (1954)
<u>A. elongatum</u>	E	Cauderon (1958)
<u>A. caninum</u>	$S X$	Cauderon (1958)
<u>A. junceum</u> var. <u>boreo-atlanticum</u>	$J_1 J_2$	Cauderon (1958)
<u>A. junceum</u> var. <u>mediterraneum</u>	$J_1 J_2 E_3$	Cauderon (1958)
<u>A. intermedium</u>	$E_1 E_2 N_1$	Cauderon (1958)
<u>A. campestre</u>	$K_1 K_2 E_4 E_5$	Cauderon (1958)
<u>A. littorale</u>	$N_2 N_3 Y_1$	Cauderon (1958)
<u>A. repens</u>	$R_1 R_2 Z_1$	Cauderon (1958)
<u>A. repens</u>	$B_1 B_2 C$	Dewey (1961b)
<u>A. desertorum</u>	$A A$	Dewey (1961b)
<u>A. trichophorum</u>	$A_1 B_1 B_2$	Dewey (1963a)
<u>A. cristatum</u>	$A A A$	Dewey (1963c)
<u>A. cristatiforme</u>	A_1	Schulz-Schaeffer et al. (1963a)
<u>A. desertorum</u>	$A_1 A_2$	Schulz-Schaeffer et al. (1963a)

Table 1 (Continued).

Species	Gametic formula	Author
<u>A. cristatum</u>	$A_1 A_1 A_2$	Schulz-Schaeffer et al. (1963a)
<u>A. spicatum</u>	B B	Dewey (1964)
<u>A. cristatum</u>	A A	Dewey (1964)

MATERIALS AND METHODS

Plants of ten strains of seven Agropyron species belonging to section Goulardia and of one species belonging to section Holopyron were used as parents in this study. The plants had been grown from seed obtained from research institutions, plant material centers, and botanic gardens. Accession numbers, names of collectors, locations where seed was collected, and observed chromosome numbers are reported in Table 2. Voucher specimens of the parents and six interspecific hybrids are located in the Montana State University Herbarium at Bozeman.

The parent plants were well established in the field nursery when hybridizations were made during the summers of 1962 and 1963. Parent plants were chosen after cytological material had been examined for regular meiosis. For each hybridization, florets were emasculated on spikes of the mother plant species. Mature spikes of the desired male parent species were collected and placed in a bottle of water attached to a stake beside the mother plant. The female culms and pollinators were covered with a waxed bag, clipped shut, and tied to the stake. There was some loss due to high winds.

The seeds were harvested and germinated on filter paper, then planted in the greenhouse. When well established they were set outside. Very few culms were collected for cytological analysis during the first summer. During the second winter the plants were divided in the greenhouse and set in the field in June. Ample material for cytological analysis was collected.

Culms for morphological comparison were collected from the parent

Table 2. Sources of 10 strains of eight *Agropyron* species used in interspecific crosses.

Species and Section		MSU	Observed	Seed Source
		field no.	2n=	
<u>A. arizonicum</u> Scribn. et Smith	Gou.	2-5	28	Received from seed collection of the Institut für Pflanzenbau und Pflanzenzüchtung Göttingen, Germany, in January 1960 as field Nos. 52-1957 and 165-1958. MONT 59,277.
<u>A. brachyphyllum</u> Boiss. et Haussk.	Gou.	3-1	42	Collected by H. S. Gentry, east base of Kuhe Zard, Charmahal Iran, Nov. 9, 1955. MONT 59,278.
<u>A. caninum</u> (L.) Beauv.	Gou.	6-3	28	Seed from Botanic Gardens, Budapest, Hungary via Gatersleben, Germany. Received through Institut für Pflanzenbau und Pflanzenzüchtung Göttingen, Germany, in January 1960 as field Nos. 4-1957 and 131-1958. MONT 59,279.
		6-4	28	
		6-5	28	
<u>A. latiglume</u> (Scribn. et Smith) Rydb.	Gou.	48-2	28	Collected by F. J. Hermann and B. M. Leese at Middle Fork of Sheeps Creek on road to Spirit Lake (Ashley National Forest) Daggett Co., Utah, at 9,000 ft. elevation, in lodgepole pine meadow during July to Sept. 1955. P.I. 232,117. MONT 59,310.
		47-3	28	Collected by F. J. Hermann and B. M. Leese, at Sentinal Peak, Coleman, Kananaskes Road, Canada, 1956. P.I. 236,637. MONT 59,279.
<u>A. riparium</u> Scribn. et Smith	Hol.	62-2	28	Received from seed collection of the Institut für Pflanzenbau und Pflanzenzüchtung, Göttingen, Germany, in January 1960 as field Nos. 60-1957 and 171-1958. MONT 59,317.
		62a-4	28	Collected by R. G. Johnson as <u>A. smithii</u> Rydb. near Canyon City, Grant Co., Oregon in area of 12 inch annual rainfall at elevation of 3000 ft. in 1933. P-2415 variety "Sodar". MONT 59,321.

Table 2. (Continued)

Species and Section	MSU field no.	Observed 2n=	Seed Source
<u>A. semicostatum</u> (Steud.) Nees	65-4	28	Received from seed collection of Institut für Pflanzenbau und Pflanzenzüchtung, Göttingen, Germany, January 1960, as field Nos. 59-1957 and 170-1958. MONT 59,321.
<u>A. subsecundum</u> (Link) Hitch.	65-1	28	Received from seed collection of Institut für Pflanzenbau und Pflanzenzüchtung, Göttingen, Germany, January, 1960, as field Nos. 59-1957 and 170-1958.
<u>A. trachycaulum</u> (Link) Malte.	93-2	28	Collected by USDA Forest Service near Beebe, Montana, 1933. Variety "Primar". P-2535. MONT 59,338.

Explanation of Accession Numbers:

MONT - Herbarium of Montana State University, Bozeman, Montana, U.S.A.

P - Seed collection of the U.S.D.A. Soil Conservation Service, Pacific Region, Plant Material Center, Washington State University, Pullman, Washington.

P.I. - Seed collection of the U.S.D.A., A.R.S., New Crops Research Branch, Crops Research Division Plant Introduction Stations, Western Region, Pullman, Washington, and North Central Region, Ames, Iowa.

species and from the hybrids, just prior to anthesis.

The most satisfactory fixing solution, giving the best spread in metaphase I, was Newcomer's (1953). Pollen mother cells of A. semicostatum x A. trachycaulum collected in the greenhouse were fixed in Farmer's solution; those collected in the field were fixed in Newcomer's solution. Pollen mother cells of A. subsecundum x A. latiglume were collected in the greenhouse and fixed in Newcomer's solution. Pollen mother cells of the other four hybrids were collected in the field and fixed in Newcomer's solution.

Observations and photographs were made from temporary mounts of acetocarmine stained pollen mother cell squash preparations. Mature pollen grains were stained with acetocarmine. Those which were wrinkled did not seem to contain much cytoplasm, and did not stain were counted as infertile.

EXPERIMENTAL RESULTS

I. Investigation of meiosis in 30 strains of 22 Agropyron species.

Forty-one plants representing 30 strains of 22 Agropyron species were analysed in meiosis. The resulting data are summarized in Table 3. The chromosome number was determined for each plant and a total of 4354 cells were interpreted. Irregularities of meiotic behavior were calculated as the percentage of cells per stage with a specific irregularity. Deviations noted were univalents, trivalents, quadrivalents or multivalents in diakinesis and metaphase I, lagging univalents or bivalents or bridges in anaphase I and telophase I, micronuclei and karyomeres in interphase and in quartet cells, and laggards or bridges in anaphase II and telophase II.

Some of the plants included in Table 3 were studied primarily to determine their chromosome number. A. cristatiforme (plants 14-8 and 16-5) were trisomic, with seven bivalents and one univalent, or six bivalents and one trivalent in metaphase I. A. dasystachyum (plant 18-5) showed counts ranging from $n=13$ to 15. A. junceum (plant 46-5) had counts of $n=34$ and 35. A. junceum (plant 46-9) had counts of $n=36$ and 1 univalent, and $n=37$ and 1 univalent. All the other plants investigated had haploid chromosome numbers of 7, 14, 21 or 28.

Thirty-six percent of the 41 plants studied showed no irregularities in the meiotic stages observed. Among these were A. cristatiforme (plants 16-1, 16-2, 16-3), A. elongatum (plant 35-1), A. pectiniforme (plants 52-8 and 56-5), A. trachycaulum (plant 95-4), and A. trichophorum (plant 104-1); the other five were the parent plants A. caninum (plants 6-3, 6-4), A. latiglume (plant 48-2), A. riparium (plant 62-2), and A. trachycaulum

Table 3. Investigation of meiosis in plants from 42 strains of 21 Agropyron species.

Species and MSU plant number	Observed chromo. number (n)	Number of cells invest.	Percent of cells with irregularities in							
			Diak.	M. I	A. I	T. I	INT.	M. II	A. II	Quart.
<u>A. acutum</u> 0.5-6	21	47		7.6U	9L	N				
<u>A. arizonicum</u> 2-5	14	36	N	N	4B 8L					
<u>A. brachyphyllum</u> 3-1(F.3)	21	30								1.4M
3-13(F.3)	21	163		10.5R						
<u>A. caninum</u> 6-3	14	72	N	N	N	N	N	N	N	N
6-4	14	100			N	N	N	N	N	N
6-5	14	385	N	N	14L	N	N		25L	6M
<u>A. ciliatiflorum</u> 9-3	14	250		N		N		N		6.6M
<u>A. cristatiforme</u> 10a-3	7	4								
14-8	7+IU	25	16T 84U		100U			83.7	11.1U	
16-1	7	25	N		N			N	N	
16-2	7	25	N							
16-3	7	25	N							

Table 3 (Continued).

Species and MSU plant number	Observed chromo. number (n)	Number of cells invest.	Percent of cells with irregularities in							
			Diak.	M I	A I	T I	INT	M II	A II	Quart.
<u>A. cristatiforme</u> (continued)										
16-5	7+IU	10	100U							
<u>A. cristatiforme</u> x A. "cristatum complex"										
80a-4	14	35	13.5U 45Q 41.5MV							
		368		100MV	11.1B	20M	9.6M	N		6M
<u>A. dasystachyum</u>										
18-5	13 14 15	65	40.4U 10.6Q 10.6U&Q	59.4U						
20-7		165			84L	7.6L				86.3M
20-8		93								48M
<u>A. elongatum</u>										
35-1	28	10			N					
<u>A. fibrosum</u>										
112-9	14	19			50B	N				
<u>A. intermedium</u>										
34a-3		354		19U		27.8L	N	N	N	9.1M

Table 3 (Continued).

Species and MSU plant number	Observed chromo. number (n)	Number of cells invest.	Percent of cells with irregularities in							
			Diak.	M I	A I	T I	INT	M II	A II	Quart.
<u>A. junceum</u>										
46-5	34,34+1U	10			10U					
46-9	36+1U 37+1U	12			100U					
<u>A. latiglume</u>										
47-3	14	57	6U 6Q	20U 10Q	6L	N	N	N	N	
48-2	14	100	N	N	N	N	N	N	N	
<u>A. lolioides</u>										
F.3-3&5		55		66.7U	20B					
<u>A. panormitanum</u>										
50a-3	14	159	22.5U		N	N	N	N		N
<u>A. pectiniforme</u>										
52-1	14	10	90N							
52-8	14	50			N					
56-5	14	10			N					
56-8	14	25	N	71U						
<u>A. riparium</u>										
62-2	14	50	N	N	N					
62a-4	14	509		10Q 5U	7L 1B	N	N			2M

Table 3 (Continued).

Species and MSU plant number	Observed chromo. number (n)	Number of cells invest.	Percent of cells with irregularities in							
			Diak.	M I	A I	T I	INT	M II	A II	Quart.
<u>A. semicostatum</u>										
64-5	21	141		33U	7.7U		N			N
65-4	14	262		N	5B	N	N			
<u>A. spicatum</u>										
	28	238		14R	9.1L					58.1M
<u>A. subsecundum</u>										
65-1	14	282	0.6U	3U	N	N	N	N	N	N
83-1	14	20		N						
<u>A. trachycaulum</u>										
93-2	14	50	N	N						
95-4	14	25	N							
<u>A. trichophorum</u>										
104-1	21	10			N					

Explanation of abbreviations:

- N - all cells normal
- L - laggards
- R - metaphase with one ring outside of equatorial plate
- M - cells with micronuclei
- B - cells with bridges
- U - cells with univalents
- T - cells with trivalents
- Q - cells with quadrivalents
- MV - cells with multivalents

(plant 93-2).

Three plants showed an increase in univalent frequency from diakinesis to metaphase I. In A. dasystachyum (plant 18-5), 40.5 percent of the diakinesis cells contained univalents and increased in metaphase I to 59.4 percent. In A. latiglume (plant 47-3), six percent of the diakinesis cells contained univalents and increased in metaphase I to 20 percent. In A. subsecundum (plant 65-1), 0.6 percent of the diakinesis cells contained univalents and increased to three percent of the cells in metaphase I. Univalents were found in diakinesis or metaphase I of several other plants, but cells in both stages were not studied.

Several plants had a percentage of cells with laggards in anaphase I but were normal and without laggards in telophase I. These were A. acutum (plant 0.5-6), with laggards in nine percent of the anaphase I cells, A. caninum (plant 6-5) with 14 percent in anaphase I, A. latiglume (plant 47-3) with six percent in anaphase I, and A. riparium (plant 62a-4) with laggards in seven percent of the anaphase I cells and none in telophase I.

The two species with the highest frequency of quartet cells containing micronuclei and karyomeres were A. dasystachyum (plant 20-7) with 86.3 percent, plant 20-8 with 48 percent, and A. spicatum with 58.1 percent.

Ten of the plants included in the survey were the parents of the six new interspecific hybrids. These were A. arizonicum (plant 2-5), A. brachyphyllum (plant 3-1), A. caninum (plants 6-3, 6-4, 6-5), A. latiglume (plants 47-3, 48-2), A. riparium (plants 62-2, 62a-4), A. semicostatum (plant 65-4), A. subsecundum (plant 65-1), and A. trachycaulum (plant 93-2).

Descriptions of their meiotic behavior are included in the sections on the comparative cytology of the parent plants and their interspecific hybrid.

II. Attempted intersectional and intrasectional hybrids within Agropyron.

Data from interspecific hybridization with preceding emasculation of the female parents during the summers of 1962 and 1963 are recorded in Table 4. These data reveal preliminary information on interspecific crossing compatibility.

In Table 4, 818 attempted crosses are reported. Fifty seeds were matured, which represents an average seed set of six percent. Twenty-one potential hybrid plants were transplanted into the field during the summer of 1964. Some of the potential hybrid seed did not germinate, some seedlings did not survive. The hybrid plants which were transplanted to the field were cloned in order to insure survival of the hybrid strains. Some of the established plants may be offspring from self-fertilized seed rather than from cross-pollination.

III. Study of four induced intrasectional hybrids in the genus Agropyron within section Goulardia.

1. A. subsecundum (plant 65-1) x A. latiglume (plant 47-3)

a. Comparative morphology

Typical spikelets and florets of A. subsecundum, A. latiglume, and their hybrid are shown in Figure 1. The average and range of measurements obtained from 10 plants each of A. subsecundum, A. latiglume, and their hybrid were scored for 11 characters and compared in Table 5.

Table 4. Interspecific hybridization with preceding emasculation during the summers of 1962 and 1963.

Female parent		Male parent		Florets emas- culated	No. of seeds	% seed set	No. hybrid plants
Species	MSU acces- sion no.	Species	MSU acces- sion no.				
<u>A. arizonicum</u>	2-5	<u>A. caninum</u>	6-4	30	3	10	3
<u>A. brachyphyllum</u>	3-6	<u>A. caninum</u>	6-1	10	0	0	0
"	3-4	<u>A. ciliatiflorum</u>	9-8	20	1	5	0
"	3-9	<u>A. dasystachyum</u>	21-2	18	3	16.7	0
"	3-3	<u>A. latiglume</u>	47-7	20	0	0	0
"	3-1	<u>A. latiglume</u>	48-2	20	0	0	0
"	3-1	<u>A. riparium</u>	62-2	12	3	25	3
"	3-6	<u>A. scabriglume</u>	1-1	50	0	0	0
"	3-1	<u>A. trachycaulum</u>	98-2	30	6	20	3
<u>A. caninum</u>	6-5	<u>A. ciliatiflorum</u>	9-1	45	0	0	0
"	6-3	<u>A. latiglume</u>	48-2	100	5	5	4
"	6-5	<u>A. riparium</u>	62a-4	25	11	44	3
"	6-2	<u>A. subsecundum</u>	83-8	30	0	0	0
<u>A. latiglume</u>	48-2	<u>A. dasystachyum</u>	18-10	22	6	27.2	0
"	48-2	<u>A. dasystachyum</u>	20-2	30	0	0	0
"	48-6	<u>A. riparium</u>	62-2	36	0	0	0
<u>A. semicostatum</u>	65-6	<u>A. caninum</u>	6-2	30	0	0	0
"	64-5	<u>A. ciliatiflorum</u>	9-1	20	2	10	0
"	65-6	<u>A. latiglume</u>	47-1	40	4	10	0
"	64-3	<u>A. latiglume</u>	47-3	20	0	0	0

Table 4 (Continued).

Female parent		Male parent		Florets emas- culated	No. of seeds	% seed set	No. hybrid plants
Species	MSU acces- sion no.	Species	MSU acces- sion no.				
<u>A. semicostatum</u>	64-3	<u>A. subsecundum</u>	83a-2	20	0	0	0
"	64-3	<u>A. trachycaulum</u>	89-6	24	0	0	0
"	64-3	<u>A. trachycaulum</u>	95-3	36	0	0	0
"	64-3	<u>A. trachycaulum</u>	98-3	20	0	0	0
"	64-3	<u>A. trachycaulum</u>	99-1	20	0	0	0
"	65-4	<u>A. trachycaulum</u>	93-2	20	3	15	2
"	65-4	<u>A. trachycaulum</u>	99-3	50	1	2	0
<u>A. subsecundum</u>	65-1	<u>A. latiglume</u>	47-3	20	2	1	1
<u>Totals</u>				818	50	6	19

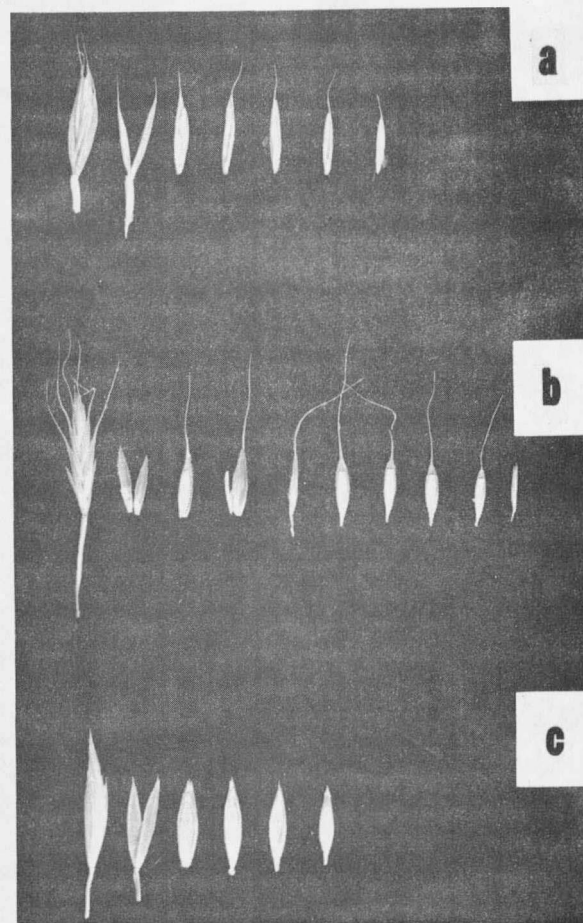


Fig. 1 Spikelets and florets of
a: A. subsecundum
b: A. subsecundum x A. latiglume
c: A. latiglume
Mag. 1X

Table 5. Morphological measurements of A. subsecundum, A. latiglume, and their hybrid (mean and range in cm.).

	<u>A. subsecundum</u>	hybrid	<u>A. latiglume</u>
Spike length	10.9 (5.3-13.5)	10.2 (6.8-14.2)	10.4 (7.8-15.2)
Internode length	0.3 (0.3- 0.4)	0.8 (0.7- 1.2)	0.4 (0.3- 0.5)
Spikelet length	1.3 (1.1- 1.6)	1.3 (1.0- 1.6)	1.5 (1.0- 2.0)
Number of spikelets per spike	22.4 (11.0-34.0)	11.5 (10.0-13.0)	19.4 (14.0-29.0)
Number of florets per spikelet	5.1 (4.0- 6.0)	6.9 (6.0- 8.0)	6.1 (5.0- 8.0)
Glume length	0.8 (0.8- 1.1)	0.5 (0.5- 0.6)	1.0 (0.8- 1.3)
Glume awn length	0.2 (0.1- 0.4)	tipped	0.1 (0.0- 0.2)
Lemma length	0.9 (0.8- 1.0)	0.8 (0.7- 1.1)	1.0 (0.8- 1.1)
Lemma awn length	0.4 (0.3- 0.5)	1.2 (0.9- 1.6)	0.1 (0.1- 1.5)
Palea length	0.8 (0.6- 0.9)	0.5 (0.4- 0.6)	0.9 (0.8- 1.1)
Anther length	0.1 (0.1)	0.1 (0.1)	0.1 (0.1)

The mean measurements of A. subsecundum were higher than those of A. latiglume for spike length, number of spikelets per spike, glume awn and lemma awn length, and anther length. A. latiglume exceeded in internode length, spikelet length, number of florets per spikelet, glume, and palea length. The lemma was slightly shorter in A. subsecundum.

The hybrid was generally intermediate between the parents in spike morphology. The spikes were shorter than those of either parent and the internode longer; consequently, the number of spikelets per spike was generally less than found for either parent. The hybrid had an awn-tipped glume, shorter than the awn of either parent glume. The range of lemma length overlapped the range of both parents. The lemma awn length of the hybrid exceeded the range of both parents. The range of the hybrid palea length was less than that of either parent, though closest to A. subsecundum. The range of anther length overlapped that of both parents.

b. Comparative cytology

A. subsecundum (plant 65-1) had a diploid chromosome number of 28. In diakinesis 0.6 percent of the cells examined contained two univalents. The other diakinesis cells showed normal bivalent pairing. Three percent of the metaphase I cells contained univalents. No irregularities were observed in the later meiotic stages (Table 3).

A. latiglume (plant 47-3) was a tetraploid plant. In diakinesis six percent of the cells contained univalents and another six percent contained quadrivalents (Table 3). In metaphase I, 20 percent contained univalents and another 10 percent contained quadrivalents (Table 3). Six percent of

the cells contained laggards at anaphase I (Table 3). No irregularities were observed in stages of the second meiotic division (Table 3).

The chromosome number of the hybrid A. subsecundum x A. latiglume was 28. The observed chromosome associations and their frequency in diplotene, diakinesis, and metaphase I are summarized in Table 6.

Chromosome associations were interpreted in ten cells of diplotene. Five different types of chromosome association were observed in this stage (Table 6). The diplotene cell shown in Figure 2a contains twelve bivalents and one quadrivalent. This particular association was found in fifty percent of ten cells in diplotene and fifty percent of the 72 cells examined in diakinesis (Table 6). Fourteen bivalents were observed in 37.5 percent of the cells in diakinesis. Bivalents averaged 12.66 per cell with a range from eight to fourteen. Univalents averaged 0.03 per cell, with a range from zero to four. Fifty-eight percent of the cells contained one or two quadrivalents with an average of 0.59 per cell. As in diplotene, no other multivalent associations were found. Eighty-one percent of the bivalents were closed.

In metaphase I, 54 percent of the 33 cells observed contained twelve bivalents and one quadrivalent; 30 percent contained fourteen bivalents, or two univalents, eleven bivalents, and one quadrivalent. The cell shown in Figure 2b shows fourteen bivalents in metaphase I. The frequency of open bivalents increased from 19 percent in diakinesis to 20 percent in metaphase I. No cell contained more than four univalents. Open quadrivalents were half as frequent in metaphase I as in diakinesis. The average frequency per cell was 0.66 univalents, 12.15 bivalents and 0.75

Table 6. Chromosome associations in diplotene, diakinesis, and metaphase I of A. subsecundum x A. latiglume.

I	II	IV	Number of cells		
			diplotene	diakinesis	metaphase I
	12	1	5	36	18
	14		1	27	7
2	11	1	2	5	3
2	13			2	2
4	8	2			2
4	12			1	1
2	9	2		1	
	10	2	1		
6	9	1	1		
Total			10	72	33

0.03 12.66 0.59 Average association in diakinesis

0.66 12.15 0.75 Average association in metaphase I

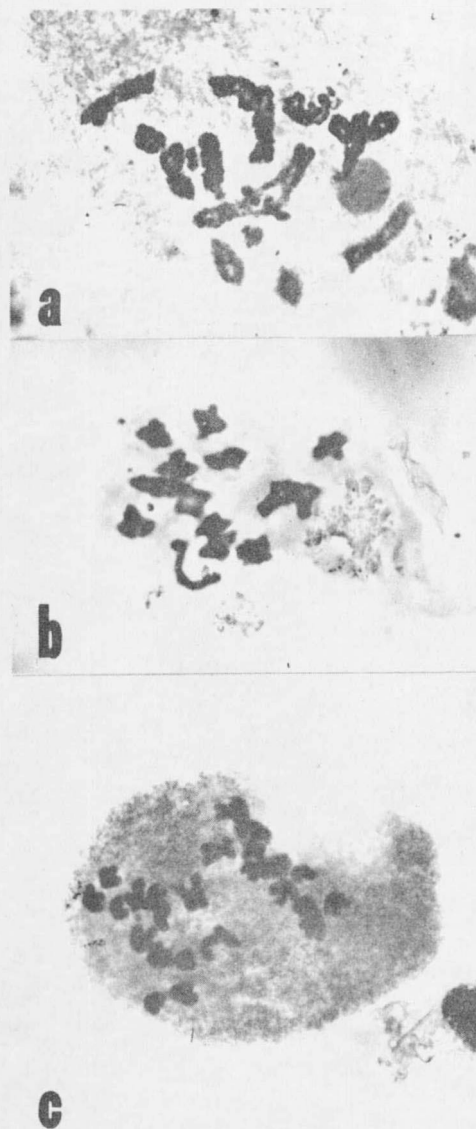


Fig. 2 Meiosis in A. subsecundum x A. latiglume
a: diplotene, 12^{II} 1^{IV}.
b: metaphase I, 14^{II}.
c: anaphase I, 3 lagging univalents.
Mag. 930X

quadrivalents. The ranges were the same as in diakinesis (Table 6).

A summary of irregularities in anaphase I and anaphase II is given in Table 7.

During anaphase I, approximately 14 percent of the 41 cells interpreted contained laggards. The laggards averaged 0.01 univalents and 0.03 bivalents per cell. In Figure 2c three lagging bivalents are seen. The 65 cells examined in anaphase II appeared completely normal. There were no laggards nor bridges (Table 7).

One hundred and ninety-four quartets were examined. Half of the cells of five quartets were shrunken and had dark cytoplasm, and one out of four cells of another five quartets were likewise shrunken and abnormal. Ninety-two percent of the cells were without irregularities.

Six hundred pollen grains were observed and were found to be empty and not stained.

2. A. caninum (plant 6-3) x A. latiglume (plant 48-2).

a. Comparative morphology

Typical spikelets and florets of A. caninum, A. latiglume, and their hybrid are shown in Figure 3. The average and range of measurements obtained from 10 plants each of A. caninum, A. latiglume, and their hybrid were scored for 11 characters and compared in Table 8.

A. caninum was a taller plant than A. latiglume. Its measurements exceeded those of A. latiglume for spike length, internode length, spikelet length, number of spikelets per spike, glume and lemma awn length, and anther length. A. latiglume exceeded A. caninum in number of florets per

Table 7. Frequency of irregularities in anaphase I and anaphase II of A. subsecundum x A. latiglume.

	Average no. of laggards per cell		Percentage of cells with laggards	Average no. bridges-fr. per cell	Total no. of cells
	I	II			
Anaphase I	0.01	0.03	14.70	0.12	41
Anaphase II	0.00	0.00	0.00	0.00	65

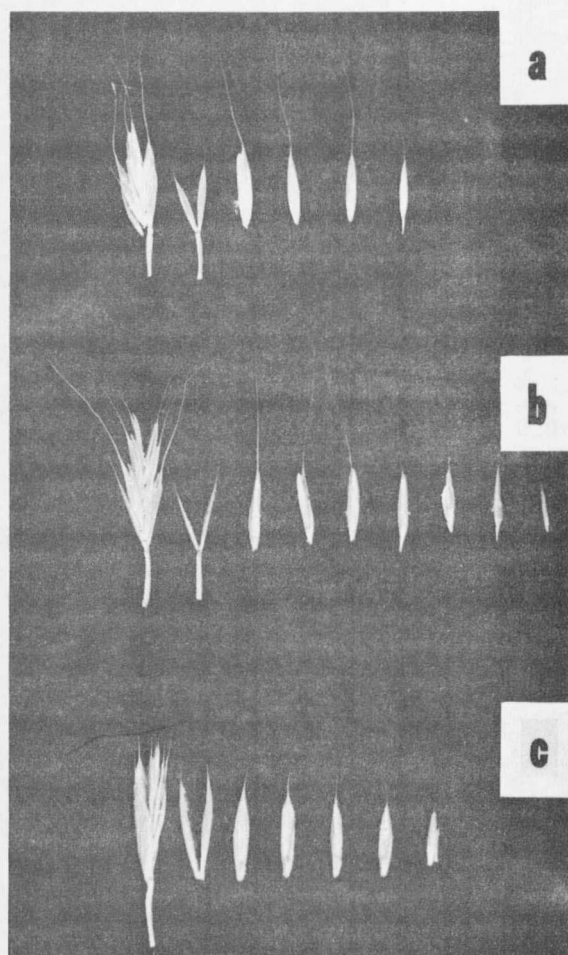


Fig. 3 Spikelets and florets of
a: A. caninum
b: A. caninum x A. latiglume
c: A. latiglume
Mag. 1X

Table 8. Morphological measurements of A. caninum, A. caninum x A. latiglume and A. latiglume (mean and range in cm.).

	<u>A. caninum</u>	hybrid	<u>A. latiglume</u>
Spike length	11.4 (8.7-14.7)	9.70 (8.4-12.5)	10.1 (9.0-12.0)
Internode length	0.6 (0.5- 0.7)	0.7 (0.5- 0.9)	0.5 (0.4- 0.6)
Spikelet length	1.5 (1.1- 1.8)	1.5 (1.2- 1.8)	1.4 (1.3- 1.5)
Number of spikelets per spike	15.7 (10.0-20.0)	12.0 (10.0-13.0)	14.1 (11.0-18.0)
Number of florets per spikelet	5.3 (5.0- 6.0)	5.9 (5.0- 7.0)	5.6 (5.0- 7.0)
Glume length	0.8 (0.7- 1.4)	0.7 (0.7- 0.9)	1.1 (0.9- 1.5)
Glume awn length	0.2 (0.1- 0.8)	0.2 (0.1- 0.4)	0.1 (0.1- 0.3)
Lemma length	1.0 (0.8- 1.2)	0.9 (0.8- 1.0)	1.1 (1.0- 1.4)
Lemma awn length	1.6 (1.1- 2.2)	1.0 (0.6- 1.7)	0.2 (0.2- 0.4)
Palea length	0.9 (0.6- 1.2)	0.9 (0.7- 1.0)	1.1 (0.9- 1.1)
Anther length	0.2 (0.2- 0.3)	0.2 (0.2- 0.3)	0.2 (0.2)

spikelét, and glume, lemma, and palea length.

The hybrid A. caninum x A. latiglume was a slightly taller plant than A. latiglume, but closer in growth habit to A. latiglume than to A. caninum. As in the A. subsecundum x A. latiglume hybrid, the spike length was shorter and internode length longer than that of either parent. The result was that there were usually fewer spikelets per spike than found in either of the parent species. The hybrid was closest to A. latiglume in number of florets per spikelet, and glume awn length. It was closest to A. caninum in spikelet length, and lemma awn length. The glume and lemma lengths were less than those of either parent. The palea and anther lengths were intermediate. The hybrid did not inherit the thin lemma edge of A. latiglume.

b. Comparative cytology

Meiosis in A. caninum (plant 6-3) was normal (Table 3). Fourteen bivalents were present in metaphase I cells. No irregularities were observed in interphase I nor in stages of the second meiotic division.

In A. latiglume (plant 48-2) meiosis was also normal. Only bivalents were present in metaphase I, and no irregularities were found in later stages (Table 3).

The chromosome associations found in diakinesis and metaphase I in A. caninum x A. latiglume are compared in Table 9.

The hybrid A. caninum x A. latiglume showed good chromosome spread in diakinesis. Fourteen bivalents were present in 66.3 percent of 110 cells. The association next in frequency was 13^{II} and 2^I, in 22.7 percent

Table 9. Chromosome associations in diakinesis and metaphase I of
A. caninum x A. latiglume.

I	II	III	IV	Number of cells	
				diakinesis	metaphase I
	14			73	20
2	13			25	28
	12		1	7	
4	12			4	24
1	12	1		1	
6	11				11
10	9				7
8	10				6
5	10	1			2
9	8	1			1
14	7				1
Total				110	100
0.60	13.55	0.01	0.06	Average association in diakinesis	
3.69	12.10	0.03		Average association in metaphase I	

of the cells. The average frequency per cell was 0.06^I , 13.55^{II} , 0.01^{III} , and 0.06^{IV} . Trivalents and quadrivalents were not present together in the same cell. Of the bivalents in diakinesis, 82 percent were closed, and 18 percent were open.

Twenty-eight percent of the metaphase I cells contained thirteen bivalents and two univalents as illustrated by Figure 4a. The frequency of cells containing fourteen bivalents dropped from 66.3 percent in diakinesis to twenty percent of those in metaphase I. Figure 4b shows a metaphase I cell with 14 bivalents. No cell contained less than seven bivalents. The average frequency of closed bivalents dropped from 82 percent in diakinesis to 54 percent in metaphase I. Only one cell contained more than 10 univalents.

Table 10 lists the frequency of irregularities in anaphase I, telophase I, and anaphase II.

Sister chromatids of lagging univalents usually showed precocious division in late anaphase I. An average of 1.21 lagging univalents per cell, with a range from 0 to 18 was observed. Figure 4c shows a cell with one lagging univalent in anaphase I. Forty-five percent of the 120 cells observed were normal. There was an average of 0.10 bridges per cell (Table 10).

The frequency of lagging univalents decreased to 0.13 per cell in telophase I (Table 10).

Of the 333 cells observed in interphase I, 77 percent appeared normal. There was an average of 0.33 karyomeres per cell.

There was an average of 0.10 laggards per cell in anaphase II, with

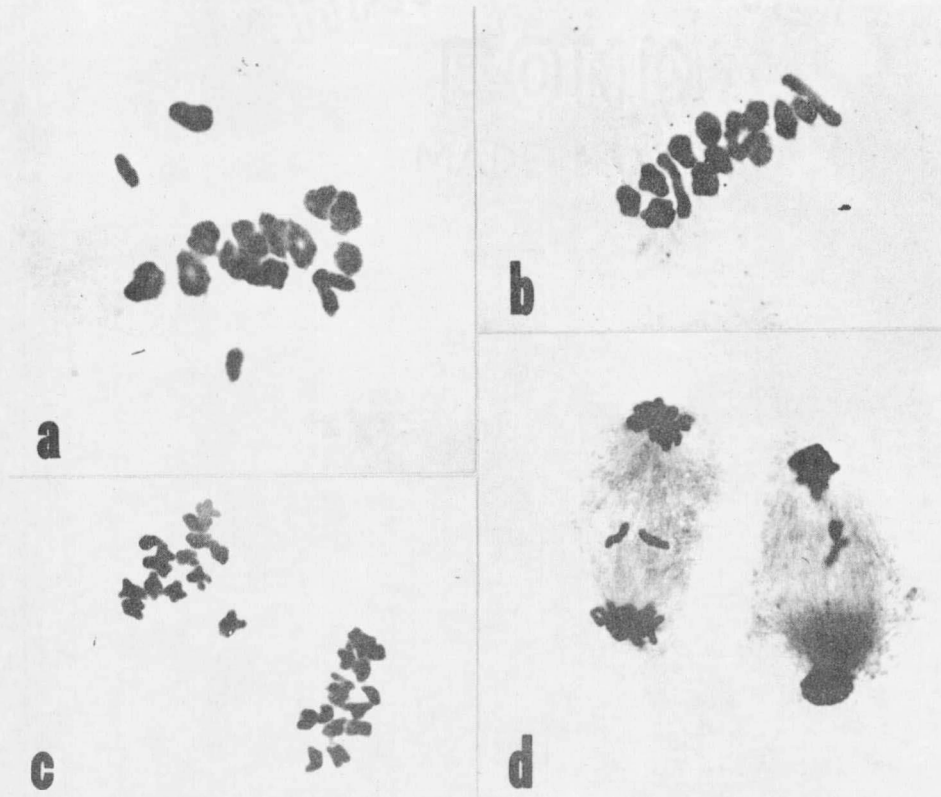


Fig. 4 Meiosis in A. caninum x A. latiglume
 a: metaphase I, 13^{II}2^I.
 b: metaphase I, 14^{II}.
 c: anaphase I, 1 lagging univalent.
 d: telophase II, 2 laggards in each cell.
 Mag. 930X

Table 10. Frequency of irregularities in anaphase I, telophase I, and anaphase II of A. caninum x A. latiglume.

	Average no. of laggards per cell		Percentage of normal cells	Average no. bridges-fr. per cell	Total no. of cells
	I	II			
Anaphase I	1.21	0.06	45	0.10	120
Telophase I	0.13		78	0.00	148
Anaphase II	0.10		89	0.03	28

an average of 0.03 bridges per cell. Eighty-nine percent of the anaphase II cells were normal. Only two cells were observed in telophase II, each cell contained two laggards. These cells are shown in Figure 4d.

There was a range from 1 to 13 micronuclei present in 35 percent of the quartets observed. Figure 5a shows four quartet cells with 0, 2, 4, and 5 micronuclei and karyomeres. The most frequent count was one micronucleus per quartet. Sixty-five percent of the quartets appeared to have four normal cells.

Mitosis was observed in 72 pollen cells at early metaphase. Of these, only seven, or nine percent contained the normal haploid number of 14 chromosomes. The others had a chromosome count ranging from 9 to 25 per pollen grain, often with fragments and micronuclei as well. Figure 5b shows 13 chromosomes in a pollen grain. The maturation of the pollen grains was abnormal, as mitosis took place before the formation of the exine and entine.

3. A. arizonicum (plant 2-5) x A. caninum (plant 6-4)

a. Comparative morphology

Typical spikelets and florets of A. arizonicum, A. caninum, and their hybrid are shown in Figure 6. The average and range of measurements obtained from 10 spikes each of A. arizonicum, A. caninum, and their hybrid, were scored for 11 characters and compared in Table 11.

A. arizonicum exceeded the morphological measurements of A. caninum in spike length, internode length, number of spikelets per spike, number of florets per spike, glume and glume awn length, lemma and lemma awn

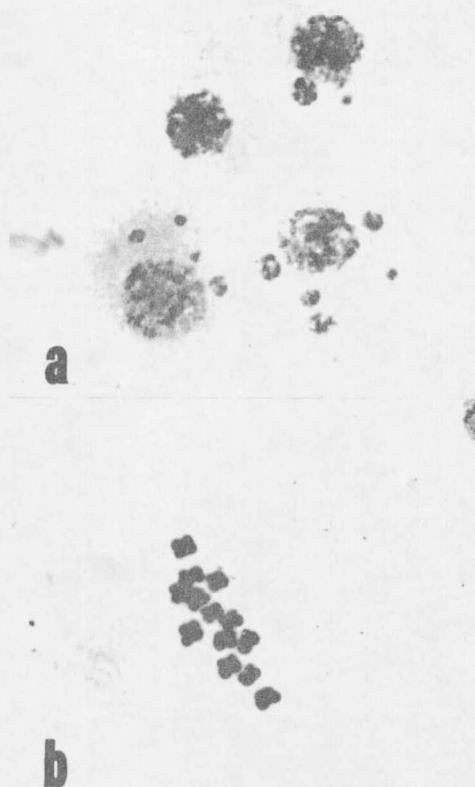


Fig. 5 Meiosis in A. caninum x A. latiglume
a: quartet with 0,2,4,5, karyomeres and micronuclei.
b: pollen mitosis, $n = 13$.
Mag. 930X

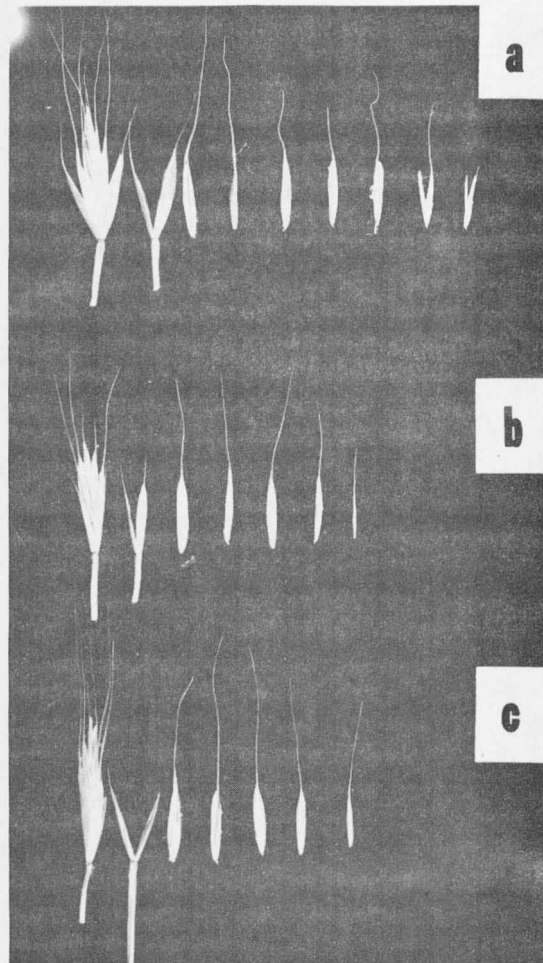


Fig. 6 Spikelets and florets of
a: A. arizonicum
b: A. arizonicum x A. caninum
c: A. caninum
Mag. 1X

Table 11. Morphological measurements of A. arizonicum, A. arizonicum x A. caninum and A. caninum (mean and range in cm.).

	<u>A. arizonicum</u>	hybrid	<u>A. caninum</u>
Spike length	14.7 (13.0-17.0)	11.3 (11.0-12.0)	11.9 (10.2-14.7)
Internode length	0.7 (0.6- 0.9)	0.5 (0.5- 0.7)	0.5 (0.5- 0.6)
Spikelet length	1.6 (1.4- 1.8)	1.4 (1.4- 1.5)	1.4 (1.1- 1.8)
Number of spikelets per spike	17.4 (14.0-23.0)	16.0 (15.0-17.0)	17.1 (13.0-20.0)
Number of florets per spikelet	6.8 (6.0- 8.0)	5.0 (5.0)	5.1 (4.0- 6.0)
Glume length	1.1 (0.9- 1.2)	0.7 (0.7- 0.9)	0.8 (0.7- 1.0)
Glume awn length	0.7 (0.5- 1.0)	0.2 (0.2- 0.4)	0.2 (0.1-0.5)
Lemma length	1.1 (1.0- 1.2)	1.0 (0.9- 1.1)	1.0 (0.9- 1.1)
Lemma awn length	1.9 (1.4- 2.3)	1.0 (0.9- 1.4)	1.5 (0.9- 2.0)
Palea length	0.9 (0.9- 1.0)	0.9 (0.8- 1.0)	0.9 (0.9- 1.0)
Anther length	0.2 (0.2)	0.2 (0.2)	0.2 (0.2- 0.3)

length, and palea length.

Morphological measurements of the hybrid showed smaller range and lower mean than either parent in spike length, number of spikelets per spike, glume length, lemma awn length, and palea length. The hybrid was closest to A. caninum in internode length, spikelet length, number of florets per spikelet, glume awn length, and lemma length.

b. Comparative cytology

A. arizonicum, (plant 2-5), was a tetraploid plant with a chromosome number of 28. There was normal bivalent pairing at diakinesis and metaphase I (Table 3). In anaphase I four percent of the cells contained bridges and eight percent contained laggards. Generally there was a low frequency of irregularities.

A. caninum, (plant 6-4), showed normal bivalent pairing in metaphase I resulting in 14 bivalents. No irregularities were observed in later meiotic stages (Table 3).

The chromosome number of the hybrid A. arizonicum x A. caninum was 28. The observed chromosome associations and their frequency in metaphase I are summarized in Table 12.

In the hybrid A. arizonicum x A. caninum 33 different chromosome associations were found in 64 cells interpreted in metaphase I. Fifty-one percent of the cells were included in eight most frequent associations. The three most frequent were four univalents and 12 bivalents; five univalents, ten bivalents, and one trivalent; and two univalents, eleven bivalents, and one quadrivalent. Figure 7a shows a metaphase I cell containing

Table 12. Chromosome associations in metaphase I of A. arizonicum x A. caninum.

Number of cells	I	II	III	IV	V	VII
1		14				
2	2	13				
3		12		1		
1	1	12	1			
6	4	12				
1		11	2			
1	1	11			1	
5	2	11		1		
3	3	11	1			
2	6	11				
1		10		2		
1	1	10				1
2	1	10	1	1		
2	2	10	2			
2	3	10			1	
2	4	10		1		
6	5	10	1			
4	8	10				
1	2	9		2		
1	3	9	1	1		
1	4	9	2			
1	5	9			1	

Table 12 (Continued).

Number of cells	I	II	III	IV	V	VII
3	6	9		1		
3	7	9	1			
1	10	9				
1		8		3		
1	4	8		2		
1	4	8	1		1	
1	9	8	1			
1	3	7	1	2		
1	6	7		2		
1	11	7	1			
1	11	4	3			
Total	64					
Average	3.98	10.13	0.48	0.45	0.08	0.015

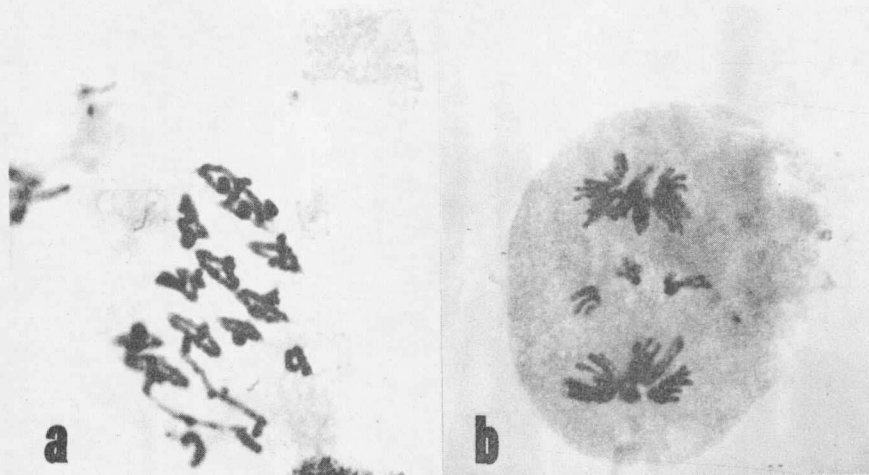


Fig. 7 Meiosis in A. arizonicum x A. caninum
 a: metaphase I, 11112111V
 b: anaphase II, 3 lagging univalents
 Mag. 930X

two univalents, nine bivalents and one quadrivalent. All multivalents and 56 percent of the bivalents were open. One fourth of the cells contained only 10 to 14 bivalents and 0 to 8 univalents. The others contained multivalents in addition to bivalents and univalents. There was an average of 3.98 univalents, 10.13 bivalents, 0.48 trivalents, 0.45 quadrivalents, 0.08 pentavalents and 0.01 hexavalents per cell (Table 12).

A summary of irregularities in anaphase I and II is given in Table 13.

During anaphase I, the lagging univalents remained on the plate and underwent precocious division. There was an average of 2.06 lagging univalents. In Figure 7b three lagging univalents can be observed. Almost 90 percent of the cells contained at least one univalent with a range from 0 to 5. There was an average of 0.18 bridges per cell.

In 26 cells at interphase I, there was an average of 1.57 micronuclei and karyomeres per cell.

There was an average of 3.6 laggards per cell during anaphase II. Bridges were more frequent than in anaphase I. There was an average of 0.44 bridges per cell. Only 16 percent of the cells appeared normal.

Micronuclei in the quartets were of varied sizes. Apparently laggards congregated to form restitution nuclei rather than forming karyomeres. There was an average of 3.60 per quartet. Only 21 percent appeared normal.

Six hundred pollen grains were counted. All were empty and not stained.

Table 13. Frequency of irregularities in anaphase I and II of A. arizonicum
x A. caninum.

	Average no. laggards per cell		Percentage of cells with laggards	Average no. bridges+fr. per cell	Total no. of cells
	I	II			
Anaphase I	2.06	0.11	89.90	0.18	68
Anaphase II	3.60		84.00	0.44	25

4. A. semicostatum (plant 65-4) x A. trachycaulum (plant 93-2)

a. Comparative morphology

Typical spikelets and florets of A. semicostatum, A. trachycaulum and their hybrid are shown in Figure 8. The average and range of measurements scored for eleven characters from ten spikes each of A. semicostatum, A. trachycaulum, and their hybrid, are compared in Table 14.

The morphological measurements for A. trachycaulum exceeded those for A. semicostatum in spike length, number of spikelets per spike, glume length, glume awn length, lemma, palea, and anther length. The spikelet length was the same in the parents and hybrid.

The hybrid plant exceeded both parent plants only in spike internode length and glume awn length. Its spike length and the number of spikelets per spike were less than that of either parent species. The hybrid was intermediate in number of florets per spikelet, glume, lemma, lemma awn, palea, and anther length.

b. Comparative cytology

A. semicostatum (plant 65-4) and A. trachycaulum (plant 93-2) were both tetraploids. Thirty-three percent of the metaphase I cells of A. semicostatum contained univalents (Table 3), while 7.7 percent of the cells in anaphase I contained lagging univalents. Cells observed in the interphase I and quartet stages appeared normal.

Diakinesis and metaphase I appeared normal in A. trachycaulum (Table 3). Seventy-five percent of the cells contained 14 closed, or one open and 13 closed bivalents in metaphase I. There were no multivalents

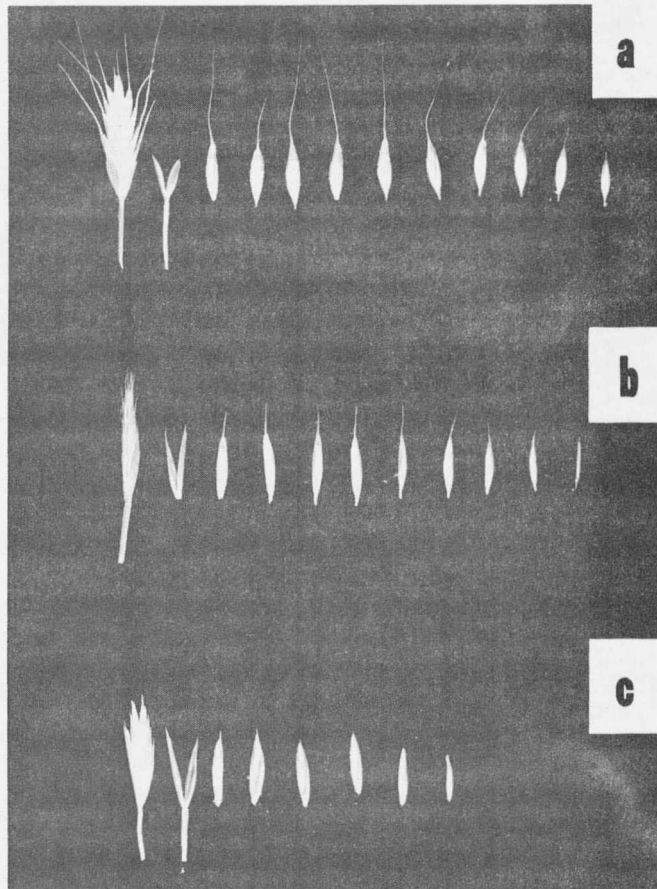


Fig. 8 Spikelets and florets of
a: A. semicostatum
b: A. semicostatum x A. trachycaulum
c: A. trachycaulum
Mag. 1X

Table 14. Comparison of morphological measurements of A. semicostatum, A. trachycaulum, and their hybrid (mean and range in cm.).

	<u>A. semicostatum</u>	hybrid	<u>A. trachycaulum</u>
Spike length	12.9 (10.5-14.5)	11.9 (9.5-15.4)	14.9 (11.5-17.0)
Internode length	0.7 (0.5- 0.9)	0.8 (0.7- 1.2)	0.6 (0.6- 0.7)
Spikelet length	1.5 (1.2- 2.0)	1.5 (1.2- 1.9)	1.5 (1.5)
Number of spikelets per spike	14.5 (13.0-16.0)	11.2 (10.0-14.0)	19.2 (15.0-23.0)
Number of florets per spikelet	10.0 (8.0-12.0)	8.1 (6.0-12.0)	7.0 (7.0)
Glume length	0.5 (0.5- 0.6)	0.8 (0.6- 1.0)	1.3 (1.0- 1.1)
Glume awn length	tipped	0.2 (0.0- 0.5)	0.1 (0.0- 0.2)
Lemma length	0.7 (0.6- 0.9)	0.8 (0.8- 1.0)	1.0 (0.9- 1.0)
Lemma awn length	1.2 (1.1- 1.5)	0.3 (0.2- 0.5)	0.1 (0.0- 0.2)
Palea length	0.5 (0.5- 0.7)	0.7 (0.6- 0.8)	0.9 (0.9- 1.0)
Anther length	0.1 (0.1)	0.1 (0.1)	0.1 (0.1- 0.2)

nor univalents observed in A. trachycaulum.

The diploid chromosome number in the hybrid A. semicostatum x A. trachycaulum was 28. Two different collections from this hybrid were studied in metaphase I. One collection was fixed in Newcomer's solution and showed fewer sticky chromosomes than that fixed in Farmer's solution. In Table 15, the observed chromosome association frequencies from the two different collections, are compared.

Chromosome associations were determined for 86 cells in metaphase I from spikes collected in Newcomer's solution from the field. Seventy-two percent of the cells contained only univalents with a range of 12 to 26 and bivalents with a range from 1 to 7. Figure 9a shows five bivalents and 18 univalents. The average chromosome association for all the cells was 18.49^{I} , 4.19^{II} , 0.33^{III} , and 0.03^{IV} . There were no pentavalents in this collection. Nineteen different chromosome associations were observed.

Chromosome associations were interpreted for 216 cells in metaphase I, in material collected in Farmer's solution from the greenhouse. Chromosome pairing again was irregular and variable. There seemed to be a degree of secondary association by univalents distant from the metaphase plate, however there was no indication that chiasmata had been formed.

Thirty-four different chromosome associations were observed in 216 cells in metaphase I. Univalents were present in all the cells, with a range from 5 to 24, and an average of 13.66 per cell. All the cells contained bivalents, with a range from 3 to 11, and an average of 6.56^{II} . Twenty-two percent of the cells contained exactly 14 univalents and 7

Table 15. Chromosome associations in metaphase I of A. semicostatum x A. trachycaulum.

Greenhouse (Farmer's solution)	Field (Newcomer's solution)	I	II	III	IV	V
Number of cells						
3		6	11			
9		8	10			
15		10	9			
29		12	8			
2		6	8	2		
1		9	8	1		
48	5	14	7			
6		11	7	1		
3		10	7		1	
2		9	7			1
1		8	7	2		
29	12	16	6			
4	2	13	6	1		
5		12	6		1	
2		11	6			1
1		10	6	2		
1		8	6		2	
1		5	6	2	1	
21	17	18	5			
2		14	5		1	
	2	12	5	2		

Table 15 (Continued).

Greenhouse (Farmer's solution)	Field (Newcomer's solution)	I	II	III	IV	V
Number of cells						
2		11	5	1	1	
1		8	5	2	1	
	10	20	4			
4	7	17	4	1		
3	1	16	4		1	
	1	13	4	1	1	
1		10	4	2	1	
1		9	4	1	2	
3	9	22	3			
3	4	19	3	1		
	1	16	3	2		
	1	12	3	2	1	
1		10	3	1	2	
1		9	3	1	3	
2	6	24	2			
	3	21	2	1		
	1	22	2			
	1	18	2	2		
1		15	2	1	2	
1		12	2	3		
	2	26	1			
	1	23	1	1		
216	86					
Average greenhouse		13.66	6.56	0.19	0.13	0.02
Average field		18.49	4.19	0.33	0.03	

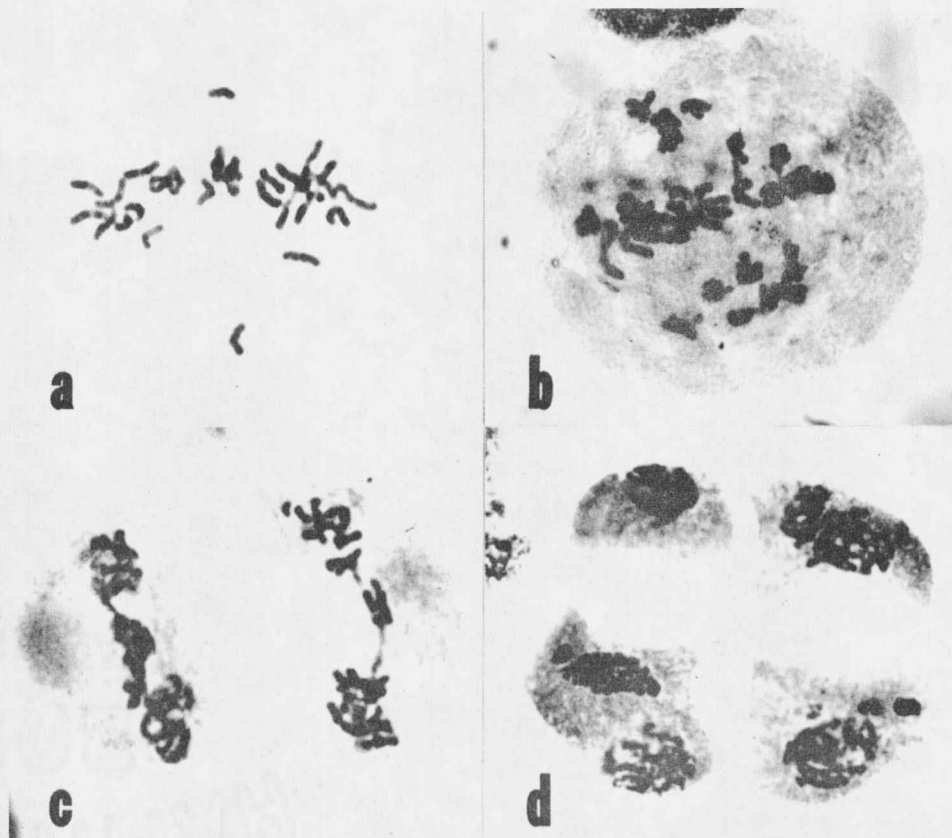


Fig. 9 Meiosis in A. semicostatum x A. trachycaulum
 a: metaphase I, 5II18I.
 b: anaphase I, approximately 14 univalents.
 c: telophase II, chromosome aggregations formed.
 d: quartet with 1,5,2,3 micronuclei and karyomeres.
 Mag. 930X

bivalents. There was an average of 0.19 ^{III}, 0.13 ^{IV} and 0.02 ^V.

One pentavalent occurred in each of 4 cells. Some of the chromosomes appeared abnormally attenuated in late metaphase.

Post-metaphase meiotic stages were investigated in the material collected in Farmer's solution from the greenhouse. Only four of the 85 anaphase I cells examined showed normal chromosome movement. The other 95.3 percent contained lagging univalents which underwent precocious division. There was an average of 8.53 lagging univalents per cell. Figure 9b shows a cell in early anaphase I, with approximately 14 lagging univalents.

Only two of the cells were normal in anaphase II. The other 97 percent contained lagging univalents, bridges, fragments, micronuclei and karyomeres. There was an average of 16.1 lagging chromosomes per cell (Table 16).

During telophase II the lagging chromosomes did not form separate karyomeres, nor aggregate into the main nucleus. They congregated into larger, but not nuclear size aggregations. Figure 9c shows two telophase II cells with these aggregations formed.

No normal quartets were seen among the 100 cells observed. Karyomeres averaged 3.05 per quartet, with a range from 1 to 11. In addition there were non-included chromosome aggregations formed in telophase II. In Figure 9d two cells of the quartet contain one of these larger aggregations, in addition to micronuclei and karyomeres. The larger aggregations averaged 1.32 per quartet. Some of the quartet cells were shrunken. All the 600 pollen grains counted were empty and nonstaining.

Table 16. Frequency of irregularities in anaphase I and II of A. semi-costatum x A. trachycaulum.

	Average no. laggards per cell	Average no. bridges+fr. per cell	Percentage of normal cells	Total no. of cells
Anaphase I	8.53	0.14	4.7	85
Anaphase II	16.10	0.36	3.2	64

IV. Study of two induced intersectional hybrids in the genus Agropyron involving sections Goulardia and Holopyron.

1. A. caninum (plant 6-5) x A. riparium (plant 62a-4)

a. Comparative morphology

Typical spikelets and florets of A. caninum, a species belonging to section Goulardia; A. riparium, a species belonging to section Holopyron; and their intersectional hybrid, are shown in Figure 10. The average and range of measurements scored for eleven characters from ten spikes each of A. caninum, A. riparium, and their hybrid are compared in Table 17.

The morphological measurements of A. caninum generally exceeded those of A. riparium in spikelet length, glume, glume awn, lemma, lemma awn, and palea length. A. riparium exceeded in internode length, number of florets per spikelet, and anther length.

The hybrid A. caninum x A. riparium was intermediate in internode length, anther length, spikelet length, number of florets per spikelet, glume awn length, lemma length, and palea length. The spike length and number of spikelets per spike were less than shown by either parent.

b. Comparative cytology

A. caninum (plant 6-5) was a tetraploid species. Only bivalents were observed in diakinesis and metaphase I (Table 3). Fourteen percent of the anaphase I cells observed contained laggards. Telophase I and interphase I appeared normal. Twenty-five percent of the anaphase II cells contained laggards, and six percent of the quartet cells

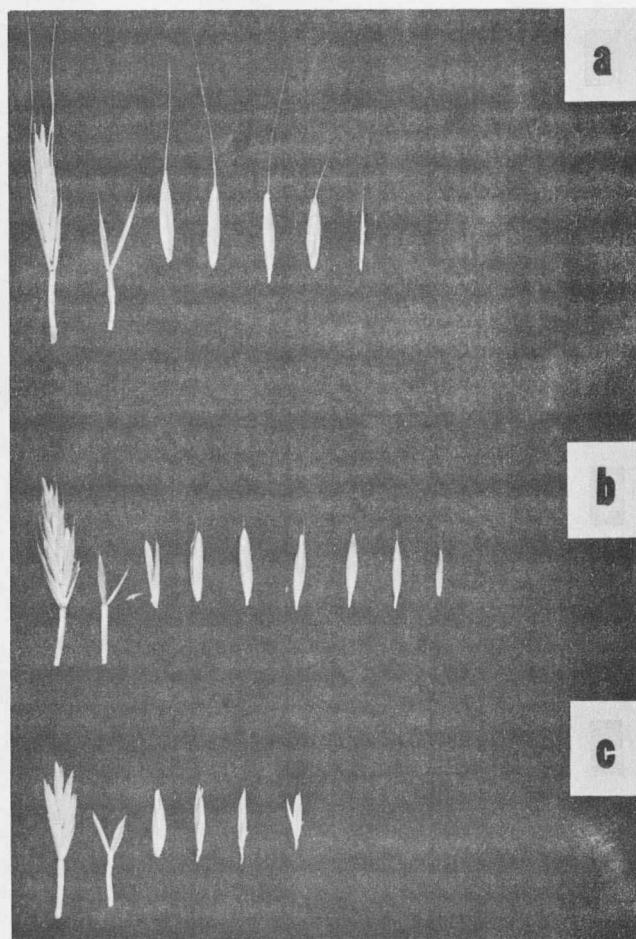


Fig. 10 Spikelets and florets of
 a: A. caninum
 b: A. caninum x A. riparium
 c: A. riparium
 Mag. 1X

Table 17. Morphological measurements of A. caninum, A. caninum x A. riparium and A. riparium (mean and range in cm.).

	<u>A. caninum</u>	hybrid	<u>A. riparium</u>
Spike length	10.7 (8.0-14.0)	10.2 (8.0-12.5)	11.0 (10.0-12.2)
Internode length	0.6 (0.5- 0.7)	0.6 (0.5- 0.7)	0.6 (0.5- 0.8)
Spikelet length	1.6 (1.4- 2.1)	1.6 (1.4- 1.8)	1.5 (1.4- 1.7)
Number of spikelets per spike	13.4 (10.0-17.0)	13.2 (11.0-16.0)	13.6 (12.0-17.0)
Number of florets per spikelet	5.6 (5.0- 8.0)	6.9 (6.0- 8.0)	6.9 (6.0- 8.0)
Glume length	0.9 (0.7- 1.4)	0.5 (0.5- 0.7)	0.5 (0.5- 0.6)
Awn length	0.2 (0.1- 0.8)	0.1 (0.0- 0.1)	0.1 (0.0- 0.1)
Lemma length	1.0 (0.8- 1.2)	0.8 (0.8- 0.9)	0.8 (0.8- 0.9)
Lemma awn length	1.6 (1.1- 2.2)	0.1 (0.1- 0.3)	0.0 (0.0-)
Palea length	0.9 (0.6- 1.2)	0.8 (0.8- 0.9)	0.8 (0.8- 0.9)
Anther length	0.2 (0.2- 0.3)	0.3 (0.2- 0.3)	0.4 (0.4- 0.5)

contained micronuclei (Table 3).

A. riparium (plant 62a-4) was also a tetraploid species. In metaphase I ten percent of the cells contained quadrivalents, and five percent contained univalents (Table 3). At anaphase I seven percent contained laggards and one percent contained a bridge. Cells observed in telophase I and interphase I appeared normal. Two percent of the quartet cells contained micronuclei (Table 3).

Figure 11a shows a diplotene cell from the hybrid A. caninum x A. riparium. This cell contained thirteen bivalents and two univalents. Chromosome association frequencies in metaphase I of the hybrid A. caninum x A. riparium are listed in Table 18. Three hundred and twenty-seven cells were interpreted in metaphase I. Fifty-six percent of the cells contained two univalents and thirteen bivalents, four univalents and twelve bivalents, or six univalents and eleven bivalents. Eighty-one percent of the cells contained one of seven most frequent associations. There was an average of 3.26 univalents, 11.62 bivalents, 0.20 trivalents, 0.22 quadrivalents, and 0.009 pentavalents. Sixty-one percent of the bivalents were closed, and 39 percent open. Seventeen percent of the cells contained trivalents and 21 percent contained quadrivalents. A metaphase I cell containing 12 bivalents and one quadrivalent is shown in Figure 11b. Only four out of 327 cells contained both a trivalent and a quadrivalent.

Seventy-three percent of 249 cells in anaphase I contained laggards. There was an average of 1.46 lagging univalents and 0.06 lagging bivalents with 0.21 bridges per anaphase I cell (Table 19). Figure 11c shows an

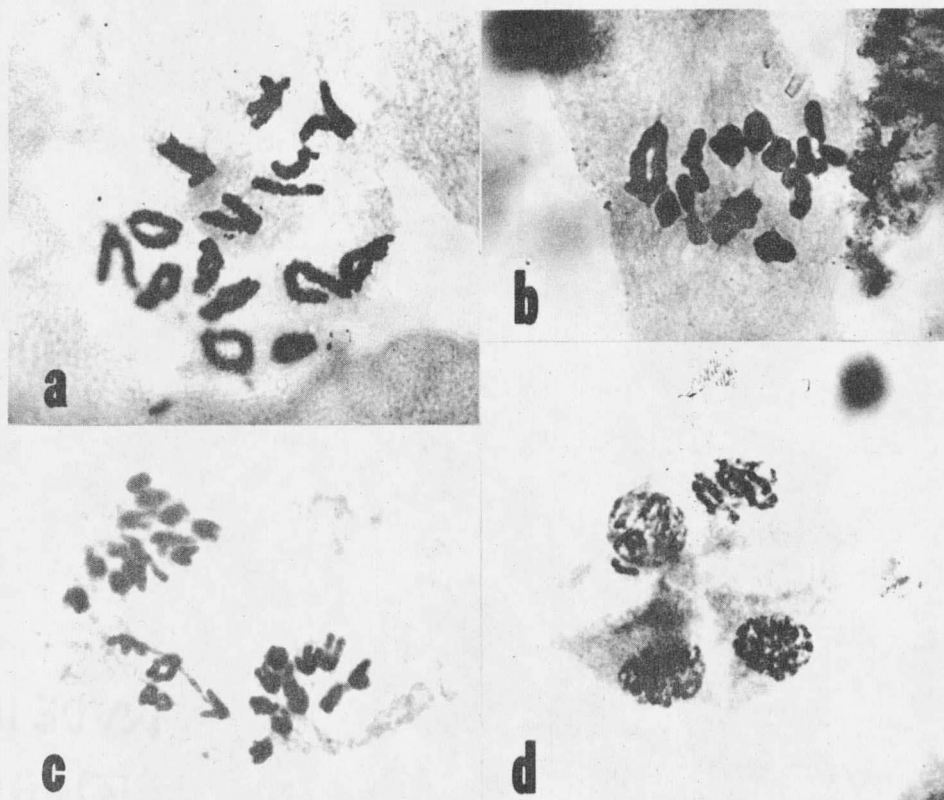


Fig. 11 Meiosis in A. caninum x A. riparium
 a: diplotene, 13II₂I.
 b: metaphase I, 12II₄IV.
 c: anaphase I, bridge and 2 lagging univalents.
 d: quartet with 0,0,1,2, karyomeres.
 Mag. 930X

Table 18. Chromosome associations in metaphase I of A. caninum x A. riparium.

Number of cells	I	II	III	IV	V
19		14			
77	2	13			
66	4	12			
10		12		1	
9	1	12	1		
34	6	11			
28	2	11		1	
22	3	11	1		
2		11	2		
1	1	11			1
22	4	10		1	
11	5	10	1		
6	8	10			
3	2	10	2		
1	1	10	1	1	
1	3	10			1
5	7	9	1		
2	6	9		1	
1	3	9	1	1	
1	10	9			
1	2	9	1		1

Table 18.(Continued).

Number of cells	I	II	III	IV	V
2	9	8	1		
2	8	8		1	
1	6	8	2		
2	7	7	1	1	
1	8	7	2		
Total	327				
Average	3.26	11.62	0.20	0.22	0.009

Table 19. Frequency of irregularities in anaphase I, telophase I and II in the synthetic hybrid A. caninum x A. riparium.

	Average no. laggards per cell		Percentage with laggards		Average no. bridges+fr. per cell	Total no. of cells
	$\frac{1}{2}$ I	I	$\frac{1}{2}$ II	II		
Anaphase I		1.46		0.06	73	0.21 249
Telophase I	2.80		0.13		74	0.07 46
Telphase II	1.97				78	0.05 84

anaphase I cell containing a bridge and two lagging univalents.

Seventy-four percent of the 46 cells in telophase I contained laggards with an average of 2.80 lagging univalents per cell, and 0.13 lagging bivalents. The average number of bridges persisting into telophase I was 0.07 per cell (Table 19).

Seventy-eight percent of the 84 cells in telophase II contained laggards. There was an average of 1.97 laggards and 0.05 bridges per cell (Table 19).

Sixty percent of 272 quartets contained karyomeres with an average of 1.17 karyomeres per quartet. Figure 11d shows a quartet with two normal cells, one cell with one karyomere, and one cell with two karyomeres.

Five hundred and ninety-nine out of 600 pollen grains were empty and nonstaining.

2. A. brachyphyllum (plant 3-1) x A. riparium (plant 62-2)

a. Comparative morphology

Typical spikelets and florets of A. brachyphyllum, a species belonging to section Goulardia; A. riparium, a species belonging to section Holopyron; and their intersectional hybrid, are shown in Figure 12. The average and range of measurements scored for eleven characters from ten spikes each of A. brachyphyllum, A. riparium and their hybrid are compared in Table 20.

The mean and range of morphological measurements for A. brachyphyllum were higher than those for A. riparium in, spike length, internode length, spikelet length, glume, lemma, lemma awn length, and palea length.

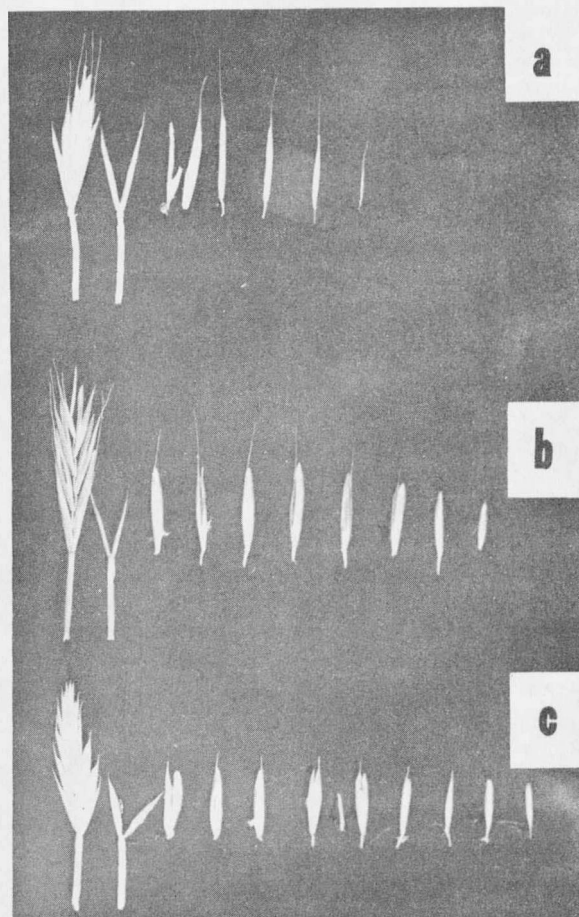


Fig. 12 Spikelets and florets of
 a: A. brachyphyllum
 b: A. brachyphyllum x A. riparium
 c: A. riparium
 Mag. 1X

Table 20. Comparison of morphological spike measurements of A. brachyphyllum (3-1) A. riparium (62-2) and their hybrid (mean and range in cm.).

	<u>A. brachyphyllum</u>	hybrid	<u>A. riparium</u>
Spike length	12.1 (9.5-14.7)	19.0 (18.5-19.5)	9.4 (7.3-12.0)
Internode length	0.1 (0.9- 1.1)	1.0 (1.0)	0.5 (0.5- 0.7)
Spikelet length	1.8 (1.5- 2.0)	2.0 (2.0- 2.1)	1.4 (1.2- 1.8)
Number of spikelets per spike	10.2 (8.0-12.0)	16.3 (16.0-17.0)	12.4 (10.0-14.0)
Number of florets per spikelet	5.6 (4.0- 6.0)	6.5 (6.0- 7.0)	6.9 (5.0- 9.0)
Glume length	1.1 (1.0- 1.2)	0.8 (0.8- 0.9)	0.6 (0.5- 0.7)
Glume awn length	0.1 (0.0- 0.2)	0.0 (0.0- 0.1)	0.1 (0.0- 0.1)
Lemma length	1.2 (1.1- 1.4)	1.2 (1.1- 1.4)	0.8 (0.7- 0.9)
Lemma awn length	0.3 (0.3- 0.5)	0.4 (0.3- 0.5)	0.1 (0.0- 0.1)
Palea length	1.2 (1.2- 1.4)	1.1 (1.1- 1.2)	0.7 (0.6- 0.8)
Anther length	0.4 (0.4)	0.3 (0.3- 0.4)	0.4 (0.4- 0.5)

A. riparium exceeded in number of spikelets per spike and glume awn length.

The hybrid received three genomes from A. brachyphyllum and two from A. riparium. Its morphological measurements were higher than those for either parent in spike length, spikelet length, and number of spikelets per spike. The glume awn was shorter than that of either parent. The hybrid was intermediate in internode length, lemma length, lemma awn length, number of florets per spikelet, glume, and palea length.

b. Comparative cytology

The diploid chromosome number of A. brachyphyllum (plant 3-1) was 42. Of the quartets observed, 1.4 percent contained micronuclei (Table 3).

No irregularities were observed in diakinesis, metaphase I and anaphase I of A. riparium, (plant 62-2). The diploid chromosome number was 28.

In the hybrid A. brachyphyllum x A. riparium, 76 cells were interpreted in metaphase I. The frequency of the associations observed is shown in Table 21. Sixty-four percent of the cells contained some combination of univalents, bivalents, and trivalents. The two most frequent associations were 6^I , 10^{II} , 3^{III} , or 9^I , 10^{II} , and 2^{III} . The average chromosome associations per cell were 7.13 univalents, 10.28 bivalents, 1.99 trivalents, 0.22 quadrivalents, 0.02 pentavalents and 0.04 hexavalents. Sticky prophase chromosomes with multivalent synapsis is shown in Figure 13a. Figure 13b shows a metaphase I cell containing 10 univalents, 11 bivalents, and one trivalent. Figure 13c shows six lagging univalents in

Table 21. Chromosome associations in metaphase I of A. brachyphyllum x A. riparium.

Number of cells	I	II	III	IV	V	VI
1	3	14		1		
2	7	14				
2	3	13	2			
2	6	13	1			
1	9	13				
1	2	12		1	1	
4	5	12	2			
3	8	12	1			
1	1	11	4			
4	4	11	3			
5	7	11	2			
1	9	11		1		
3	10	11	1			
1	2	10	3	1		
1	3	10	4			
1	4	10	2		1	
1	5	10	2	1		
7	6	10	3			
1	6	10	1			1
1	8	10	1	1		
1	9	10				1
7	9	10	2			
1	11	10		1		
1	12	10	1			
1	4	9	3	1		

Table 21 (Continued).

Number of cells	I	II	III	IV	V	VI
1	4	9	2			1
2	7	9	2	1		
3	8	9	3			
3	10	9	1	1		
4	11	9	2			
1	4	8	5			
1	10	8	3			
1	12	8	1	1		
1	8	7	3	1		
1	13	7	2			
1	7	6	4			
1	13	6	2	1		
1	7	4	3	2		
TOTAL	76 PMC					
AVERAGE	7.13	10.28	1.99	0.22	0.02	0.04

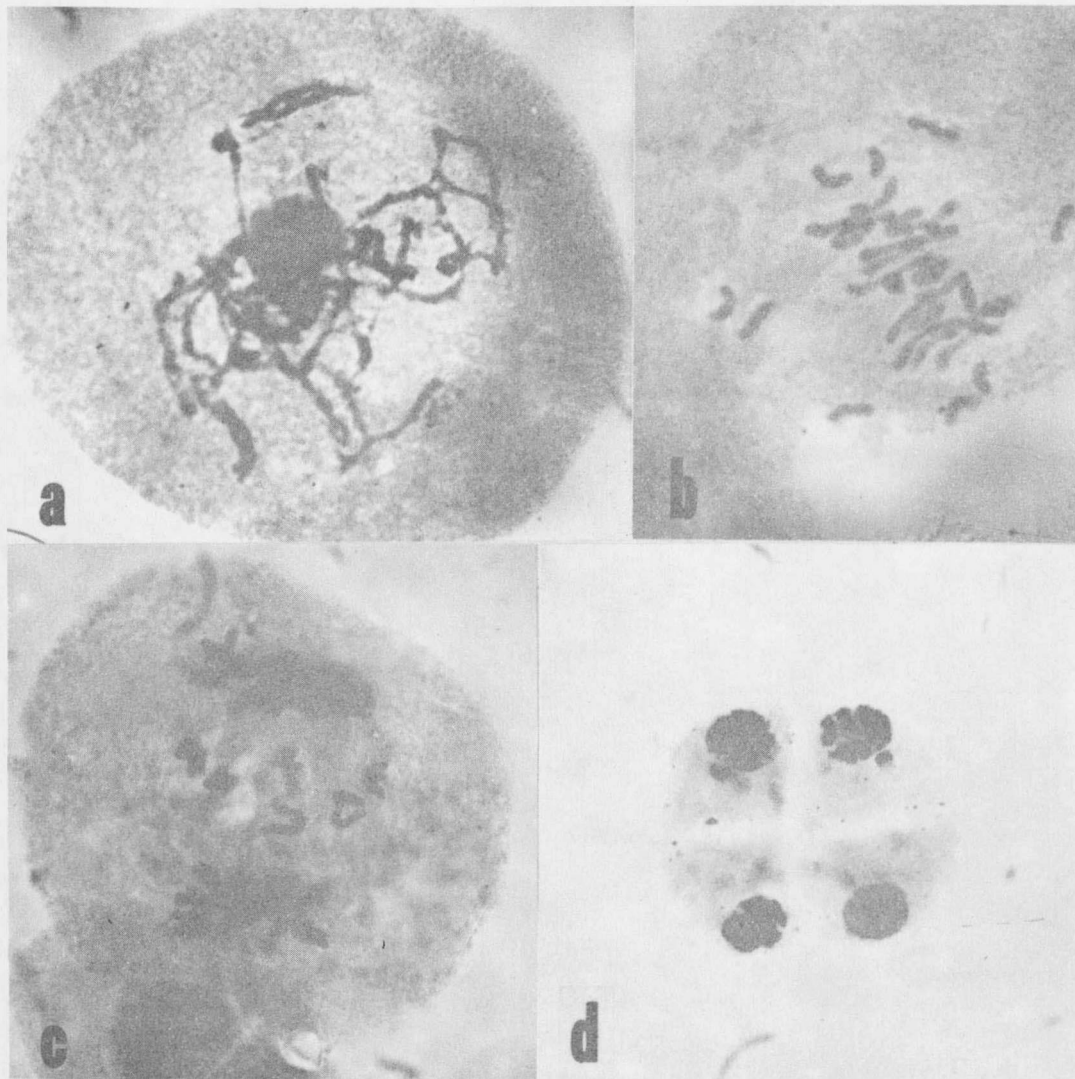


Fig. 13 Meiosis in A. brachyphyllum x A. riparium
 a: sticky chromosomes and multivalent formation.
 b: metaphase I, 10^I 11^{II} 1^{III}.
 c: anaphase I, 6 lagging univalents.
 d: quartet with 1,1,0,1 karyomeres.
 Mag. 1000X

anaphase I.

In telophase I, 91 percent of the cells contained laggards. There was an average of 5.36 lagging chromatids and 0.12 lagging univalents in 69 cells. The univalents had divided precociously during anaphase I. There was an average of 0.17 bridges per cell.

There was an average of 2.61 laggards in 13 telophase II cells. Laggards were present in 68 percent of the cells in this stage (Table 22).

Sixty percent of the quartets contained micronuclei and karyomeres. There was an average of 2.27 per quartet. Figure 13d shows a quartet, with three cells each containing a karyomere, and one normal cell.

Twelve hundred pollen grains were observed, only three were full and stained.

Table 22. Frequency of irregularities in telophase I and II of A. brachyphyllum x A. riparium.

	Average no. laggards per cell		Percent of cells with laggards	Average no. bridges+fr. per cell	Total no. of cells
	chromatids	I			
Telophase I	5.36	0.12	91	0.17	69
Telophase II		2.61	68	0.07	13

DISCUSSION

The plants investigated in meiosis are potential parents for the interspecific hybridization program being carried out at Montana State University. It is necessary to be familiar with the meiotic behavior of the parent species in order to accurately interpret the meiotic changes in the hybrid. Some of the irregularities present in the parent species may cause sterility, or reduced seed production. As the strains studied are a cross-section of the material used in the current grass breeding programs, Schulz-Schaeffer et al. (1963a) suggested that an increase in seed production could be achieved through careful plant selection. Schulz-Schaeffer et al. (1963a), after study of meiosis in a hybrid from the crested wheatgrass complex wrote "Despite close morphological resemblance of the (so called) species in this complex, important isolation mechanisms are operative resulting in different degrees of sterility in mixed populations. Unless breeding material is carefully selected on the basis of biosystematic characteristics, and unless seed is increased in isolated nurseries, no real progress in breeding will be made with this material."

This study was primarily concerned with the determination of genome distribution amongst species of the genus Agropyron, from sections Holopyron and Goulardia. The problem was approached through study of meiosis in six synthetic interspecific hybrids. Plants of A. arizonicum, A. brachyphyllum, A. caninum, A. latiglume, A. riparium, A. semicostatum, A. subsecundum and A. trachycaulum were used as parents.

In general during meiosis in hybrids between closely related species the chromosomes are synapsed to varying degrees, depending on their

homology, during prophase and metaphase I. Characteristic abnormalities of the later stages are laggards, bridges, fragments, karyomeres and micronuclei. Interspecific hybrids in the Triticineae are usually partially or completely sterile. The frequency of chromosome synapsis and chiasmata formation is generally used as an indication of the degree of genome homology. In order to interpret the chromosome associations it is necessary to determine the presence of allosyndesis, autosyndesis or a combination of both.

Stebbins and Pun (1953) concluded from cytogenetic study of interspecific and intergeneric Agropyron hybrids that a genome essentially homologous to that of A. spicatum and A. inerme is wide spread among diploid species and a component of many polyploid species of Agropyron and related genera. The spicatum genome was referred to as A_1 by Stebbins and Singh (1950) and as S by Cauderon (1958). The most reasonable designation for genomes derived from diploid A. spicatum appeared to be S. This nomenclature was used in the present study.

The spicatum genome has been determined to be present in A. caninum of Eurasia, and A. trachycaulum and Sitanion hystrix of North America through cytogenetic study of three triploid hybrids, A. caninum x A. spicatum, A. trachycaulum x A. spicatum, (Stebbins and Snyder 1956) and A. spicatum x S. hystrix (Dewey 1964a). The most common metaphase I association per cell in these hybrids was seven bivalents and seven univalents. The chromosome pairing was interpreted as allosyndetic on the basis of normal bivalent pairing in the parent species during metaphase I. Further

evidence of allosyndesis in the latter hybrid came from the almost complete absence of synapsis and chiasmata in meiosis of the hybrid Elymus cinereus x S. hystrix. The common occurrence of 28 univalents indicated that there was no autosyndesis of the S. hystrix genomes in the hybrid.

Stebbins and Vaarama (1954) identified the genome in Sitanion hystrix which was derived from A. spicatum, as A_2 , and the genome of unknown diploid origin as E_s . A. trachycaulum genomes were identified as A and B by Boyle and Holmgren (1955), the average bivalent pairing of 12.20 in A. trachycaulum x S. hystrix (Boyle 1963) indicated that A. trachycaulum and S. hystrix possess derivations of the same two genomes. The average metaphase I chromosome associations are compared for these hybrids in Table 23.

Evidently the allotetraploids A. trachycaulum and S. hystrix each contained a second genome in addition to the spicatum genome indicated by the univalents at metaphase I in A. trachycaulum x A. spicatum (Stebbins & Snyder 1956) and in A. spicatum x S. hystrix (Dewey 1964a). This second genome formed bivalents in A. trachycaulum x S. hystrix (Boyle 1963). In the present study this second genome will be referred to as B. Previously proposed genome formulae for four of the species involved in these hybrids are listed in Table 24, and correlated with S and B genome nomenclature. No hybridizations have given a definite clue to the origin of the B genome. However, cytogenetic studies have indicated that modifications of both the S and B genomes are present in the allotetraploids A. parishii, A. trachycaulum, S. hystrix, S. jubatum and Elymus glaucus (Stebbins et al. 1946a, Stebbins and Singh 1950, Stebbins and Vaarama 1954). The

Table 23. Average metaphase I chromosome associations in hybrids involving A. caninum, A. spicatum, A. trachycaulum, Sitanion hystrix and Elymus cinereus.

Hybrid	2n	Average association per cell				Author
		I	II	III	IV	
<u>A. caninum</u> x <u>A. spicatum</u>	21	7.09	5.87	0.30	0.27	Stebbins & Snyder (1956)
<u>A. trachycaulum</u> x <u>A. spicatum</u>	21	7.74	5.98	0.24	0.20	Stebbins & Snyder (1956)
<u>A. spicatum</u> x <u>S. hystrix</u>	21	7.18	6.84	0.05		Dewey (1964a)
<u>Elymus cinereus</u> x <u>S. hystrix</u>	28	26.80	0.60			Dewey & Holmgren (1962)
<u>A. trachycaulum</u> x <u>S. hystrix</u>	28	1.13	12.20		0.60	Boyle (1963)

Table 24. Genome formulae for A. spicatum, A. caninum, A. trachycaulum and Sitanion hystrix.

Species	2n	Correlated	Published	Author
		genome formula	genome formula	
<u>A. spicatum</u>	14	S ₁ S ₁	A ₁ A ₁	Stebbins & Singh (1950)
<u>A. caninum</u>	28	S ₂ S ₂ B ₂ B ₂	S S X X	Cauderon (1958)
<u>A. trachycaulum</u>	28	S ₁ S ₁ B ₁ B ₁	A A B B	Boyle & Holmgren (1955)
<u>S. hystrix</u>	28	S ₁ S ₁ B ₁ B ₁	A ₂ A ₂ E _s E _s	Stebbins & Vaarama (1954)

average metaphase I chromosome associations of the hybrids involving these species are listed in Table 25.

Genome formulae have been proposed for species involved in the hybrids compared in Table 25, and are presented in Table 26 together with correlated formulae.

Sitanion hystrix and A. trachycaulum have been identified as allotetraploids (Dewey and Holmgren 1962, Boyle 1963) possessing derived S and B genomes. After consideration of the reported metaphase I chromosome associations in A. trachycaulum x A. subsecundum (Robinson 1930) and in A. subsecundum x S. hystrix (Witte 1956), one concludes that A. subsecundum also possesses modified S and B genomes. These genomes may then be traced through the allotetraploid species A. latiglume and A. scribneri, as indicated by the figures for average metaphase I chromosome associations in the hybrids A. trachycaulum x A. scribneri (Dewey 1963) and A. scribneri x A. latiglume (Collins 1965). The metaphase associations found in these hybrids are compared in Table 27.

Dewey (1963) proposed $A_1A_1B_1B_1$ as the genome formula for A. scribneri, corresponding to $S_1S_1B_1B_1$. Having compared the metaphase I chromosome associations, Collins (1965) proposed $A_2A_2B_2B_2$ for A. latiglume which corresponds to $S_2S_2B_2B_2$. Through comparison of the average metaphase I chromosome associations in the hybrids just mentioned in Tables 23, 25, and 27, evidence accumulated showing that three of the species investigated in this study were closely related allotetraploids, each possessing derived S and B genomes. The three were A. latiglume, A. subsecundum, and A. trachycaulum.

Table 25. Average metaphase I chromosome associations in hybrids involving A. inerme, A. parishii, A. spicatum, A. trachycaulum, Elymus glaucus, Sitanion hystrix, and S. jubatum.

Hybrid	2n=	Average association per cell						Author
		I	II	III	IV	V	VI	
<u>A. inerme</u> x <u>E. glaucus</u>	21	9.30	5.60	0.14				Stebbins & Singh (1950)
<u>E. glaucus</u> x <u>S. jubatum</u>	28	0.88	13.56					Stebbins & Vaarama (1954)
<u>E. glaucus</u> x <u>S. hystrix</u>	28	1.13	13.13					Stebbins & Vaarama (1954)
<u>A. trachycaulum</u> x <u>E. glaucus</u>	28	1.55	10.40	0.33	0.40	0.04	0.48	Stebbins & Vaarama (1954)
<u>A. parishii</u> x <u>A. spicatum</u>	21	6.90	6.30	0.31				Stebbins & Singh (1950)
<u>A. parishii</u> x <u>S. jubatum</u>	28	1.55	11.81	0.15	0.59			Stebbins <u>et al.</u> (1946a)

Table 26. Genome formulae for A. inerme, A. parishii, A. spicatum, A. trachycaulum, Elymus glaucus, Sitanion hystrix, and S. jubatum.

Species	2n	Correlated genome formula	Published genome formula	Author
<u>A. inerme</u>	14	$S_1 S_1$	$A_1 A_1$	Stebbins & Singh (1950)
<u>A. spicatum</u>	14	$S_1 S_1$	$A_1 A_1$	Stebbins & Singh (1950)
<u>A. parishii</u>	28		$A_1 A_1 E_1 E_1$	Stebbins & Singh (1950)
<u>A. trachycaulum</u>	28	$S_1 S_1 B_1 B_1$	$A A B B$	Boyle & Holmgren (1955)
<u>E. glaucus</u>	28	$S_1 S_1 B_1 B_1$	$A A E_1 E_1$ $2 2 1 1$	Stebbins & Singh (1950)
<u>S. hystrix</u>	28	$S_1 S_1 B_1 B_1$	$A_2 A_2 E_s E_s$	Stebbins & Vaarama (1954)
<u>S. jubatum</u>	28	$S_1 S_1 B_1 B_1$	$A A E E$ $2 2 s s$	Stebbins & Vaarama (1954)

Table 27. Average metaphase I chromosome associations in hybrids involving A. latiglume, A. scribneri, A. subsecundum, A. trachycaulum, and Sitanion hystrix.

Hybrid	2n=	Average association per cell						Author
		I	II	III	IV	V	VI	
<u>A. subsecundum</u> x <u>S. hystrix</u>	28	1.90	12.30		0.07			Witte (1956)
<u>A. trachycaulum</u> x <u>A. subsecundum</u>	28		14*					Robinson (1930)
<u>A. trachycaulum</u> x <u>A. scribneri</u>	28	1.54	11.74	0.24	0.27	0.03	0.17	Dewey (1963)
<u>A. scribneri</u> x <u>A. latiglume</u>	28		12*					Collins (1965)

*approximately

Supporting evidence for the presence of the spicatum genome are the karyotypes published for 20 Agropyron species from sections Holopyron and Goulardia by Schulz-Schaeffer and Jurasits (1962). They found one type of indicator chromosome in all investigated species from section Goulardia, and in 10 of 15 from section Holopyron. They concluded that this F-type of satellite chromosome is the indicator for the spicatum genome. Included in their study were A. caninum, A. latiglume and A. subsecundum, which had previously been shown through hybridizations to possess the spicatum genome. They also predicted that the spicatum genome would be found in A. brachyphyllum and A. riparium. The present study bears this out.

In Table 28, the average metaphase I chromosome associations found in the hybrids in this present study, are compared.

Study of the associations from hybrids involving A. arizonicum, A. brachyphyllum, A. caninum, A. riparium, A. latiglume, A. subsecundum, and A. trachycaulum led to the conclusion that the spicatum genome is present in each of these allopolyploid species. It seemed most likely that the genome pairing in the hybrid A. semicostatum x A. trachycaulum was between the spicatum genomes, as Jurasits (1961) found the spicatum genome in hexaploid A. semicostatum. Hexaploid A. brachyphyllum was shown to possess the S and B genomes, and a third genome, as yet unidentified as to origin or distribution. Generalized genome formulae for the species studied through the hybrids listed in Table 28, are compared in Table 29.

Following will be a discussion of the hybrids:

Table 28. Average metaphase I chromosome associations in hybrids involving A. arizonicum, A. brachyphyllum, A. caninum, A. latiglume, A. riparium, A. semicostatum, A. subsecundum, and A. trachycaulum.

Hybrid			Average association per cell					
			I	II	III	IV	V	VI
<u>A. subsecundum</u>	x <u>A. latiglume</u>	28	0.66	12.15		0.75		
<u>A. caninum</u>	x <u>A. latiglume</u>	28	3.69	12.10	0.03			
<u>A. arizonicum</u>	x <u>A. caninum</u>	28	3.98	10.13	0.48	0.45	0.08	0.02
<u>A. caninum</u>	x <u>A. riparium</u>	28	3.26	11.62	0.20	0.22	0.01	
<u>A. brachyphyllum</u>	x <u>A. riparium</u>	35	7.13	10.28	1.99	0.22	0.02	0.04
<u>A. trachycaulum</u>	x <u>A. semicostatum</u> (greenhouse)	28	13.66	6.56	0.19	0.13	0.02	
<u>A. trachycaulum</u>	x <u>A. semicostatum</u> (field)	28	18.49	4.19	0.33	0.03		

Table 29. Genome formulae for A. arizonicum, A. brachyphyllum, A. caninum, A. latiglume, A. riparium, A. semicostatum, A. subsecundum, and A. trachycaulum.

Species	2n=	Correlated genome formula	Published genome formula	Author
<u>A. arizonicum</u>	28	S ₄ S ₄ B ₄ B ₄		
<u>A. brachyphyllum</u>	42	S ₅ S ₅ B ₅ B ₅ X X		
<u>A. caninum</u>	28	S ₂ S ₂ B ₂ B ₂	S S X X	Cauderon (1958)
<u>A. latiglume</u>	28	S ₂ S ₂ B ₂ B ₂	A ₂ A ₂ B ₂ B ₂	Collins (1965)
<u>A. riparium</u>	28	S ₃ S ₃ B ₃ B ₃		
<u>A. semicostatum</u>	28	S ₁ S ₁ (or B ₁ B ₁) Y Y		
<u>A. subsecundum</u>	28	S ₂ S ₂ B ₂ B ₂		
<u>A. trachycaulum</u>	28	S ₁ S ₁ B ₁ B ₁	A A B B	Boyle & Holmgren (1955)

A. subsecundum x A. latiglume

A. subsecundum and A. latiglume, both species from section Goulardia, have been shown through previous hybridizations (Table 27), to be allotetraploids, each possessing modified S and B genomes (Table 29). The data collected from A. subsecundum x A. latiglume on chromosome pairing in diplotene, diakinesis, and metaphase I, strongly emphasized the close relationship of the parent species within the "Slender Wheatgrass Complex". Dewey (1963) characterized the species of this complex as those with self-fertility, small anthers, meiosis in the very early boot and pollen shedding in the morning; and listed A. trachycaulum, A. subsecundum, A. latiglume and A. scribneri. The most frequent chromosome association in metaphase I of the hybrid A. subsecundum x A. latiglume, was 12 bivalents and one quadrivalent, indicating that there may be a reciprocal translocation. At least one quadrivalent was present in 42 out of 72 cells in diakinesis and in 23 out of 33 cells in metaphase I, averaging 0.75 quadrivalents per cell in metaphase I (Table 6). Boyle (1963) found an average of 0.60 quadrivalents per cell in the hybrid A. trachycaulum x S. hystrix. Because quadrivalents were absent in about half the cells and occurred in variable numbers when present, he postulated that these quadrivalents were less likely to be the result of a reciprocal translocation, than to be an indication that the homologies between the genomes involved were very great.

Pre-anaphase bivalent separation was noted in A. trachycaulum x A. scribneri (Dewey 1963). Bivalent desynapsis was observed in both A. subsecundum (plant 65-1) and A. latiglume (plant 47-3). In

A. subsecundum there were univalents in 0.6 percent of the diakinesis cells. A. latiglume showed univalents in 6 percent of the diakinesis cells and in 20 percent of the metaphase I cells (Table 3). These plants were the parents of the A. subsecundum x A. latiglume hybrid, whose meiotic data reported here also suggested desynapsis of bivalents. The quadrivalent frequency hardly changed from diplotene to diakinesis to metaphase I, but the frequency of cells containing 14 bivalents dropped from 37.5 percent of the cells in diakinesis to 21.21 percent of the cells in metaphase I. Li (1945) studied meiosis in Triticum aestivum. He observed genetically controlled failures of metaphase I pairing characterized by (1) quite complete zygotene and pachetene chromosome synapsis, (2) gradual separation of the chromosomes at diplotene, (3) lowered chiasmata frequency, and finally, (4) lowered bivalent frequency subject to environmental variation. He found the extent of desynapsis to increase with higher temperature.

The univalent frequency of 0.66 per cell in metaphase I of A. subsecundum x A. latiglume was extremely close to the 0.67 frequency found by Stebbins and Pun (1953) in the diploid hybrid A. inerme x A. caespitosum. They speculated that the genomes present in this hybrid, are separated only by cryptic structural hybridity.

The complete absence of trivalents in A. subsecundum x A. latiglume emphasized the fact that autosynapsis is unlikely. In the hybrid A. intermedium x Secale cereale (Stebbins and Pun 1953) trivalents were present in 80 percent of the cells. It was suggested that autosynapsis took place between the Agropyron genomes present. In this hybrid there was a size

distinction between the Secale and Agropyron chromosomes. In A. subsecundum x A. latiglume, however, the chromosomes were not morphologically distinguishable, and the bivalent pairing was interpreted on the basis of chromosome association in other closely related hybrids (Tables 23, 27).

The frequency of laggards was very low in anaphase I, and there were none in anaphase II, indicating that meiotic chromosome movement was very little disturbed in this hybrid (Table 7). The bridges observed in anaphase I cells showed that the genomes though very similar, included chromosomes heterozygous for inversions, and that crossovers within the inversions took place. The frequency of micronuclei was 0.04 per quartet. As no laggards were seen in anaphase II, these micronuclei were most likely formed from anaphase I laggards. In spite of the high bivalent pairing and the absence of major meiotic irregularities, the pollen was sterile, and the hybrid bore no seed. In addition to the possible reciprocal translocation, the genomes of A. subsecundum and A. latiglume must differ by cryptic structural hybridity.

Creel (1964) found a serological difference index of 0.14 between A. latiglume and A. trachycaulum (Table 30). He considered that any difference of about 0.12 or less was indicative of close serological similarity. Collins (1965) concluded from his serological study that A. latiglume is apparently closely related to A. subsecundum and somewhat more distantly related to A. trachycaulum.

On the basis of the serological and cytogenetic data discussed for the parent species and the meiotic data obtained from study of the synthetic hybrid, the genome formula $S_2S_2B_2B_2$ was given to A. subsecundum,

Table 30. Difference indices from serological comparison of selected Agropyron species
(from Greel, 1964).

Species	MSU field no.	<u>Agropyron</u> section	2-8	3-2	47-3	62a-2	64-8	93-4
<u>A. arizonicum</u>	2-8	Goulardia	0.00	0.16	0.20	0.14	0.08	0.18
<u>A. brachyphyllum</u>	3-2	"		0.00	0.08	0.06	0.08	0.10
<u>A. latiglume</u>	47-3	"			0.00	0.06	0.12	0.14
<u>A. riparium</u>	62a-2	Holopyron				0.00	0.06	0.08
<u>A. semicostatum</u>	64-8	Goulardia					0.00	0.10
<u>A. trachycaulum</u>	93-4	"						0.00

A. latiglume, and to their interspecific hybrid.

A. caninum x A. latiglume

A. caninum of section Goulardia, a species of Eurasian origin, has previously been identified as an allotetraploid (Stebbins and Snyder 1956), and has been shown to possess S and B genomes (Tables 23, 24, pgs. 83, 84). during meiosis in A. caninum x A. latiglume as well as in closely related hybrids suggests that genomes closely homologous to the B genome of A. trachycaulum may prove to be as widespread as the S genome. The unknown diploid from which the B genome was derived would show slight or no pairing when hybridized with diploid A. spicatum, and would form approximately seven bivalents and seven univalents when hybridized with allo-tetraploid species containing the B genome.

As in the early meiotic stages of A. trachycaulum x A. scribneri and A. subsecundum x A. latiglume, there was an apparent decrease in average bivalent pairing from prophase to metaphase I, in A. caninum x A. latiglume. In the latter hybrid there was an average of 13.55 bivalents in diakinesis which dropped to an average of 12.10 bivalents in metaphase I. This desynapsis or decrease in pairing has been suggested to indicate a lack of true chromosome homology or precocious separation as a result of imbalance in the hybrid's genic control of chromosome movement (Dewey 1963). Li's (1945) observations on desynapsis in wheat also apply here.

The frequency of bridges was low in anaphase I and dropped in anaphase (Table 10, pg. 43). Richardson (1936) showed that when bridge-fragment configurations are the result of crossing over in inverted chromosome

segments they will appear in anaphase II only if there have been at least two crossovers in the same chromosome arm with one between the centromere and the inverted segment and one within the inverted segment.

In contrast to the 65 percent of the quartets that appeared normal, only nine percent of the 72 pollen grains observed during mitosis contained the expected 14 chromosomes. Ninety percent of the mature pollen stained and appeared to have been fertile.

After consideration of the serological and cytogenetic data available on the parent species and the meiotic data obtained from study of the synthetic hybrid, the genome formula $S_2S_2B_2B_2$ was given to A. caninum, A. latiglume and to their interspecific hybrid (Table 29, pg. 91).

A. arizonicum x A. caninum

Data from metaphase I chromosome association in A. arizonicum x A. caninum showed the two genomes of allotetraploid A. arizonicum to be closely related to the S and B genomes of A. caninum (Table 28). There was an average of 24.02 out of 28 chromosomes per cell associated in bivalents or multivalents. Seventy-five percent of the cells contained at least one multivalent, but only eight percent contained more than one multivalent. The number of chromosomes associated in multivalents ranged from 3 to 12, with an average of 3.76 per cell. Chromosome bridges indicated that the genomes differ by inversions. The average frequency of bridges more than doubled from anaphase I to anaphase II (Table 30) as a result of crossovers between the centromere and the inverted segment as

well as in the segment (Richardson 1936).

The average frequency of 3.98 univalents in metaphase I was followed by an average frequency of 2.06 univalents in anaphase I. The average frequency of laggards in anaphase II was close to the average number of micronuclei and karyomeres in the quartets. All of the anaphase II laggards did not form separate karyomeres. Some were united in micronuclei. A similar phenomenon was observed in quartets of A. semicostatum x A. trachycaulum during this study. In meiosis of the hybrid A. spicatum var. inerme x A. caespitosum, Stebbins and Pun (1953) reported non-congregation of the chromosomes at one pole and attributed it to a disharmony between the species in regard to genetic control of chromosome movement, and possibly a reflection of genic imbalance in the hybrid. Creel (1954) found a high difference index between A. arizonicum and A. trachycaulum (Table 30). He did not study A. caninum. Judged by the average metaphase I chromosome associations of this hybrid, one would expect a low difference index on Creel's scale between A. arizonicum and A. caninum.

Having considered the serological and cytological data available on the parent species and the cytogenetic data obtained from study of the synthetic hybrid, the following genome formulae were proposed: A. arizonicum $S_4S_4B_4B_4$, A. caninum $S_2S_2B_2B_2$ and $S_2S_4B_2B_4$ for the interspecific hybrid (Table 29, pg. 91).

A. caninum x A. riparium

From the analysis of meiotic chromosome behavior in A. caninum x

A. riparium and A. brachyphyllum x A. riparium; it was postulated that A. riparium was an allotetraploid species and that it contained a genome derived from spicatum, and the B genome (Tables 28, 29). A. caninum was previously identified as containing an S genome (Stebbins and Synder 1956), and the second genome has been identified as the B genome through the hybridization of A. caninum x A. latiglume (Table 28, 29, pgs. 90, 91).

A higher percentage of the bivalents had formed chiasmata in both chromosome arms in A. caninum x A. riparium in which 61 percent of the bivalents were closed than in A. arizonicum x A. caninum with 44.17 percent closed bivalents, or in A. caninum x A. latiglume with 53.75 percent closed bivalents. This greater frequency suggested that there is slightly more complete homology between individual chromosomes of A. caninum and A. riparium, than between the chromosomes of A. caninum, and A. arizonicum or A. latiglume. The average univalent frequency was very close in the three hybrids involving A. caninum (Table 28, pg. 90).

Bridges in anaphase I occurred with close to the same frequency in A. caninum x A. riparium as in A. arizonicum x A. caninum (Tables 13, 19). The laggard frequency of 1.45 per cell in anaphase I of A. caninum x A. riparium correlated well with the average of 2.80 lagging univalents in telophase I because precocious chromatid separation took place.

Cryptic structural hybridity was probably responsible for the sterility of this hybrid. There must also have been some larger translocations or inversions in the S and B genomes, resulting in formation of multivalents in the hybrid.

Through serological comparison Creel (1964) found a low difference index of 0.08 between hexaploid A. riparium and tetraploid A. trachycaulum. An even lower difference index was found by Creel between hexaploid A. riparium and tetraploid A. latiglume, indicating that hexaploid A. riparium is more closely related serologically to tetraploid A. latiglume than to tetraploid A. trachycaulum (Table 30, pg. 95).

After consideration of available serological and cytological data on the parent species and the cytogenetic data obtained from study of the synthetic hybrid, the following genome formulae were proposed: A. caninum $S_2S_2B_2B_2$, A. riparium $S_3S_3B_3B_3$, and $S_2S_3B_2B_3$ for the interspecific hybrid (Table 29, pg. 91).

A. brachyphyllum x A. riparium

Data from previous hybridizations support the contention that A. riparium an allotetraploid containing modified S and B genomes (Tables 28, 29). A. brachyphyllum was a hexaploid species with normal bivalent pairing in metaphase I. Most likely it was an allopolyploid. From analysis of meiosis in A. brachyphyllum x A. riparium and in previous hybrids (Tables 28, 29) these genomes were identified as derived from the S and B genomes and a genome of unknown source or distribution, genome X.

Stebbins (1946b) found an average frequency of 0.63 trivalents per cell in Hordeum nodosum x Elymus glaucus. He stated that the nature of the bivalent pairing determines also the nature of the trivalent chromosome relationship. All the trivalents in Hordeum nodosum x Elymus glaucus and in A. brachyphyllum x A. riparium were open configurations. Stebbins considered these to be characteristic of those resulting from structural

hybridity and concluded that most likely there was no autosyndesis in the Hordeum x Elymus hybrid (Stebbins et al. 1946b). Open trivalents likewise may be evidence against autosyndesis of A. brachyphyllum genomes. There was an average frequency of 1.99 trivalents per cell in A. brachyphyllum x A. riparium. The open trivalents and chain configurations of higher chromosome number suggested rather that small translocations have taken place, resulting in the modification of the S, B, and X genomes in A. brachyphyllum when compared to the S and B genomes of tetraploid A. riparium. The average frequency of univalents was 7.13 in A. brachyphyllum x A. riparium, extremely close to the difference in gametic chromosome number between the two parent species. A few chromosomes from the X genome, possessing translocated segments could have sometimes formed open trivalents or higher associations through synapsis with chromosomes from genomes S and B during hybrid meiosis.

The average number of bridges at telophase I of 0.17 per cell in A. brachyphyllum x A. riparium, was higher than the frequency in A. caninum x A. riparium. This indicated that there were more short inverted segments in the former than in the latter hybrid. The percentage of cells containing laggards and the frequency of laggards was also higher in A. brachyphyllum x A. riparium (Tables 19, 22, pgs. 70, 79.)

Greel (1964) reported a difference index of 0.06 between hexaploid A. riparium and hexaploid A. brachyphyllum. This serological evidence suggested that the hexaploid species are very closely related (Table 30, pg. 95.)

Two cells were seen in metaphase I of A. brachyphyllum x A. riparium containing seven bivalents and seven univalents. If the bivalents in such

a cell formed from allosyndesis and had proceeded during anaphase I to separate normally, the X genome might have been left out of one of the quartet cells, and a fertile SB pollen grain might have resulted. Three out of 1200 pollen grains stained and appeared fertile, however, no seeds were produced by the hybrid.

From synthesis of available serological and cytogenetic data on the parent species and the meiotic data obtained from the hybrid the following genome formulae were proposed: A. brachyphyllum $S_5S_5B_5B_5X$ X, A. riparium $S_3S_3B_3B_3$, and $S_3S_5B_3B_5X$ for the interspecific hybrid, (Table 29, pg. 91).

A. semicostatum x A. trachycaulum

It has previously been demonstrated that A. trachycaulum from section Goulardia behaves meiotically as an allotetraploid (Boyle and Holmgren 1955). Additional support for this conclusion comes from the average chromosome association frequencies in the hybrid A. semicostatum x A. trachycaulum. Meiosis in the tetraploid A. semicostatum was normal with 14 bivalents formed in metaphase I. It may be assumed that A. semicostatum is also most likely an allotetraploid. Had it shown autosyndesis, the bivalent frequency which averaged 4.19 and 6.56 in the hybrid and the trivalent frequency which averaged 0.19 and 0.33, would have been higher.

The multivalents in A. semicostatum x A. trachycaulum were open chains. Stebbins et al. (1946a) explained open chains in interspecific hybrids with A. trachycaulum as a result of structural hybridity. Schulz-Schaeffer et al. (1963a) stated that multivalents with lower numbers of chromosomes can arise as a result of failure of chiasma formation in

certain paired segments of multivalent associations in prophase.

The average frequency of bivalents in the collection from the greenhouse was 6.56, very close to the 5.98 average reported for the triploid A. trachycaulum x A. spicatum (Stebbins and Snyder 1956), and to the 6.30 average bivalent frequency found in the tetraploid A. trachycaulum x Hordeum jubatum (Boyle and Holmgren 1955). In metaphase I the average univalent frequency was 13.66 in A. semicostatum x A. trachycaulum, 13.80 in the tetraploid A. trachycaulum x Hordeum jubatum (Boyle and Holmgren 1955), and 7.74 in the triploid A. trachycaulum x A. spicatum (Stebbins and Snyder 1956). In each of these hybrids the univalent and bivalent frequency indicated that the parent species shared only one common genome.

The difference in average metaphase I configurations between the greenhouse and field material collected from A. semicostatum x A. trachycaulum may have been partially environmental. An even wider difference in average association was found in separate studies of A. trachycaulum x H. jubatum. The average frequency found by Boyle and Holmgren (1955) was 6.93 bivalents while that found by Gross (1960) was 3.61.

The bivalents in the hybrid A. semicostatum x A. trachycaulum were sometimes abnormally elongated and stretched in late metaphase, as were some bivalents in Hordeum nodosum x Elymus glaucus (Stebbins et al. 1946b). Stebbins referred to McClintock (1941) and proposed it might be the result of difficulty in unravelling of chiasmata. One result of this stretching would be chromosome breakage. Walters (1950) showed that bridge-fragment configurations in hybrids might be due to chromosome breakage followed by reunion of sister chromatids. She demonstrated that if chiasmata frequency

in metaphase I is relatively low, most of the bridges seen in anaphase II are probably due to this phenomenon.

As in the A. arizonicum x A. caninum hybrid, there were micronuclei of various sizes. They were even more varied and frequent than in the other hybrid. It seemed most likely that they were formed from the lagging univalents of the two non-homologous genomes.

Through analysis of meiotic behavior in A. semicostatum x A. trachycaulum it appeared that tetraploid A. semicostatum possessed a modified set of one of the A. trachycaulum genomes. Without further hybridizations it was not possible to identify this genome as derived from spicatum or B. However, on the basis of the indicator chromosome type in hexaploid A. semicostatum (Jurasits 1961), it would seem most likely that a modified A. spicatum genome is the common denominator between the tetraploids A. semicostatum and A. trachycaulum. If this is so, a hybrid between tetraploid A. semicostatum and diploid A. spicatum would average near seven bivalents and seven univalents in metaphase I. If the spicatum genome is not present in tetraploid A. semicostatum, there would be little or no pairing in this postulated hybrid.

Matsumura (1942) in hexaploid A. semicostatum x diploid A. cristatum found an average chromosome association of 23.19 univalents, 219 bivalents, 0.13 trivalents and 0.001 quadrivalents in metaphase I. Possibly the bivalents formed because of the presence of translocations. On the whole the three genomes from A. semicostatum appeared to be non-homologous to the genome present in diploid A. cristatum.

Sakamoto (1964) concluded from interspecific hybridizations that

the two different genomes of the Nepalese species A. semicostatum are basically homologous to those found in the Japanese allotetraploids A. ciliare, A. gmelini and A. yezoense. The tetraploid hybrid A. ciliare x A. trachycaulum had a metaphase I average of 5.33 bivalents, with very slight multivalent formation (Sakamoto 1964). Apparently the allotetraploids A. ciliare and A. trachycaulum had close to one genome in common. As in the case of A. semicostatum, a hybrid between one of the tetraploid Japanese species A. ciliare, A. gmelini or A. yezoense, and diploid A. spicatum would determine whether or not the common genome had been derived from the spicatum genome.

On the basis of the serological and cytogenetic data available on the parent species and the meiotic data obtained for the synthetic hybrid the following genome formulae were proposed: A. semicostatum S_1S_1 (or B_1B_1)Y Y, A. trachycaulum $S_1S_1B_1B_1$, and $S_1S_1B_1Y$ for the hybrid if the common genome was derived from spicatum, or $S_1B_1B_1Y_1$ if B is the common genome. (Table 29, pg. 91).

CONCLUSIONS

The three dimensional model shown in Figure 14 was constructed using the serological difference indices (Table 30, pg. 95) determined by Greel (1964) as relative distance between the species. The model shows a cluster of three hexaploid *Agropyron* species: A. riparium, A. semicostatum, and A. brachyphyllum. The tetraploids A. latiglume and A. trachycaulum are nearer to the hexaploid species cluster than is tetraploid A. arizonicum.

Meiotic difference indices calculated from average metaphase I chromosome association in hybrids were used as another means of evaluating the relationship between species. The meiotic difference index was calculated for a hybrid by dividing the average frequency of bivalents observed per cell by the number expected from perfect genome homology. This fraction was then subtracted from one. Complete bivalent pairing within a hybrid would have resulted in a difference index of 0.00. The difference indices calculated for fourteen hybrids are listed in Table 31, and were used as relative measurements in building the three dimensional model shown in Figure 15.

The three dimensional model in Figure 15, shows two species clusters. In the case of A. brachyphyllum, A. arizonicum, and A. semicostatum only one hybridization for each species was known, making it impossible to determine their position relative to more than one species. However, their meiotic difference indices were high compared to those of the other species. The meiotic difference index for A. brachyphyllum x A. riparium was 0.27, for A. arizonicum x A. caninum 0.28, and for A. semicostatum x A. trachycaulum 0.23, suggesting more distant relationships. The genome

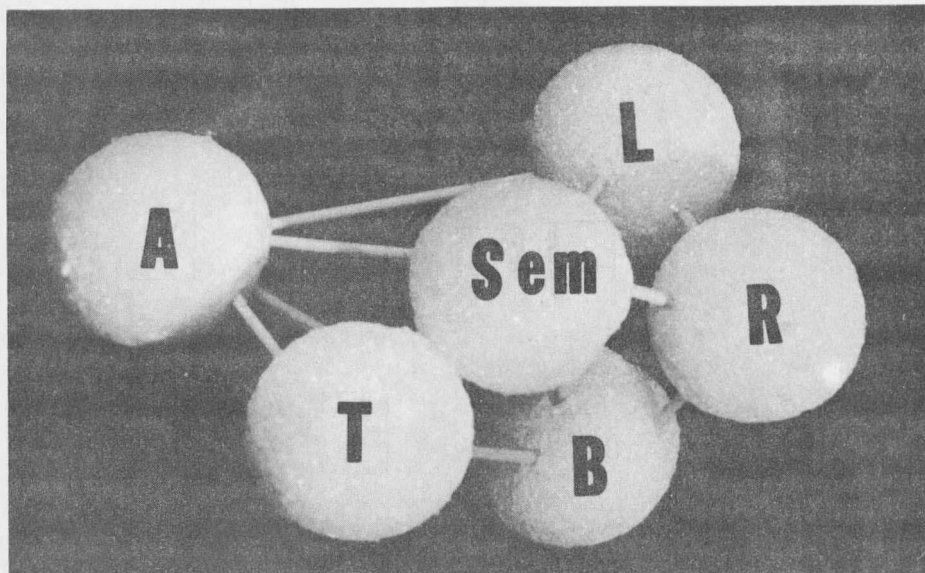


Fig. 14 Model showing relative serological relationships of, A: A. arizonicum, T: A. trachycaulum
 Sem: A. semicostatum, L: A. latiglume
 R: A. riparium, B: A. brachyphyllum (Creel 1964).

Table 31. Meiotic difference indices calculated for fourteen hybrids.

Parent species	A. spicatum	E. glaucus	A. subsecundum	A. trachycaulum	A. caninum	A. brachyphyllum
<u>S. hystrix</u>	0.03	0.02	0.12	0.13		
<u>A. latiglume</u>			0.13		0.14	
<u>A. scribneri</u>				0.16		
<u>A. caninum</u>	0.16					
<u>A. trachycaulum</u>	0.15	0.26				
<u>A. riparium</u>					0.17	0.27
<u>A. semicostatum</u>				0.23		
<u>A. arizonicum</u>					0.28	

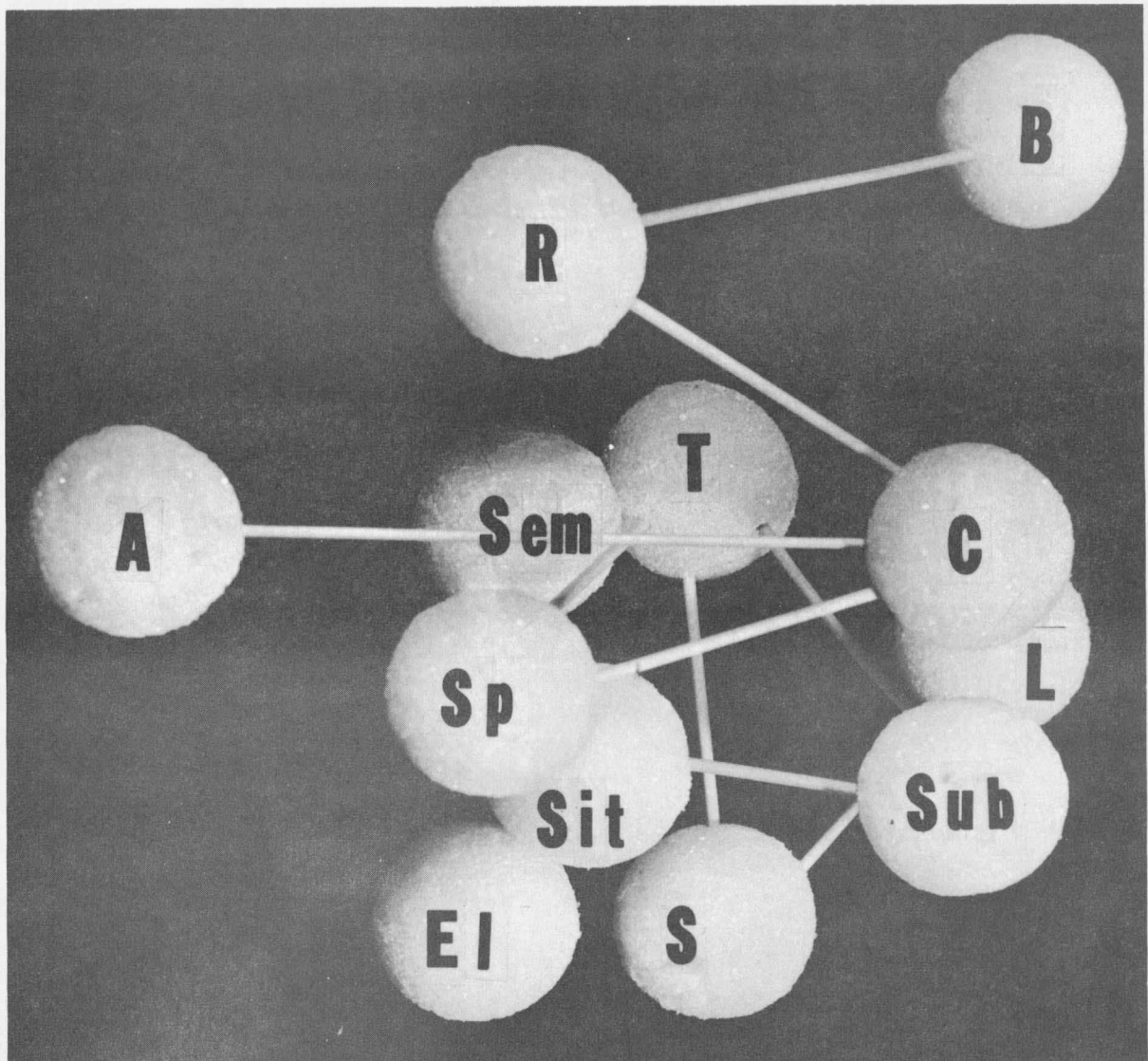


Fig. 15. Model showing cytotenetic relationship of twelve species, calculated from meiotic chromosome association data of fourteen hybrids. R: A. riparium, B: A. brachyphyllum, A: A. arizonicum, Sem: A. semicostatum, T: A. trachycaulum, C: A. caninum, Sp: A. spicatum, L: A. latiglume, Sit: Sitanion hystrix, Sub: A. subsecundum, El: Elymus glaucus, S: A. scribneri.

formula given A. brachyphyllum was $S_5S_5B_5B_5X X$, and that given A. semi-costatum was S_1S_1 (or B_1B_1) $Y Y$. (Table 29, pg. 91).

The tightest species cluster was A. spicatum x S. hystrix 0.03 (Dewey 1963) and Elymus glaucus x S. hystrix 0.02 (Stebbins and Vaarama 1954). Apparently the spicatum genome shared by these species is very little differentiated.

A second cluster included A. caninum, A. latiglume, A. scribneri and A. subsecundum. Each of these species contain derivatives of the S and B genomes and have been given the genome formula $S_2S_2B_2B_2$ (Table 29). The difference indices between these species ranged from 0.12 to 0.16 when calculated from their interspecific hybrids (Table 31). A. trachycaulum was also in this cluster. Meiotic difference indices for hybrids involving A. trachycaulum ranged from 0.13 to 0.23, and the genome formula given was $S_1S_1B_1B_1$ (Table 29). With the exception of A. caninum, these species have previously been recognized as being very closely related through both morphological and cytogenetic analysis (Dewey 1963).

The difference index between A. caninum and A. riparium was 0.17. Both species have been shown to possess derivatives of the S and B genomes; however, further hybrids must be studied before the relative position of A. riparium can be determined. The genome formula given A. riparium was $S_3S_3B_3B_3$ (Table 29, pg. 91).

As in the model built from the serological difference indices (Figure 14) calculated by Creel (1964), A. arizonicum is distant from the other species in the cytological model, though it, too, contains the S and

B genomes. The formula given was $S_4S_4B_4B_4$ (Table 29, pg. 91).

The results of this and previous investigations have demonstrated the world-wide, intergeneric and intersectional distribution of derivatives of the spicatum genome S and the B genome of unknown diploid origin. The failure of attempted hybridizations and the lack of bivalent pairing in hybrids between species in section Goulardia and section Agropyron support the conclusion of Schulz-Schaeffer and Jurasits (1962) and of Creel (1964) that the section Agropyron is valid and distinct.

SUMMARY

Cytogenetic study of six interspecific Agropyron hybrids has given further evidence of the world-wide and intersectional distribution of genomes derived from the spicatum genome. Modifications of the S genome have been shown to be present in each of eight Agropyron species: hexaploid A. brachyphyllum of section Goulardia, from Iran; the tetraploids A. caninum, and A. semicostatum of section Goulardia from Eurasia; and the North American tetraploid species, A. arizonicum, A. latiglume, A. subsecundum, and A. trachycaulum of section Goulardia and A. riparium of section Holopyron.

Derivatives of a genome of similar origin to the B genome present in tetraploid A. trachycaulum were traced through previously reported hybrids of species from sections Goulardia and Holopyron and through the species involved in the newly studied hybrids, with the exception of tetraploid A. semicostatum of section Goulardia. This species contains one genome of undetermined origin.

No indication was found of the possible origin of the third genome in A. brachyphyllum. The two known genomes were one of spicatum derivation and one derived B genome.

The failure of attempted hybridizations and the absence of normal bivalent pairing in hybrids between species of sections Goulardia and Agropyron support the conclusion of Schulz-Schaeffer and Jurasits (1962) and of Creel (1964) that the section Agropyron is valid and distinct.

The results of the study suggest that the number of attempted hybrids involving one diploid parent should be increased. Modified genomes

from the diploids A. spicatum, A. cristatum, and A. elongatum apparently have contributed to varied degree in the evolution of our present-day Agropyron species. When more polyhaploids are discovered and more hybridizations are made between species with distinct chromosome size, invaluable information on the frequency of autosyndesis in individual species will be obtained, and contributions will be made to the basic understanding of genome distribution and homology in Agropyron species.

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