



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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in

Genetics

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TABLE OF CONTENTS

	<u>Page</u>
VITA	ii
ACKNOWLEDGMENT	iii
TABLE OF CONTENTS	iv
LIST OF TABLES	v
LIST OF FIGURES	viii
ABSTRACT	ix
INTRODUCTION	1
REVIEW OF LITERATURE	7
MATERIALS AND METHODS	19
EXPERIMENTAL RESULTS	21
I. Investigation of meiosis in 30 strains of 21 <u>Agropyron</u> species	21
II. Attempted Inter- and Intra-sectional hybrids within <u>Agropyron</u>	27
III. Study of 4 induced intra-sectional hybrids in the genus <u>Agropyron</u> within section Goulardia	27
IV. Study of 2 induced inter-sectional hybrids in the genus <u>Agropyron</u> involving sections Goulardia and Holopyron	63
DISCUSSION	80
CONCLUSIONS	106
SUMMARY	112
LITERATURE CITED	114

LIST OF TABLES

Table	Page
1. Published genome formulae for <u>Agropyron</u> , <u>Sitanion</u> , and <u>Elymus</u> species studied through intergeneric and interspecific hybrids.	15
2. Sources of 10 strains of 8 <u>Agropyron</u> species used in interspecific crosses.	18
3. Investigation of meiosis in plants of 21 <u>Agropyron</u> species.	22
4. Interspecific hybridization with preceding emasculation during the summers of 1962 and 1963.	28
5. Morphological measurements of <u>A. subsecundum</u> , <u>A. subsecundum</u> x <u>A. latiglume</u> and <u>A. latiglume</u> .	31
6. Chromosome associations in diplotene, diakinesis and metaphase I of <u>A. subsecundum</u> x <u>A. latiglume</u> .	34
7. Frequency of irregularities in anaphase I and II of <u>A. subsecundum</u> x <u>A. latiglume</u> .	36
8. Morphological measurements of <u>A. caninum</u> , <u>A. caninum</u> x <u>A. latiglume</u> and <u>A. latiglume</u> .	38
9. Chromosome associations in diakinesis and metaphase I of <u>A. caninum</u> x <u>A. latiglume</u> .	40
10. Frequency of irregularities in anaphase I, telophase I, and anaphase II of <u>A. caninum</u> x <u>A. latiglume</u> .	43
11. Morphological measurements of <u>A. arizonicum</u> , <u>A. arizonicum</u> x <u>A. caninum</u> and <u>A. caninum</u> .	47
12. Chromosome associations in metaphase I of <u>A. arizonicum</u> x <u>A. caninum</u> .	49
13. Frequency of irregularities in anaphase I and II of <u>A. arizonicum</u> x <u>A. caninum</u> .	53
14. Morphological measurements of <u>A. semicostatum</u> , <u>A. semicostatum</u> x <u>A. trachycaulum</u> and <u>A. trachycaulum</u> .	56
15. Chromosome associations in metaphase I from collections in the greenhouse and field, of <u>A. semicostatum</u> x <u>A. trachycaulum</u> .	58

Table	Page
16. Frequency of irregularities in anaphase I and II of <u>A. semicostatum</u> x <u>A. trachycaulum</u> .	62
17. Morphological measurements of <u>A. caninum</u> , <u>A. caninum</u> x <u>A. riparium</u> and <u>A. riparium</u> .	65
18. Chromosome associations in metaphase I of <u>A. caninum</u> x <u>A. riparium</u> .	68
19. Frequency of irregularities in anaphase I, telophase I and II of <u>A. caninum</u> x <u>A. riparium</u> .	70
20. Morphological measurements of <u>A. brachyphyllum</u> , <u>A. brachyphyllum</u> x <u>A. riparium</u> and <u>A. riparium</u> .	73
21. Chromosome associations in metaphase I of <u>A. brachyphyllum</u> x <u>A. riparium</u> .	76
22. Frequency of irregularities in telophase I and II of <u>A. brachyphyllum</u> x <u>A. riparium</u> .	79
23. Average metaphase I chromosome associations in hybrids involving <u>A. caninum</u> , <u>A. spicatum</u> , <u>A. trachycaulum</u> , <u>Sitanion hystrix</u> and <u>Elymus cinereus</u> .	83
24. Genome formulae for <u>A. spicatum</u> , <u>A. caninum</u> , <u>A. trachycaulum</u> and <u>Sitanion hystrix</u> .	84
25. Average metaphase I chromosome associations in hybrids involving <u>A. inerme</u> , <u>A. parishii</u> , <u>A. spicatum</u> , <u>A. trachycaulum</u> , <u>Elymus glaucus</u> , <u>Sitanion hystrix</u> and <u>S. jubatum</u> .	85
26. Genome formulae for <u>A. inerme</u> , <u>A. spicatum</u> , <u>A. parishii</u> , <u>A. trachycaulum</u> , <u>E. glaucus</u> , <u>S. hystrix</u> and <u>S. jubatum</u> .	87
27. Average metaphase I chromosome associations in hybrids involving <u>A. latiglume</u> , <u>A. scribneri</u> , <u>A. subsecundum</u> , <u>A. trachycaulum</u> and <u>S. hystrix</u> .	88
28. Average metaphase I chromosome associations in hybrids involving <u>A. arizonicum</u> , <u>A. brachyphyllum</u> , <u>A. caninum</u> , <u>A. latiglume</u> , <u>A. riparium</u> , <u>A. semicostatum</u> , <u>A. subsecundum</u> and <u>A. trachycaulum</u> .	90

Table	Page
29. Genome formulae for <i>A. arizonicum</i> , <u><i>A. brachyphyllum</i></u> , <u><i>A. caninum</i></u> , <u><i>A. latiglume</i></u> , <u><i>A. riparium</i></u> , <u><i>A. semicostatum</i></u> , <u><i>A. subsecundum</i></u> and <u><i>A. trachycaulum</i></u> .	91
30. Difference indices from serological comparison of selected <u><i>Agropyron</i></u> species (from Creel 1964).	95
31. Meiotic difference indices calculated for 14 hybrids.	108

LIST OF FIGURES

Figure	Page
1. Spikelets and florets of <u>A. subsecundum</u> , <u>A. subsecundum</u> x <u>A. latiglume</u> and <u>A. latiglume</u> .	30
2. Meiosis in <u>A. subsecundum</u> x <u>A. latiglume</u> .	34a
3. Spikelets and florets of <u>A. caninum</u> , <u>A. caninum</u> x <u>A. latiglume</u> and <u>A. latiglume</u> .	37
4. Meiosis in <u>A. caninum</u> x <u>A. latiglume</u> .	42
5. Meiosis in <u>A. caninum</u> x <u>A. latiglume</u> .	45
6. Spikelets and florets of <u>A. arizonicum</u> , <u>A. arizonicum</u> x <u>A. caninum</u> and <u>A. caninum</u> .	46
7. Meiosis in <u>A. arizonicum</u> x <u>A. caninum</u> .	51
8. Spikelets and florets of <u>A. semicostatum</u> , <u>A. semicostatum</u> x <u>A. trachycaulum</u> and <u>A. trachycaulum</u> .	55
9. Meiosis in <u>A. semicostatum</u> x <u>A. trachycaulum</u> .	60
10. Spikelets and florets of <u>A. caninum</u> , <u>A. caninum</u> x <u>A. riparium</u> and <u>A. riparium</u> .	64
11. Meiosis in <u>A. caninum</u> x <u>A. riparium</u> .	67
12. Spikelets and florets of <u>A. brachyphyllum</u> , <u>A. brachyphyllum</u> x <u>A. riparium</u> and <u>A. riparium</u> .	72
13. Meiosis in <u>A. brachyphyllum</u> x <u>A. riparium</u> .	77
14. Model showing relative serological relationships of <u>A. arizonicum</u> , <u>A. trachycaulum</u> , <u>A. semicostatum</u> , <u>A. latiglume</u> , <u>A. riparium</u> and <u>A. brachyphyllum</u> .	107
15. Model showing cytogenetic relationship of twelve species, calculated from meiotic chromosome association data of fourteen hybrids.	109

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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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.

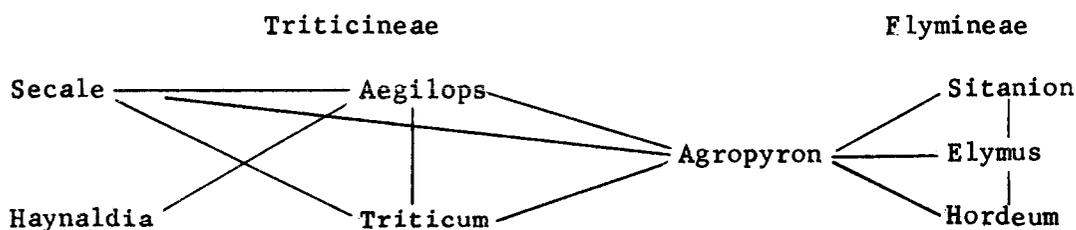
Hexaploid A. brachyphyllum possessed derivatives of the S and B genomes and a third genome of undetermined origin and distribution.

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.

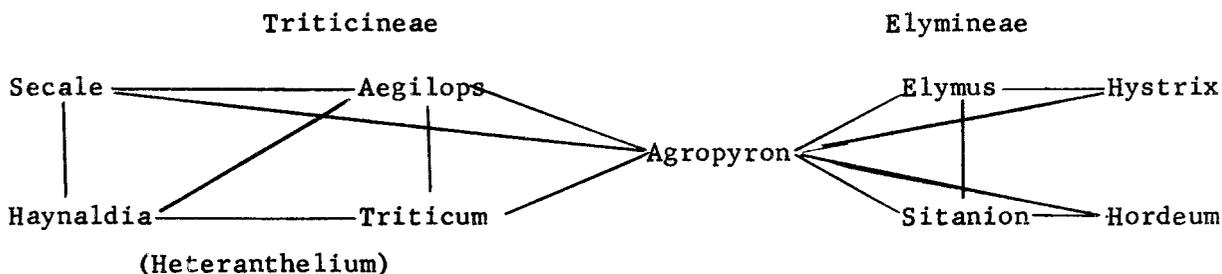
Though morphologically closer to the subtribe Triticineae than to Elymineae, intergeneric hybridizations have indicated that the genus Agropyron biosystematically occupies an intermediate position between the two subtribes. The relationship of the genera within the tribe was diagrammed in the following 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. Creel (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 $J_1 J_2$ (Ostergren 1940); J. originating from an unknown diploid species. A. junceum var. mediterraneum n=21, $J_1 J_2 E_3$; A. intermedium n=21, $E_1 E_2 N_1$ (Stebbins 1953); A. campestre n=28, $K_1 K_2 E_4 E_5$ (K could be close to N); A. littorale n=21, $N_2 N_3 Y_1$ (Y could be close to J); A. repens n=21, $R_1 R_2 Z_1$ (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 Creel (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 $B_1 B_2 C$ 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 autosynopsis of the spicatum chromosomes, which resulted in seven bivalents, with the crisatum chromosomes present as seven univalents. The gametic genome formulae assigned were: A. spicatum B B, and A. crisatum A. A. spicatum has morphological characteristics of species of section Holopyron (Stebbins 1961) and A. crisatum 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 field no.	Observed 2n=	Seed Source
<i>A. arizonicum</i> 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.
<i>A. brachyphyllum</i> 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.
<i>A. caninum</i> (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	
<i>A. latiglume</i> (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
<i>A. riparium</i> 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

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 4.5Q 41.5MV								
		368		100MV	11.1B	20M	9.6M	N		6M	23
<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	

