

CYTOGENETIC, MORPHOLOGIC AND AGRONOMIC ASPECTS
OF THE
BOREAL NORTH AMERICAN BROMOPSIS AND CLOSELY RELATED TAXA

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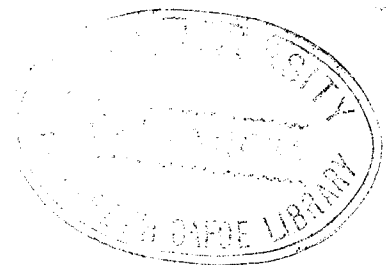
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A THESIS

Submitted to the Faculty of Graduate Studies
in Partial Fulfilment of the Degree of
Doctor of Philosophy

University of Manitoba

October, 1965



ACKNOWLEDGEMENTS

The author wishes to acknowledge financial aid from the National Research Council of Canada and the Rockefeller Foundation. He would like to thank Dr. A. H. Mick, Director of the Alaska Agricultural Experiment Station for use of Station facilities; and to express gratitude to the various staff members of the University of Manitoba and the Alaska Agricultural Experiment Station for assistance given generously in innumerable ways. Valuable assistance was contributed to the thesis by the late J. G. Dickson who, as head of the Alaskan Rockefeller project, authorized the study on plant materials collected by him. Particular thanks are due Mr. R. L. Taylor for collecting plant materials from the Palmer nursery and forwarding them to Winnipeg; to Dr. A. K. Storgaard for collection of agronomic data at Winnipeg during the author's absence; to Mr. D. N. Fox who assisted with the photography; and to Dr. J. D. Truscott for guidance and assistance throughout the investigation.

ABSTRACT

A collection of Alaskan bromegrasses was examined cytologically, morphologically, and agronomically. The various taxa were inter-crossed, and several were hybridized with Bromus inermis Leyss. Another species of Russian origin was also examined.

An attempt to reduce B. inermis to a lower ploidy level and so facilitate further cytogenetic investigations was unsuccessful. No polyhaploids were found among over 300 sets of B. inermis twin seedlings, but a diplo-triplo combination was found.

Cytological investigations showed that B. inermis, Alaskan B. pumpellianus, Scribn. and Russian B. ornans, Kom. were octoploids; a previously unreported taxon discovered in Alaska, and Alaskan B. pacificus Shear were diploids. Aneuploids, and plants possessing possible accessory chromosomes, were found among the B. pumpellianus. Studies of satellited chromosomes indicated that the octoploids were alloautoploids, and the Unknown Taxon was an allopolyploid. Satellite chromosomes of the Unknown Taxon, B. pumpellianus, B. inermis, and B. ornans were similar, and differed from satellite chromosomes of B. pacificus and B. ciliatus.

B. pumpellianus was shown to be morphologically variable but separable from B. inermis on the basis of degree of spread, awn length and leaf height in midsummer.

Crosses of B. inermis with most B. pumpellianus plants and B. ornans were successful as were those between the Unknown Taxon and B. pumpellianus and tetraploid B. inermis. Crosses between the Unknown Taxon and B. pacificus and B. ciliatus were unsuccessful. Certain villous B. pumpellianus plants could not be crossed with B. inermis.

Vivipary was recognized for the first time in B. pumpellianus and

in the Unknown Taxon. It occurred sporadically but widely in B. pumpellianus but not in B. inermis. This interspecific difference, morphological differences, and results of hybridization experiments indicated that most Alaskan forms of B. pumpellianus have diverged considerably from cultivated B. inermis. The Unknown Taxon was closely related to these two species but not to B. pacificus and B. ciliatus. B. pumpellianus appeared to be agronomically inferior to B. inermis in seed production and quality of forage. Desirable characteristics such as lack of spread and early forage production may be transferred from B. pumpellianus to B. inermis through artificial hybridization.

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GENERAL INTRODUCTION

Smooth brome grass, Bromus inermis, Leyss. is widely distributed and used. Originating in central Europe, it is now found over nearly all of that continent, in Eurasia, and in North America. Congeneric and consectional species also occur in these areas. Particularly close relatives are found among both Russian and North American bromes (Komarov, 1934; translated 1963).

Of the indigenous North American species B. pumpellianus Scribn. is undoubtedly the most closely related, having been proposed as the North American counterpart of B. inermis (Elliott, 1949a). Morphologically diverse forms of B. pumpellianus occur in large numbers in the northern part of the continent and are particularly abundant in Alaska (Hulten, 1942; Anderson, 1959; Wiggins and Thomas, 1962).

Speculation arises as to the phylogenetic relationships of these morphological types. Do the various octoploid forms belong to one population? Is their variability indicative of varying genotypic relationships with B. inermis? Are they segregating offspring after hybridization of the two species, or is the variability merely a demonstration of natural ecotypic variation? The answers to these questions have direct agronomic ramifications. Certain forms of B. pumpellianus possess traits that might be desirably incorporated into B. inermis (Hanna, 1961; Johnston and Bezeau, 1962). The relative genotypic affinities of these forms with cultivated brome may determine the probability of success of any hybridization program involving the two species. The degree of ecotypic variation in the

native bromegrass may determine the amount of sampling of the native population required to find agronomically useful traits. If B. pumpe-
lianus is to be used as a source of germ plasm to improve B. inermis its phylogenetic position should be determined more exactly.

Since it is thought that some Russian bromegrasses are close relatives of the above species (Elliott, 1949a; Wagnon, 1952) they may also represent a potential source of germ plasm for the improvement of smooth bromegrass. Their phylogenetic position is therefore also of importance.

The present study has been an examination of a morphologically and geographically diverse group of Alaskan bromegrasses to determine their relationship to B. inermis and to assess partially their agronomic potential. At the same time, the relationship of two Russian bromegrasses to B. inermis was investigated.

The first section of the thesis was devoted to a study of a technique designed to facilitate the later phylogenetic investigation. Since this forms a separate entity within the thesis it will be reported separately.

PART A
A CYTOLOGICAL AND MORPHOLOGICAL STUDY OF TWIN
SEEDLINGS IN BROMUS INERMIS LEYSS.

INTRODUCTION

B. inermis Leyss. and B. pumpellianus Scribn. are octoploids ($2n = 56$) and the former is believed to be an alloautoploid (Carnahan and Hill, 1960; Ghosh and Knowles, 1964). Both species, possibly because of their complex polyploid nature, are considered difficult to study either genetically or cytogenetically (Elliott, 1949b; Nielsen, 1951; Barnett, 1955). A reduction in the chromosomal complement to the haploid or, more correctly, polyhaploid ($2n = 28$) level would facilitate studies of: karyology, because the fewer chromosomes present the easier that they might be separated for measurement and the less chromosomal pre-shrinking required; genetics, because the approach to homozygosity is much quicker at lower ploidy levels than at higher ones; and chromosomal pairing relationships, because meiotic configurations might be more easily discerned at the lower levels of ploidy. Also, fertility or sterility of the polyhaploid might be suggestive of allo- or autopoloidy in the species. Hybridization and breeding of these bromegrasses might be facilitated since, as pointed out by Chase (1964), desirable germ plasm might be selected at the lower ploidy levels and through hybridization and doubling of the chromosome complement combined into amphiploids. A concentrated effort was therefore made to find haploids. It was decided to investigate B. inermis first and later to study B. pumpellianus if the first experiment was successful.

LITERATURE REVIEW

The frequency of twinning varies in different species within the Gramineae. Wilson and Ross (1961) found the frequency of twins to be one in 3,200 seedlings of winter wheat (Triticum aestivum). Kihara (1940) reported no twins in progeny of Triticum monococcum from self- or open-pollination but a frequency of 0.78 percent after cross-pollination. In a delayed pollination experiment he observed three pairs of twins among 195 seedlings of T. monococcum. Smith (1946) failed to obtain twins from 2,855 seedlings from naturally self-pollinated spikes of T. monococcum. Nielsen (1951) found an average of only one set of twins in 550 seeds of B. inermis.

The frequency with which haploids occur in pairs of twins also is quite variable. In T. aestivum frequencies of one haploid in 426, 58 and 38 twins have been found by Muntzing (1938), Namikawa and Kamakami (1934), and Wilson and Ross (1961), respectively. Among nine perennial grasses Muntzing (1938) found varying frequencies according to genera and species. No haploids were found in Festuca pratensis, ovina or rubra, or in Lolium perenne, Agrostis stolonifera, Cynosurus cristatus; but frequencies of one in 99, 135 and 148 twins occurred in Dactylis glomerata, Poa pratensis and Phleum pratense, respectively. Dewey (1961, 1964) found one haploid for each 281 twin plants of Agropyron desertorum and two haploids from 200 plants of Agropyron intermedium. Only one case has been reported of a polyhaploid occurring in twins of B. inermis (Nielsen, 1951) although a presumed polyhaploid

of unknown origin has been reported (Elliott and Wilsie, 1948).

In some species triploids are found more frequently than haploids among twins. Muntzing (1938) found 77 triploids and only 11 haploids among the 16 species of 11 genera that he examined. Wilson and Ross (1961) found five triploids and four haploids in a population of T. aestivum. Yamamoto (1936) also reported that triplo-diplo twins were more common than haplo-diplo twins in T. aestivum, but suggested that there might be a variety difference in this regard.

MATERIALS AND METHODS

Seed from the B. inermis varieties Wisconsin 81, Lancaster, Manchester, Achenbach, Wisconsin 55, Lyon, Homesteader, Saratoga, and Southland was received from the Agricultural Research Service, United States Department of Agriculture, Beltsville, Maryland. Canadian commercial bromegrass was obtained from a local seed company.

Seed was placed on moist blotting paper in 10- x 20- inch metal trays and germinated in a standard germinator set at 65°F. When the seedlings were approximately 2 inches in height the frequency of twins was recorded. The twins were removed from the tray, planted in vermiculite for 1 week, separated, cytologically analyzed and replanted in 3 inch clay pots. Because the varieties Saratoga and Wisconsin 81 appeared to be good sources of twins, additional seedlings of these varieties were screened.

For cytological analysis, root-tips were pre-treated with tap

water at 32-34°F. for 24 hours and fixed in Farmer's solution. After fixation the root-tips were hydrolyzed for 12 minutes in N HCL, stained with Feulgen, and squashed in aceto-carmin.

All observations of morphological characteristics were made on living material grown in the greenhouse. Height and degree of pubescence were recorded when seedlings were 1 month old. Where a difference in the level of ploidy was noted, stomata were measured on each twin according to the method outlined by Sarvella et al. (1961) with the exception that the upper surface of the leaf was studied instead of the lower surface, since more stomata are present on the upper surface of leaves of grasses (Wagnon, 1952). To be consistent, measurements were taken 2 cm from the ligule on the fifth leaf of each culm. Five stomata were measured from individual leaves of 25 culms of each plant.

RESULTS AND DISCUSSION

Twins were found to occur in varying ratios according to the variety from 1:85 for Saratoga to 1:1000 for Southland (Table 1). In addition 1 triplet was found in the total of 80,492 germinated seeds examined.

TABLE 1. Frequency of twinning in germinated seed lots of varieties of Bromus inermis

Variety	No. of seeds germinated	No. of sets of twins	Ratio of twins/seeds
Commercial	10,398	18	1:578
Wisconsin 81	40,594	87	1:467
Lancaster	5,254	8	1:657
Manchar	2,499	6	1:416
Achenbach	3,395	9	1:377
Wisconsin 55	5,393	9	1:599
Lyon	2,670	5	1:534
Homesteader	5,869	8	1:734
Saratoga	12,420	146	1:85
Southland	2,000	2	1:1000
Total	80,492	298	1:545 ¹

¹Weighted ratio

Variability in frequency of B. inermis twins is not a new phenomenon. Nielsen (1951) reported variation in frequency of twinning within separate populations of B. inermis and noted one extreme case in which a plant had 8 percent twins among its offspring. One or two such plants in the ancestry of Saratoga could easily account for the relatively high frequency of twins observed in this variety. The weighted ratio of total number of twins to seedlings examined was 1:545 (Table 1) which is remarkably similar to the ratio of 1:550 reported by Nielsen. A single seed lot of B. pumpellianus investigated produced one twin among 572 seeds.

Eighty pairs of twins from the variety Saratoga were classified according to the degree of pubescence (Table 2). In about two-thirds of the pairs, the numbers were similar in this characteristic. If these were identical twins they could have arisen through sporophytic embryony or cleavage of the zygote. Dewey (1964) has pointed out that in crosses, monozygotic twins are usually observable as coming from a single coleoptile. In the present study occasional seedlings arose in this manner. The high frequency of seemingly identical twins suggests that in some cases at least, sporophytic embryony may have occurred.

TABLE 2. A comparison of pubescence on leaves of 80 sets of twins in the variety Saratoga

Comparison	Classification of the 80 sets for pubescence on	
	Sheath	Blade
Twin members similar		
Both glabrous	5	1
Both sparsely pubescent	3	4
Both pubescent	43	49
Both pilose	5	3
Sub-total	56	57
Twin members different		
One glabrous, one sparsely pubescent	2	5
One glabrous, one pubescent	10	15
One glabrous, one pilose	5	2
One sparsely pubescent, one pilose	4	0
One pubescent, one pilose	3	1
Sub-total	24	23
Total	80	80

In the remaining third of the population however, where the twin pairs differed in appearance, it would appear that either two pollen

grains had been involved in fertilization (fertilizing one egg and some other cell of the gametophyte); or two cells of the gametophyte had fused to produce one plant parthenogenetically and the egg had been fertilized in the normal manner to produce the other. If the latter were true, then one would expect that parthenogenetic development of a single haploid cell would also take place occasionally to produce haploid plants. Since there was none, it seems more likely that two pollen grains fertilized the gametophyte simultaneously.

A total of 556 seedlings derived from the 298 pairs of twins were cytologically checked for chromosome number. The remaining 40 seedlings died before they could be examined. It should be pointed out that these seedlings were lost at the beginning of the experiment before the environmental conditions were controlled or techniques well developed. In view of subsequent results it seems improbable that these plants were haploid. All of the seedlings examined, including those from B. pumpellianus, were diploid except for one pair which contained 57 and 83 chromosome individuals (Figure 1). At the time this was found it was the first report of 12-ploid B. inermis plant. Since then Ghosh and Knowles (1964) have reported finding another. B. arizonicus, a natural hybrid between B. cartharticus and B. trinii, is the only naturally occurring 12-ploid Bromus species, although higher ploidy has been induced artificially in several cases (Stebbins, 1949).

There are several possible explanations for the formation of the diplo-triplo combination. Two of these are presented here: Since aneuploidy is involved it is probable that non-disjunction of one chromosome occurred in a mitotic division in the formation of the nuclei in

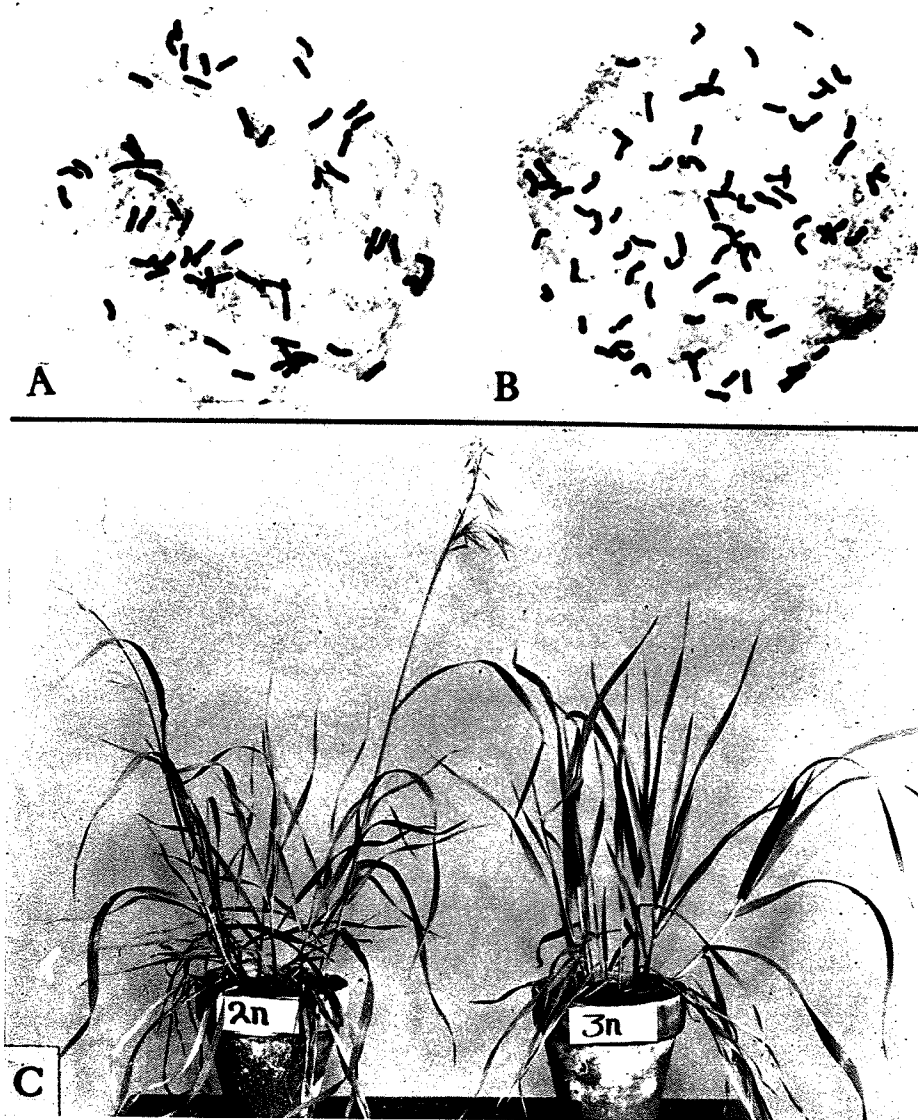


FIGURE 1. Aneuploid diplo-triplo twins in *Bromus inermis*:

A. Mitotic metaphase in root-tip cell of diploid twin having $2n = 57$ chromosomes.

B. Mitotic metaphase in root-tip cell of triploid twin having $2n = 83$ chromosomes.

C. Diploid twin on left; triploid on right.

the female gametophyte. One of the synergids could have received 27 chromosomes and the egg 29. If these were fertilized by two pollen grains, one male gamete might fuse with the egg to produce the 57-chromosome seedling and two male gametes might fuse with the synergid to produce the 83 chromosome seedling. The fourth gamete might fertilize the endosperm.

A second possibility could have been that non-disjunction in mitotic division might have resulted in the egg having 29 chromosomes and one of the polar nuclei having 27. A single pollen grain then, after mitotic division, could fertilize both the egg and the polar nuclei to give the zygote and endosperm, respectively, 57 and 83 chromosomes. The endosperm could then split to form the triploid seedling and normal endosperm. The possibility of such a cleavage is given more credence as a result of Dewey's previously mentioned work on Agropyron. However, since only a few seedlings were shown to be of monozygotic origin the possibility of fertilization by two pollen grains seems the more likely of the two explanations. This seems all the more believable when one considers the many dissimilar twins (Table 2) which had to be fertilized by two pollen grains.

Some outstanding differences between the diploid and triploid twin plants are presented in Table 3. In general, the triploid was leafier, more upright, coarser and more pubescent than the diploid. In the first year's growth it produced 110 vegetative culms but no panicles; the diploid produced 66 culms of which three flowered. The diploid flowered in 3 subsequent years and was fertile; the triploid flowered only once and produced an abnormal panicle with only a few florets and

rather shrivelled seed. The leaves of the triploid were relatively thick, coarse, pilose on the upper surface, and scabrous on the edges. Stomata and pollen grains were much larger in the triploid than in the diploid, the difference being sufficiently large that determination of their size might well be used to screen large seedling populations for triploids.

TABLE 3. Morphological comparison of diploid (8x) and triploid (12x) plants of Bromus inermis

Gross Morphology							
Ploidy	Chromosome number	Height (cm.) 14 days	No. of culms 1 year	Heading frequency	Pubescence	Panicle	Seed
Diploid	57	10	66	4	sparse	normal	normal
Triploid	83	6	110	1	pilose	few florets	shrunken

Detailed Morphology					
Ploidy	Chromosome number	Stomata length μ		Pollen grain size μ	
		Average	Range	Average	Range
Diploid	57	40.5	27-47	39.0	33-43
Triploid	83	49.3	44-62	50.6	46-53

Great differences in size can occur between diploid members of a pair of twins. Measurements at 4 weeks of age showed that, in 54 out of 269 sets, one member was less than half as tall as its twin; 53 were half

to two-thirds as tall, and 162 were two-thirds to equally as tall. Although this is not proof that haploids do not occur among the smaller progeny, it does indicate the lack of significant relationship between seedling size and ploidy.

On the basis of the present investigation it must be concluded that screening twin seedlings of B. inermis for haploids will prove no more successful than screening a large natural population.

PART B
PHYLOGENETIC RELATIONSHIPS AND AGRONOMIC POTENTIAL
OF B. PUMPELLIANUS AND RELATED TAXA

INTRODUCTION

Evidence of phylogenetic relationship is necessarily diverse, often coming from several different disciplines and sources. The present investigation of Alaskan B. pumpellianus was conducted along the following various lines: classification of morphological variability of B. pumpellianus and comparison of its morphology with that of B. inermis; hybridization of the species with B. inermis; karyological studies of B. pumpellianus and other Bromopsis members in Alaska; investigation of a possible progenitor of the species; and studies of vivipary, a reproductive anomaly.

The purpose of studying morphology and interfertility is obvious. In a widely diverging population phenotypic relationships should, on a broad basis, parallel genetic relationships. This would be expected to be particularly true where introgression was operative. Since there have been numerous introductions of B. inermis into Alaska (Irwin, 1945) introgression could have occurred in some localities.

Karyotypic investigation involving the determination of the chromosome number, the size and shape of the chromosomes, the number of satellite chromosomes, and the size and shape of satellites are useful in determining phylogenetic relationships. In the present study all of these factors were investigated except chromosome size and shape, which are difficult to determine in Bromus octoploids. Because of the significance of aneuploidy in taxonomic relationships (Heslop-Harrison, 1956) attempts were made to determine the exact chromosome number.

During the early part of these investigations evidence came to light

suggesting that one of the plants being studied might be a member of an unreported taxon. Its resemblance to B. pumpellianus suggested that the taxon could be a progenitor of that species or at least a very close relative. The plant could also conceivably have been a polyhaploid form of B. pumpellianus or have originated from earlier polyhaploids out of the species. The possible presence of such a closely related form suggested that much morphological variability among northern plants of B. pumpellianus might be entirely unrelated to introgression from recent introductions of B. inermis. The securing of evidence as to origin and relationships of the taxon therefore became one of the major objectives of the thesis.

Apomictic forms of reproduction curtail genetic recombination and encourage the formation of distinct, vigorous and possibly heterozygous biotypes. Meiotic abnormalities, aneuploidy, and chromosomal structural deviations, and the spread of low seed producing plants are encouraged. Variability within a taxon is greater where facultative apomixis occurs (Nygren, 1954; Heslop-Harrison, 1946). Proliferations indicative of vivipary, a form of apomixis, were observed in B. pumpellianus early in the present investigation. An attempt was made to determine whether these were truly viviparous, if they would root and form mature plants, what environment was required for their formation, and how widespread was the anomaly in the B. pumpellianus population. It was questioned also if proliferations occurred with equal frequency in B. inermis and B. pumpellianus.

Finally, since the purpose of studying the phylogeny of B. pumpellianus was to see if it was closely enough related to B. inermis

that agronomic characteristics could be freely transmitted, the native species was agronomically evaluated. Although it was recognized that complete appraisal of agronomic factors was presently impossible, important information could be gathered, and guide lines to further investigations laid down.

LITERATURE REVIEW

Morphology of Alaskan Bromopsis

Members of the Bromopsis found in Alaska may be divided broadly into spreading and non-spreading taxa (Anderson, 1959). All spreading taxa are now included as varieties of B. pumpellianus although at one time one of these, var. arcticus Shear, was considered to be a species (Scribner and Merrill, 1910). Although Wagnon (1952) relegated all B. pumpellianus to sub-species of B. inermis the present paper will follow Hitchcock (1951) who considered it to be a species.

B. inermis may be briefly described as a rhizomatous perennial, generally glabrous throughout, panicle erect to somewhat open, branches ascending, awns 0 to 2 mm long. B. pumpellianus resembles B. inermis except that it has pubescent nodes, leaves, and lemmas and awns 2 to 3 mm long (Hitchcock, 1951). Anderson (1959) separated these two bromes on much the same lines except that he also recognized the two northern variants, var. arcticus Shear Porsild and var. villosissimus Hult. Var. arcticus was reported to have purplish panicles, spreading branches, spikelets 20 to 45 mm long, and coarsely pubescent glumes. Hulten (1942) described var. villosissimus as being distinguished by a thin gray indument covering the glumes and lemmas, and by the pubescence on both

upper and lower leaf surfaces. Wagnon (1952) after reducing B. pumpellianus to sub-specific level, followed Hulten in dividing it into two varieties, var. purpurascens and var. arcticus. He separated these in his key on the basis that var. arcticus had more pubescent lemmas and glumes than var. purpurascens. Hulten (1942) said that var. villosissimus corresponded to B. ornans Kom. found on the Kamchatka Peninsula in Siberia. Komarov (1934, translated to English, 1963) described B. ornans as relatively low growing, densely villous, and having a strict, contracted panicle.

The non-spreading Bromopsis members reported in Alaska previous to the completion of this investigation include B. ciliatus., B. richardsonii Link, and B. pacificus Shear (Hulten, 1942; Anderson, 1959). All of these species have conspicuously nodding panicles (Hulten, 1942; Hitchcock, 1951).

Scribner and Merrill (1910) assigned all B. ciliatus type plants to B. richardsonii in their treatment of the grasses of Alaska. Ryberg (1922, 1932) reduced B. richardsonii to synonymy with B. ciliatus. Anderson (1959) followed this approach in his Flora of Alaska. Recently it has been shown that the two species in Alaska are morphologically and cytologically distinct, and relatively distinct genetically (Mitchell and Wilton, 1965).

B. ciliatus is easily recognizable by its distinctly fringed lemmas, its relatively short culms (50-95 cm), and its very short anthers (1-1.4 mm). B. richardsonii differs from B. ciliatus in having much larger plant parts, being taller (65-145 cm) and coarser; having an almost completely glabrous sheath with only a tuft of hair at the auricle position, nodes completely glabrous, and a slight amount of pubescence on the backs

of the lemmas on the upper spikelets (Mitchell and Wilton, 1965). It has anthers 1 to 2 mm long. B. pumpellianus differs from these species in having anthers 4.5 to 7 mm long and in not possessing fringed lemmas, and tufted growth habit (Anderson, 1959; Wagnon, 1952).

B. sibiricus from Russia is also said to have a conspicuous pubescent margin on its lemmas but is rhizomatous (Komarov, 1934). Elliott (1949a) and Tzvelev (personal correspondence) both mentioned that B. sibiricus was similar to B. pumpellianus.

B. pacificus, superficially at least, resembles B. pumpellianus more closely than does any of the tufted perennials just described. Its anthers are 3 to 4 mm long; it has a long ligule, pubescent nodes, pilose leaves and lemmas completely pubescent on the back (Hulten, 1942; Wagnon, 1952).

Phytogeographic and fertility investigations

The distribution of B. pumpellianus on the North American continent was investigated by Elliott (1949a) and Wagnon (1952). The species occurs predominantly in the western part of the continent, angling down from Alaska, along the Rocky Mountains to central United States, with occasional populations reported in the east. These investigations also showed that B. inermis is now distributed over nearly all of North America. Elliott, using lemma pubescence as the main variable, showed that introgression had occurred from B. inermis into B. pumpellianus wherever the population of the cultivated species was sufficiently large.

In a concurrent investigation Elliott (1949b) reciprocally crossed B. inermis with B. pumpellianus from Alaska, Northern Alberta, and Washington to obtain seed sets varying from 24.4 to 34.5 percent. Hanna

(1959) crossed a single B. pumpellianus plant of Alaskan origin with B. inermis and obtained seed sets of 8.0 and 8.2 percent in reciprocal crosses. Of these, 35 and 40 percent were later characterized as hybrids (Nielsen, Hanna and Drolsom, 1962). These hybrids produced comparatively few seedlings with many physiological deviants compared with populations of the parental species (Nielsen, Drolsom, and Jalal, 1962). No evidence was found in the literature for introgression among any of the species mentioned in the preceding section although one, B. ciliatus, is more widespread on this continent than B. pumpellianus (Wagnon, 1952).

Wagnon believed B. ciliatus to be a surviving type because of its widespread but sporadic distribution in the three mountain systems of the continent. B. richardsonii, recognized as a species by the same author, was found mainly along the Rocky Mountains. B. pacificus the remaining species under consideration, is distributed in moist thickets and ravines along the coast from southern Alaska to western Oregon (Hitchcock, 1951; Wagnon, 1952). Wagnon found no evidence of inter-relationship of B. pacificus with any of the species presently under consideration.

Cytogenetics of the Bromopsis in the North

B. inermis and B. pumpellianus have been found to be predominantly octoploids ($2n = 56$) although various investigators have found euploid counts of $2n = 28$, $2n = 42$, and $2n = 70$ for B. inermis, and one obtained $2n = 42$ for B. pumpellianus (Darlington and Wylie, 1955). The hexaploid count by Stahlin (1929) apparently is under some suspicion since the origin of the seedlings on which the count was made was not clearly

stated, nor has the count even been confirmed (Wagnon, 1952).

Of the northern varieties of B. pumpellianus, Wagnon (1952) and Bowden (1961) each reported one plant of var. arcticus to be an octoploid ($2n = 56$). No reports could be found of the chromosome number of var. villosissimus or of any of the Russian species (Darlington and Wylie, 1955; Cave, previous to 1956, 1956-1963).

Of the three Alaskan indigenous non-creeping Bromopsis members, B. ciliatus, is usually reported as a diploid ($2n = 14$), B. richardsonii a tetraploid ($2n = 28$) (Elliott, 1949b; Wagnon, 1952; Barnett, 1955), and B. pacificus has once been reported as a hexaploid ($2n = 42$) (Stahlin, 1929). Elliott (1949b) also reported a tetraploid form of B. ciliatus. The situation is somewhat confused since some taxonomists considered the two taxa as one species (Hitchcock, 1951; Anderson, 1959). In any event, Alaskan members of B. ciliatus have been found to be diploid and the members of B. richardsonii tetraploid (Mitchell and Wilton, 1965). The single hexaploid count for B. pacificus by Stahlin is suspect for the same reasons given for doubting his B. pumpellianus count (Wagnon, 1952).

Various irregular counts have been reported for the octoploid bromegrasses. Aneuploids with counts ranging from 49 to 58 have been obtained in B. inermis (Schertz and Murphy, 1958; Sigurbjornsson, Mochizuki and Truscott, 1958; Hanna, 1961). Hanna (1959) also found one B. pumpellianus clone bearing a B. chromosome. Chromosome fragments have been reported in B. inermis (Hill and Myers, 1948; Nielsen, 1953; Schertz and Murphy, 1958).

Investigations of meiosis in both B. inermis and B. pumpellianus have generally proved to be rather fruitless as far as determining

intergenomic relationships are concerned (Hanna, 1961; Nielsen, Hanna and Drolsom, 1962; Nielsen, Drolsom and Jalal, 1962). Chromosome clumping, stickiness, and the relatively high chromosome number were given as reasons for the difficulties in observing pairing relationships in B. inermis. Stickiness was observed in B. pumpellianus (Hanna, 1961). Nielsen (1962) suggested that there were two types of lethality in these species: prophase lethality, brought about by an imbalance in the synthesis system that accompanied the transfer of certain nuclear genes into foreign cytoplasm, and conventional meiotic irregularity. Hanna (1961) found B. pumpellianus to be more regular meiotically than B. inermis. Meiotic irregularity was significantly and positively correlated to the percentage of non-stainable pollen in B. pumpellianus but not in B. inermis.

Chromosome satellites have been investigated in B. inermis but not in B. pumpellianus. Schulz-Schaeffer (1960) and Ghosh and Knowles (1964) found two pairs of chromosomes with large satellites and one pair with small ones in B. inermis. Hanna (1961) reported finding six satellites in plants of B. inermis but made no reference to their size. Schulz-Schaeffer (1960) attempted to categorize all the satellite chromosomes in the Bromopsis. Of the species under consideration he listed B. inermis as possessing types I and III and B. ciliatus as having type IV. He concluded that B. ciliatus was not closely related to B. inermis.

Some evidence is available on the genomic origin of B. inermis but none on the origin of B. pumpellianus. Elliott and Wilsie (1948) concluded on the basis of a presumed polyhaploid that B. inermis arose through the doubling of two closely related allotetraploids. Regularity

of tetraploids of similar origin, of octoploids, and irregularity of a hexaploid hybrid suggested to Hill and Carnahan (1957) that B. inermis was autopolyploid for two basic genomes. As a result of later work with the same plant material Carnahan and Hill (1960) concluded that B. inermis was an alloautopolyploid, AAAABBBB. Ghosh and Knowles (1964) found a tetrasomic ratio for the inheritance of chlorophyll production in plants of B. inermis indicating that the species was an alloautopolyploid. They pointed out that Carnahan and Hill had failed to take into account the satellite chromosomes in the species and that the number of satellites suggested the genomic formula AAAABBBCC, with the B and C genomes differing slightly.

Vivipary

Vivipary has been reported infrequently in the Bromopsis. Moore (1941) and Knowles (1962) reported on individual viviparous plants of B. inermis. Fernald (1950) mentioned a B. inermis forma proliferus in which spikelets were changed to bulblets and leafy tufts. Nielsen (1941) also reported the phenomenon in B. purgans.

Vivipary is common in other genera. According to Nygren's review (1954) vivipary has been reported in 16 species of six genera of Gramineae. Only one of these 16 was at the diploid level. B. purgans, not mentioned by him, is also diploid ($2n = 14$). According to Nygren's review the frequency of vivipary increased with the level of ploidy. As an illustration of this he cites Deschampsia alpina in which the diploid form ($2n = 26$) is non-viviparous, a triploid form D. alpina ($2n = 39$) had only weakly developed bulblets, and above this level aneuploid plants

between $2n = 39$ and $2n = 52$ were facultatively-obligately viviparous.

The causes and methods of inheritance of vivipary are not generally known. Nygren (1954) suggested, without supporting genetical data, that vivipary in Poa alpina might be conditioned by recessive genes. In the same species no relationship was established between meiotic irregularity and the occurrence of vivipary. Nygren (1950) studied 13 viviparous clones of this species in which chromosome numbers ranged from $2n = 36$ to $2n = 57$. Meiosis in two clones, $2n = 37$ and $2n = 46$, was fairly regular.

MATERIALS AND METHODS

Origin of plant material

Fifty Bromus clones, taken from a nursery of plants originating in central Alaska, were brought to Winnipeg, Manitoba, for study and evaluation. All except two clones possessed morphological characteristics suggesting they were some form of B. pumpellianus. One of these, henceforth called the Unknown Taxon, had taxonomic affinities with both B. pacificus and B. pumpellianus; the other was a B. inermis plant. The B. pumpellianus ranged morphologically from plants with very villous leaves, sheaths, glumes and lemmas, to plants whose gross appearance suggested they might not be far removed genetically from B. inermis. Six golden-leaved B. inermis plants from Saskatoon, Saskatchewan, were also used.

The following additional plant material was introduced later into the study: 10 seedlings brought from Russia as B. sibiricus, but later identified as B. ciliatus; 2 B. ornans plants from Russia; 14 B. pacificus clonal and herbarium specimens from Sitka, Alaska; several B. ciliatus seedlings from the Plant Introduction Station, Ames, Iowa; the B. inermis tetraploid plant Ia 554-39, reported by Elliott; 6 B. inermis plants of typically northern strain from R. P. Murphy, New York; and 32 plants of the Unknown Taxon. The 32 plants were from seed collections made in 1957 and 1960 and planted at Palmer, Alaska in succeeding years.

This Unknown Taxon was found at Bishop Mountain, Alaska (Yukon River, 64° 43' N latitude; 157° 50' W longitude). The B. pumpellianus from which the original Alaskan nursery was established came from seed collected throughout the central part of the state (Figure 2).

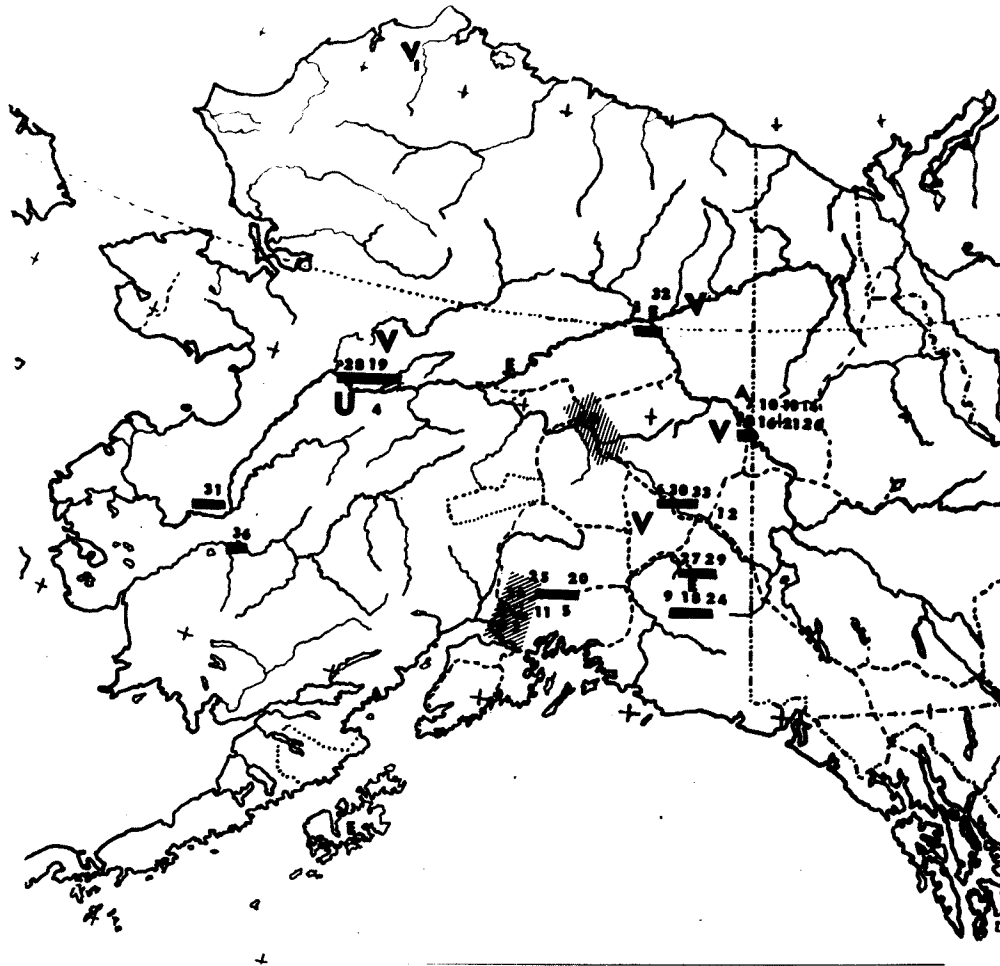




Figure 2. Map of Alaska showing collection sites of indigenous bromegrasses 1 - 36, of the Unknown Taxon U, and of viviparous plant material V; and showing factors affecting bromegrass distribution: population centers , old abandoned army posts A, experimental stations E, and mountain or park areas .

1. Northern collections made by H. J. Hodgson in a concurrent investigation

Taxonomic and agronomic tests

An attempt was made to separate the 50 indigenous bromegrass plants on a morphological basis according to the amount of pubescence on their glumes, lemmas, nodes and leaves; characters previously used by agrostologists (Hulten, 1942; Hitchcock, 1951; Anderson, 1959). On this basis 28 plants, thought to represent all morphological types in the original group, were selected for further evaluation. A clonal nursery was established as a source of taxonomic specimens, to compare plant development under uniform conditions, and to get an indication of the agronomic usefulness of the Alaskan B. pumpellianus.

This nursery was located at Winnipeg, Manitoba. It consisted of six clonal replicates of 36 plants in a randomized block design. In addition to the above 28 B. pumpellianus plants there were included one B. inermis plant, one plant from the Unknown Taxon, and five golden leafed B. inermis plants from Saskatoon, Saskatchewan.

The following agronomic characters were investigated: (1) open-pollinated fertility; (2) self-pollinated fertility; (3) seed yield; (4) spring leafiness; (5) leaf height in mid-summer; (6) weight of dry matter; (7) approximate area of spread after one year's growth; and (8) aftermath.

Indexes of both open-and self-pollinated fertility were obtained by counting the number of seeds present in florets from 12 spikelets from each of two to five panicles per plant. The indexes were statistically analyzed. Clones with more than one missing plot were eliminated from the test and individual missing plots were calculated by the missing plot technique (Snedecor, 1946). The test of self fertility was

unreplicated since wind damage precluded getting complete data from all replicates of the nursery.

Methods of obtaining other agronomic characteristics were as follows: plants were scored (1-10) for leafiness in the spring, one considered to be best; dry weights were taken on mature brome plants after seed harvest; plant spread, the product of two measurements at right angles across the plant, was taken after seed harvest in the second year of growth; and regrowth or aftermath was measured in late October of the second year of growth.

Morphological evidence was gathered from herbarium mounts and live plants of B. pumpellianus from the Winnipeg nursery. These plants were finally described according to six key characters, four determined from the herbarium sheets, and two from the data collected in the nursery. The six characters were spread, leaf height, lemma awn length, sheath pubescence, lemma pubescence, and glume pubescence.

In order to give equal weight to each character, Andersen's hybrid index was used (Benson, 1962). Measureable scores were placed in four classes, and observed characteristics were given a rating from one to four. Number one represented the B. inermis type and four its anti-thesis. By totalling scores for all characteristics in a single plant an index of its resemblance to B. inermis was obtained.

Herbarium specimens of plants of the Unknown grown in a growth chamber (temperature: 72° F, light 20 hours per day) were forwarded to the Botanical Institute of the Academy of Science, U.S.S.R.; the U.S. National Museum, Smithsonian Institution; and the Plant Research Institute, Central Experiment Farm, Ottawa, Ontario.

Further taxonomic comparisons were based on detailed measurements of nine herbarium specimens of the Unknown Taxon, including one plant from the 1957 seed collection and eight from the 1960 seed collection; on the examination of 28 B. pumpellianus plants from the Winnipeg nursery, 14 B. pacificus plants and several B. ciliatus plants from Palmer, Alaska, and other B. ciliatus plants grown from increase seed from Ames, Iowa. The Ames B. ciliatus originated in New Hampshire and an unknown location in Canada.

Confirmations of plant identities were as follows: B. pacificus by D. G. Dore, taxonomist, Ottawa, and by W. W. Mitchell, taxonomist-ecologist, Alaska; B. pumpellianus by W. W. Mitchell; and Russian B. ciliatus by N. Tzvelev of the Botanical Institute of the Academy of Science, U.S.S.R.

Hybridization experiments

Of the B. pumpellianus, 25 plants from the 28 selected for detailed evaluation were cross pollinated with bulk pollen from the northern strain of B. inermis. Separate panicles of 14 of these plants were also pollinated with bulk pollen from B. pumpellianus. The two pollinations were made simultaneously. Plants were emasculated with scissors (Ghosh and Knowles, 1960) shortly before anthesis when the anthers were still slightly green, and mutually bagged with more than one pollen parent. During sunny periods throughout the day the plants were periodically agitated to stimulate cross pollination. The total number of seeds were counted for each panicle and the percent fertility recorded.

The Unknown Taxon was crossed with all species listed except B.

ornans. Two different forms of B. pumpellianus were used in the crosses: one with long awned lemmas and medium amounts of pubescence on plant parts, the other with short awned lemmas and extremely villous plant parts. B. ornans was pollinated by plants of B. inermis and B. pumpellianus. The same techniques were used in making these crosses as was used in crossing B. pumpellianus and B. inermis except that these hybridizations were made in the growth chamber (temperature: 65° - 70°F; light 20 hours per day). It was found, as reported by Knowles (1961), that by suddenly increasing the air temperature to 80 - 85°F. at time of anthesis, anthers could be made to exert themselves for easy pollination and the maximum number of stamens made to protrude and dehisce. In all tests, because the indigenous bromes flowered much earlier than other bromes, the commencement of growth was staggered to bring about simultaneous flowering.

Cytological methods

Chromosome counts were taken of all available plant material. Detailed studies of satellite chromosomes were made on each of the species and where possible, on morphological variants of the species. The cytological techniques used are those reported in Section A except that when satellites were to be viewed much less pre-shrinking was required. Microphotographs were taken of cells under oil immersion, projected on the screen, and measurements of satellites and satellite chromosomes were made of the projected image. Since minute satellites were not always visible and chromosomes varied in extent of shrinkage during cytological pre-treatment, satellite length was considered as a percent of the whole chromosome length. Measurements were made of the

long arm; the segment between the centromere and the satellite (interstitial segment), and of the satellite. The percentage of the whole chromosome making up the satellite was plotted on a graph against the length of the chromosome making up the interstitial segment of the chromosome.

RESULTS AND DISCUSSION

Ecology and distribution of the Alaskan bromes

The area from which Alaskan indigenous bromes were collected (Figure 2) is about 100,000 square miles. It varies physiographically from wooded, mountainous terrain to open flat areas, and to sandy sites along swampy wide rivers. The collection sites range in altitude from sea level to about 3,000 feet; in climate from the relatively moist, moderate temperatures of the coast to the dry environment of the interior. About half of the collection came from central Alaska, an area unglaciated during Pleistocene time; the remainder from areas recently glaciated or where glaciers are still active. Such varied environments might affect the development of these bromes and might be conducive to much morphological variation.

Earlier epochs in Alaskan history undoubtedly also affected plant development. The whole central area of the state was completely unglaciated when much of North America was covered with ice (Heusser, 1957). Fossil flora of plants now occurring much further south indicate a hypsithermal period when climate of the area was moderate (Hopkins and Benninghoff, 1965). A land bridge approximately 1,000 miles wide once connected Asia and America (Haag, 1962). These happenings may have permitted the introduction of Eurasian bromegrasses to North America (Hulten, 1937) and may also have contributed to genetic change within the grass after its arrival on this continent.

Relatively recent introductions of B. inermis to experimental stations and to an army post (Figure 2 E,A) and the consequent possibility

of introgression of that species into B. pumpellianus could have contributed to the variability within the native brome population.

The bromegrasses used in this investigation came from the general vicinity of the collection sites in the map in Figure 2. Although only a small group of plants are reported there, they represent samplings of much of the Alaskan bromegrass population. Their variability represents the effects of many of the environmental factors just mentioned. With such a background one would expect these bromegrass plants to be extremely variable morphologically. The following investigation was conducted to study their morphology and to determine whether any variability present could be classified.

Morphological characteristics of Alaskan bromes

The population of bromegrasses studied is graphically described in Figure 3; photographs of some variable characteristics are presented in Figure 4; and measurable characteristics are shown in Table 4.

Attempts to classify these bromegrasses as presently recognized species and varieties were quite successful at the specific level but much less so at the varietal level. The B. pumpellianus plants were more pubescent than the B. inermis, had shorter rhizomes, less leaf height at midsummer, and usually possessed longer awns (Table 4). None of the plants were sufficiently pubescent to be classified as var. villosissimus (Figure 4) which, according to Hulten (1942), has extreme lanate pubescence equal to that of B. ornans. Eleven plants, mainly because of their pubescent glumes, might have been referred to var. arcticus (Anderson, 1959); and the remainder would have had to be

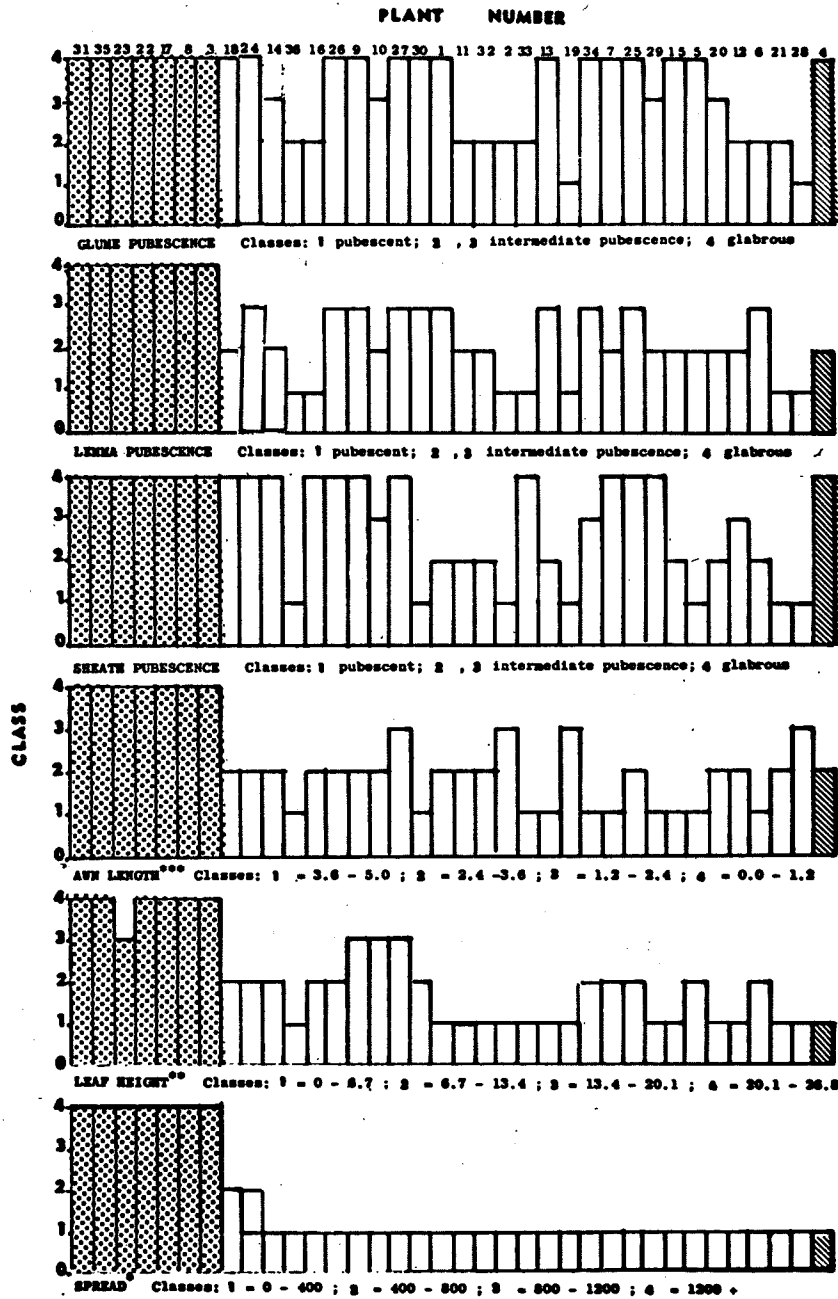


Figure 3. Morphological characteristics of *B. inermis*, *B. pumpellianus* and the Unknown Taxon ; in classes of 1 to 4, with 1 most like *B. inermis*.

- * Plants arranged in descending order of spread from left to right, although class size precluded showing this in all cases
- ** Height in inches of the tallest non-flowering culm in July
- *** Measurements in mm from tip of lemma to end of awn

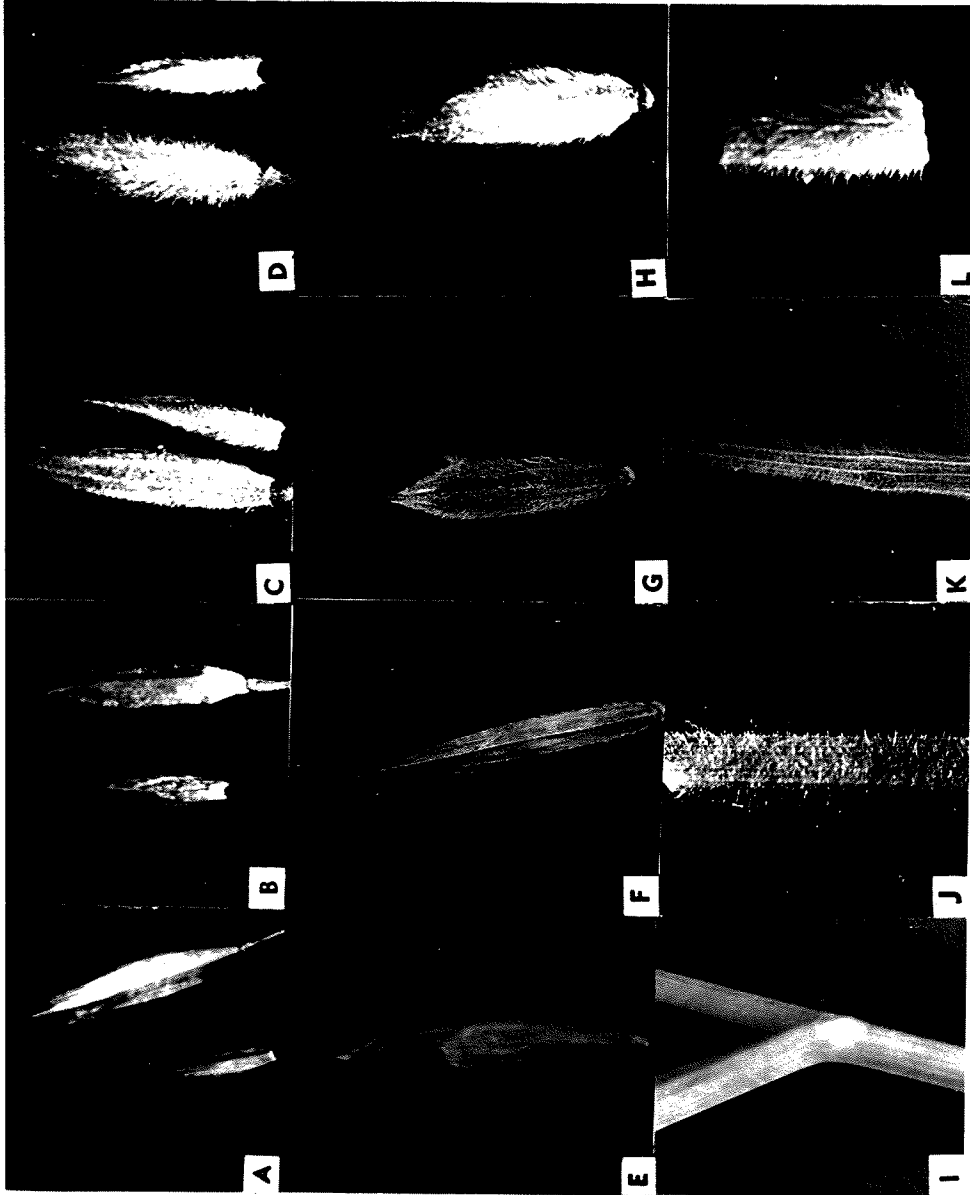


Figure 4. Pubescence on glumes, lemmas and leaf sheath on *B. inermis* A, E, I; moderately pubescent *B. pumpellianus* B, F, J; villous *B. pumpellianus* C, G, K; and *B. ornans* D, H, L.

TABLE 4. Morphological index, awn length, area covered by plant after one year's growth, and leaf height at maturity of B. pumpellianus, B. inermis and of the Unknown Taxon.

Plant number	Morphological index	Awn length (cm.)	Area of cover (sq.in.)	Leaf height (in.)
<u>B. pumpellianus</u>				
1	13.0	2.5	264.0	6.0
2	9.0	1.5	225.0	5.8
5	11.0	4.0	172.0	7.2
6	11.0	5.0	113.0	6.6
7	15.0	3.7	192.0	7.3
9	17.0	3.0	314.0	14.3
10	14.0	3.0	310.0	14.2
11	10.0*	3.0	291.0	5.0*
12	11.0	2.5	138.0	4.0
13	12.0	4.5	211.0	6.0
14	14.0	3.5	384.0	7.2
15	11.0	5.0	176.0	6.2
16	12.0	2.5	328.0	12.0
18	16.0	3.5	558.0	12.3
19	8.0	1.5	199.0	5.3
20	11.0*	3.5	156.0	4.9*
21	8.0	3.0	94.0	6.7
24	16.0	2.5	381.0	7.0
25	16.0	2.5	189.0	7.0
26	16.0	2.5	324.0	7.2
27	18.0	1.5	302.0	17.1
28	8.0	1.5	76.0	4.6
29	12.0	4.5	184.0	4.5
30	12.0	4.5	292.0	4.5
32	10.0	2.5	244.0	4.0
33	10.0	4.5	221.0	4.3
34	14.0*	4.5	193.0	8.0*
36	7.0*	4.0	366.0	4.0*
Avg.	12.2	3.2	246.3	7.2
<u>B. inermis</u>				
3	24.0	1.0	1434.0	20.0
8	24.0	0.0	1472.0	21.8
17	24.0	0.0	1751.0	22.2
22	24.0	0.0	2071.0	22.2
23	23.0	1.0	2275.0	16.7
31	24.0	0.0	5148.0	26.7
35	24.0	1.0	2655.0	20.7
Avg.	23.8	0.4	2400.8	21.4
<u>Unknown Taxon</u>				
4	14.0	3.5	47.0	6.2

* Compiled from data incomplete in all replicates.

considered var. purpurascens (Wagnon, 1952).

It was difficult to make clear distinctions between these varieties, however. Plant variation was suggestive of mixed genetic heritages but not necessarily mixtures of B. pumpellianus and B. inermis genotypes. For example, B. pumpellianus plants 19 and 27 (Table 4, Figure 4) were the most villous, and typically were non-spreading but possessed short awns as does B. inermis and B. ornans. They differed from these species in their rhizome development. (Komarov, 1934, translated to English, 1963; Hitchcock, 1951). Various other combinations of these three characters were found on pubescent plants. Plant 2 was villous, short awned, and moderately spreading. Plant 21 was villous, moderately long awned, and non-spreading. Number 36 was villous, long awned and quite spreading. The extremely pubescent types did not originate in any particular area but were distributed throughout Alaska (Figure 2). Some of those with short awns (plants 2, 19, and 28) originated at sites to which it is doubtful that B. inermis could have been introduced. The long awned plant, number 36, was from an area in which B. inermis was found (Figure 2).

If an associated group of morphological characters are found in different forms it usually indicates that these forms are closely related. Among the bromegrasses investigated, four characters were associated at the specific level. B. inermis was constantly more spreading, had greater leaf height in midsummer, had shorter awns, and had less hairy lemmas than B. pumpellianus (Figure 3). No form or variety of B. pumpellianus could be separated out in such a manner. B. pumpellianus plants were arranged in Figure 3 from left to right in descending order

of spread. If there was an association of characters within the species one would expect a corresponding gradual decrease in leaf height, and an increase in awn length and lemma pubescence from left to right across the graph. No such gradation was shown.

No clear morphological evidence was found to indicate introgression of B. inermis into B. pumpellianus, though some plants were more like B. inermis than others. Six clones: 9, 18, 24, 25, 26 and 27 were most similar to B. inermis and had a morphological index of from 15 to 18; five clones: 2, 19, 21, 28 and 36 were unlike B. inermis and had morphological indexes of 7, 8 or 9; the remaining 16 clones had indexes graduating between the two levels. Despite the gradation, the separation between an index of 18, the highest for B. pumpellianus, to one of 23, the lowest for B. inermis, suggests that if introgression has occurred in these plants it must be of a minor nature. Three plants could have been construed as backcrosses to B. inermis: clones 18 and 24, spread 557 and 502 inches, respectively; and clone 27, had a morphological index of 18.

The spreading characteristic deserves further mention since the two species differed so strongly in this regard. Clones of the native species covered areas varying from 76.0 to 557.0 square inches. The B. inermis plants covered areas varying from 1434.0 to 5148.0 square inches. This character, because it can be observed only in a plant nursery, is of little use to a taxonomist but it is mentioned because this wide difference is quite strong evidence of genetic deviation between the Alaskan B. pumpellianus and cultivated B. inermis.

Included also in Table 4 and Figure 3 is one plant of the Unknown

Taxon. This plant did not follow the description of B. pumpellianus or B. inermis; being like B. pumpellianus in spread, leaf height and awn length; and like B. inermis in sheath and glume pubescence.

Inter- and intraspecific compatibility and its relationship to morphological index

An attempt to correlate the morphological indexes just determined, with the ability of B. pumpellianus plants to set seed when hybridized with B. inermis was unsuccessful on an individual plant basis (Table 5). In a test under a relatively uniform environment in the greenhouse at Palmer, Alaska, seven plants out of 25 produced practically no seed after crossing. Three of these were eliminated from consideration when it was discovered in open pollinated tests, that they were somewhat infertile. The other four plants: 5, 19, 20 and 26 varied in their similarity to B. inermis and had respective indexes of 11, 8, 11 and 16. The morphological indexes for the whole test ranged from 8 to 18 among the B. pumpellianus plants. Plant 27 had the highest index but only 9.2 percent of the pollinated florets produced seeds. Number 31, the only B. inermis plant in the test, produced seed on 29.6 percent of its florets after pollination by other plants of that species. The average seed set for B. pumpellianus in this test was 8.8 percent but ranged from 0.0 to 35.0 percent. A statistical analysis showed a low non-significant correlation coefficient ($r = +.369$) between morphological index and percent compatibility of the B. pumpellianus with B. inermis.

Despite this, the percent seed set on all florets in plants having low morphological indexes (8 - 12) was much less than in plants with a high index (14 - 18), being 5.2 in the first instance and 12.0 in the

TABLE 5. Seed production in 24 crosses of B. pumpellianus x B. inermis, and in one cross of B. inermis x B. inermis related to their morphological indexes.

Morpho- logical index	Maternal plant number	Palmer greenhouse crosses			Total of all crosses		
		Florets polli- nated	Seed set	Percent seed set	Florets polli- nated	Seed set	Percent seed set
<u>B. pumpellianus</u> x <u>B. inermis</u>							
8	19	313	1	0.3	868	1	0.0
	21	93	22	23.6	93	22	23.6
	28	336	8	2.4	358	8	2.2
9	2 ¹	224	22	9.8	273	22	8.0
10	11 ²	70	0	0.0	610	6 ⁶	1.0
	33	56	4	7.1	56	4	7.1
11	5	120	0	0.0	174	0	0.0
	6	129	5	3.9	173	5	2.9
	12	-	-	-	18	0	0.0
	20 ²	91	0	0.0	91	0	0.0
12	13 ³	123	7	5.7	170	12	7.0
	16	153	8	5.2	169	8	4.7
	29	194	9	4.6	330	15	4.5
	37 ⁴	50	4	8.0	50	4	8.0
	38 ⁴	192	17	8.8	192	17	8.8
8 - 12		2044	107	5.2	3625	124	3.4
14	10	109	17	15.6	208	22	10.6
	14	58	16	27.6	76	16	21.0
15	7 ⁵	60	0	0.0	100	0	0.0
16	18	196	15	7.6	196	15	7.6
	25	60	21	35.0	60	21	35.0
	26	84	1	1.2	84	1	1.2
17	9	106	2	1.9	141	2 ⁶	1.4
	24	85	22	25.9	85	22	25.9
18	27	98	9	9.2	98	9	9.2
14 - 18		856	103	12.0	1048	108	10.3
<u>B. inermis</u> x <u>B. inermis</u>							
24	31	152	45	29.6	152	45	29.6

¹2n = 54 + 2B

²Compiled on the basis of data incomplete in all replicates

³2n = 58

⁴Extremely villous plants, morphological index could not be calculated

⁵2n = 55

⁶Shrunken seeds



second.

Prior to the greenhouse test at Palmer, many relatively unsuccessful attempts have been made to cross villous B. pumpellianus with B. inermis. Fifteen plants with morphological index below 12 produced only 124 hybrid seeds from 3625 pollinated florets, (3.4 percent fertile). The nine plants with an index from 14 to 18 yielded 108 seeds from 1048 pollinated florets (10.3 percent fertile). Plants 11, 19, 28 and 29, although crossed extensively, produced very few hybrid seeds (30 seeds from 2166 florets). It is somewhat doubtful that this low seed set of plants, dissimilar to B. inermis is mainly due to inherent low fertility of maternal plants, although this could be a factor. Under open-pollinated conditions these clones were respectively 35.2, 28.8, 19.2 and 41.2 cross fertile.

In order to determine whether variations in cross fertility were due to inherent low fertility panicles of 14 of the above B. pumpellianus clones were crossed among themselves. Also, in an attempt to show further interspecific relationships B. ornans was hybridized with B. inermis and B. pumpellianus. Results of these crosses are presented in Tables 6 and 7.

Twelve clones produced seed from both crosses and two did not set seed (Table 6). In this experiment plants unlike B. inermis (index: 8 - 12) were inherently lower seed yielders than plants more like B. inermis. Hybridizing with B. inermis reduced seed production of the 8 - 12 group still further. This group produced about one and one-half times as much seed from intraspecific pollinations as from interspecific

TABLE 6. Relationship of morphological index to percent fertility on B. pumpellianus plants after inter- and intraspecific crosses

Morpho- logical index	Plant number	Percent fertility	
		<u>B. pump- ellianus</u> pollen	<u>B. inermis</u> pollen
8	28	2.0	2.4
9	2	29.6	9.8
10	11	0.0	0.0
	33	2.5	7.1
12	13	12.0	5.7
	29	22.0	4.6
	37*	1.2	8.0
	38*	8.6	8.8
8 - 12		9.7	5.8
14	10	39.3	15.6
15	7	0.0	0.0
16	18	13.0	7.6
	25	26.0	35.0
17	9	6.4	1.9
	24	5.6	25.9
14 - 17		15.0	14.3

* Villous plants for which a morphological index could not be calculated

TABLE 7. Compatibility of B. pumpellianus (B. pu.) and B. ornans (B. or.) with B. inermis (B. in.)

Cross	Number of florets pollinated	Percent of florets pro- ducing seed	Average percent seed set per plant
B. pu. x B. in.	1861	7.95	9.46*
B. pu. x B. pu.	1398	13.38	12.01*
B. or. x B. in.	79	13.92	13.92
B. or. x B. pu.	96	00.00	00.00

* Not significantly different

pollinations. The 14 to 17, or B. inermis like group, yielded about the same amount of seed from either pollination.

Over the whole test seed set per plant of B. pumpellianus was slightly less following pollination by B. inermis than following pollination by B. pumpellianus, but not significantly so (Table 7).

Approximately the same percentage of B. ornans florets produced seed following B. inermis pollinations as did B. pumpellianus florets following B. inermis pollination. The cross B. ornans x B. pumpellianus was unsuccessful. Two of the eight seeds from the B. ornans x B. inermis crosses germinated and were recognized as hybrid seedlings by their golden colored leaves inherited from the B. inermis parent. Although a complete morphological analysis was not made because the hybrids failed to produce panicles, it was noted that their foliage was intermediate in pubescence between the two parents. As far as can be determined, this is the first recorded cross between B. ornans and B. inermis. It is further evidence for the correctness of Elliott's thesis that a close relationship exists between B. inermis, B. ornans and B. pumpellianus (Elliott, 1949a).

Karyology of B. pumpellianus, B. inermis and B. ornans

Karyological studies of these three species confirmed the above conclusions and contributed to the general karyological description of the taxa. B. ornans, B. inermis, and B. pumpellianus were octoploids and predominantly possessed 56 chromosomes. (Figure 5 A,B,C). As far as could be determined from the available literature this count is the first reported for B. ornans (Darlington and Wylie, 1955; Cave, previous

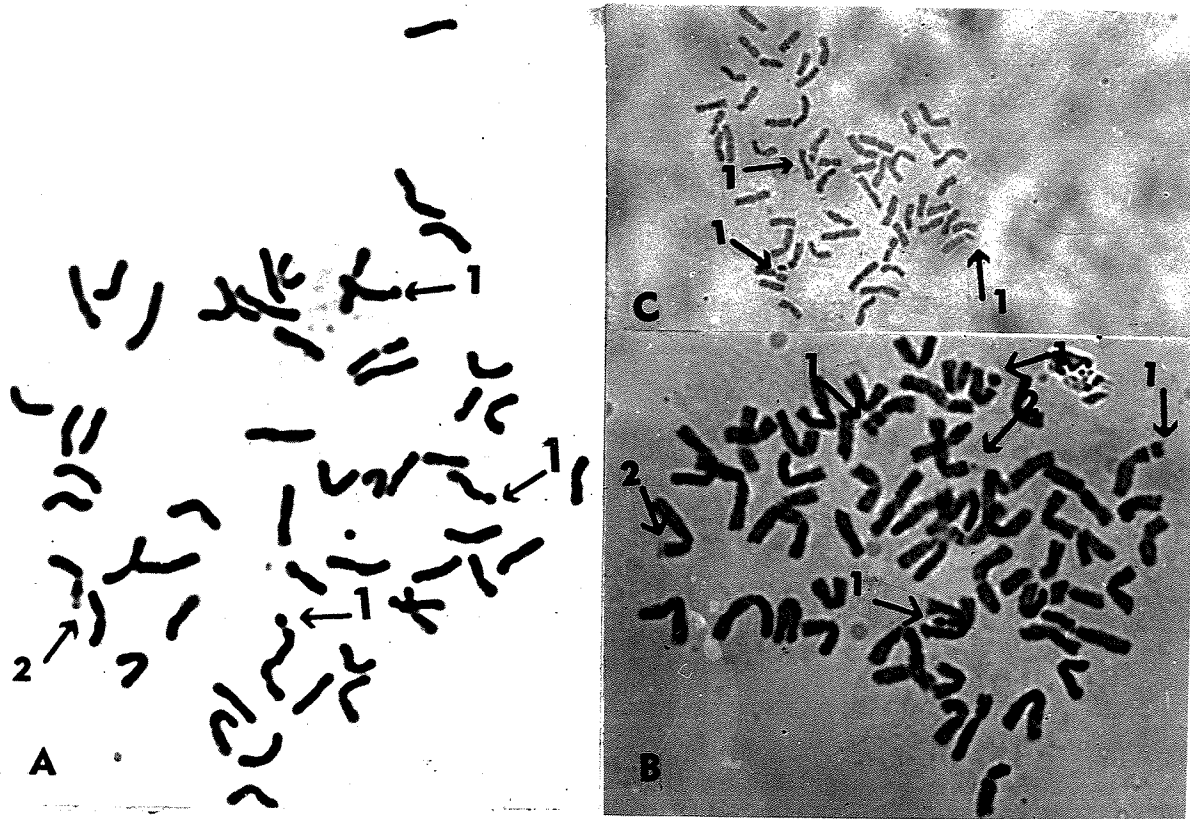


Figure 5. Somatic cells of B. inermis, A; B. pumpellianus, B; B. ornans, C; showing their octoploid chromosome complement ($2n = 56$) and their large (1), and small (2), satellites.

to 1956, 1956-1962; Love and Love, 1961). The 56 count on B. pumpellianus material from diverse locations in the far north confirmed that northern forms of that species as well as those found further south were octoploids (Table 8). Previous to these determinations, and in addition to Elliott's report, only eight plants of B. pumpellianus from north of 60° latitude, the northern provincial boundary, had been checked cytologically (Wagnon 1952; Bowden, 1960; Hanna, 1961). Both Elliott (1949b) and Hanna (1961) studied plants from about 60° N latitude, South-Central Alaska. Some of the material in the present study was from 350 miles farther north. This collection covered about 550 miles in an east-west direction.

The presence of three definite aneuploids and seven suspected aneuploids was quite interesting (Table 8; Figure 6 A,B,C). Since six of these were from one location (Eagle, Alaska) they probably represent a genetically disturbed population of B. pumpellianus plants. Since B. inermis has also been found at that collection site, having been introduced there early in the present century (Dickson, 1958), introgression might account for the genetic disturbances.

The counts obtained on the plant from Eklutna (Table 8, and Figure 6 A) were puzzling. The chromosome numbers ($2n = 54, 55$ and 56) were obtained at various times on the one plant. When crossed with a tetraploid bromegrass two 40 chromosome hybrids resulted indicating the nullisomic or double monosomic count ($2n = 54$) was correct. The plant may be a nullisomic with two accessory chromosomes. The doubtful counts obtained on some other plants might also be due to the presence of accessory chromosomes.

TABLE 8. Chromosome numbers of one B. inermis plant and 49 B. pumpellianus plants from Alaskan locations

Location	North latitude	West longitude	Chromosome number ¹
Eklutna	61° 24'	149° 09'	(1) 54-56 ²
Chitina	61° 32'	144° 27'	(4) 56
Sleetmute	61° 40'	157° 05'	(1) 56
Mile 55 Glenn Hwy.	61° 45'	148° 55'	(2) 56
Mile 70 Glenn Hwy.	61° 47'	148° 31'	(2) 56
Mile 85 Glenn Hwy.	61° 49'	148° 13'	(2) 56
Hick's Creek	61° 50'	148° 00'	(1) 56
Gakona	62° 18'	145° 16'	(1) 56-57 ³
Anvik	62° 30'	160° 05'	(1) 56 ⁴
Tanacross	63° 24'	143° 19'	(4) 56 (1) 56-57 ³
Mile 8, Taylor Hwy.	63° 40'	142° 30'	(1) 56
Eagle	64° 46'	141° 12'	(1) 55 (7) 56 (4) 56-57 ³ (1) 58
Campion	64° 46'	156° 55'	(5) 56
Fort Yukon	66° 35'	145° 15'	(9) 56 (1) 56-57 ³

¹Bracketed numbers are the number of chromosomes examined

²More than one chromosome count on the same plant

³Where chromosome number could not be determined with certainty but extra chromosomes were believed to be present

⁴B. inermis

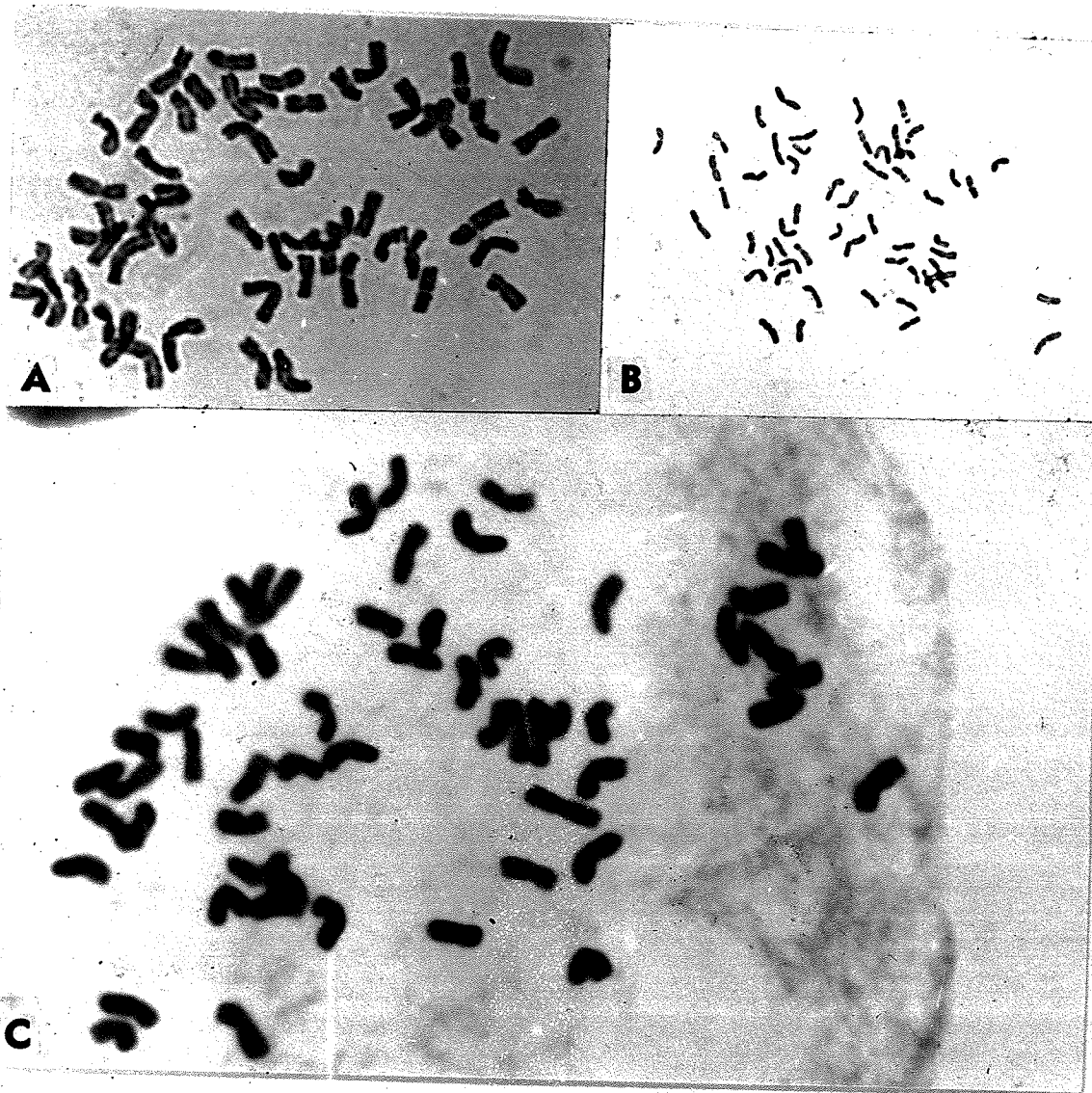


Figure 6. Aneuploid cells of three *B. pumpellianus* plants: A - $2n = 55^*$, B - $2n = 55$, and C - $2n = 58$.

* counts of $2n = 54$ and 56 were also obtained on this plant

Proven aneuploids showed some effects of their irregularity. The 54 chromosome plant produced slightly less seeds per panicle than most of the other B. pumpellianus in an open pollinated test; the 55 chromosome plant (Figure 6 B) was almost completely sterile; and the 58 chromosome plant (Figure 6 C) was characterized by having weaker culms when growth was at a maximum in the greenhouse, but under normal field conditions was indistinguishable from other plants.

In contrast to the genetic differences between B. pumpellianus and B. inermis suggested by the aneuploid investigations the satellite chromosome studies indicated interspecific homology. Satellites of B. pumpellianus, B. inermis and B. ornans were grossly similar. Each species possessed 2 pairs of large satellites and each had small satellites (Figure 5 A,B,C: 1,2). Although these were difficult to find not more than two per cell were seen in either B. inermis or B. pumpellianus. A minute satellite was seen only once in B. ornans.

Precise measurements of satellites and interstitial segments failed to clearly separate species or the two forms of B. pumpellianus (Figure 7). Size of satellites varied only slightly. B. ornans possessed the biggest of the large satellites and B. inermis the biggest of the smaller ones. In both instances satellite size is relative to the total chromosome length. It could not be shown that the satellite chromosomes of the villous, short-awned plant 19 were materially different from those of the lightly pubescent long-awned plant 27, although small satellites were easier to find in the latter.

The investigation of satellites shows that the species; B. ornans, B. inermis, and B. pumpellianus are somewhat similar karyotypically and

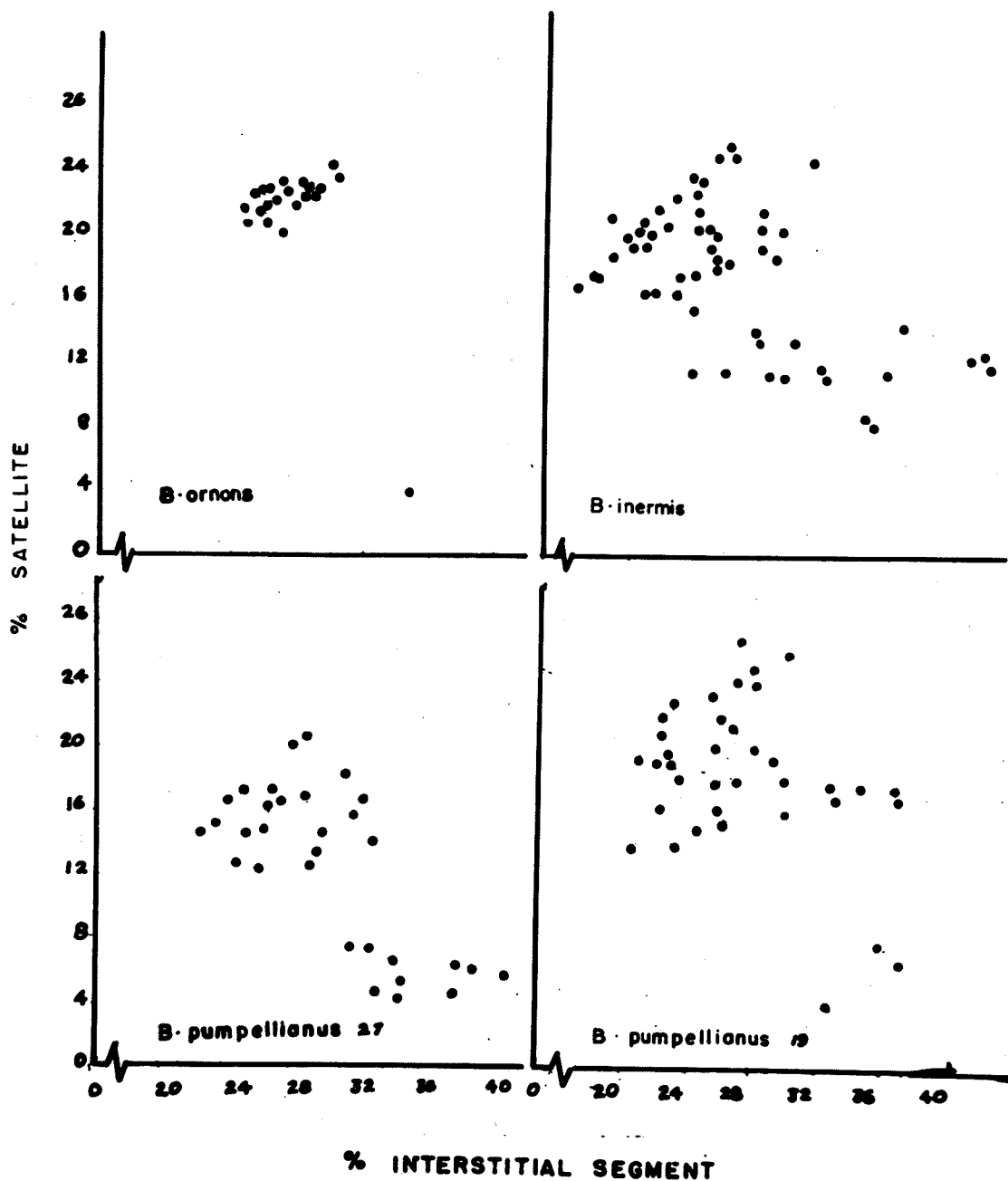


Figure 7. Graph showing percentage of the whole chromosome making up satellite plotted against the interstitial segment of the satellite chromosomes of B. ornans, B. inermis, and plants 27 and 19 - intermediate pubescent and villous B. pumpellianus plants.

suggests that they may have a similar phylogenetic origin. Because they each had four large satellites and a normal somatic complement of 56 chromosomes they should all be considered alloautoploids. The genomic formula AAAABBB₁B₁ is suggested for B. inermis and the long-awned non-villous B. pumpellianus plants. B. ornans and the villous form may possibly also have this genomic formula.

The Unknown tetraploid Bromus

The genetic diversity of the Alaskan bromegrass population was further illustrated by the finding of the hitherto undescribed taxon mentioned briefly in the preceding pages. The investigation of this taxon and its genetic implications were the subject of considerable study and are reported as follows:

Description and comparative morphology

The Unknown Taxon may be described as a non-spreading to very weakly spreading perennial; culms 82-119 cm, ascending with a nodding panicle; nodes 4-6, glabrous to slightly pubescent; 5-10 mm wide; auricles absent; ligule erose, 2-4 mm long; panicle 20-30 cm long; spikelets 15-31 mm long, 3-7 per node; florets 3-12 per spikelet; rachilla pubescent; glumes, lower 5-10 mm long and 1-1.5 mm wide, upper 8-12 mm long and 1.5-2.0 mm wide, acuminate, puberulent along the nerve; lemma, pubescent all over, 5-9 nerves, 5-17 mm long, 2-3.5 mm wide, awn 4-8 mm; palea 8-10 mm long, 1.2-2.0 mm wide, 2 nerved chartaceous ciliate; and anthers 4-6 mm long.

The Unknown Taxon resembles B. pumpellianus in that both taxa have pubescence on the leaf sheath and upper leaf blade; both have large pubescent spikelets with long, lanceolate, awned lemmas (Figure 8 A) and

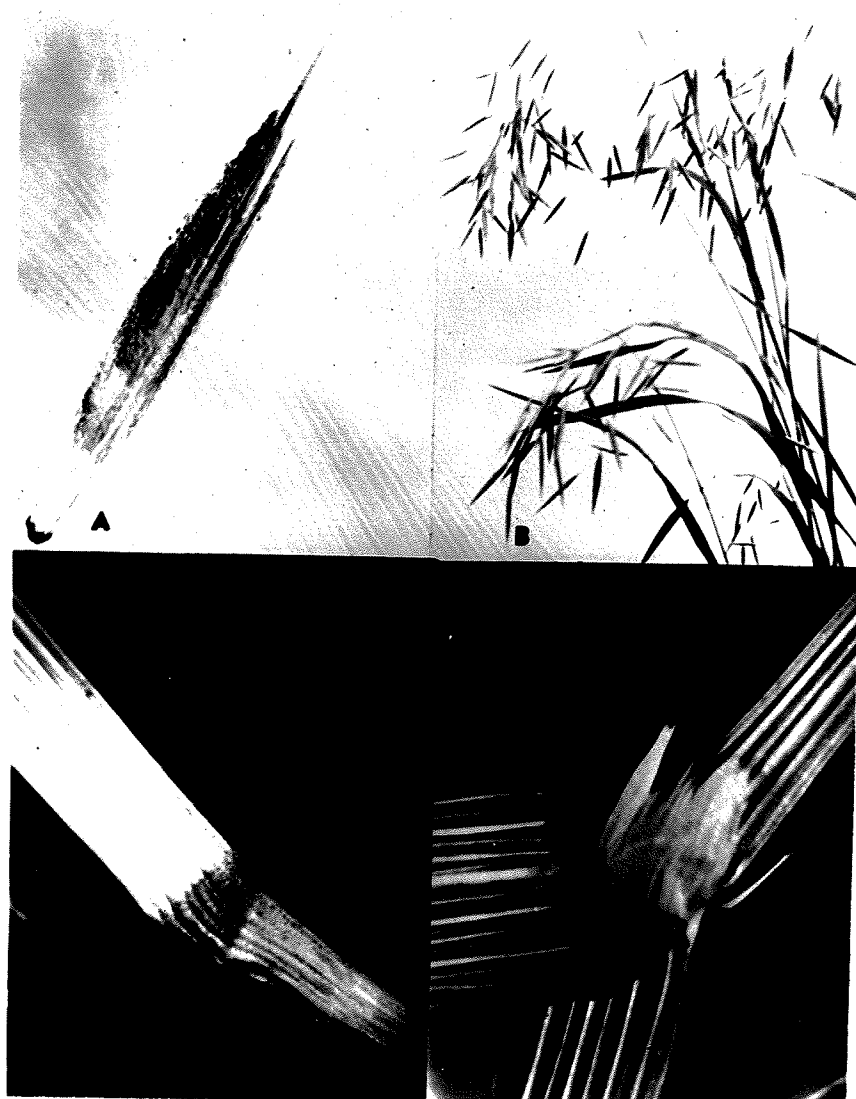


Figure 8. Notable morphological features of the Unknown
Taxon: A - lemma, B - drooping panicle, C - puberulent
node, D - long ligule.

anthers from 4-6 mm long, It differs from B. pumpellianus in that it is non-spreading to very weakly spreading; exhibits a more ascending growth habit, becoming decumbent at maturity; has longer, laxer and more drooping panicles; frequently lacks nodal pubescence; and, in nearly all cases, possesses a longer ligule (Figure 8, B,C,D).

The Unknown Taxon resembles B. pacificus in its very drooping panicle, its lemma pubescence, lack of rhizomes, and in its very long ligule. It differs from the species in that it is less robust, has finer less rigid leaves, and does not exhibit the extremely retrorse nodal pubescence of B. pacificus.

J. R. Swallen of the U. S. National Herbarium and N. Tzvelev of the Academy of Science, U.S.S.R. also noted morphologic similarities between the Unknown Taxon and both B. pumpellianus and B. pacificus. In addition, Swallen confirmed that the Unknown Taxon was no other North American species, and Tzvelev that it was no known Eurasian species (personal correspondence). The plants did not fit any of the morphological descriptions in Komarov (1934, translated to English, 1963).

Karyology

In addition to the octoploid B. pumpellianus previously mentioned, two further levels of ploidy were found in the Bromopsis occurring in Alaska (Table 9). The Unknown Taxon and B. pacificus plants were tetraploids ($2n = 28$) (Figure 9 A,B) and B. ciliatus plants were diploids ($2n = 14$) (Figure 9 F,G). The two tetraploid counts as far as the writer is aware, have not been previously reported. The diploid count on B. ciliatus confirms many previous records (Darlington and Wylie, 1955;

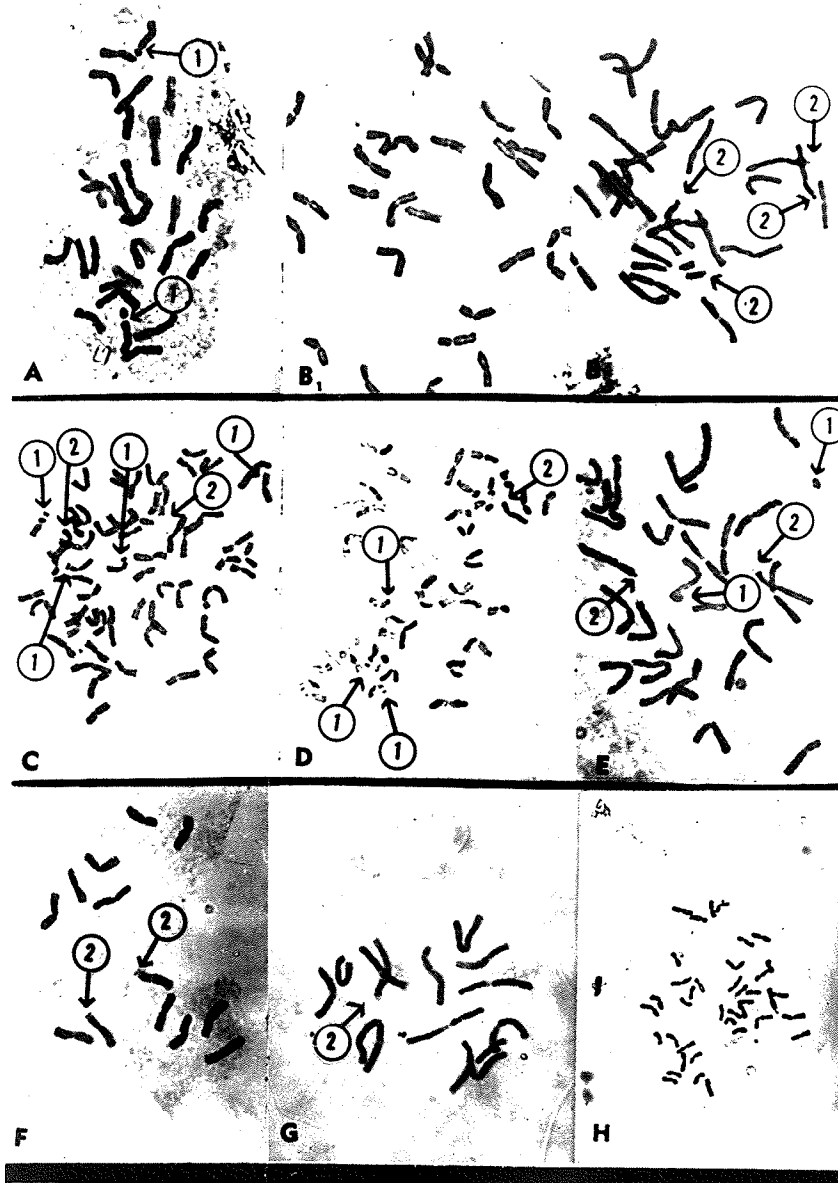


Figure 9. Cells of northern Bromopsis and related taxa: A - Unknown $2n = 28$; B - B. pacificus $2n = 28$; B₁ - section of B. pacificus cell; C - B. pumpellianus $2n = 56$; D - B. pumpellianus x Unknown $2n = 42$; E - Iowa B. inermis $2n = 28$; F - B. ciliatus (1 chromosome lost); G - Russian B. ciliatus $2n = 14$; H - B. pumpellianus x Unknown $2n = 40$.

(1) indicates large satellite and (2) small satellites

Cave, previous to 1956, 1956-1962). Plant 554-39 of B. inermis was a tetraploid, as reported by Elliott and Wilsie (1948); the other plants of B. inermis were octoploids, as reported in numerous other investigations (Darlington and Wylie, 1955; Cave, previous to 1956, 1956-1962).

TABLE 9. Chromosome numbers and satellites observed in tetraploid B. inermis and four taxa indigenous to Alaska

Taxa	Chromosome number 2n	Number of satellites	
		Large	Small
<u>B. ciliatus</u>	14	0	2
Unknown	28	2	0 or 2*
<u>B. pacificus</u>	28	0	4
<u>B. inermis</u> **	28	2	2
<u>B. pumpellianus</u>	56	4	2

* Satellites were observed in only one instance

** This plant is the polyhaploid, Ia 554-39, reported by Elliott and Wilsie (1948)

Similarities exist between the satellited chromosomes of some of these taxa (Table 9). The Unknown Bromus, B. inermis and B. pumpellianus possess either one or two pairs of large satellites depending upon the level of ploidy (Figure 9 A,C,E:1). Large satellites were not found in B. pacificus or B. ciliatus (Figure 10).

All taxa possessed identifiable small satellites, although these were found only once in the Unknown Taxon. These satellites were so minute as to render comparisons between taxa difficult but some differences were noted. B. pacificus possessed the largest of the small satellites (Figure 9 B₂:2), and Russian B. ciliatus had smaller satellites than

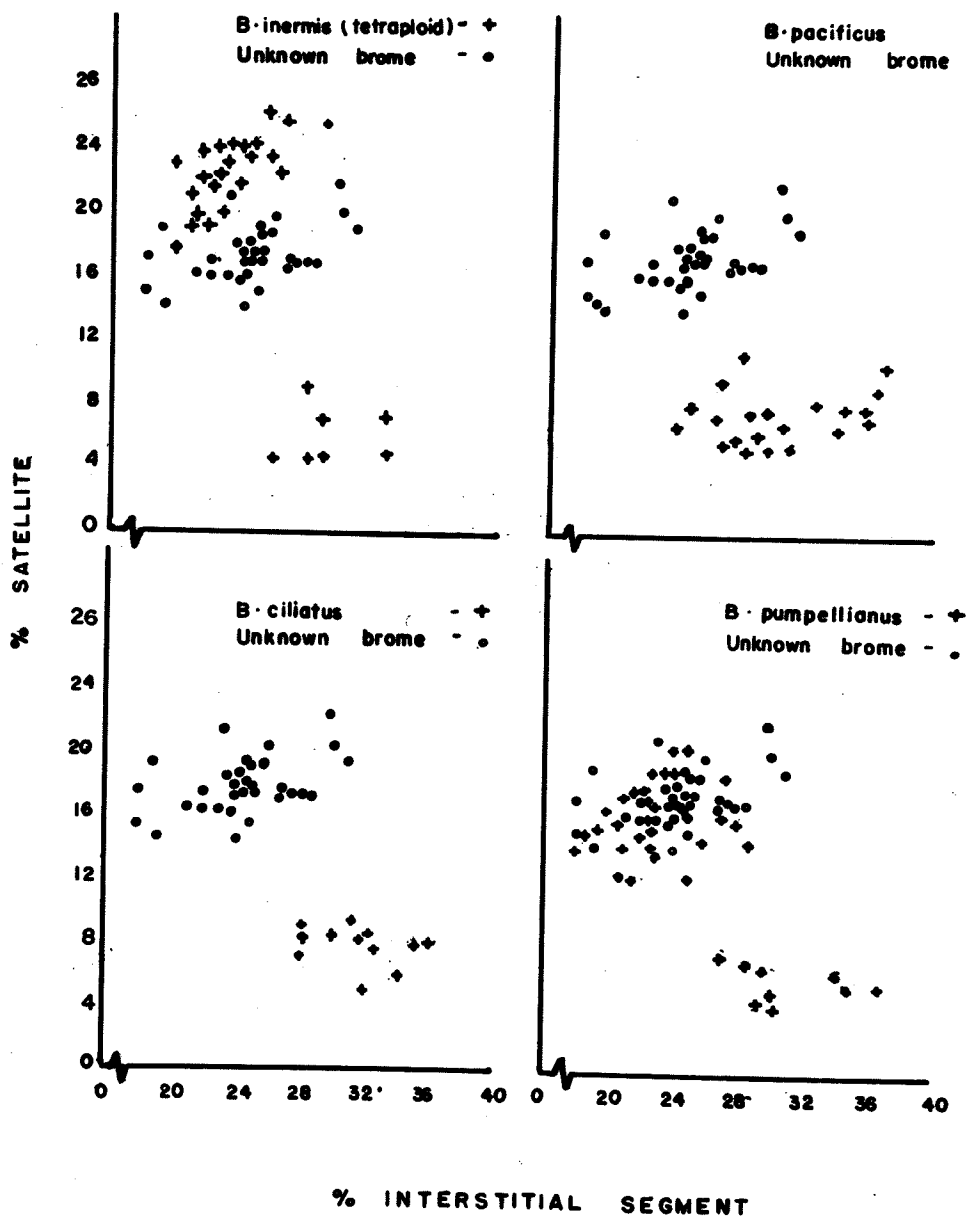


Figure 10. Comparison of satellite chromosomal segments of the Unknown Taxon and possible close relatives. Percentage of each chromosome consisting of the satellite is plotted against the percentage of the chromosome consisting of the interstitial segment.

North American B. ciliatus (Figure 9, F, G). This latter difference was noted in several cytological preparations despite varying amounts of chromosome shrinkage. The pair of small satellites seen once in the Unknown Taxon was also minute.

B. pacificus satellites were clearly distinguishable from the large satellites in the Unknown Taxon. The average ratio of lengths of chromosomal segment to satellite was 4.15 (sd. 0.83) in B. pacificus and only 1.40 (sd. 0.15) in the Unknown Taxon.

The number of observable satellites corresponded to the number of basic genomes in B. pacificus and in B. ciliatus and in the one instance, to the number in the Unknown Taxon. As pointed out previously there were fewer observable satellites than basic genomes in octoploid B. pumpellianus, there being four large and two small satellites.

Interspecific compatibility and fertility

Attempted hybridizations of the Unknown Taxon with B. ciliatus and B. pacificus were unsuccessful (Table 10). No hybrid seeds developed from 583 crosses of B. ciliatus on to the Unknown nor from 98 of the Unknown Taxon on to B. pacificus. Two seeds developed from the B. ciliatus cross, but since they possessed the chromosome number of the maternal parent, $2n = 28$, instead of the expected $2n = 21$, these likely resulted from self pollination.

In contrast to this, nearly all crosses between the Unknown Taxon and both forms of B. pumpellianus, and between the Unknown Taxon and tetraploid and octoploid B. inermis, produced a large number of

TABLE 10. Attempted hybridizations of the Unknown Taxon (Un.) with B. ciliatus (B. cil.); B. pacificus (B. pac.); B. pumpellianus (B. pu.) and B. inermis (B. in.)

	Number florets polli- nated	Number seeds	Percent seed set	Number germ- inated seeds	Number hybrid plants	Chrom- osome number
Un.(28) x B. cil.(14)	583	2	0.003	2	0	
Un.(28) x B. pac.(14)	98	0				
Un.(28) x B. pu.(56,54)*	460	166	36.1	0	0	
B. pu.(56,54) x Un.(28)	334	162	48.5	9	5	42,40*
Total	794	328	41.3	9	5	42,40*
B. in.(56) x Un.(28)	111	24	21.0	0		
B. in.(28) x Un.(28)	126	0				
Un.(28) x B. in.(28)	349	95	27.2	56	51**	28

* A nullisomic inadvertently included as a parental plant produced two nullisomic offspring

** Three plants were recognized as hybrids on morphological grounds seeds. From 460 florets of the Unknown Taxon pollinated by two forms of B. pumpellianus, 166 seeds were formed. In the reciprocal cross, 162 out of 334 florets produced seeds. In crosses on to the Unknown Taxon by tetraploid and octoploid B. inermis, respectively, 95 out of 349, and 24 out of 111 of the florets produced seed. The reciprocal cross, with tetraploid B. inermis as the maternal parent, was surprisingly unsuccessful, and despite two separate attempts no seed was produced.

A higher percentage of florets developed seeds in crosses of the Unknown Taxon with B. pumpellianus than in crosses with B. inermis: an overall average of 41.3 percent in B. pumpellianus as opposed to 21.6 and 27.2 percent in octoploid and tetraploid B. inermis.

Despite very low seed germination, possibly brought about by chromosome imbalance, hybrid seedlings developed from crosses of the Unknown Taxon with both B. pumpellianus and B. inermis. In the first cross nine out of 238 seeds germinated and lived. Five of these proved to be hexaploid hybrids, but the other four were octoploids, presumably the result of self pollinations. Of the five hexaploids, three from a somewhat B. inermis-like B. pumpellianus were euploids ($2n = 42$), and two more, from a villous aneuploid B. pumpellianus, were aneuploids ($2n = 40$) (Figure 9, D,H). None of the seeds germinated from crosses of the Unknown Taxon with octoploid B. inermis ($2n = 56$) but when these were repeated with tetraploid B. inermis ($2n = 28$) 56 out of 95 seeds germinated and 51 were judged to be hybrids.

In the above crosses with B. pumpellianus ($2n = 56$), seeds germinated only when the octoploid was the maternal parent. A barrier apparently exists precluding gene flow to the tetraploid or to the lower chromosome number.

Six plants of a clone of the Unknown Taxon were grown with B. pumpellianus and octoploid B. inermis clones at Winnipeg, Manitoba. Their open pollinated fertility indices varied from 2 to 15 percent. When a single plant of the same clone was grown with other Unknown Taxon and B. pumpellianus clones, at Palmer, Alaska, its open-

pollinated fertility index was 47 percent. Although many factors affect compatibility, it is assumed in this instance that the presence of pollen from other clones of the Unknown Taxon at Palmer increased seed production.

Twenty of the seeds from Palmer were planted. All proved to be tetraploids and were therefore not interspecific hybrids.

Stability of the Unknown Taxon and its hybrids

Counts of micronuclei per quartet and of stainable pollen showed that the Unknown Taxon was fairly regular meiotically and is evidence in favor of its being a relatively stable taxon (Table 11). Ninety-seven percent of the quartets examined were free of micronuclei and ninety-four percent of the pollen was stainable and probably viable.

TABLE 11. Pollen and micronuclei studies of the Unknown Taxon (Un.) and its hybrids: Unknown Taxon (Un.) x tetraploid B. inermis (B. in.) and Unknown Taxon (Un.) x B. pumpellianus (B. pu.)

Plants examined	Chromosome number 2n	Number plants	Number cells	Percent stainable pollen	Number quartets	Percent quartets without micronuclei
Un. (28)	28	5	300	94	444	97
Un. (28 x B in. (28)	28	4	370	55	413	45
B. pu. (56) x Un. (28)	42	2	136	45	189	11

Stability was demonstrated further in the following experiment. Cross fertility indices were taken on 18 clones of the Unknown Taxon and 28 B. pumpellianus clones growing in the field at Palmer, Alaska. Indices

of the Unknown Taxon ranged from 29.8 to 84.7 percent, averaging 55.7 percent, and B. pumpellianus indices ranged from 8.8 to 86.8 percent, averaging 46.9 percent. All indices are well within the expected range of unselected polyploids.

Hybrids between the Unknown Taxon and both B. pumpellianus and tetraploid B. inermis were less regular and presumably were less stable than the parental plants (Table 11). B. inermis x Unknown Taxon hybrids had 45 percent of their quartets free of micronuclei and had 55 percent stainable pollen. Euploid hybrids from the B. pumpellianus cross had only 11 percent of their quartets free of micronuclei but had 47 percent stainable pollen. No panicles were produced on aneuploid hybrids with 40 chromosomes.

The significance of the Unknown Taxon

The discovery of an Unknown Taxon and the demonstration that both it and B. pacificus were tetraploids was rather surprising. As pointed out previously, tetraploids are rather rare in the north and low chromosome taxa are not generally found. The present study accounts for two more tetraploids in Alaska. B. pacificus was previously said to be a hexaploid although the chromosome number was never verified, nor was the origin of the plant material on which it was taken clearly indicated (Stahlin, 1929). The tetraploid count for B. pacificus also separates that species from B. vulgaris, a morphologically similar diploid occurring further south in mountainous regions (Wagnon, 1952).

Despite the morphological similarity of the Unknown Taxon to B. pacificus and their identical chromosome numbers, they obviously are non conspecific and probably are not closely related. The Unknown

Taxon possessed a pair of chromosomes with large satellites that were absent from B. pacificus. Small satellites were not usually visible in the Unknown Taxon but, in the one instance in which they were seen, they were smaller than those in B. pacificus. Also, the two species were completely incompatible.

No clear evidence could be found for a close affinity between the Unknown Taxon and North American B. ciliatus although the discovery of minute satellites in the Unknown Taxon and in Russian B. ciliatus suggested this possibility. The large satellite shown in the Unknown Taxon were absent from B. ciliatus and, according to another investigation (Mitchell and Wilton, 1965), from B. richardsonii. The incompatibility shown between the Unknown Taxon and B. ciliatus was very strong evidence against close relationship of these taxa.

B. pumpellianus apparently is closely related to the Unknown Taxon. The two taxa were similar morphologically; both possessed large satellites; both were quite compatible; and despite their different chromosome numbers, produced hybrids when crossed. The presence of a high level of stainable pollen in their hexaploid hybrids suggests considerable interspecific homology. On the other hand, the occurrence of a high frequency of quartets with micronuclei in their hybrids may be due to lack of pairing between chromosomes of parental genomes or to non-disjunction resulting from crossing between the two levels of ploidy.

The Unknown Taxon and B. inermis were found to be closely related. Both taxa possessed large satellites, were compatible, and produced hybrids in crosses at the tetraploid level with the Unknown Taxon as the maternal parent. It is not known why the reciprocal of this cross was

unsuccessful.

Vivipary in *B. pumpellianus*

Viviparous clones of *B. pumpellianus* have now been found in diverse morphological forms of that species that originated at widely separated points throughout much of this State (Figure 1 V). Proliferations were first seen on six plants growing in the greenhouse at Winnipeg, Manitoba, under 12 hours of daylight extended by 4 hours artificial light, at temperatures from 60° to 70°F. Growing under the same environmental conditions were over 100 plants of four varieties of *B. inermis* and 43 other plants of *B. pumpellianus*, also of Alaskan origin. None of these plants were viviparous although one culm of *B. inermis* showed an abnormal growth somewhat resembling vivipary. A total of 20 proliferations were excised from the viviparous plants, planted in vermiculite, replanted in soil 2 weeks later, and grown to maturity.

Vivipary has been observed by the author on four further occasions in *B. pumpellianus* (Table 12). First, under an environment similar to that reported above, five of the original plants again became viviparous; later when two original plants and two propagules were grown under a 20 hour day with artificial light, vivipary occurred; subsequently another plant growing under a 16 hour day with artificial light proliferated; and finally an additional plant proliferated when grown under wet conditions in the field at Winnipeg, Manitoba (latitude 49° 55'). None of these clones have proliferated in the field at Palmer, Alaska (latitude 61° 35') although they have been observed for five seasons. It is interesting that one plant showing vivipary in the field at Winnipeg was an aneuploid

with the somatic chromosome number of 55. Another aneuploid with 58 or 59 chromosomes was found in the clone which proliferated under the 16 hour day. In addition, the author has found vivipary once in a plant of the Unknown Taxon, and Dr. Hodgson, a co-worker, also found it on a B. pumpellianus plant from the extreme north (Figure 2 V₁). In both of these instances the plants were growing in the greenhouse under an 18 hour day with artificial light.

TABLE 12. Presence (+) or absence (-) of vivipary in panicles of B. pumpellianus grown under four environments

Environment	Plant ⁴							
	1	2	3	4	5	6	7	8
Winnipeg								
Greenhouse 1961 ¹	-	+	+	+	+	+	+	*
Greenhouse 1962 ¹	-	+	+	+	*	+	+	*
Field (1 season)	+	-	-	*	*	-	*	*
Palmer								
Light chamber ²	*	+	+	*	*	+	+	-
Light chamber ³	*	*	*	*	*	*	+	+
Field (5 seasons)	-	-	-	-	-	-	-	-

* No observation recorded

³ 16 hours artificial light

¹ 12 hours natural light plus 4 hours artificial light

⁴ plant numbers are unrelated to previous numbers in this thesis

² 20 hours artificial light

There are apparently several environmental conditions which predispose a plant to vivipary. Light, temperature, and moisture have all been suggested as possible causes of viviparous development in different

species (Nielsen, 1941; Nygren and Almgard, 1962). The present observations show that vivipary in some plants of this species occurred where there were long days, relatively moist conditions, and moderate temperatures. The atmosphere in both greenhouse and light chamber were relatively moist, and wet conditions prevailed in the field where vivipary occurred. The culm showing vivipary in the field at Winnipeg showed evidence of being in contact with the wet soil. However, humid atmosphere and wet soil conditions frequently prevail at the time of anthesis in the field at Palmer where no vivipary occurred. Summer temperatures are usually higher at Winnipeg than at Palmer, and this may have a bearing on the occurrence of vivipary.

Clausen and Hiesey (1958) cited an example in which non-viviparous plants of tufted hairgrass, Deschampsia caespitosa, were moved from native habitats at 56, 60 and 68 degrees latitude northern Europe, to 38 degrees north latitude, western United States. When grown at this lower latitude some plants from all three latitudes became viviparous. It is implied, although not specifically stated that change in day length is a major factor in promoting vivipary in this grass.

Nygren and Almgard (1962) found that changing to a long day treatment combined with high temperatures counteracted vivipary in Poa bulbosa and Poa alpina, while changing to short day treatments and moderate temperatures stimulated the formation of propagules in P. chaixii. In contrast to this, the above observations show vivipary to occur in B. pumpellianus under quite long days although this is the natural day length for anthesis and seed production in Alaska where they originated. Change in day length therefore does not appear to be a major factor in promoting vivipary in

this species.

There is little doubt, however, that a particular environment is required for the expression of vivipary (Table 12). Furthermore, the fact that relatively few plants of the population exhibited this condition suggests that only certain genotypes will respond to the inducing environment.

It was originally considered that the proliferations might be of parthenogenetic origin and possess the reduced chromosome complement of an unfertilized egg. However, somatic chromosome numbers were determined on propagules from plants numbers 2 to 6 and it was found that they had 56 somatic chromosomes as did their parents. Proliferations from plant number one were only seen on dried material and it was not possible to determine the chromosome number. Mature plants grown from propagules were similar to the mother plants in gross morphology, indicating that the genotype probably was of maternal origin.

The manner of proliferation also indicates maternal origin of propagules. As shown in Figure 11A the first proliferating leaflet always arose from the terminal floret of a spikelet. In plants where this was not readily apparent closer examination revealed that the leaflet was never subtended by a lemma alone but instead a palea, and usually a pistil and anthers, were found between it and the next lower lemma (Figure 11B).

Dissection of a proliferation other than the ones shown revealed that of its three leaflets, the outer enclosed a pistil, anthers, and palea; and the inner and youngest leaf only enveloped a palea. It seems that when plants possessing the genetic potential for proliferation were

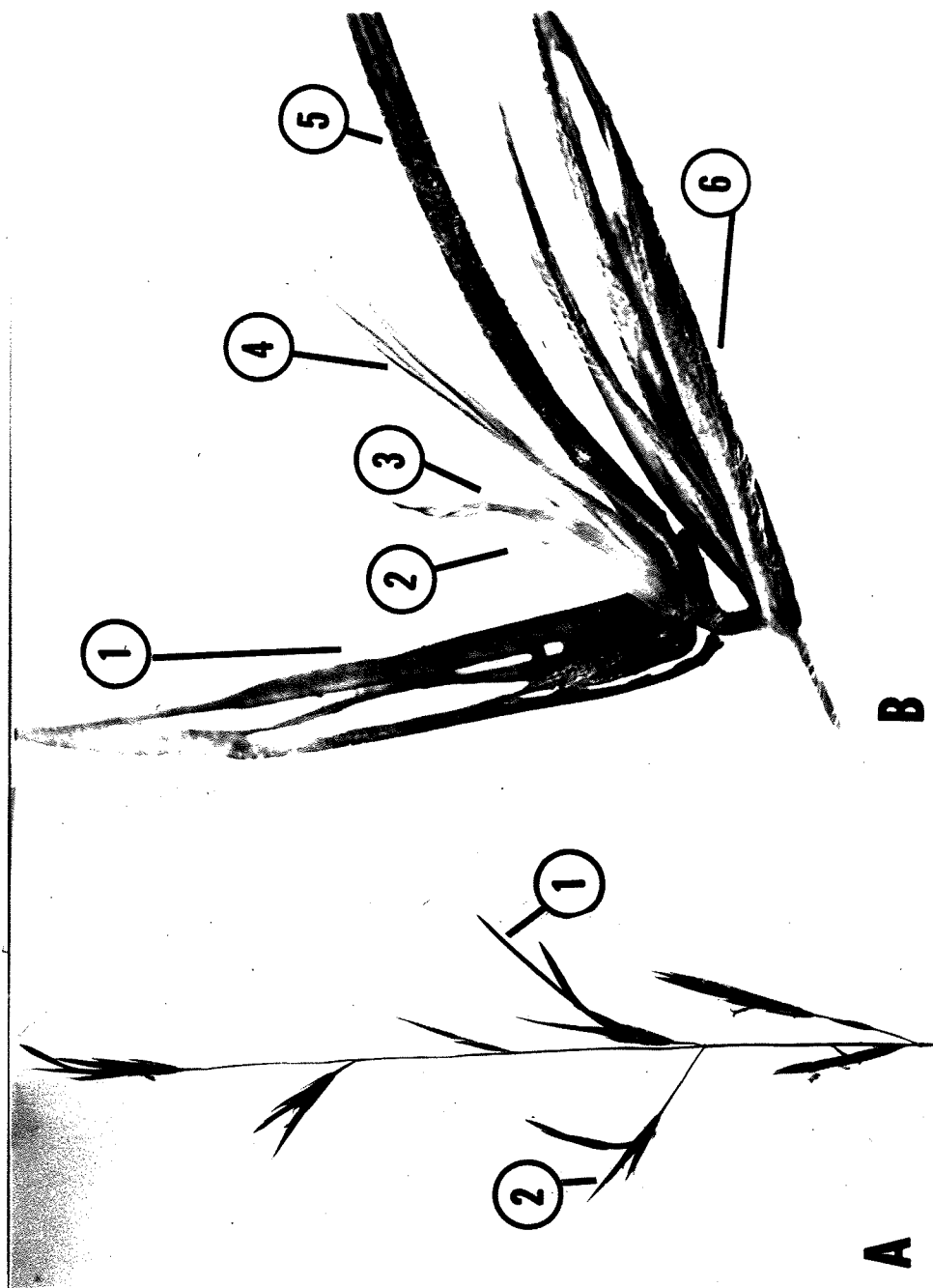


Figure 11. Leafy proliferations in a panicle of *B. pumpellianus* showing A, whole panicle with (1) leafy proliferations and (2) elongated lemmas; B - single proliferation with (1) enveloping lemma, (2) two of the anther filaments, (3) pistil, (4) palea, (5) proliferating leaflet arising from outside of the floret and (6) next lower floret.

grown under long days, relatively moist conditions, and moderate temperatures, the lemmas continued to grow and became leaflets of the viviparous propagules.

The viviparous plants varied considerably in morphological type and in plant fertility. Complete records were available on four of them. These had morphological indexes of 8, 11, 15 and 16 covering nearly the complete range of types found in the present investigations. The average open-pollinated fertility indexes in one year at two locations were 16.7, 28.8, 38.2 and 64.1. Plants exhibiting vivipary were therefore not necessarily infertile although seed yields were low in three of four viviparous plants.

Nygren (1954) has pointed out that vivipary is usually associated with aneuploidy, meiotic irregularity and is common in the higher ranges of polyploidy. In this investigation aneuploidy has been noted among these plants, they are polyploids, and judging by the lower seed yields on three of the four plants they may, on the whole, be somewhat irregular meiotically. However, since one of the viviparous plants was relatively fertile, low seed production is not a prerequisite for the occurrence of vivipary. Vivipary has occurred frequently in the octoploid B. pumpellianus but only once in the tetraploid Unknown Taxon. Although it has not been shown in this investigation that the Unknown Taxon and B. pumpellianus are the same species, the more common occurrence of vivipary at higher ploidy levels of closely related species has been demonstrated.

The demonstration that the proliferations will root under the proper environment suggests that vivipary may have been a means of survival in

this species under certain extreme environmental conditions.

The total absence of the phenomena in B. inermis in the present experiment and its widespread occurrence in northern B. pumpellianus is suggestive of genetic difference between the two species. It is not known whether vivipary occurs in B. pumpellianus plants from farther south. Despite occasional reports of vivipary in B. inermis its failure to occur here or in any other greenhouse, light chamber or field experiment seen by the author, indicates that it is not common in the species.

It was concluded that this anomaly, which at first seemed somewhat unrelated to the main aims of the thesis, was indeed valuable phylogenetic evidence. It provided additional evidence to their gross morphological differences for the continued taxonomic separation of B. pumpellianus from B. inermis.

Agronomic aspects

Judging by the variable cross-compatibility and the presence of much aneuploidy in B. pumpellianus that species probably has a varied genetical background, in turn suggesting that it would be variable in its agronomic performance. On the other hand, in view of the distinct morphological differences the two species would be expected to be quite distinct from one another. The following results confirm this line of reasoning.

Rhizome development

B. pumpellianus and B. inermis are quite different in degree of rhizome development. The greatest area covered by a plant of B. pumpel-

lianus was 558 square inches, the area covered by the least spreading B. inermis was 1434 square inches (LSD 495 square inches)(Table 13). At Palmer, B. pumpellianus spread less than B. inermis. After one year's growth these B. pumpellianus clones covered an average area of 199 square inches. Fifteen B. inermis plants growing among them covered an average area of 744 square inches. A significant correlation ($r = +0.51$) was found between the B. pumpellianus clonal averages at Winnipeg and the individual plant measurements at Palmer. Considering the lack of replication in the Palmer test and the difference in latitude, this is a reasonably close correlation. A statistical analysis of the amount of spread in B. pumpellianus at Winnipeg showed highly significant clonal differences. These results suggested that spread of B. pumpellianus, although it is affected to some degree by environment, is largely under genotypic control. The B. inermis clones also varied in the amount that they spread when tested at Winnipeg; the one originating in Alaska covering much more area than the others. This was rather surprising since northern strains of B. inermis are usually thought to be non-spreading (Hughes, Heath and Metcalfe, 1951). Knowles and White (1949), however, failed to find a significant difference between northern commercial B. inermis and the southern strain Achenbach.

Although the extremely spreading B. inermis plant was not particularly rhizomatous when grown at Palmer the lack of rhizome development there could have been caused by winter injury since it was growing in a rather low spot in the field.

The single clone of the Unknown Taxon was non-spreading at either location.

TABLE 13. Area of cover and forage production of B. pumpellianus and B. inermis plants and one plant from the Unknown Taxon.

Plant number	Morphological index	Area of cover (sq.in.)	Forage production			
			Leafiness (1-10) 12-6-62	Leaf height (in.) 30-7-62	Ratio of DM/area of cover 30-7-62	Leaf height (in.) 3-10-62
<u>B. pumpellianus</u>						
1	13.0	264.0	5.0	6.0	0.49	9.7
2	9.0	225.0	8.2	5.8	0.19	4.8
5	11.0	172.0	9.0	7.2	0.15	6.8
6	11.0	113.0	6.7	6.6	0.94	10.3
7	14.0	192.0	4.2	7.3	0.48	7.7
9	17.0	314.0	2.7	14.3	0.69	12.2
10	14.0	310.0	2.0	14.2	1.58	11.3
11	10.0*	291.0	9.2	-	-	6.5
12	11.0	138.0	9.3	4.0	-	-
13	11.0	211.0	6.0	6.0	0.54	9.3
14	14.0	384.0	5.0	7.2	0.47	8.7
15	11.0	176.0	3.1	6.2	0.51	9.0
16	12.0	328.0	4.2	12.0	0.67	10.7
18	16.0	558.0	1.8	12.3	0.80	15.3
19	8.0	199.0	6.5	5.3	0.40	6.7
20	11.0*	156.0	8.8	-	0.19	-
21	8.0	94.0	5.2	5.0	0.65	6.7
24	17.0	502.0	6.3	7.0	0.21	7.0
25	16.0	186.0	6.3	7.0	0.64	-
26	16.0	324.0	5.0	7.2	0.52	9.0
27	18.0	302.0	5.5	17.1	1.70	11.8
28	8.0	76.0	7.2	4.6	-	5.5
29	12.0	184.0	8.8	6.3	0.20	8.5
30	12.0	292.0	3.5	6.8	0.41	9.0
32	10.0	236.0	1.5	4.0	0.42	7.5
33	10.0	217.0	7.8	4.3	0.19	5.5
34	14.0	193.0	-	-	-	6.9
36	7.0	391.0	-	-	-	-
Avg.	12.2	246.3	5.7	7.6	0.57	8.6

TABLE 13 CONTINUED

Plant number	Morphological index	Area of cover (sq.in.)	Leafiness (1-10) 12-6-62	Leaf height (in.) 30-7-62	Ratio of DM/area of cover 30-7-62	Leaf height (in.) 3-10-62
<u>B. inermis</u>						
3	24.0	1434.0	5.0	20.0	-	9.0
8	24.0	1472.0	6.0	21.8	0.18	11.8
17	24.0	1751.0	3.5	22.2	0.24	12.8
22	24.0	2071.0	3.2	22.2	0.21	11.0
23	23.0	2275.0	3.3	16.7	0.06	5.8
31	24.0	5148.0	2.8	26.7	0.28	14.3
35	24.0	2655.0	5.3	20.7	0.09	8.0
Avg.	23.8	2400.8	4.1	21.5	0.18	10.4
<u>Unknown Taxon</u>						
4	14.0	47.0	4.5	6.2	1.18	6.5
LSD 1%	**	653.0	2.9	4.6	0.40	2.8
	***	133.0				
5%	**	495.0	2.2	3.5	0.30	2.6
	***	100.0				

- * Compiled from data incomplete in all replicates.
 ** Including B. inermis in the analysis
 *** Excluding B. inermis from the analysis

Forage production

Data on forage production of B. pumpellianus is presented in Table 13. B. pumpellianus plants varied in their ability to produce spring forage and scores for leafiness in the spring taken upon B. pumpellianus considerably overlapped similar records of B. inermis plants. The average scores among the B. pumpellianus ranged from 1.50, very leafy, to 9.30, sparsely leafy; scores among the B. inermis ranged from 2.83 to 6.00. The Unknown Taxon had an average rating of 4.50 (LSD 1% = 2.90).

The height of leaves on non-flowering culms in midsummer, on the other hand, was clearly different in the two species. All of the B. pumpellianus plants except one, had leaves that were significantly shorter at maturity than those of B. inermis. The average leaf heights of B. pumpellianus in midsummer varied from 4.00 to 17.1 inches. B. inermis plants ranged from 16.7 to 26.7 inches and the Unknown Taxon was 6.2 inches (LSD 1% = 4.6 inches). Similar results have been obtained on other plants of B. pumpellianus and B. inermis in Alaska.

An index of productivity was obtained by dividing the total amount of dry matter by the area of plant cover. The B. inermis produced less forage per unit area than most of the B. pumpellianus. Production in B. inermis ranged from 0.06 to 0.24 grams per square inch; and in B. pumpellianus from 0.19 to 1.70 (LSD 1% = 0.40 grams per square inch). The very dense growth usually seen on B. pumepllianus plants probably partially accounted for their favorable showing.

One might expect this method of measuring yield would penalize the more rhizomatous plants because of limited access of the central culms to nutrients, but this did not happen. Some of the more rhizomatous of the

B. pumpellianus, plants 10, 27 and 18, had the greatest amount of forage per square inch. Similarly, among the B. inermis, the most spreading plants produced the most forage per unit area.

Regrowth after harvest was similar in the two species. Fall plant height varied from 5.8 to 14.3 inches in B. inermis and from 4.8 to 15.3 inches in B. pumpellianus.

On the whole, the B. pumpellianus plants produced coarser, less leafy forage but were satisfactory as far as the total amount of production was concerned. There appeared to be some possibility for improvement of B. pumpellianus through selection for early forage production.

Fertility and seed production

Although self-fertility data was of necessity unreplicated and therefore not analyzable certain trends were evident (Table 14). B. pumpellianus, in general, was self-sterile, appearing to be more so than B. inermis. Self-fertility indexes in clones of the native species ranged from 0.0 to 14.5 percent, whereas, those of B. inermis ranged from 0.0 to 32.5 percent. Five out of the 24 B. pumpellianus plants were more than five percent self-fertile and three of the six B. inermis plants were more than five percent self-fertile.

The analyses of cross fertility indexes were significant. At the five percent level of significance cross-fertility indexes of three out of 23 B. pumpellianus plants from Winnipeg were significantly lower than the index of the least fertile B. inermis plant, one was significantly more fertile than the most fertile B. inermis, and two were not significantly different than the most fertile B. inermis plant (Table 14). In the same test the range of cross fertility among the B. pumpellianus plants varied

TABLE 14. Ratios of yield of dry matter to yield of seed, and fertility indexes of B. pumpellianus and B. inermis and one plant of the Unknown Taxon.

Plant number	Morphological index	Winnipeg			Palmer			Avg. 2 locations OP 1962
		Yield DM/ seed 1962	Percent fertility		Percent fertility			
			Self 1962	OP 1962	OP 1962	OP 1963	2 yr. avg.	
<u>B. pumpellianus</u>								
1	13	29.3	0.0	76.6	47.3	45.0	46.0	62.0
2	9	105.8	0.2	42.5	12.4	17.5	15.0	27.4
5	12	-	2.7	39.7	72.0	53.0	62.5	55.8
6	13	58.8	0.5	8.3	8.8	6.0	7.4	8.6
7	15	50.5	1.4	11.5	21.5	19.0	20.2	16.5
9	17	28.9	2.4	20.4	64.9	34.0	49.4	42.6
10	14	7.5	2.6	44.7	45.8	29.0	37.4	45.3
11	9	-	0.0	10.4	60.0	32.5	-	35.2
12	11	-	8.0	9.2	15.8	10.8	13.3	12.5
13	11	58.9	1.9	23.7	84.6	69.0	76.8	54.2
14	14	80.8	14.5	14.3	32.8	12.0	22.4	23.5
15	11	51.2	5.8	45.8	82.4	96.0	89.2	64.1
16	12	16.9	3.3	20.5	44.3	27.0	35.6	32.4
18	16	25.8	0.0	37.3	19.3	5.0	12.1	28.3
19	8	208.8	-	27.1	30.6	22.5	26.6	28.8
20	12	8.1	-	55.0	86.8	52.5	69.6	70.9
21	8	21.4	6.2	22.0	29.6	33.0	31.3	25.8
24	16	13.0	-	57.5	66.7	86.0	76.4	62.1
25	16	-	6.1	38.6	22.2	23.0	22.6	30.4
26	16	6.8	-	41.3	35.1	23.0	29.0	38.2
27	18	21.2	0.0	44.2	30.6	20.0	25.3	37.4
28	8	-	0.6	11.1	27.3	17.5	22.4	19.2
29	12	104.9	3.1	21.4	61.0	27.5	44.2	41.2
30	12	49.7	0.0	56.7	84.8	54.5	69.6	70.7
32	10	48.4	-	37.4	46.3	25.0	35.6	41.8
33	10	58.2	1.4	35.7	52.2	24.5	38.2	43.9
34	14*	-	1.9	48.8	34.6	29.0	31.8	41.7
36	7*	-	2.6	28.5	30.8	7.0	18.9	29.6
Avg.		50.2	2.8	33.2	44.6	32.5	38.1	38.9

TABLE 14. CONTINUED

Plant number	Morpho- logical index	Winnipeg			Palmer			Avg. 2 loca- tions OP 1962
		Yield DM/ seed 1962	Percent fertility		Percent fertility			
			Self 1962	OP 1962	OP 1962	OP 1963	2 yr. avg.	
<u>B. inermis</u>								
3	24	-	0.1	-	-	-	-	-
8	24	10.2	0.0	40.3	-	-	-	-
17	24	5.4	32.5	59.7	-	-	-	-
22	24	14.7	0.5	25.2	-	-	-	-
23	23	13.0	0.7	25.2	-	-	-	-
31	24	15.0	7.4	50.7	2.1	9.0	5.5	-
35	24	5.1	14.9	41.4	-	-	-	-
Avg.		10.6	8.0	40.4	-	-	-	-
<u>Unknown Taxon</u>								
4	14	94.9	0.7	8.0	46.0	48.0	-	27.0
	LSD 1%			16.6**			26.5***	
	5%	35.3**		12.5**			19.6***	13.8

* Compiled on the basis of data incomplete in all replicates

** Leaving out plots 11, 20, 23, 28, 33, 34 and 36 in the analysis since they had more than one missing plot

*** Only B. pumpellianus clones analyzed

from indexes of 8.3 to 76.6 percent, and from 25.2 to 59.7 percent among the B. inermis plants. In a test of the same lines of B. pumpellianus at Palmer, Alaska, as had been grown in Winnipeg, open-pollinated fertility indexes ranged from 8.8 to 86.8 in 1962 and from 5.0 to 96.0 in 1963. All differences were highly significant. In these tests, therefore, there was not a wide difference between B. inermis and B. pumpellianus in cross fertility but the native species possessed a number of very infertile plants.

Differences in latitude, soil, or pollen parents were not the major factors contributing to variation in open pollinated fertility among this group of native brome. In the combined analysis of 1962 cross fertility data at Winnipeg and Palmer, clones of B. pumpellianus differed by a significant amount. Fertility varied from 8.6 to 70.9 (LSD 5% = 19.6). The significance of clonal differences in combined analysis suggests that factors in the maternal plants themselves govern their fertility more than does their cross compatibility. The causes of the lowered fertility in some plants are not understood except in the one instance where an infertile plant, number 7, was shown to be an aneuploid.

The villous plants were as a group rather infertile. Plants numbered 2, 19, 21, 28 and 36, all with a morphological index below 9, had respective cross fertility indexes of 27.4, 28.8, 25.8, 19.2 and 29.6 when averaged over two locations in 1962. The remainder of the plants had fertility indexes varying from 8.6 to 70.9. Plant number 27, with an index of 18, had a cross fertility index of 37.4; plant 32, with a morphological index of 10 had fertility index of 41.8.

From the tests of seed production at Winnipeg it seems apparent that unselected B. pumpellianus plants are rather low seed producers. Ratios of

dry matter to seed yield were much lower for B. inermis than for B. pumpel-
lianus in most instances (Table 14); indicating that the cultivated brome
was a much superior seed producer.

The total seed yield was closely allied to the fertility index. Thus,
plants with a low fertility index under open pollination were, as a rule,
poor seed producers (high DM/seed ratio); where the fertility index was
high better seed production usually occurred (low DM/seed ratio). Among
this group of plants one would conclude that genetic factors rather than
physiological adaptation might have had a major effect on seed production.

CONCLUSIONS

It was concluded from this investigation that two major Bromopsis members, B. inermis and B. pumpellianus, and one Eurasian species, B. ornans were closely related. This conclusion is dictated, not only by their gross morphological similarities, but by their similar karyology and by the results of hybridization experiments. Except for the introduced polyhaploid B. inermis all plants of the three species proved to be octoploids, all had two pairs of large satellites and had small satellites; most crosses between B. inermis and the other two species were successful. The common ability of B. inermis and two forms of B. pumpellianus to cross with the Unknown Taxon is evidence of their interrelationship.

The Unknown Taxon apparently also belongs to the B. inermis, B. pumpellianus, B. ornans complex although it is from a lower level of ploidy. It had one pair of large satellites, and one plant possessed small ones; it crossed with B. pumpellianus despite their different chromosome numbers, and also crossed with the polyhaploid B. inermis. On the other hand, there was evidence that it was not closely related to other Bromopsis members.

One may draw some tentative conclusions as to the phylogeny of Alaskan bromegrasses. The common alloautoploid status of the octoploids, suggested by the presence of two pairs of large satellites in cells of each species, indicates a common heritage. The proven relationship of B. pumpellianus to two species from Eurasia, B. inermis and B. ornans, suggests that B. pumpellianus or its progenitor could also have come from there. The Unknown Taxon could either have originated in Eurasia and then

migrated to North America to remain as a relic species, or it could have begun as a polyhaploid form of B. pumpellianus. Relic status for the Unknown Taxon seems the most probable for the following reasons: (1) It is morphologically variable and possesses characters not as prominent in B. pumpellianus, i.e. drooping panicle, non-spreading growth habit, and a long ligule. (2) It crosses with B. pumpellianus with great difficulty. (3) The Unknown Taxon is relatively self-sterile and unless polyhaploidy occurred simultaneously in several plants it is doubtful if a polyhaploid form could have persisted. (4) The recent paper by Johnson and Packer (1965), showing a preponderance of polyploid over diploid monocotyledons in an area adjacent to the collection site of the Unknown Taxon suggests that that environment has been most favourable to the higher levels of ploidy. Evolution must have progressed from lower to higher chromosome numbers in the species that they studied. There seems no good reason why its direction should be reversed in the present instance.

Although the octoploids are closely related and possibly all originated in Eurasia they appear to have diverged considerably and here do not appear to be conspecific, as intimated by Elliot (1949b), and Wagnon (1952). A minor incompatibility barrier hindered, and in some instances, prevented crossing of some B. pumpellianus with B. inermis. There was no difficulty in separating the species morphologically. Under the conditions of the experiment vivipary was found in all forms of B. pumpellianus but not in B. inermis, signifying that the species differed physiologically. B. pumpellianus

is variable indicating an infusion of outside germplasm. The presence of a close relative, the Unknown Taxon, to which B. pumpellianus might cross suggest that genetic changes may be occurring within the octoploid entirely unrelated to those brought about by recent introgression of B. inermis.

One must conclude however, since some plants of all forms of B. pumpellianus will cross with B. inermis, and since this form of B. ornans will cross with it, that B. pumpellianus and B. ornans are potential breeding material. The closeness of their relationship would govern the method of breeding to be used. Since forms of B. pumpellianus similar to B. inermis are easiest to cross and at the same time possess desirable agronomic attributes these would be most useful in a breeding program. However, all forms of the species spread very little, produce early forage and are reasonably productive.

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