CYTOLOGICAL AND MORPHOLOGICAL VARIATIONS IN *BROMUS INERMIS* LEYSS.

by

BJÖRN SIGURBJÖRNSSON

A Thesis

Submitted to the Committee on Graduate Studies

in Partial Fulfilment of the Degree of

Master of Science

The University of Manitoba

May 1957

THE UNIVERSITY OF MANITOBA LIBRARY

ABSTRACT

Cytological and Morphological Variations
in *Bromus inermis* Leyss.

by

Björn Sigurbjörnsson

A cytological investigation was conducted, involving several varieties and strains of southern and northern brome grass.

Data on some morphological and agronomical characters were also obtained on some of the plants used in the cytological investigations.

Most of the plants were found to have a chromosome complement of 2n = 56. However, one plant was observed to have 49 somatic chromosomes, while 35.29% of the population studied were found to be aneuploid with the chromosome numbers 54, 55, 57, and 58. A limited meiotic study revealed considerable irregularities. Some of the morphological and agronomical characters were found to be correlated with chromosome numbers, varieties, and ecotypes.

TABLE OF CONTENTS

LIST OF TABLES

TABLE		PAGE

LIST OF PLATES

ACKNOWLEDGMENTS

This study was made possible by a bursary granted by the National Research Council of Canada. The project was carried out in the Plant Science Division at the University of Manitoba.

The writer is indebted to Prof. J. D. Truscott for guidance and encouragement throughout the course of study, and to Dr. Anna Storgaard for assistance in preparing the manuscript.

He also wishes to express sincere appreciation to Dr. A. Mochizuki for his assistance with the cytological investigations and his constructive criticisms and suggestions.

Thanks is also extended to Dr. B. C. Jenkins for reading the manuscript and for his assistance in preparing the photographs.

INTRODUCTION

Brome grass, Bromus inermis Leyss. is commonly considered to be the most important forage species in North America. It is a long-lived, perennial, cross-pollinated species with strong creeping rhizomes which form a dense and heavy sod with an abundance of vegetative growth. It was introduced into this continent in the late nineteenth century and has since been grown widely throughout Canada and the United States, especially in regions of moderate rainfall and low to moderate summer temperatures.

Two distinct types of brome grass, differing in morphology, growth behavior, and adaptation, are generally recognized. The "northern type" was introduced from Siberia and has been found to be well adapted to Canada and the Northern Great Plains. The "southern type" was introduced from central Europe and is particularly well adapted to the Corn Belt States and some parts of the central Great Plains.

Much of the seed supply for Canada and the United States is produced from the northern type brome grass in the prairie regions of Canada. Because the northern type is not as well adapted to the southern regions as the southern type, plant breeding programs are under way for the purpose of developing varieties of brome grass that will give high seed yields in northern regions, and yet produce satisfactory yields of forage in southern regions. Programs of this sort may require the use of plants of both the southern and the northern types. The present study includes a cytological survey of a number of varieties and strains of both the southern and northern types with the intention of isolating clones which might prove suitable for use in the development of strains adapted to both northern and southern regions.

LITERATURE REVIEW

A. Ecotypes

The division of native and cultivated plants within the species *B. inermis* into two rather distinct types was done by Zherebina (36) in 1931. He collected brome grass from a wide area in the U.S.S.R., and studied this material together with small collections from western Europe and northern America. He recognized two main ecologic-geographical groups: a northern climatype, the "meadow" group, (*climatypus borealis*), and a southern climatype, the "steppe" group, (*climatypus australis*). Descriptions of these two types have been found to correspond with the "northern" and "southern" types subsequently discovered within the United States (25).

Differences between the two types were found to be mainly of an ecological and morphological nature (36). The morphological differences were in root development, height, and number of vegetative versus reproductive tillers. Leaves of the steppe group were coarser in texture, shorter, narrower, and more erect than of the meadow group. Short, narrow panicles were more common in the steppe group, but no differences in seed characters were found. He also found the steppe group to be more susceptible to rust, (*Puccinia graminis bromi* Erikss.) and the meadow group more susceptible to brown spot, (*Pyrenophora bromi* Died.).

Ecological differences in the native collections were found in the respective area of distribution of the two types (36).

The meadow type was found to be distributed from Arkhangelsk in the north to the Ukraine and the Caucasus in the south. In the southern regions, however, south of the Central Chernozyom Region, this type was generally confined to valleys and more moist habitats. The steppe type predominated in the dry steppe areas of the mid- and lower-Volga districts, Kazakstan, the northern Caucasus, the Eastern Ukraine, and the southern Altai districts of Asia. The steppe type accompanied the meadow type in the Central Chernozyom Region.

Collections of cultivated brome grass both from America and most of the U.S.S.R. were found (36) to be of the northern or meadow type. True steppe types were only rarely found in the cultivated material, but some intermediate types were found among the Russian grasses that closely approached the steppe type. These were confined to the Central Chernozyom Regions, the central and lower Volga districts, and Western Siberia. Zherebina (36) described two subspecies of *B. inermis*, subsp. *borealis*, and subsp. *australis*, corresponding to the two types, based on his investigations on wild populations, but he stated that this division only partially applies to cultivated brome grass.

In North America Newell and Keim (25) made comparative studies at Lincoln, Nebraska, of several brome grass strains obtained from northern and southern seed sources, and found strains from southern areas to be much higher in hay production than strains from northern areas. Seed production of the southern strains was higher in the second year, but lower than the northern ones in the third year. Seedlings of the southern strains were found to be more vigorous in Nebraska and better able to withstand heat and drought conditions. Southern strains were also found to grow more rapidly in the spring and were, therefore, considered earlier than the northern strains. This is contradictory to Zherebina's finding (36) that the meadow group is earlier.

Knowles and White (19) reported in 1949 that other investigators working in the States of Kansas, Iowa, Ohio, and Missouri have produced additional evidence of the superior adaptation and forage productivity of the southern strains in these states. Churchill (5) in 1947 observed that the advantages of southern type strains are somewhat less evident in Michigan.

Newell and Keim (25) reviewed the history of the introductions of brome grass into the United States. They concluded that southern strains arose from introductions through France from Hungary around 1880, while northern strains came from importations from Russia during the period 1896-1899. Because introductions from Russia were on a large scale and widely distributed, brome grass of the northern type soon predominated in the American seed trade. Fletcher, according to Knowles and White (19), reported an introduction from Germany around 1888 to Canada which became distributed throughout the country and represents the main foundation of brome grass in Western Canada.

Knowles and White (19) reported on various tests and comparisons between southern and northern type strains conducted at nine Dominion Experimental Stations in the three prairie provinces. They found forage production of southern strains of brome grass to be similar to that of northern commercial brome grass. On the other hand they found seed production of southern strains at three of the stations to be inferior to that of the northern commercial strain. Differences between the types in minor morphological characters, such as nature of the leaf and panicle shape, were found to correspond reasonably well with the differences observed by Zherebina (36) between the meadow and the steppe type. Southern strains were two to three days later in flowering than northern strains, and showed more resistance to fall and spring frosts. Southern strains possessed superior resistance to certain leaf spot diseases. The degree of self-fertility and the distribution of plants for level of self-fertility was similar for the two types. The southern and northern types were also found to be interfertile in crosses. Unpublished data from yield tests conducted at the University of Manitoba by Truscott (35), indicate that some southern strains equal or exceed northern strains in both forage and seed yield.

B. Cytology

1. Chromosome Numbers

Sections within *Bromus* are characterized by the presence of euploid series with the basic chromosome number x = 7 (10). The genus includes species with a diploid chromosome number of 2n = 14 to a duodecaploid number of 2n = 84 (3, 7, 10, 11, 32). Chromosome numbers reported recently by Schulz-Schaeffer (29) for four *Bromus* species do not conform to a basic number of 7. Stebbins (32) has reported that species within the section Bromopsis in North America are mostly diploid.

Chromosome numbers for *B. inermis* have been reported by many investigators. A chromosome complement of 2 = 56 has been most commonly found (2, 6, 10, 11, 12, 13, 16, 17, 18, 21, 26, 27, 28).

Hill and Myers (14) in 1948 reported chromosome counts of 163 plants of *B. inermis*. They found 159 plants with 56 chromosomes, 3 with approximately 56, and one plant with 0-11 fragments in addition to a normal complement of 56. They proposed that the fragments were analogous to inert B-chromosomes.

A chromosome complement of 2n = 42 for *B. inermis* has been reported more rarely (1, 16, 27, 31). Nielsen in 1939 (26) reported a chromosome complement of 2n = 70. Polyhaploid 28-chromosome plants of *B. inermis* were reported by Elliott and Wilsie (13), Nielsen (28), and Knobloch (17).

Schulz-Schaeffer (29) counted n = 31 chromosomes in his brome grass material in 1956. He found the chromosomes to be of two types, 21 of one type and 10 of the other, possibly involving B-chromosomes.

In discussion of their results, Hill and Myers (14) state that "the possibility of aneuploid plants cannot be excluded." This is the only reference to aneuploidy in *B. inermis* that the writer has been able to find.

Aneuploidy has been reported frequently in many other grasses. Stuckey and Banfield (34) in 1946 reported the following chromosome numbers for *Agrostis alba* on Rhode Island: 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 39, 41, 42. They found the species to represent a very diversified group with variations in morphological characters. They drew no conclusions as to relationships between aneuploidy and the morphological variations.

Myers (24) reported aneuploid numbers in *Poa pratensis*, *P. alpina*, *Alopecurus alpinus*, *A. antarcticus*, *Lolium perenne*, *Agropyron cristatum*, *Arrhenatherum elatius*, *Festuca arundinacea*, and *Phleum pratense*. In *Poa pratensis* somatic chromosome numbers of 14 and 21 to 147, inclusive, have been reported. Aneuploidy in this species is related to its apomictic behavior

The majority of *Poa pratensis* plants have chromosome numbers in multiples of 7.

Myers (24) found the incidence of aneuploidy in *D. glomerata* to be higher in progenies of aneuploids than in progenies of euploids. He hypothesized that aneuploidy might be expected in plants showing meiotic behavior characteristic of autopolyploids.

Müntzing (23) found progressive increase in vigor in 2n = 14, 21, 28, and 35 chromosome *Dactylis* but less vigor in plants with intermediate chromosome numbers.

In his study on morphologic and agronomic variations in *Poa pratensis* Kramer (20) found his material to have somatic chromosome numbers from 50 to 85. He found spreading rate and mildew susceptibility to be positively associated with increase in chromosome number, but no other significant associations. He concluded that chromosome numbers in *Poa pratensis* may vary over a considerable range without having an appreciable effect on morphological characters or agronomic behavior.

Sharp (30) states that autopolyploids are frequently found with a chromosome missing as a result of irregular disjunction of multivalents, but the loss of a single member causes less unbalance than in diploids. De Robertis *et al.* (8) attribute the occurrence of aneuploidy to a faulty separation of chromosomes in the meiotic process.

2. Meiotic Behavior

It has been found very difficult to study meiotic behavior in brome grass due to the fact that preparations of metaphase I are not made readily and are difficult to interpret. Chromosomes at the metaphase I stage exhibit much stickiness and clumping. This is reported by Elliott and Love (12), Nielsen (28), Elliott (10), and Barnett (3). Consequently, most cytological analyses have been made by observing late diakinesis which, aside from not being a satisfactory stage for the study of pairing associations *per se*, exhibits also varying degrees of stickiness and clumping of the chromosomes (3). The high polyploidy of the species and the high frequency of multivalent associations also adds to the difficulty of analysis (3, 10, 28).

Elliott and Love (12) feared that bias may result in studying frequency of multivalent associations because the cells that are most easily studied are possibly those with the least complicated associations. They found that structural hybridity of the chromosome complement is evidenced by anaphase bridges, lagging univalents, and various multivalent associations. They reported some ring-shaped univalents indicating duplication on either side of the centromere. The occurrence of bridge formations in 22% of anaphases observed in one of the clones studied suggests the presence of an inversion heterozygote. The chromosomal associations were found to indicate extensive segmental interchange which may be expected to result in complex genetic segregations. They felt, however, that the inversion heterozygote may be advantageous because it may hinder many harmful recombinations; on the other hand the meiotic irregularity present in B. inermis introduces limitations in obtaining desirable gene combinations, in addition to linkage group effect in inbred and crossbred populations. Elliott and Love (12) studied 7 to 10 cells in each of six clones of brome grass in their investigations. They found a very high frequency of multivalent associations, with as many as six quadrivalents in a single cell. Occasional cells exhibited 28 bivalents. As many as five univalents were found in one cell. The behavior exhibited in these clones was unlike what might be expected in normal diploids.

These investigators (12) felt that "during its lifetime an exceptionally vigorous clone may spread extensively asexually and possess a high adaptive value in spite of meiotic irregularity." Natural selection may favour an individual that is vegetatively vigorous, and it may thus spread, even though excessive irregularity at meiosis impairs its sexual reproduction.

Barnett (3), studying several Bromus species, found species with lower chromosome numbers to have regular meiosis. Higher polyploids exhibited a number of irregularities at diakinesis. He reported that B. inermis showed marked meiotic irregularity with fairly high frequency of univalents and multivalents, although more than half of the chromosomes were usually paired as bivalents. Quadrivalents were found to represent the predominant class of multivalents. Occasional octovalents were the largest configurations observed. A high frequency of laggards was observed at anaphase I. These consisted mainly of univalents which in many cases were dividing. Barnett also found interplant differences in brome grass. Some plants exhibited almost complete bivalent pairing and were relatively regular in later meiotic stages. Others showed high frequencies of univalents and multivalents at diakinesis, marked irregularity at anaphase I, and high frequencies of tetrad micronuclei.

Knobloch (16) has also reported on meiotic irregularity in brome grass, with laggards present in both anaphase I and II.

Elliott (11) found varying multivalent associations in brome grass, from univalents to octovalent associations.

Nielsen (28) in his study of twin plants in brome grass found differences in meiotic irregularities both between and within twins. He observed quadrivalent, hexavalent, and octovalent associations in his material, and found that often the entire chromosome complement was joined by sticky threads extending between the chromosomes.

He also found translocation configurations, duplications, and inverted loops.

In their study of a 28-chromosome polyhaploid Elliott and Wilsie (13) found much internal pairing at meiosis, and chromosomal behavior generally normal. They felt that the high fertility of the plant, and the relatively normal meiotic behavior is indicative of a high degree of duplication of the genetic material at the octoploid level. The additional evidence of the presence of many closed bivalents in the polyhaploid might suggest that the species was nearly an autopolyploid which arose by chromosome doubling of a hybrid between two closely related autotetraploids. They also suggested that forces other than homology may be responsible for certain of the observed pairing complications.

Love (22) suggested that due to losses of chromosomes resulting from lagging of univalents, Mendelian segregation cannot be expected in the offspring of such plants.

Stebbins (33) observed that autotetraploids are usually characterized by the presence of multivalents at meiosis, tetrasomic ratios, and sometimes by slower development and reduced fertility. In segmental allopolyploidy multiple associations are less common. There is no way of distinguishing between autopolyploidy and segmental allopolyploidy except by finding out through systematic studies and experimental verification the actual origin of the polyploid in question. Allopolyploids resemble diploids in meiotic behavior. He suggested that perhaps all the natural polyploids in Bromus in California are allopolyploids rather than autopolyploids. Bromopsis species, native to North America, are mostly diploid (2n = 14). Hexaploids in South America of the section Ceratochloa are all strict allopolyploids with the genomic formula AABBCC (32). Some of the octoploids of Ceratochloa in North America are allopolyploids. Stebbins (33) found in allopolyploid species of Ceratochloa from 6-10 quadrivalents regularly at meiosis. Hexavalent, octovalent, and duodecavalent species of Ceratochloa and Neobromus in California are all allopolyploids. Stebbins contended that a large proportion, if not a majority of the hexaploids, octoploids, and higher polyploids within Bromus likely represents some variant of the autoalloploid condition - autopolyploids, segmental allopolyploids, and true allopolyploids combined in different ways. Stebbins (33) considered *Bromus inermis* to be a segmental allopolyploid. Nielsen (28) considered it to be an allopolyploid involving a related species, whereas Elliott (11) and Knobloch (17) regarded it an autopolyploid.

3. Micronuclei and Pollen Fertility

Micronuclei consist of one or more chromosomes or fragments that fail to be incorporated in the young pollen nuclei and can be seen as small dots in the tetrad.

Usually much sterility results from this because of deficiency in chromatin material.

Love (22) stated that a very simple method of investigating meiotic stability in parental strains is to examine young pollen tetrads for micronuclei. He advocated that no plant with a higher number of micronuclei than the mean for that particular species should be used as a parent in crossings.

Elliott and Love (12) recommended tetrad analysis for the discovery of clonal lines with abnormal meiosis. They obtained a negative correlation between percentage of tetrads with micronuclei and pollen stainability. They found no clear cut association of univalents, bivalents, or other chromosomal configurations with normal tetrads and pollen stainability.

Elliott and Wilsie (13) in their study of the polyhaploid found a high proportion of stainable pollen and 87% of the tetrads to be free of micronuclei. This, they felt, was indicative of normal meiosis.

Elliott (10) in 1949 found a highly significant correlation between expected percentage of normal eggs as predicted by micronuclear analyses and seed set under open-pollination in the greenhouse.

Cheng (4) found a negative correlation between percentage of aborted pollen and number of viable seeds set under open pollination conditions, but no relationship between the diameter of pollen grains and seed setting ability.

MATERIALS AND METHODS

The plant material used in this study consisted of varieties and strains of <u>Bromus inermis</u> Leyss. shown in Table I.

TABLE I

LIST AND DESCRIPTIVE NOTES OF THE VARIETIES AND STRAINS OF BROME GRASS USED IN THE INVESTIGATIONS

Name	Type	Kind*	Source	Origin
Canadian Comm.	Northern	OP		Unity, Sask.
53-7	"	"	Superior	Saskatoon, Sask.
175-44	"	"	Can. Comm.	" "
216-20	"	"	" "	" "
194-12	"	"	" "	" "
175-29	"	"	" "	" "
Southland	Southern			Oklahoma, U.S.A.
Fischer	"			Iowa, "
Lancaster	"			Nebraska "
Lyon	"			"
561	"	PC	Achenbach	Ames, Iowa, "
383	"	"	Lincoln	" " "
567	"	"	Achenbach	" " "
BR-3 Syn. 2	"			" " "
510	"	PC	Fischer	" " "
475	"	"	"	" " "

*OP = open pollination seed
PC = polycross seed

A space-planted nursery of these varieties and strains was established in the spring of 1954. The seeds were germinated in a greenhouse in paper cups contained in greenhouse flats. The seedlings were thinned down to one seedling per cup, and when the plants were well established they were transplanted to the field.

The distance between plants and rows was three feet, each plant thus occupying nine square feet. Alfalfa was seeded on the centreline between the plants. Ten replications were used with 10 to 20 spaced plants in each replicate, giving a total of from 100 to 200 spaced plants per variety.

In 1955 field notes were taken on hay vigor, height, width of leaf, panicle production, seed yield, and fertility ratio. The plants were rated for creeping vigor and general vigor in 1956.

The plants were scored from 1 to 10 for hay vigor, 10 indicating the most vigorous plant. The height measured in feet, represents the height of the average panicle. Leaf width was obtained by averaging the measurement in millimeters of the widest point of three leaves picked at random. Panicle production represents the estimated number of panicles at harvest time, based on the thickness of the culm bundles as compared to a set of standard bundles in which the culms had been counted. Seed yield is the threshed seeds from each plant, measured in grams. Fertility ratio represents the ratio between the weight of the unthreshed panicle and the weight of seeds threshed from that panicle, based on a sample of five panicles from each plant. Creeping vigor was scored from 1 to 10 with 10 representing the most vigorous creeper. General vigor is represented as a score, the most vigorous plant receiving the score 10.

In the fall of 1955, root cuttings of a total of 120 plants were transferred to a greenhouse. This material included 55 plants of 10 different strains of the southern type and 65 plants of 6 different strains of the northern type.

The root cuttings were potted in 4" pots and allowed to become well established. When new roots had developed, root tips were removed with a pair of tweezers and placed in glass vials containing water. These were placed in a refrigerator and left overnight.

After this pretreatment, the root tips were transferred to a Farmer's solution fixative, (3 parts 95% ethyl alcohol: 1 part glacial acetic acid), and left there for a minimum of three hours. Root tips that were not used immediately were stored in the fixative and kept in a refrigerator.

After fixation the root tips were washed in water, dried on blotting paper, and hydrolysed in one normal hydrochloric acid. Hydrolysis was carried out in a water bath at a constant temperature of 60° for 7 to 10 min. The time required for hydrolysis was found to vary with length of storage in the fixative. The longer the root tips had been stored, the longer was the time required for hydrolysis.

After hydrolysis the root tips were washed in water thoroughly, dried on blotting paper, and placed in a Feulgen stain, (leuco-basic fuchsin), and kept in the stain in darkness for a minimum of ten minutes. Root tips that could not be worked on immediately were stored in the stain in the refrigerator for a maximum of three days.

The stained root tips were mounted in 45% acetic acid, the chromosomes spread by tapping on the coverslip and heating intermittently.

Semi-permanent slides were made by sealing with gum mastic.

For establishing chromosome numbers of 56 or higher at least three well spread metaphases were counted. In cases where the chromosome number was 55 or lower at least five metaphase preparations were studied in order to eliminate the possibility of counting incomplete complements.

After the preliminary survey, it was decided to narrow the field of investigations down to four varieties. Two of each of the southern and northern types respectively were selected, investigating a larger number of plants from each variety.

The selection of the four varieties was based on the extent of their previously established aneuploid variations. One variety of each type was assumed to be a "stable" or a reasonably stable type as revealed by the preliminary survey, while the other showed more extreme variation.

Root cuttings of a number of plants of the four varieties were transferred to the greenhouse in the summer of 1956. The plants were handled in the same way as in the preliminary survey, and the cytological procedure was identical.

Meiosis was studied in pollen mother cells. Panicles were collected in the field in the summer of 1956. The correct stage for meiotic observations was found to be when the panicle had emerged far enough to reveal about two to three cm. of the culm. Bulk fixation was used, the panicles being placed in 8 oz. bottles containing Farmer's solution as a fixative, and stored as such in a refrigerator.

The stage of meiosis was checked by squashing a portion of an anther under a coverslip in a drop of 1% acetocarmine. Semipermanent mounts were made by the squash method, using acetocarmine for staining and an iron needle as a mordant. Late diakinesis was generally employed for pairing observations, but metaphase I was utilized for this purpose whenever possible.

Mature pollen was obtained immediately prior to anthesis by collecting anthers and placing them in small vials containing Farmer's solution. Pollen was mounted in acetocarmine for nuclear analysis. Pollen from a total of 27 plants was classified into 14 categories, depending upon size and nuclear condition (15). Two sizes were recognized: normal and small. The nuclear classification was based on the number and shape of the pollen nuclei. Seven classes of normal sized pollen and seven classes of small pollen were established. The nuclear classes were: I, one round-shaped and two thread-shaped nuclei; IIa, a borderline class where the exact shape of one or two of the thread-shaped nuclei could not be determined; II, three nuclei, either all round, two round and one thread-like, or all thread-like; III, only two nuclei present; IV, one nucleus present; V, pollen devoid of nuclei; VI, four or more nuclei present.

Cytological differences between northern and southern brome grass were tested by using contingency tables and the statistic X^2 (9).

Analysis of variance was used in studying the relationship between chromosome numbers and morphological and agronomical characters.

RESULTS AND DISCUSSION

A. Chromosome Numbers

Table II presents the chromosome counts that were obtained in the preliminary survey of a few plants of a number of varieties, including both northern and southern strains. The figures in the columns indicate the number of plants within each variety found to have the designated chromosome number.

TABLE II

CLASSIFICATION OF PLANTS ACCORDING TO THEIR CHROMOSOME NUMBERS

Varieties	Somatic Chromosome Numbers					Total
	54	55	56	57	58	
Canadian Comm.		1	7	2		10
53-7			5	4	1	10
175-44		1	5	5		11
216-20		2	5	1	2	10
175-29		2	6			8
194-12		1	9			10
Total Northern		7	37	12	3	59
Southland			5			5
Fischer	1		3	1		5
Lancaster			4			4
Lyon			2	1	1	4
567			6			6
561		1	3	1		5
383	1	1	2		1	5
BR-3	1	2	2			5
510			4			4
475		1	4			5
Total Southern	3	5	35	3	2	48
Grand total	3	12	72	15	5	107

The preliminary survey of a total of 107 plants in the species Bromus inermis showed that 35 of them were aneuploids. This was felt to be a rather striking result in view of the fact that according to available literature aneuploidy has not been reported previously for this species. Aneuploidy is fairly common, however, among grasses, especially the higher polyploids (7).

The preliminary survey indicated that some varieties exhibited less aneuploid variation than others. Some varieties, such as Southland, Lancaster, 567, and 510, appeared to have a "stable" chromosome complement, others, such as 194-12 and 475 only a slight variation, whereas still others, 216-20 and Fischer, seemed to exhibit a more extreme type of variation.

Some differences appeared to exist between the two types in the extent of the variation, the northern type having 37.29% aneuploidy while the southern type had only 27.09%.

The purposes of the second part of the investigations were to find out whether the assumed "stable" varieties were really more stable in their chromosome numbers than the others when a larger number of plants were examined, to study the extent of the aneuploid variation, and to detect real differences, if present, between the varieties and between the ecotypes in the extent of the aneuploid variation.

Of the southern types, Southland was selected to represent the "stable" type, while the variety Fischer represented the variable one. None of the northern varieties appeared to be stable. Canadian Commercial was selected because it represents the most common "variety" of northern brome grass, and in addition appeared to be somewhat more stable than the others. A strain 53-7 which promises to be of agronomic value, exhibited considerable variation in chromosome number, and was selected to represent the variable type.

The total aneuploid variation found within each of the four varieties is presented in Table III.

TABLE III

CLASSIFICATION OF PLANTS ACCORDING TO THEIR CHROMOSOME NUMBERS *

(Second Survey)

Varieties	Somatic Chromosome Numbers 49	54	55	56	57	58	Total
Canadian Comm.		1	5	19	5	1	31
53-7			1	22	7	2	32
Total Northern		1	6	41	12	3	63
Southland		4	6	24	2		36
Fischer	1	1	11	26	6	1	45
Total Southern	1	5	17	50	8	1	81
Grand total	1	6	23	91	20	4	145

*Plants from the same four varieties studied in the preliminary survey are included in this classification.

After extensive and thorough testing one plant of the Fischer variety was found to have 49 somatic chromosomes. This plant is not included in the aneuploid analysis, because it is not known whether its chromosome number represents aneuploidy or euploidy.

It is evident from Table III that the assumption that some of the varieties are more stable than others in their chromosome numbers is unfounded. The Southland variety which did not exhibit any variation in the preliminary survey showed variation from 54-57 chromosomes, similar to that of the other varieties.

The extent of the variation in chromosome number was found to be the same as revealed in the preliminary survey with the exception of the one plant which had 49 chromosomes. The total range found includes the following numbers: 49, 54, 55, 56, 57, and 58 (see Plates I, II, III, IV, V, and VI). According to available literature, all these numbers, with the exception of 56, are reported here for the first time. The numbers 28, 42, 62, and 70, which have been reported previously for this species were not found in any of the 222 plants examined.

Comparisons between the varieties are made in Table IV.

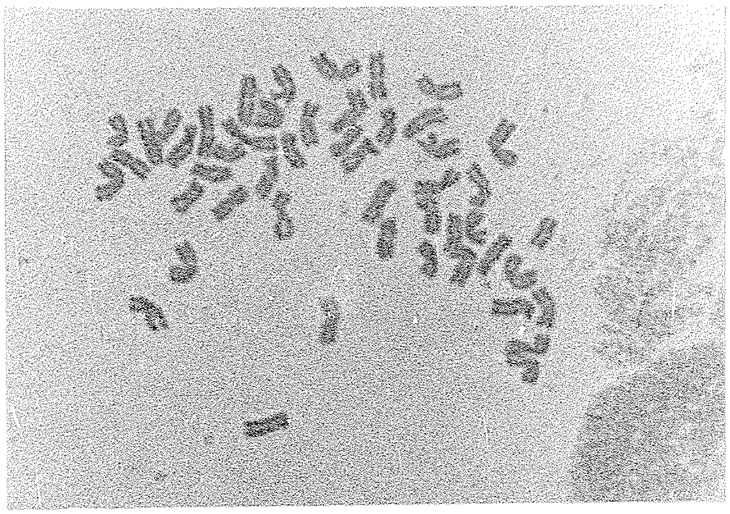

Plate I Mitotic metaphase,
49-chromosome plant (x 1600).

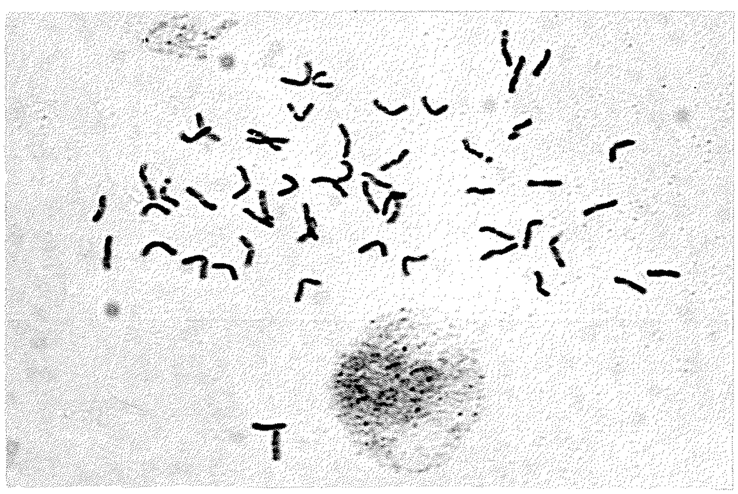

Plate II Mitotic metaphase,
54-chromosome plant (x 1200).

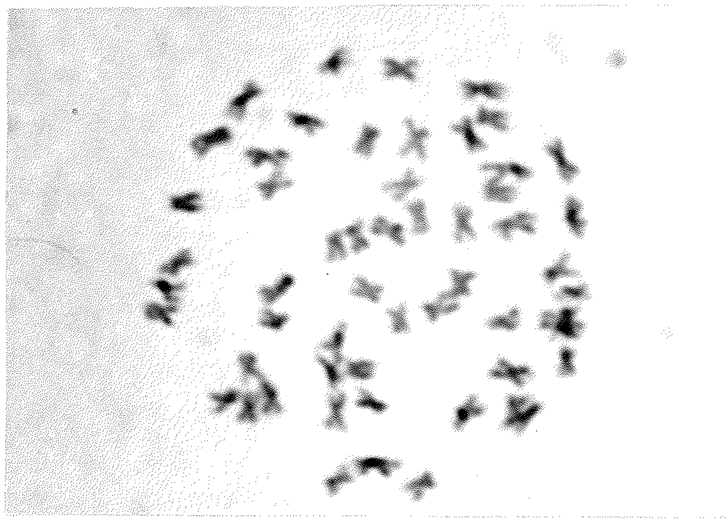

Plate III Mitotic metaphase,
55-chromosome plant (x 1600).

Plate IV Mitotic metaphase,
56-chromosome plant (x 1600).

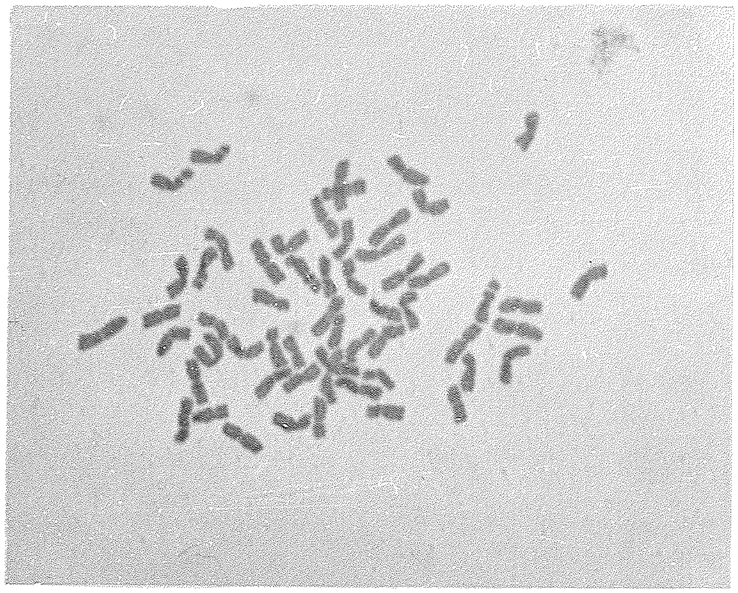

Plate V Mitotic metaphase,
57-chromosome plant (x 1100).

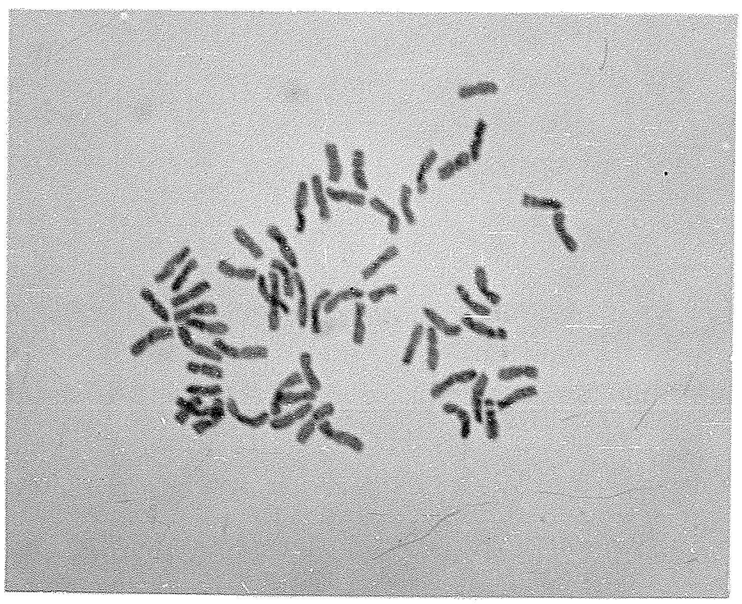

Plate VI Mitotic metaphase
58-chromosome plant (x 1100).

TABLE IV

ANALYSIS OF THE FREQUENCY AND DISTRIBUTION OF ANEUPLOIDY OF THE PLANT POPULATION STUDIED IN THE SECOND SURVEY

Varieties	Total Plants	Aneuploids		< 56		> 56	
		No.	%	No.	%	No.	%
Can. Com.	31	12	38.71	6	19.35	6	19.35
53-7	32	10	31.25	1	3.13	9	28.12
Southland	36	12	33.33	10	27.78	2	5.55
Fischer	45	19	42.22	12	26.67	7	15.55
Total	144	53	36.81	29	20.14	24	16.67

While there are some differences between the varieties in the percentage of aneuploidy, it is probably no more than an insignificant variation around the mean due to the relatively small numbers employed.

In comparing the frequency of aneuploidy of the southern and northern types of brome grass the results of the preliminary survey and the second phase of the study may be pooled. Table V shows the range in the aneuploid variation of the total population of 221 plants studied.

TABLE V

CLASSIFICATION OF ECOTYPES ACCORDING TO THEIR CHROMOSOME NUMBERS (RESULTS OF THE PRELIMINARY AND THE SECOND SURVEY COMBINED)*

Ecotypes	Somatic Chromosome Numbers					Total
	54	55	56	57	58	
Northern	1	12	66	18	5	102
Southern	7	22	77	10	3	119
Total	8	34	143	28	8	221

*The 49-chromosome plant is excluded.

The relative frequency of aneuploidy is shown in Table VI

TABLE VI

ANALYSIS OF THE FREQUENCY OF ANEUPLOID PLANTS WITH RESPECT TO ECOTYPES

Ecotypes	Number of plants			Percentage
	Euploid	Aneuploid	Total	
Northern	66	36	102	35.29
Southern	77	42	119	35.29
Total	143	78	221	35.29

It is evident that there is no difference in the frequency of aneuploidy between the northern and southern types of brome grass.

In Table VII the type of aneuploidy is considered, the classifications being based on whether the plants have one or two chromosomes missing, or one or two additional chromosomes. Since the proportion of aneuploidy was found to be equal within each of the northern and southern types, this feature can be examined directly without consideration of the euploid plants. The percentage of classified aneuploidy shown in Table VII is, therefore, based on the number of aneuploid plants within each type only.

TABLE VII

ANALYSIS OF THE DISTRIBUTION OF TYPES OF ANEUPLOIDY WITH RESPECT TO ECOTYPES

Ecotypes	Total Plants	Aneuploidy Number < 56	Percent < 56	Number > 56	Percent > 56
Northern	36	13	36.11	23	63.89
Southern	42	29	69.05	13	30.95
Total	78	42	53.85	36	46.15

It is obvious that the two ecotypes, northern and southern, do differ in the type of aneuploidy, the northern one exhibiting a higher frequency of plants having chromosome numbers in excess of 56, while the southern type shows the reverse situation.

In order to test the statistical significance of this difference, contingency tables were set up and the data tested for independence, using the statistic X^2.

The distribution of aneuploid chromosome numbers was found to be significantly different at the 1% level between northern and southern brome grass. Northern type brome grass has a significantly larger number of plants with chromosome numbers in excess of 56, while the southern type brome grass has a significantly larger number of plants having chromosome numbers lower than 56. No cytological difference between the two types of brome grass has been reported previously.

No information has been obtained in this study as to whether plants having additional chromosomes conform more specifically to the northern type of brome grass than plants with 56 chromosomes or less, or whether plants lacking one or two chromosomes conform more closely to the concept of the southern type than other plants do. It is of interest, however, at this point to refer to Table IV. In the last four columns the type of polyploidy is classified in the same way as in the aneuploid analysis, described earlier. The Canadian Commercial variety is a very heterogenous population of plants which cannot be recognized as belonging to one variety. It may be termed the "wild type" of northern brome grass, consisting of plants of diverse description. The strain 53-7 is an open-pollinated selection from the variety Superior which, in turn, is a selection from Canadian Commercial. Both Superior and strain 53-7 were selected and tested in areas where northern types are better adapted.

Thus the selection of 53-7 was presumably based on the typically northern characters found in Canadian Commercial. The data on Canadian Commercial presented in Tables III and IV indicate that the aneuploid distribution is symmetrical. The strain 53-7 is very different. Only one plant out of a total of 32 had a chromosome number lower than 56, but nine had chromosome numbers in excess of 56. The actual breakdown is shown in Table III. Whether the strain 53-7 conforms more closely to the northern type of brome grass than does the commercial variety is not known, and no further attempt to substantiate this hypothesis was made in this study.

The relationship between the two southern varieties, Southland and Fischer, is not very clear. Fischer originated as a local strain that appeared different from other types of brome grass, perhaps as a result of an introduction from a different area. Southland is of a more recent origin, resulting from bulking of strains from Oklahoma. It is likely that the relationship between these two southern strains is somewhat similar to that of the northern ones. The aneuploid distribution of these two varieties does not seem to be strikingly different. Southland, however, seems to have a preponderance of plants with missing chromosomes. Only eight of the 222 plants studied had 54 chromosomes. Four of these plants are from the Southland variety.

B. Meiotic Behavior

The study of meiotic behavior was generally found to be very difficult. The main difficulties encountered were: 1. rarity of suitable stages for the observation of pairing, 2. extreme stickiness and clumping of the chromosome at metaphase I, 3. inadequacy of the diakinesis stage for distinguishing between various types of chromosomal associations, although little stickiness occurred at this stage. These difficulties have been reported previously by most investigators of the cytology of brome grass. It was found, however, that meiotic chromosomes in a few plants that had flowered in the greenhouse during the winter showed much less stickiness and clumping than did chromosomes from plants that had flowered in the field during the summer (see Plates VII and VIII). All the material intended for this study was fixed during the summer flowering with the result that meiotic analysis became extremely difficult. The results reported here are thus incomplete, and can only give a very general picture of the meiotic behavior of the material studied. Due to lack of adequate data, no attempt has been made to analyse the results statistically in relation to the aneuploid distribution.

Meiosis was generally irregular. Bivalents ranged from 7 to 28. Only a few cells in one of the 56-chromosome plants was observed to have 28 bivalents. Univalents were

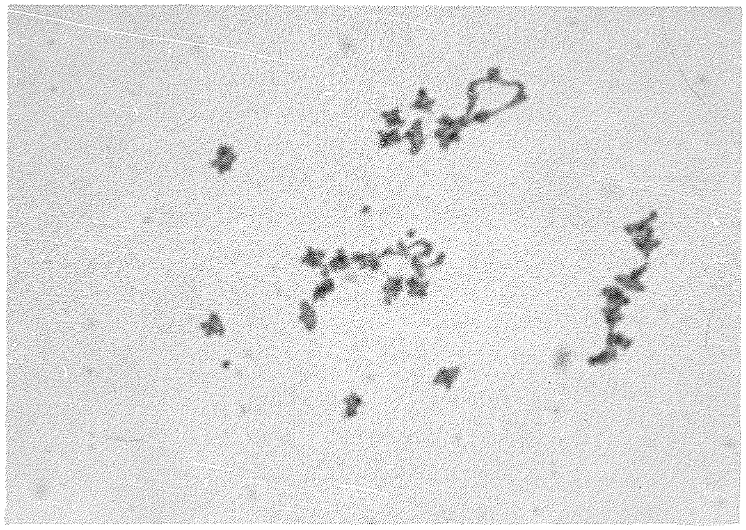

Plate VII Meiotic metaphase I, greenhouse material (x 1000).

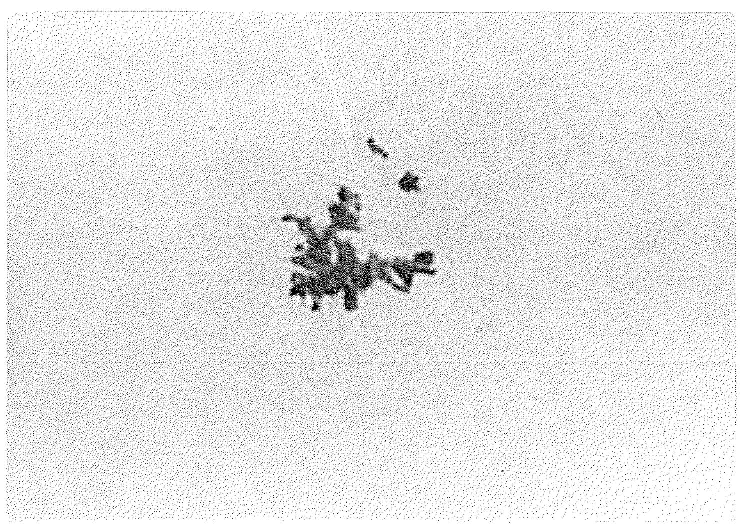

Plate VIII Meiotic metaphase I, field material (x 1000).

rather frequent, ranging from 1 to 3 per cell. Quadrivalents were found to be the most frequent type of multivalents. Only a few trivalents were observed. In many cells pairing was so complicated that sometimes the whole chromosome complement seemed to be involved. Sticky threads were noticeable in many diakinesis preparations, sometimes joining two or three bivalents or, occasionally, extended between all the chromosomes. The threads appeared to have the same color intensity as the chromosomes but were much narrower and appeared like projections from them.

Seven plants were studied at anaphase I and II for the occurrence of laggards. Two of these plants appeared to be free of laggards, but the remaining five had laggards in 25 to 50% of their anaphase cells. The number of laggards per cell ranged from 1 to 4 with their frequency declining in that order. Bridge formations were also noted occasionally (see Plate IX).

A study was made of the frequency of micronuclei in tetrads in the same seven plants. Micronuclei appeared on the average in 12.5% of tetrad cells, with a range from 0.1-22.4%. The four plants with the lowest percentage of micronuclei were the same four plants with the lowest frequency of laggards at anaphase.

Meiotic study in the 49-chromosome plants was hampered by the difficulties encountered in all the meiotic analyses.

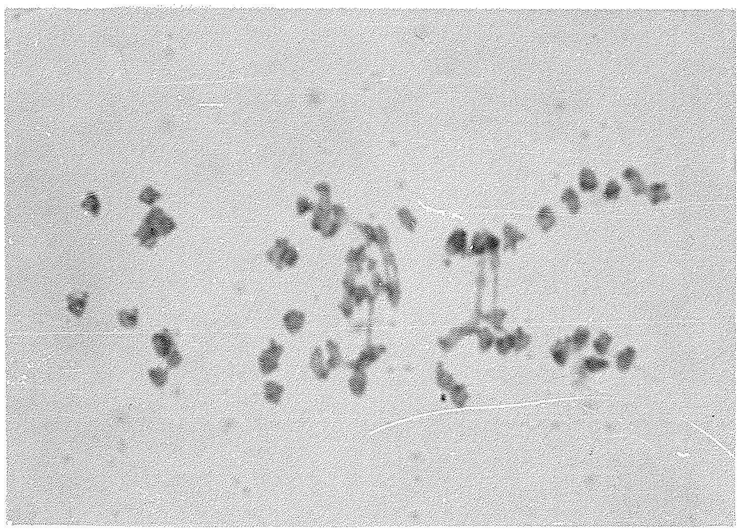

Plate IX Meiotic anaphase I, bridge formations evident (x 1000).

The question arises whether the complement of 49 chromosomes is due to one of the following possibilities:

1. Any seven chromosomes missing. Such a plant would be a true aneuploid.
2. A whole genome of seven chromosomes missing, or
3. The plant is a cross between a hexaploid, 42-chromosome and an octoploid, 56-chromosome brome grass. Such a plant would be euploid with a heptaploid chromosome complement. Only a thorough and extensive study of meiotic behavior, including crosses with other brome grass plants with different chromosome numbers, would reveal the origin of this plant. This was not done in the present study.

For meiotic study in the 49-chromosome plant only the diakinesis stage could be used, and this stage was found particularly inadequate for distinguishing between univalents and bivalents. A quadrivalent was observed in three of the 26 cells studied, the remaining cells having exclusively bivalents and univalents. In one cell it was possible to detect 21_{II} and 7_{I}. In another cell six univalents were positively identified. In each of the remaining 21 cells 28 chromosome entities were counted, but no distinction between univalents and bivalents was possible.

A total of 29 anaphase cells were studied, all having one or more laggards. The total breakdown is shown in Table VIII.

TABLE VIII

LAGGARD DISTRIBUTION, 49-CHROMOSOME PLANT

Laggards	No. of cells
1	8
2	7
3	5
4	2
5	4
6	1
7	2
Total	29

The frequency of laggards is high and the presence of up to seven laggards per cell reflects the high number of univalents found at diakinesis.

C. Pollen Analysis

Table IX presents the frequency and percentage of pollen in each class for 27 plants with six different chromosome numbers. All the pollen of class I and half the pollen of class II for the normal size were considered fertile. The percentage of fertile pollen for each chromosome number, and for all 27 plants, is also presented in Table IX. The percentage of fertile pollen is very low, with an average of 32.24% and a range in fertility among individual plants of from 12-61%. The absence of one chromosome, or the presence of one or two additional chromosomes does not appear to lower the fertility of the pollen. The fertility is, in fact, higher for the 57- and 58-chromosome plants than for the euploid 56-chromosome plants studied. The fertility of the four plants with two missing chromosomes appeared to be lower, and the 49-chromosome plant exhibited the lowest fertility recorded in this study.

The amount of abnormal pollen in all 27 plants indicates a high incidence of meiotic irregularity which is probably more affected by chromosomal abberations commonly present in allogamous plants, than by the degree of aneuploidy.

THE UNIVERSITY OF MANITOBA LIBRARY

TABLE IX POLLEN ANALYSIS

Chromosome Number	Number of Plants	Pollen Classes* Normal Size I	IIa	II	III	IV	V	VI	Smaller Size I	IIa	II	III	IV	V	VI	Total	Percent Fertile Pollen
49	1																
Total		38	101	403	160	5	0	1	38	0	4	11	5	0	0	766	11.55
Percent		5.0	13.9	52.6	20.9	0.6	0	1.1	5.0	0	0.5	1.4	0.6	0	0	100	
54	4																
Total		127	147	253	133	96	5	0	67	94	139	58	29	14	0	1162	17.26
Percent		10.9	12.6	21.8	11.6	8.3	0.4	0	5.8	8.1	12.0	5.0	2.5	1.2	0	100	
55	4																
Total		226	182	272	45	12	2	2	64	26	44	30	2	8	0	915	34.64
Percent		24.7	19.9	29.7	4.9	1.3	.2	.2	7.0	2.8	4.8	3.3	.2	.9	0	100	
56	11																
Total		1208	430	525	243	36	2	1	309	142	356	343	209	242	8	4054	35.10
Percent		29.8	10.6	13.0	6.0	.9	.1	.02	7.6	3.5	8.8	8.5	5.2	6.0	.2	100	
57	4																
Total		376	144	218	70	17	2	1	29	33	74	32	15	3	0	1014	44.18
Percent		37.1	14.2	21.5	6.9	1.7	.02	.1	2.9	3.2	7.3	3.2	1.5	0.3	0	100	
58	3																
Total		271	179	196	76	11	5	3	37	9	241	26	23	30	0	890	40.50
Percent		30.4	20.1	22.0	8.5	1.2	.6	.3	4.2	1.0	2.7	2.9	2.6	3.4	0	100	
Total (all plants)	27	2246	1183	1867	727	177	6	8	544	304	641	500	283	297	8	8801	32.24
% (all plants)		25.5	13.4	21.2	8.3	2.0	.2	.1	6.2	3.4	7.3	5.7	3.2	3.4	.1	100	

* I: one round-shaped and two thread-shaped nuclei (see Plates X, XII, and XIII).
IIa: a borderline class where the exact shape of one or two of the thread-shaped nuclei could not be determined (see Plate XIV).
II: three nuclei, either all round, two round and one thread-like, or all thread-like (see Plate XI).
III: only two nuclei present (see Plate X).
IV: one nucleus present (see Plate X).
V: pollen devoid of nuclei (see Plates XII and XIII).
VI: four or more nuclei present (see Plate XIV).

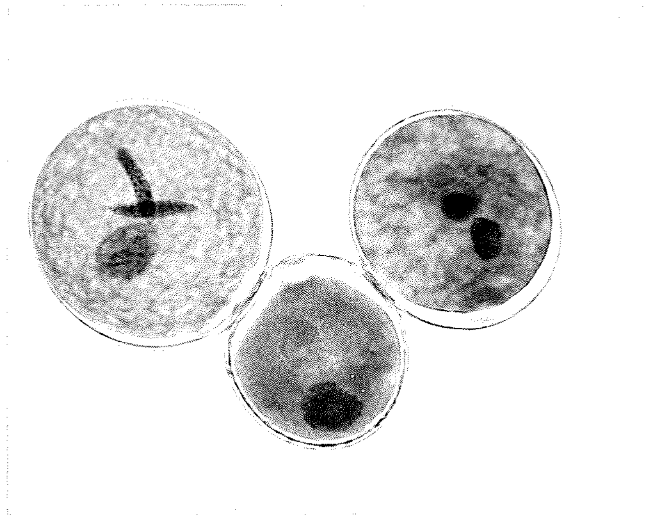

Plate X Pollen grains.
Class I Class IV ClassIII(x 1000).

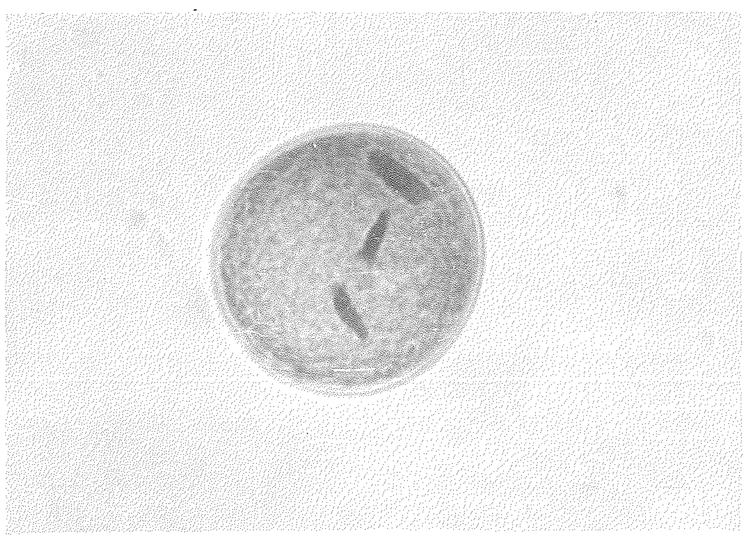

Plate XI Pollen grain.
Class II (x 1000).

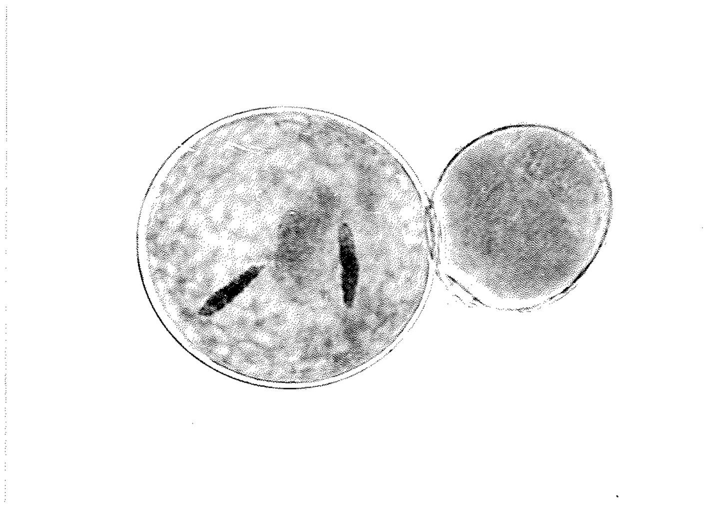

Plate XII Pollen grains.
Class I Class V (x 1000).

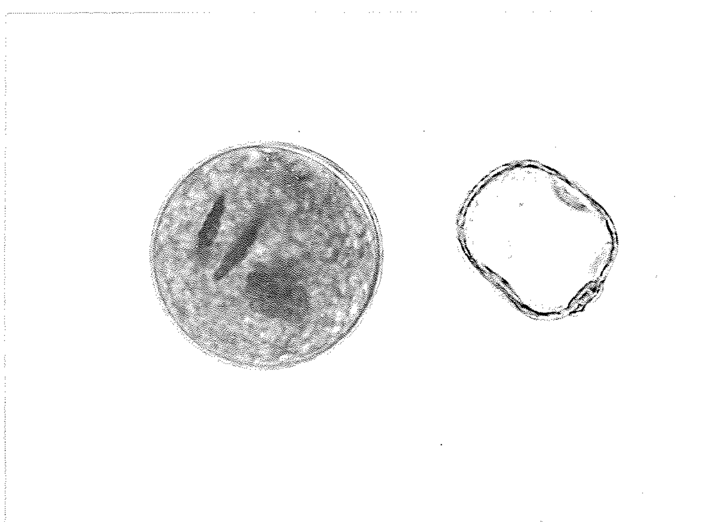

Plate XIII Pollen grains.
Class I Class V (x 1000).

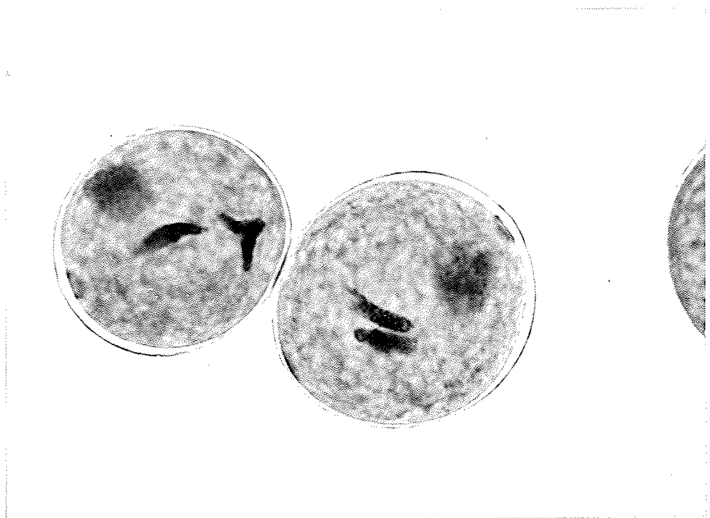

Plate XIV Pollen grains.
Class VI Class IIa (x 1000).

D. Morphological and Agronomical Characters in Relation to Chromosome Numbers, Varieties, and Ecotypes.

Eight morphological and agronomical characters were studied to determine the relationships, if any, between these characters and chromosome numbers, varieties or ecotypes. It would not be possible to make strictly valid comparisons in all instances, due to the fact that the numbers of plants in the various chromosome groups are extremely variable. This is evident from Table X, where the number of plants in each chromosome group is reported. It would be impossible to obtain the same number of plants with 49 chromosomes as with 56 chromosomes. Similarly it would be difficult to locate equal numbers of plants in the other aberrant chromosome groups, 54, 55, 57, and 58.

TABLE X

FERTILITY RATIO VS. CHROMOSOME NUMBERS

Chromosome Number	Number of Plants	Fertility Ratio* Range	Mean
49	1	-	.067
54	6	.027-.557	.342
55	23	.068-.636	.450
56	91	.009-.692	.470
57	20	.328-.664	.513
58	4	.105-.503	.303

*Ratio of cleaned seed weight to weight of unthreshed panicle.

The data in Table X with respect to fertility ratio means of the various chromosome groups show a definite trend to lower fertility ratios for those groups most divergent in chromosome number from the normal complement of 2n = 56.

The data indicate that the presence of one extra chromosome or the absence of one chromosome does not appear to materially affect the fertilitly ratio of such abnormal plants. However, the fertility ratio of plants with either two extra or two missing chromosomes is considerably lower, and the fertility ratio of the one 49-chromosome plant is very low. When the individual plants were considered, it was found that four 56-chromosome and one 54-chromosome plant had lower fertility ratios than the 49-chromosome plant. This suggests the influence of other factors besides chromosome number in modifying the fertility ratio.

Comparisons of chromosome groups with respect to plants having fertility ratios higher than .500 and .600 respectively may be made from Table XI.

TABLE XI

CLASSIFIED FERTILITY RATIOS

Chromosome Number	Fertility Ratio* % over .500	% over .600
54	17	0
55	50	18
56	55	24
57	55	15
58	25	0

*Ratio of cleaned seed weight to weight of unthreshed panicle.

Six 56-chromosome plants were also found to have a higher fertility ratio than any of the other plants. This fact, and the greater percentage of 56-chromosome plants shown in Table XI to have a fertility ratio higher than .600, suggests that a majority of highly fertile panicles occurs in the euploid portion of the population. The distribution of highly fertile plants puts further emphasis on the fact that two additional or two missing chromosomes may modify the fertility ratio to a greater degree than does one additional or one missing chromosome.

The fertility ratio was found to be significantly different with respect to varieties and ecotypes. For this comparison, the data are more reliable since the number of plants in each class is more nearly equal. The fertility ratios for the varieties and ecotypes are shown in Tables XII and XIII.

TABLE XII

FERTILITY RATIO VS. VARIETIES

Varieties	Number of plants	Fertility Ratio* Range	Fertility Ratio* Mean
Can. Comm.	31	.232-.692	.509
53-7	32	.105-.678	.523
Southland	36	.010-.666	.424
Fischer	46	.009-.667	.420
L.S.D. (5%)			.08
(1%)			.10

*Ratio of cleaned seed weight to weight of unthreshed panicle.

TABLE XIII

FERTILITY RATIO VS. ECOTYPES

Ecotypes	Number of plants	Fertility Ratio* Range	Mean
Northern	63	.105-.692	.516
Southern	82	.009-.667	.422
L.S.D. (5%)			.06
(1%)			.08

*Ratio of cleaned seed weight to weight of unthreshed panicle.

The northern ecotype, which includes the varieties Canadian Commercial and 53-7, has a significantly higher fertility ratio than the combined ratio of the southern varieties Soutland and Fischer. This agrees with other findings that southern varieties are less fertile than northern ones under northern conditions (19).

The panicle heights of plants with the different chromosome numbers are presented in Table XIV.

TABLE XIV

HEIGHT VS. CHROMOSOME NUMBERS

Chromosome Number	Number of Plants	Height* Range	Mean
49	1	-	2.83
54	6	2.83-4.33	3.45
55	23	2.50-4.08	3.38
56	91	2.25-4.17	3.33
57	20	2.58-4.17	3.47
58	4	2.25-3.08	2.65

*Height is given in feet.

The variable numbers of plants in the different chromosome groups precludes the possibility of a statistical analysis of height data. The data indicate, however, that height tends to be lower in the plants represented in the chromosome groups of 2n = 49 and 2n = 58.

Differences between the four varieties and between the two ecotypes with respect to creeping vigor were found to be significant. The mean scores and ranges for creeping vigor with respect to varieties and ecotypes are presented in Tables XV and XVI.

TABLE XV

CREEPING VIGOR VS. VARIETIES

Varieties	Number of Plants	Creeping Vigor* Range	Mean
Can. Comm.	31	4-9	7.03
53-7	32	4-9	6.53
Southland	36	5-9	7.69
Fischer	46	4-9	7.02
L.S.D. (5%)			0.55
(1%)			0.73

*Least vigorous: 1, most vigorous: 10.

TABLE XVI

CREEPING VIGOR VS. ECOTYPES

Ecotypes	Number of Plants	Creeping Vigor* Range	Mean
Northern	63	4-9	6.78
Southern	82	4-9	7.36
L.S.D. (5%)			0.40
(1%)			0.52

*Least vigorous: 1, most vigorous: 10.

The data indicate that the mean creeping vigor of the Southland plants is considerably higher than that of the other varieties.

The statistical analyses of the data on general vigor indicate that significant differences also exist between the varieties and between the ecotypes with respect to this character. From Table XVII it may be seen that the strain 53-7 is a considerably more vigorous variety than the remaining three,

TABLE XVII

GENERAL VIGOR VS. VARIETIES

Varieties	Number of Plants	General Vigor* Range	Mean
Can. Comm.	31	2-10	7.19
53-7	32	5-9	7.97
Southland	36	2-9	5.95
Fischer	46	2-9	6.52
L.S.D. (5%)			0.87
(1%)			1.15

*Least vigorous: 1, most vigorous: 10.

Although the northern varieties do not spread as much as the southern varieties, it may be seen in Table XVIII that they are generally more vigorous.

TABLE XVIII

GENERAL VIGOR VS. ECOTYPES

Ecotypes	Number of Plants	General Vigor* Range	Mean
Northern	63	2-10	7.58
Southern	82	2-9	6.22
L.S.D. (5%)			0.63
(1%)			0.84

*Least vigorous: 1, most vigorous: 10.

In contrast to this, no significant differences were found with respect to the hay vigor score given to the plants in 1955. Furthermore, no significant differences between either chromosome number, varieties, or ecotypes were found with respect to seed yield, panicle production, or leaf width.

CONCLUSIONS AND SUMMARY

The thesis project was centered around a study of somatic chromosome numbers in the species *Bromus inermis* Leyss. Special emphasis was placed on the detection of possible differences between northern and southern types of brome grass with respect to somatic chromosome numbers. The project included, in addition, a study of some morphological and agronomical characters and their relation to chromosome numbers, varieties, and ecotypes. A limited study of meiotic behavior and pollen fertility of the species was also carried out.

The study of somatic chromosome numbers consisted of two main phases; a preliminary survey of ten varieties and strains of southern brome grass and six varieties and strains of northern brome grass, and a secondary survey involving a larger number of plants of each of two northern and two southern types. The morphological and agronomical data were obtained from the plants studied in the secondary survey, and the limited meiotic study involved a few plants also belonging to the four varieties under investigation in this survey.

The following results are reported:

A. Somatic chromosome numbers:

1. In the preliminary survey of somatic chromosome numbers, 35 out of 107 plants studied were found to be aneuploid

with the following chromosome numbers reported: 54, 55, 56, 57, and 58. This appears to be the first report on aneuploidy in brome grass. The preliminary survey suggested that some varieties exhibited less aneuploidy than others. That is, some varieties appeared to be more "stable" with respect to their chromosome complement than others.

2. All four varieties included in the more intensive second survey appeared to exhibit the same degree of aneuploidy; no variety appeared more stable than the others.

3. In addition to the chromosome numbers found in the preliminary survey, one plant of the southern variety Fischer was found to have 49 somatic chromosomes. This has apparently not been reported previously for this species.

4. When chromosome numbers of plants from both the preliminary and the second survey were studied together, the two ecotypes, northern and southern brome grass, were found to show a highly significant difference with respect to the type of aneuploidy exhibited by each. A greater proportion of the northern aneuploids had chromosome numbers higher than 56 while a majority of the southern aneuploids had one or two missing chromosomes. This difference was found to be significant at the 1% level. This appears to be the first report of any cytological difference between the two ecotypes.

5. The percentage of total aneuploidy was found to be

equal for southern and northern brome grass.

B. Meiotic study.

1. The meiotic study was found to be very difficult due to extreme stickiness and clumping of the chromosomes at metaphase I. A limited study of diakinesis indicated irregular meiosis, often exhibiting extremely complex pairing.

2. Laggards were found to be frequent at anaphase, and the number of micronuclei per tetrad cell appeared to be high and related to the number of laggards at anaphase, although the numbers observed were not sufficient to minimize the occurrence of such a relationship by chance.

3. Pollen fertility was found to be generally low, and the number and shape of nuclei variable, reflecting the irregularities at meiosis.

C. Morphological and agronomical characters in relation to chromosome numbers, varieties, and ecotypes.

Eight morphological and agronomical characters were studied: Fertility ratio, seed yield, panicle production, leaf width, creeping vigor, general vigor, and hay vigor.

1. Some evidence is presented that fertility ratio may be related to chromosome number, varieties, and ecotypes. The presence of one additional chromosome or the absence of one chromosome from the normal complement of 56 did not appear to interfere with the fertility ratio. The presence of two

additional chromosomes or the absence of two chromosomes resulted in reduced fertility. The fertility ratio of the 49-chromosome plant was found to be very low. A greater percentage of plants with highly fertile panicles was found among the euploid portion than among the aneuploid portion of the plants. The two southern varieties are less fertile than the northern ones under Manitoba conditions.

2. Some evidence is presented that height may be related to chromosome number. Plants with the extreme variation in chromosome number were shortest.

3. Creeping vigor was found to be significantly related to varieties and ecotypes, the southern varieties being more vigorous than the northern ones.

4. In contrast to this, the northern type varieties had a significantly higher mean for general vigor than the southern ones.

LITERATURE CITED

1. Atwood, S. Cytogenetics and breeding of forage crops. Advances in Genetics I: 1-67, 1947.

2. Avdulov, N. P. Karyo-Systematische Untersuchung der Familie Gramineen. Bull. Appl. Bot. Gen. and Plant Br., Suppl. vol. 44: 1-428, 1931.

3. Barnett, F. L. A karyological survey of several Bromus species. Agron J. 47: 88-91, 1955.

4. Cheng, C. Self-fertility studies in three species of commercial grasses. J. Am. Soc. Agron. 38: 873-881, 1946.

5. Churchill, B. R. Productiveness of brome grass strains from different regions when grown in pure stands and in mixtures with alfalfa in Michigan. J. Am. Soc. Agron. 39: 750-765, 1947.

6. Cugnac, A. and Simonet, M. Les nombres de chromosomes de quelques espéces du genre Bromus (Graminées), C. R. Soc. Biol. Paris. 135: 728-731, 1941.

7. Darlington, C. D. and Wylie, A. P. Chromosome atlas of flowering plants. George Allen and Unwin Ltd. 1955.

8. De Robertis, E. D. P., Nowinski, W. W., and Saez, F. A. General cytology. W. B. Saunders Company, 1954.

9. Dixon, W. J. and Massey, F. J. Introduction to statistical analysis. McGraw-Hill Book Co., Inc., 1951.

10. Elliott, F. C. The cytology and fertility relations of Bromus inermis and some of its relatives. Agron. J. 41: 298-303, 1949a.

11. Elliott, F. C. Cross-fertility and cytogenetics of selected Bromopsis section members within the genus Bromus L. Iowa State Coll. Journal Sci. 24: 44-45, 1949b.

12. Elliott, F. C. and Love, R. M. The significance of meiotic behavior in breeding smooth brome grass, _B. inermis_ Leyss. J. Am. Soc. Agron. 40: 335-341, 1948.

13. Elliott, F. C. and Wilsie, C. P. A fertile polyhaploid in _Bromus inermis_. J. Heredity 39: 376-380, 1948.

14. Hill, H. D. and Myers, W. M. Chromosome numbers in _Bromus inermis_ Leyss. J. Am. Soc. Agron. 40: 466-469, 1948.

15. Kihara, H. Genome Analyse bei Triticum und Aegilops VII Kürz Übersicht über die Ergebnisse der Jahre 1934-36. Mem. Coll. Agric. Kyoto Imp. Univ. 41: 1-61, 1937.

16. Knobloch, I. W. Morphological variations and cytology of _Bromus inermis_. Bull. Torr. Bot. Club 70: 467-474, 1943.

17. Knobloch, I. W. Tetraploid smooth brome grass. Bull. Torr. Bot. Club 80: 131-135, 1953.

18. Knowles, P. F. Interspecific hybridization in _Bromus_. Genetics 29: 128-140, 1944.

19. Knowles, R. P. and White, W. J. The performance of southern strains of brome grass in Western Canada. Sci. Agr. 29: 437-450, 1949.

20. Kramer, H. H. Morphologic and agronomic variation in _Poa pratensis_ in relation to chromosome numbers. J. Am. Soc. Agr. 39: 181-191, 1947.

21. Löve, Áskell and Löve, Doris. Cyto-taxonomical studies on boreal plants III. Some new chromosome numbers of Scandinavian plants. Archiv. för Botanik. 31, no. 12: 1-23, 1944.

22. Love, R. M. Interspecific and intergeneric hybridization in forage crop improvement. J. Am. Soc. Agr. 39: 41-46, 1947.

23. Müntzing, A. The effects of chromosomal variation in _Dactylis_. Hereditas 23: 113-227, 1937.

24. Myers, W. M. Cytology and genetics of forage grasses. Bot. Review 13: 319-421, 1947.

25. Newell, L. C. and Keim, F. D. Field performances of brome grass strains from different regional seed sources. J. Am. Soc. Agr. 35: 420-434, 1943.

26. Nielsen, E. L. Grass studies III. Additional somatic chromosome complements. Am. J. Bot. 26: 366-371, 1939.

27. Nielsen, E. L. Macrosporogenesis and fertilization in B. inermis. Am. J. Bot. 34: 431-433, 1947.

28. Nielsen, E. L. Cytology and breeding behavior of twin plants of B. inermis. Bot. Gazette 113; 23-54, 1951.

29. Schulz-Schaeffer, J. Cytologische Untersuchungen in der Gattung Bromus Leyss. Zeitschrift für Pflanzenzüchtung 35: 297-320, 1956.

30. Sharp, L. W. Fundamentals of cytology. McGraw-Hill Book Company, Inc., 1943.

31. Stählin, A. Morphologische und zytologische Unterzuchungen an Gramineen. Wissenschaftliches Archiv. F. Landwirtsch. Abt. A. Pflanzenbau. 1: 330-398, 1929.

32. Stebbins, G. L. Jr. The evolutionary significance of natural and artificial polyploids in the family Gramineae. Proc. 8th International Congr. Gen. (Hereditas supp. vol.). 461-485, 1947.

33. Stebbins, G. L. Jr. Types of polyploids. Their classification and significance. Adv. in Gen. I: 403-429, 1947.

34. Stuckey, I. H. and Banfield, W. G. The morphological variations and the occurrence of aneuploids in some species of Agrostis in Rhode Island. Am. J. Bot. 33: 185-190, 1946.

35. Truscott, J. D. Unpublished data. Plant Science Div., U. of Man.

36. Zherebina, Z. N. Botanical-agronomical studies of awnless brome grass (*Bromus inermis* Leyss.). Bull. Appl. Bot. Gen. and Plant Br. 25: 201-352, 1931.