NOTE TO USERS

CROSS-FERTILITY AND CYTOGENETICS OF SELECTED BROMOPSIS SECTION MEMBERS WITHIN THE GENUS BROMUS L.

by

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INTRODUCTION

Bromus inermis Leyss is one of the most promising perennial forage grasses in temperate regions of the United States and Canada. The improvement of this and other perennial species for pasture, hay and conservation purposes, looms as a stimulating challenge to the plant breeder.

Significant varietal improvement has not been made in *B. inermis* to date. Certain features of this apparent failure have been explored cytologically in this study. Progress in the synthesis of outstanding varieties cannot as yet be compared to that attained in cereal and corn breeding programs. The Fischer, Lincoln, and Achenbach varieties, grown extensively at present, represent merely the increase of seed harvested from surviving members in old established fields.

There are, from an agronomic point of view, a number of problems needing special attention in the improvement of grasses. These are:

1. Greater yields of higher quality forage in grass-legume associations.
2. Seedling vigor.
3. Various seed and seed-setting characters.

In addition, resistance to organisms causing foliage diseases and to certain soil borne fungi may become pressing pathological problems. A serious objection to certain sod-forming grasses

alone or in combinations with legumes is the aggressive spreading habit of these grasses. Pure stands may rapidly become sod-bound and unproductive with depleted nitrogen supplies. Some grasses may tend to crowd out the legume component of the forage. Selection for a non-spreading growth habit in some instances may prove advantageous.

In an intensive breeding program the closely related taxonomic entities may deserve attention as potential sources of germplasm in the improvement of parental species. The successful transfer of black stem rust from Triticum durum to T. aestivum by Hayes (16) and of T. Dicoccum to T. aestivum by McFadden (33) may be cited as outstanding interspecific transfers which later may be repeated in kind among the forages.

The possibility of successful transfer of specific genes for disease resistance, or other agronomic characters, from one species to another is dependent, however, upon the fertility relations and possible extent of recombination. The success of interspecific hybridization cannot be measured in terms of F_1 fertility. Recombination may have been seriously impaired and succeeding hybrid generations may rapidly return to the parental types. Simply inherited characters such as resistance to specific races of certain obligate organisms may be transferred more readily than characters inherited in a quantitative manner.

Information concerning fertility relations between species is a necessary adjunct to the attempted transfer of specific

genes from one member to the other. These data correlated with a cytological analyses of the parents and progenies also may yield important phylogenetic information.

The Bromopsis section of the genus Bromus, although its representatives are distributed rather widely in temperate regions of four continents, is unique in that one species has completely dominated the other members from an agronomic point of view. B. inermis is particularly well adapted to a great many conditions in the temperate regions of North America, Asia, and Europe. It has now become of great importance in the northern sections of the United States. High yields of forage under a wide range of environmental conditions have established the species as one of the leading grass components of forage in this area.

A survey of fertility relations among the available section members, as well as a cytological analyses, would seem desirable to predict the possibility of gene exchange or transfer from the unimportant members to B. inermis.

REVIEW OF PERTINENT LITERATURE

General reviews of forage breeding

Two reviews rather thoroughly covering the subject of forage breeding recently have appeared in print. Atwood (1), in a comprehensive review, summarized the available cytogenetic literature pertinent to forage breeding. He emphasized the role of cytogenetics in the forage breeding program and postulated increasing emphasis on the correlation of cytological information and breeding behavior. Myers (35), in a review of the cytology and genetics of forage grasses, emphasized the cytogenetic approach as an aid in breeding and an adjunct to morphological data in studies of taxonomy and phylogeny. In addition to the extensive literature review the available chromosome numbers of numerous grasses have been tabulated by Myers (35).

The North American history of B. inermis

In a recent review of the early history of B. inermis in North America, Nielsen (38) has attempted to trace the vague and inadequately recorded introductions of this species into this country and Canada. In view of his treatment literature of historical interest largely has been omitted in this review.

The cytology and breeding of B. inermis

In contrast to the extensive researches under way to improve B. inermis, relatively little cytological information is available of significance to the breeding program. In early papers Avdulov (2) reported a 2n=42 chromosome number which was reported also by Knobloch (26). This count could not be confirmed in a study by Elliott and Love (9) involving some of the clones used by Knobloch (26). Additional cytological analyses reported in this study have failed to verify a 2n=42 chromosome number in the nursery material examined. In a recent survey of chromosome numbers in B. inermis, Hill and Myers (18) reported on the chromosome number of 193 plants representing 111 seed lots from widely separated sources. No instance of a 2n=42 chromosome number was reported in their study. They did, however, report the occurrence of one plant with 8 to 11 accessory chromosomes in addition to the normal complement of 2n=56.

On the basis of observed cytological irregularities, Elliott and Love (9) postulated the association of a selective advantage with certain cytological irregularities. They postulated additionally that the maintenance and selective advantage imparted to a clone by these mechanisms might impose restrictions on the sampling of potential gamete populations. The postulates presented in that paper have been enlarged upon and extended in the present study.

Nielsen (39) recently has reported on megasporogenesis and fertilization in B. inermis. He concluded that embryo sac development was of the "normal" type.

Cheng (3), in his study of self-fertility in three commercial grasses, reported a close relation between the percentage of aborted pollen in clones of B. inermis and the frequency of quartets with micronuclei. This relation was not consistent in the study by Elliott and Love (9), nor in the present report. In clones of brome grass, as well as crested wheat-grass, Cheng (3) found a significant negative correlation between the percentage of aborted pollen and the number of viable seeds produced per sample under open-pollination. He obtained a significant correlation between seed-setting under bags and under space isolation in addition to significant differences in seed-setting between clonal lines within B. inermis under open-pollination and under parchment bags.

Smith (43), from a summary of results of numerous investigations on self- and cross-fertility, reported that both B. erectus and B. inermis were low in self-fertility. He also reported that selfed and open-pollinated seed of both species germinated approximately 50%. For a more recent and thorough summary of the available literature on self-fertility and progeny performance in B. inermis the reader is referred to the review by Hawk (15).

Pertinent morphology and physiology of B. inermis

In 1913 Keyser (25) reported the presence of extensive morphological variability in available B. inermis clones under observation at the Colorado station. In 1921 Waldron (47) reported on physical and chemical studies of brome grass seedlings and clones at the North Dakota station. The analyses presented in this bulletin provide evidence of considerable morphological and physiological variability within the available material of the species.

The extensive botanical-agronomical study of B. inermis by Zherebrina (49) emphasized the tremendous intraspecific variability of the species in Russia as well as the possible variability resulting from introgressive hybridization with B. erectus.

Knobloch (26), in 1943 found even greater variability of certain morphological characters than previously reported.

Studies by Newell and Keim (37), Wilsie, Peterson, and Hughes (48), and a study reported by Evans and Wilsie (10) have emphasized differences in performance of clones from separate regional sources. The morphological and physiological differences of Canadian strains, particularly when grown south of central Iowa in comparison with such strains as Lincoln, Fischer, and Achenbach, justified their treatment by these authors as northern and southern types respectively.

Evans and Wilsie (10) noted also that clones of varying

maturities responded differently to temperature and fertility levels under greenhouse culture.

A recent study by Gall (13) of flowering in B. inermis under certain environmental conditions, reemphasized the necessity for long day photoperiods to induce flowering in this species. Although floral primordia were present in field-grown plants at Chicago in April a subsequent 13 hour photoperiod inhibited normal development and elongation.

In a companion paper Dibbern (6) reported the vegetative responses of the species to environmental variation. He noted that clipping was more adverse to root growth than top growth with this effect carrying over into the next season. In a comparison of various soil containers in the greenhouse culture of B. inermis, Dibbern (6) noted that the clones in 8 inch glazed pots were as vigorous as those in clay pots two and one half times larger.

Interspecific fertility relationships of certain bromes

The available literature on interspecific hybridization in Bromopsis section of Bromus L. is quite restricted. Knowles (29), in his studies of the improvement of B. mollis by interspecific hybridization, was unable to cross this species of the Bromium section with either Bromopsis or Neobromus section members. Where hybrids were obtained between B. mollis and Eubromus section members they generally were intermediate between the parental species in morphological characters.

Knowles (29) found a distinct parallel relation between the morphological similarity of parent species and the degree of chromosome association in the F_1 hybrids between them.

Stebbins and Tobgy (46) were able to hybridize representatives of the B. carinatus complex with the South American species B. catharticus. On the basis of cytological and morphological evidence they postulated that the B. carinatus complex arose as a result of hybridization between B. catharticus, or a similar related species, and some diploid species ancestral to the west coast species of the Bromopsis section. Because of the extreme polymorphism of this complex, Stebbins and Tobgy (46) admit the possibility of more than one Bromopsis species entering into the ancestry.

Pertinent cytogenetic literature

The two extensive reviews mentioned earlier (1)(35) have emphasized the presence of meiotic irregularity, particularly among long-lived polyploid grasses. Myers (35) mentioned specifically the common occurrence of inversion hybridity, as well as univalent and multivalent chromosome associations with the accompanying occasional disjunctive irregularities. The consequences of these irregularities are an important issue in this study. A few specifically applicable papers are, therefore, reviewed.

Katterman (24), in one of the most significant earlier

reports on multivalent formation in several grasses, commented on certain observed meiotic features which have, so far, remained unsolved. Within plants of Anthoxanthum odoratum (2n=20) he observed chromosome associations varying from 10 bivalents to an extreme multivalent association of 12 chromosomes. Assuming the validity of Darlington's (5) concept of homology as the sole criterion of pairing, this observation is difficult to interpret, as Katterman (24) pointed out. Extensive segmental interchange and, or, non-homologous to partially homologous associations seem necessary to account for the data obtained. In diakinesis stages of B. erectus var. eu-erectus, Katterman (24) observed that satellited members were not always in the same position in certain multivalent configurations. This condition has occasionally been observed in the present study.

Flovik (12), in a cytological study of several arctic grasses, reported that meioses in Festuca rubra var. arenaria (Osb.) E. Fries (2n=42), in spite of univalents, trivalents, quadrivalents, and sexivalents an unequal numerical distribution of the chromosomes seemed to be an exceptional occurrence despite the non-disjunctional configurations occurring at Metaphase I. In Puccinellia phryganodes (Trin) Scribn. et Merr. (2n=28) he reported very abnormal meioses with a high frequency of univalents and multivalents at Metaphase I. A single extreme association of 12 chromosomes was observed at pro-meta-phase in this

species. In Calamagrostis neglecta univalents, trivalents, and quadrivalents frequently were observed by Flovik (12). Here, too, no certain case of unequal distribution of anaphase chromosomes was observed.

Nordenskiold (40), in crosses between Phleum pratense (2n=42) and P. alpinum (2n=28), obtained 2n=35 hybrids exhibiting some characters of both parents. Two of the hybrids had 90 percent pollen fertility. In the hybrids obtained, univalents, bivalents, and multivalents of various kinds including both chain and ring quadrivalents, were observed.

MATERIALS AND METHODS

Hitchcock's (19) classification of the genus into sections and species has been adopted for the most part in the present study. The Komarov key by Nevski and Sochava (36) was employed in the delimitation of the B. riparius Rehm material used in the study. This material, originally collected by members of the United States Department of Agriculture in European Russia, has been rather widely distributed in this country as B. erectus PI No. 98, 277. The same flora (36) was consulted for the Asiatic distributions of certain species morphologically similar to B. pumpellianus as will be noted in the distributions of parental species.

Herbarium specimens examined for the distribution of B. inermis and B. pumpellianus in North America were obtained from the following herbaria by Dr. Ada Hayden, curator of the Iowa State College herbarium:

Gray Herbarium, Harvard University
New York Botanical Garden
National Museum of Canada
United States National Museum
Missouri Botanical Garden
Rocky Mountain Herbarium, University of Wyoming
Colorado A & M College
University of California
University of Wisconsin
University of Minnesota

The parental clonal material of B. inermis was provided by the Iowa Agricultural Experiment Station through the

courtesy of Dr. C. P. Wilsie. Seed of the Fischer and Lincoln varieties was obtained from the Soil Conservation Service at Ames, Iowa. Certain seed collections were made personally in the Intermountain Region as noted elsewhere in this paper. Seed of diploid and tetraploid members was provided largely by Dr. G. Ledyard Stebbins, Jr. whose extensive collections of Bromopsis section material were generously made available. Dr. G. H. Turner, Fort Saskatchewan, Alberta and Dr. P. F. Knowles, Edmonton, Alberta provided a variety of B. pumpellianus collections made in their respective locations. The individual panicles from which the various lemma pubescence classes were established in the distribution of introgressive forms between B. inermis and B. pumpellianus also were supplied by Dr. P. F. Knowles from a parkland area off the campus of the University of Alberta. M. A. Hein, Beltsville, Md. supplied seed of several members involved in the study.

Cultural techniques employed

Seed of parental species and hybrids obtained were germinated in the greenhouse in flats containing a soil mixture of two parts loam and one part sand. Freshly harvested seed of various crosses made during the study was allowed to dry a few days and then subjected to several cycles of warm (20°C) and cold (0°C) temperatures of 24 hour duration. Fair to good germination usually resulted after such treatment. Difficulty was encountered in germinating greenhouse seed of B. texensis

on several occasions. Soaking the seeds for two or three days on moistened filter paper followed by removal of the adhering lemma and pales and germination at 20°C gave material improvement in most cases.

Well established seedlings of parents and hybrids were transplanted to 4 inch pots and placed on greenhouse benches. Because of space restrictions in the greenhouse a large proportion of the material was grown to maturity in 4 inch clay pots. Five and 6 inch clay pots were used for some material, however. The soil mixture used generally was the same as that for germinating seed. In order to sustain vigorous growth during the winter season supplementary nutrient solutions were added. At about weekly intervals the pots were watered with about 40 ml's of a mineral nutrient solution made up as follows, per gallon of water:

5 grams ..... KNO_3
5 grams ..... KH_2PO_4
2 grams ..... $MgSO_4$

The $MgSO_4$ was desirable in the greenhouse at Ames where the winter water supply contained a rather high percentage of calcium salts.

General field and greenhouse crossing techniques

The initial series of crosses were made in the greenhouse at Iowa State College during the winter of 1945-46. For these crosses parental clones of B. inermis from the nursery were moved to the greenhouse late in October and

placed on benches with the seedlings of other species planted one month earlier. A second series of crosses was made in the greenhouse at the College of Agriculture, Davis, Calif., and at Gill Tract in Berkeley, Calif., during the winter of 1946-47. The third series of crosses was made at Iowa State College during the winter of 1947-48. Summaries of these crosses appear elsewhere in this paper. The crossing techniques, in general, were the same in each series.

Crosses involving B. inermis, B. pumpellianus, and B. riparius as female parents were made by emasculating immediately prior to anthesis. Under Iowa and California greenhouse conditions pollen shedding in these members began around 4:00 PM if light conditions were favorable. Prior to pollen shedding in the afternoons the panicles were rubbed gently by hand to hasten flower opening and extrusion of the anthers. As soon as the flowersopened slightly emasculation was begun and continued for about two minutes after complete opening of the flowers without pollen shedding. Under low light intensities on cloudy days flower opening sometimes was considerably delayed. After light conditions became favorable the delayed anthers occasionally dehisced before complete flower opening. Usually only the lower two or sometimes four flowers on each spikelet were emasculated and the remainder were removed.

Emasculation in the diploids and tetraploids was accomplished one to two days prior to anthesis and the lemma and

palea tips cut off to facilitate pollination. Glassine bags were used to protect the flowers from chance cross-pollination.

In many cases removal of anthers in B. inermis, B. pumpellianus, and B. riparius much in advance of pollen shedding resulted in failure of lodicule stimulation and normal stigma development at anthesis. Whether this was due to mechanical injury of the delicate flower parts or to removal of specific growth regulating substances in the anther which control lodicule stimulation and stigma development was not ascertained.

Pollination was effected in some cases by collecting pollen in glassine bags at anthesis from selected male panicles and applying to the emasculated female flowers. In other instances the potted plants were so arranged that female panicles were directly below selected male panicles in glassine bags. Thus, pollen might fall on the female flowers over a period of several days. In certain cases this method was quite effective although in one experiment the hand-pollinated flowers set a greater percentage of seed on the average than those which were enclosed with male panicles. Other factors may have been involved since the seed set from a single pollination germinated considerably higher than the seed set obtained from the other method of pollination.

Clones of B. inermis, B. pumpellianus, and B. riparius brought in from the nursery late in the fall usually flowered sometime after the latter part of December, provided the photoperiod was artificially extended. An 18 hour photoperiod

generally was used although a 23 hour period was employed for the B. pumpellianus material from Alaska. Under natural conditions this source material normally flowered under nearly 24 hours of light.

Among the clones were exceptional plants which failed to flower during the entire winter season. This condition was more serious among plants grown from seed during the current greenhouse season. With an extended photoperiod, a high level of nutrition, and temperatures between 60° and 70°F approximately half of the seedlings of these species could be brought into flowering during the winter season. Failure of flowering in the remainder may be ascribed to the failure of thermal induction or to some other physiological reaction conditioned by certain genetic factors. The seedlings which failed to flower appeared more or less at random throughout the various crosses.

Vegetative culms were cut out at about monthly intervals during the growth of the seedlings forcing newly differentiated shoots to grow. As long as the older vegetative shoots were allowed to remain new culms with differentiated flower panicles did not appear in these small pots.

Plants from seed of B. auleticus from Uruguay, South America, B. porteri from southwest Utah, B. ciliatus from Colorado Springs, Colorado, and B. purgans from Gray's Summit, Missouri, failed to flower under the ordinary greenhouse conditions during the winter. Seedlings of the latter did reach

flowering stages, however, in the greenhouse at Berkeley, Calif., during the winter of 1946-47.

To facilitate establishment of potential hybrids obtained from crossing B. inermis and B. pumpellianus with B. texensis it appeared desirable to study the possibility of embryo culture. The potential F_1 hybrids of these crosses previously obtained failed in several ways. In some the caryopses remained shrunken, in others the caryopses was normal but failed to germinate, and in still others the seedlings were chlorophyll deficient or, if not, failed to grow past the first seedling leaf stage.

A series of crosses among plants of B. inermis were set up and at daily intervals starting at 8 days after fertilization, excision of the embryos was attempted. Techniques and materials employed were similar to those outlined by Randolph (42) in the culture of iris embryos. Embryos of 12 day pollinations or less could not be manipulated physically. After 12 days the embryo tissue was sufficiently firm to facilitate easy removal of the scutellum and embryonic axis as a unit. The caryopses approached maturity 20 days after pollination and no further excision was attempted. The Randolph (42) media was satisfactory for culture of embryos from 16 to 20 days after pollination. Nutrient deficiencies existed among the cultured embryos under 16 days, however. The 13 day embryos, which were the youngest to live in the experiment, grew only very slowly and failed to reach a transplanting stage. Growing embryos were transplanted to two inch pots of sand on the greenhouse bench

and watered with the mineral nutrient solution mentioned previously. They made satisfactory growth but were slightly chlorotic, indicating deficiencies. Later they were transplanted to four inch pots of soil and the chlorotic symptoms disappeared.

The embryo culture techni ue was initially attempted as a means of establishing hybrid embryos between B. inermis and B. texensis and also between B. pumpellianus and B. texensis. On previous occasions hybrid seeds failed to develop fully, to germinate, or to grow past the first seedling leaf stage. No normal embryos were obtained in the final series of pollinations between B. inermis or B. pumpellianus with B. texensis.

Cytological techniques

Cytological emphasis was placed upon chromosome association and behavior at meioses in the parent species and hybrids. Particular attention was paid to fixation and smearing of the material since analyses were made from temporary aceto-carmine smears. Polyploid members of this section are unfavorable subjects for meiotic studies. Only rarely were diakinetic and first metaphase cells found which could be analyzed. No doubt there has been some bias injected into the analyses although it is likely to be of a conservative nature. Those cells with more complex associations might contain entangled multivalents which spread poorly upon smearing while those possible of analyses might contain the least complex associations.

On this basis one might expect estimates of bivalent association to be higher and multivalent associations minimized in actual meiotic analyses of these species. The most serious difficulty imposed on meiotic analyses of the complex polyploids of this section is the interpretation of extremely complex multivalent associations.

A late diakinesis stage in which maximum contraction of the chromosomes and dispersal about the nucleus is attainable was found most satisfactory for association analyses. Metaphases offer little possibilities for critical study because of multivalents and other factors probably including fixation.

In most of the material examined meioses did not occur until the panicles were completely emerged from the boot. There was a period of several days during which meioses occurred in progressively younger flowers of each spikelet.

Rapid killing was obtained by placing individual flowers severed at the base in one of the following Carnoy solutions:

3 parts 95% ethyl alcohol
1 part glacial acetic acid

6 parts 95% ethyl alcohol
3 parts chloroform
1 part glacial acetic acid

4 parts 95% ethyl alcohol
3 parts chloroform
1 part glacial acetic acid

Fixation in the 3:1 solution was inferior to that obtained in either of the two containing chloroform for diakinesis stages. No critical differences, however, were observed in favor of

one or the other solutions containing chloroform. After 12 to 24 hours in the killing solutions the flowers were transferred to 70% alcohol in vials and stored at 50°F until smeared.

Quartet stages were smeared in the ordinary manner as outlined by Love (31). Diakinesis stages, however, required special attention. Individual anthers in a drop of stain on a slide were cut in several cross-sections with a scalpel and needle under a 15 X wide field binocular. These sections were flattened with a small rounded chisel and sections of anther walls retrieved with a needle and small spade before affixing the cover slip. Slides were heated over a steam bath 15 to 20 seconds before pressing and sealing.

By diluting aceto-carmine with 45% acetic acid to a standard maintained for color comparisons it was possible to effect a more critical staining of chromosome details than with ordinary stain. Several days at room temperatures were required to attain optimum staining of the chromosomes. During this period temporary mounts required careful cover-slip sealing with paraffin or sealing wax to pevent drying out. During warm weather the temporary slides were placed in a glass jar with an air-tight lid. Addition of 45% acetic acid at intervals to toweling in the bottom maintained a humid atmosphere in the jar. Occasionally, slides were made permanent by the technique outlined by Love (31).

Root-tip mitosis smears were employed in the analyses of a number of clones. Prefixation for a 4 to 6 hour period in a

saturated aqueous solution of paradichlorobenzene at 50°F proved beneficial in most cases. Fixation was accomplished using a 3:1 Carnoy solution for a three day period at 50°F. Storage in 70% alcohol for a few hours at least prior to smearing facilitated staining. A 1% orcein stain in 45% acetic acid proved satisfactory for chromosome counting in this material. Some difficulty was encountered in obtaining conditions favorable for root-tip mitoses. Various nutritional treatments were attempted with only erratic success. The same treatments of various clones failed to result in comparable numbers of cells undergoing mitoses.

Photomicrographic techniques

The initial photomicrography for the study was made in the agronomy laboratory at Davis, Calif., at magnifications of 580X and 820X using a Zeiss binocular research microscope equipped with a 1.4 N.A. pancratic condenser, apochromatic objectives, compensated oculars, and a Zeiss bellows camera unit.

The photomicrography at Iowa State College was done with a Bausch and Lomb binocular research microscope with a 1.4 N.A. aplanatic condenser, achromatic low and high power objectives, a 100X Fluorite oil-immersion objective, and compensated oculars. To this unit a Voightlander double extension bellows camera was adapted affording magnifications from 800X to 1100X. Blue filters were used in both instances.

Contrast Process Panchromatic and Contrast Process ortho Eastman Kodak film were used. Eastman Kodak Panatomic X film was used for certain morphological photographs. Developing and printing were carried out using Kodak chemicals, various grades of Velox paper, and Kodak formulae.

RESULTS

Distribution of parental species

The species of the Bromopsis section, as indicated earlier, are native to Europe, Asia, North America, and South America. The diploid members of concern here are natives of western United States. Little specific research other than that contained in the various floras of the United States is available concerning the distributions of these relatively uncommon section members. The manual by Hitchcock (19) lists the distributions by states of the various section members and may serve as an approximate guide. B. auleticus from Uruguay was included in the study.

B. inermis is native to Asia, European Russia, and Central Europe (17,19,36). Since its introduction into California in 1884 (8) it has been planted extensively on ranges and pastures throughout the area indicated in Fig. 1.

The closest North American counterpart of B. inermis is B. pumpellianus which ranges northward from the Intermountain region into Canada and Alaska as far as the Seward Peninsula as indicated in Fig. 2. During the past few years it has become difficult to locate typical plants of this species in the United States. This phenomenon will be treated in detail later.

B. riparius Rehm, used quite extensively in crossing

FIG. 1. NORTH AMERICAN DISTRIBUTION OF BROMUS INERMIS FROM HERBARIUM SPECIMENS

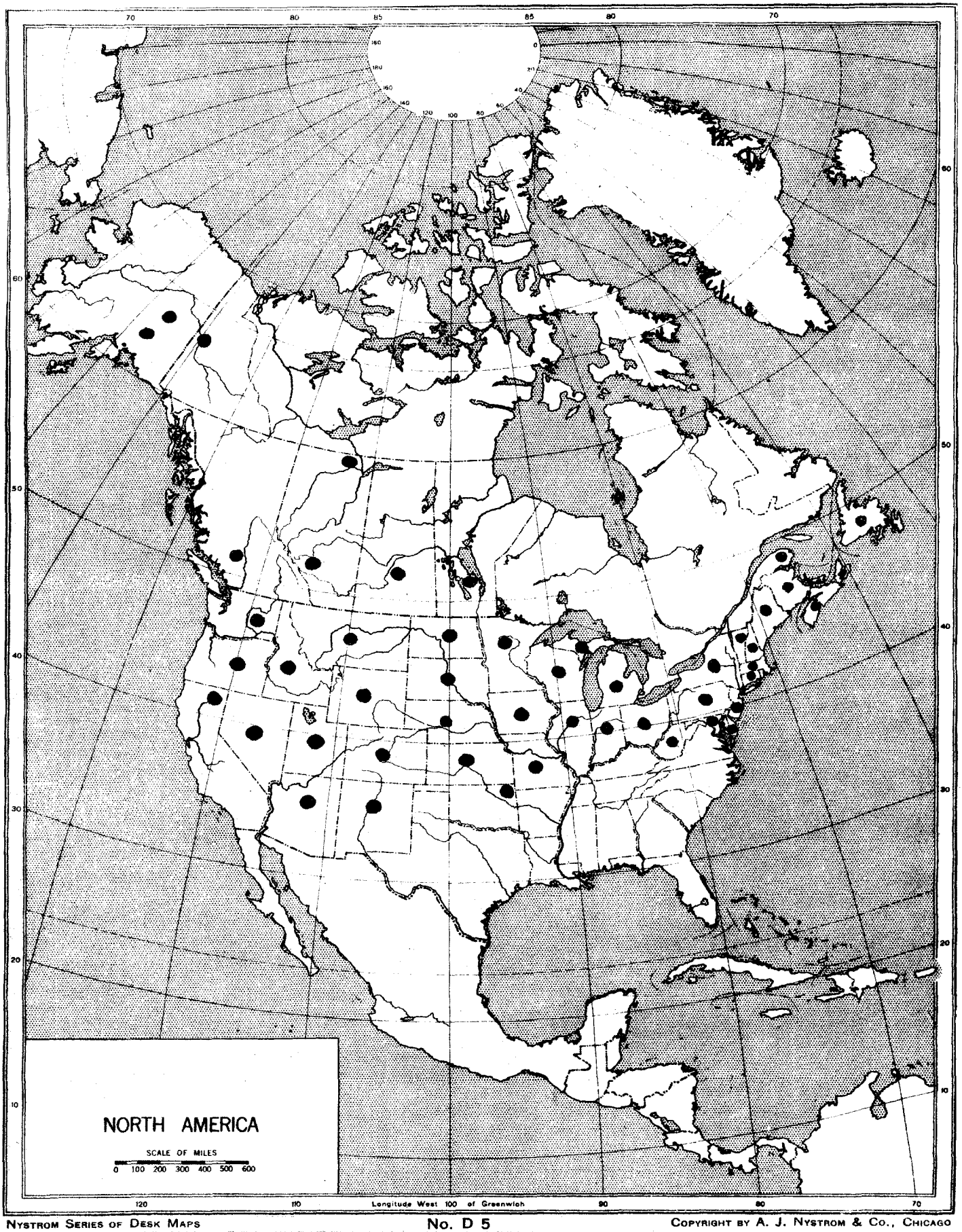
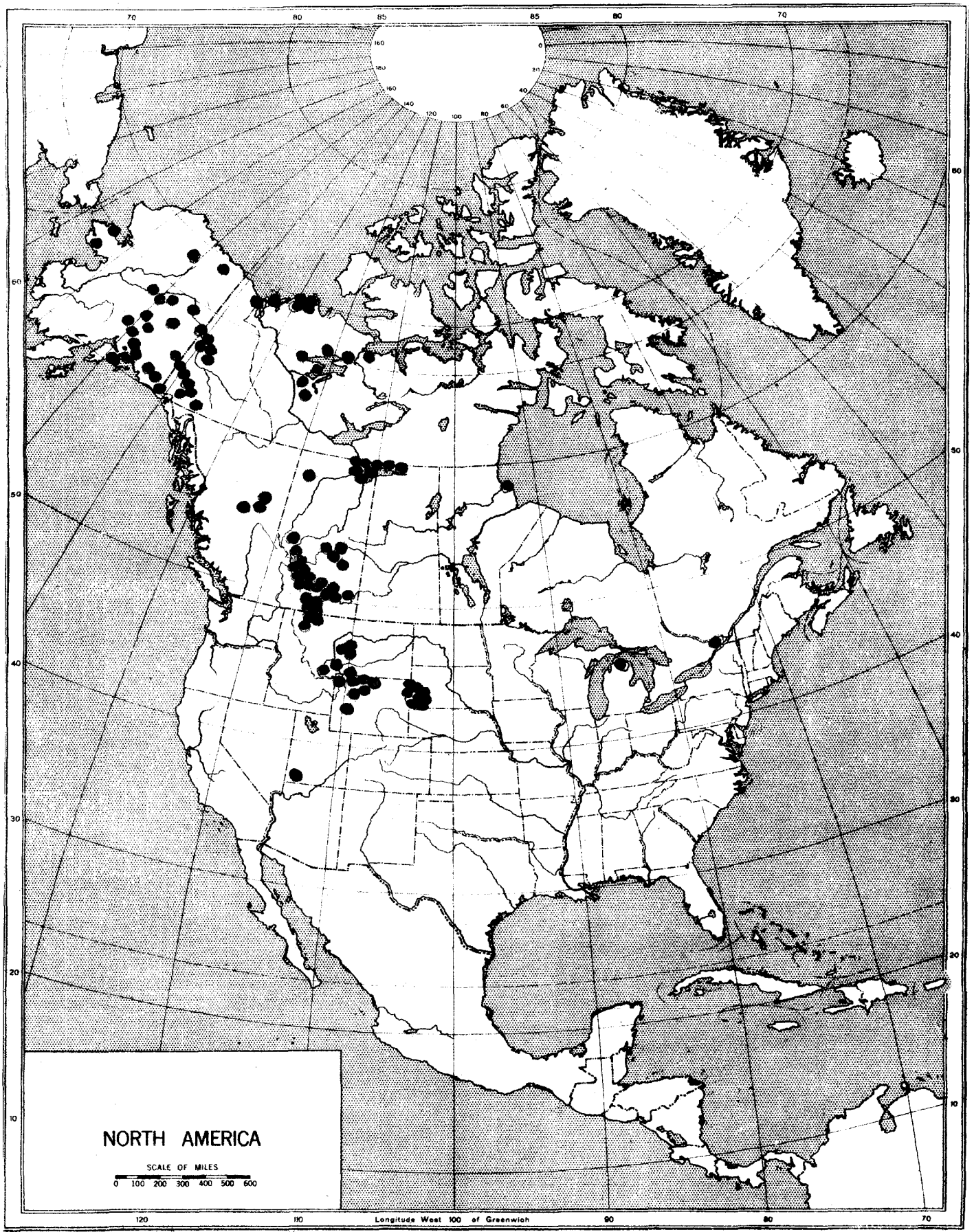


FIG. 2. DISTRIBUTION OF B. PUMPELLIANUS IN NORTH AMERICA AS INDICATED BY SPECIMENS COLLECTED DURING THE PERIOD, 1860-1947



experiments in this study, is native to parts of Russia.

Morphology of the parental species

The Bromopsis section, according to Hitchcock (19), is characterized by terete spikelets and lemmas which are not compressed and keeled. They predominantly are perennials, although B. texensis grew as an annual in greenhouse experiments reported here.

The sparse habits of growth and general adaptation of the majority of the uncommon section members to poor, infertile places has relegated them to a rather inconspicuous role as forage species.

B. inermis and B. pumpellianus are the only rhizomatous members in this section listed by Hitchcock (19). They are particularly interesting from a morphological point of view (see Figs. 3-15). The presence of pubescence on the margins of the lemmas in B. pumpellianus has been employed by several taxonomists as the chief delimiting characteristic. In commenting on the habits of B. inermis and B. pumpellianus at Fort Saskatchewan, Alberta, Dr. G. H. Turner wrote: "Bromus inermis, as we have it here, is very aggressive, and now occurs widely and generally in more or lessdense stands, in any soil where it can obtain a footing. B. pumpellianus is very modest in its habits, and remains to be found only on bits of native sod that remain here and there along roadsides, river banks, etc. Usually it is much less caespitose than B. inermis, the stems

Fig. 3. Spikelet of Alaskan B. pumpellianus 25-15. 3Xca.

Fig. 4. Spikelet of clone 26-1 from Fort Saskatchewan, Alberta. 3X ca.

Fig. 5. Lemma with class 7 pubescence. 7X ca.

Fig. 6. Lemma of typical class 6 pubescence. 7X ca.

Figs. 7-8-9. Node and culm characters in various clones.

Fig. 10. Leaf sections of characteristic clones of B. pumpellianus.

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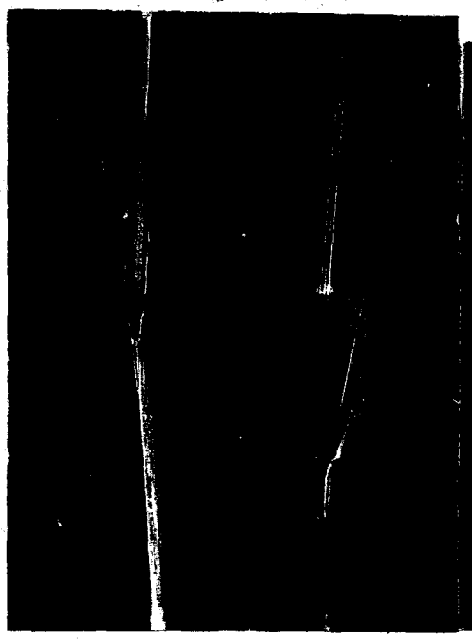

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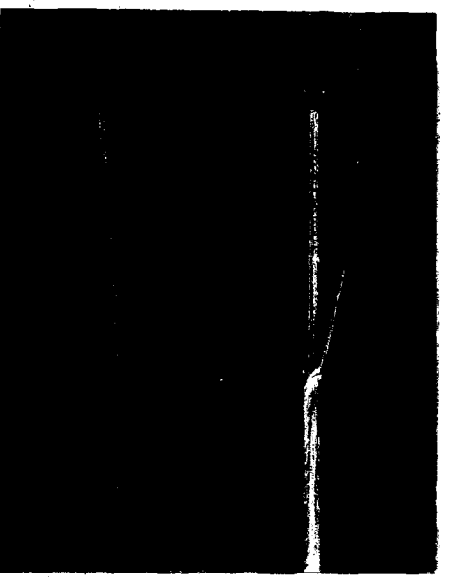

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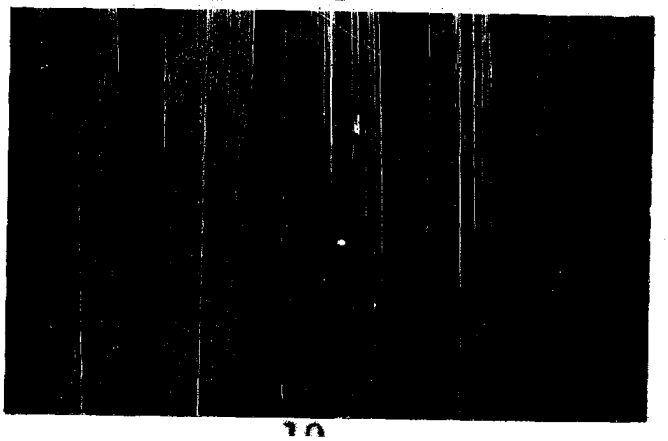

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Fig. 11. Spikelet of clone 548-26. 3X ca.

Fig. 12. Class 3 lemma observed in Fischer B. inermis. 7X ca.

Fig. 13. Class 1 lemma. 7X ca.

Fig. 14. Culm and node characters of clone 269-44. 1X ca.

Fig. 15. B. inermis embryos in vitro 8 days after excision. Embryos excised 17 days after pollination. 1X ca.

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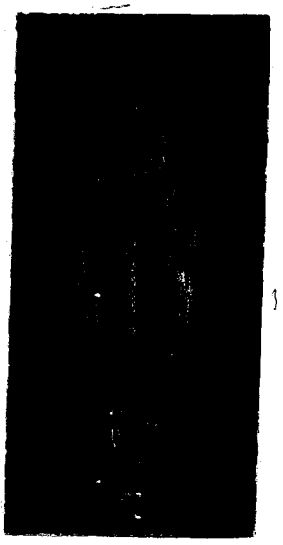

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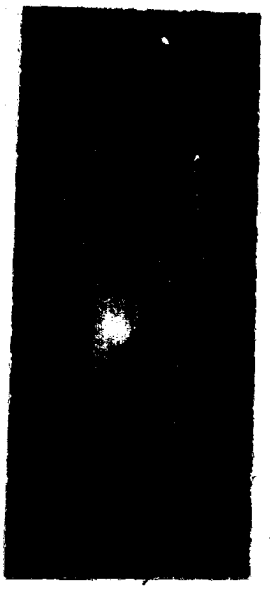

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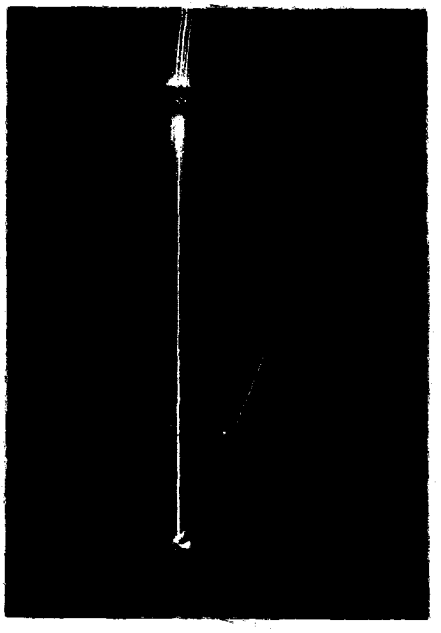

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being very discretely spread in such colonies as we have left--B. pumpellianus remains easily recognized by its erect panicles and larger spikelets in addition to its pubescence." The diversity of forms probably related to B. pumpellianus is shown by previous taxonomic analyses.

Hulten (22) has included the varieties arcticus and villosissimus in B. pumpellianus. He classified specimens with pilose glumes and lemmas as var. arcticus and those with lemmas covered with dense, villous, gray indumentum as var. villosissiumus. Hulten (22) regarded B. pumpellianus as closely related to B. inermis, differing only by its longer awns, pubescent culm nodes, and more pubescent lemmas. On this basis he postulated that B. pumpellianus was the American counterpart of the Eurasian B. inermis and that its vars. arcticus and villossissimus, as well as the Kamchatkan B. ornans, were northern variations of this series.

Nevski and Sochava (36), in their treatment of the sub-genus Zerna, described B. ornans as endemic to sandy ridges of river valleys and volcanic sands on the Kamchatkan peninsula. They describe B. richardsonii as a rhizomatous perennial with very similar morphology distributed in meadows of eastern Siberia, in Kamchatka, Sakhalin, Udsk, Okhotsk, Zu-Bureya, Ussuria, and also generally distributed in North America, Japan-China (Manchuria) and the eastern part of Mongolia. Hitchcock (19) reduced B. richardsonii to a

robust form of B. ciliatus, common in the Rocky Mountains, since he reported it graded freely into this species. A robust form of B. ciliatus collected in Moraine Park, Colorado, in 1945 by the writer is tetraploid, whereas two collections of this species examined by Stebbins (44), one from Maine and one from California, are both diploid. Neither the diploid nor tetraploid forms are rhizomatous, however, and cannot, therefore, be regarded morphologically equivalent to B. richardsonii as recognized by Nevski and Sochava (36).

B. irkutensis was described by these authors (36) from the Irkut River valley in eastern Siberia and generally distributed in Mongolia. B. korotkyi is endemic to sandy shores in eastern Siberia and was described from sands along the Ulan-Buri River. B. sibiricus, in this key, was distributed in the Northern and Central Urals, eastern and western Siberia, and Mongolia.

B. ornans, B. korotkyi, B. irkutensis, B. vogulicus, B. richardsonii, and B. sibiricus are not sufficiently delimited from a morphological point of view to warrant exclusion from a complex which would include the various forms of B. pumpellianus.

Cytology of parental species

Plants of the diploid and tetraploid species examined, in the main, exhibited regular meioses. There were rare instances where sporocytes were deficient in chromosome number and there were also occasional lagging univalents and bridges as shown in the accompanying photomicrographs. (Figs. 16-33). The later meiotic irregularities shown in B. laevipes, Figs. 18-21, probably were due to the presence of at least one, and perhaps two, heterozygous inversions in one chromosome arm as shown in the pachytene, Fig. 17. Failure to observe the same configurations in adjacent cells as shown in Fig. 16 were interpreted as evidence of non-homologous pairing. Analysis of this plant revealed 94 percent normal anaphase I stages, 1 percent with 1 lagging chromosome, 2 percent with more than one lagging, and 3 percent with single bridges. One tetraploid B. ciliatus plant was observed in which an accessory chromosome was present (Fig. 33). The quartets of the plants examined in this group were free of micronuclei in excess of 90 percent. Chromosome numbers and associations observed here are similar to those observed by Stebbins (44) for members of the group. The mean chromosome associations per sporocyte of the species representatives examined are presented in Table 1.

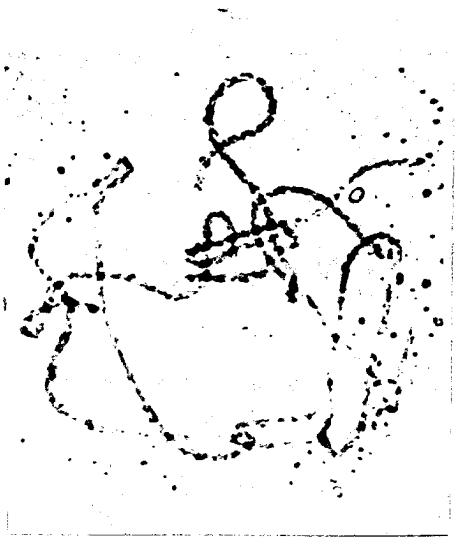
Figs. 16-17. Pachytene stages in B. laevipes. Note heterozygous inversion configuration in Fig. 16. 1200X ca.

Figs. 18-19. Metaphase I stages in B. laevipes. Note the presence of two univalents in Fig. 19. 1200X ca.

Fig. 20. Anaphase I in B. laevipes showing division of two lagging univalents. 1200X ca.

Fig. 21. Early anaphase I in B. laevipes with a bridge which presumably arose from crossing-over in the heterozygous inverted segment shown in Fig. 17. 1200X ca.

Figs. 22-23-24. Diakinesis in B. ciliatus. 820X.



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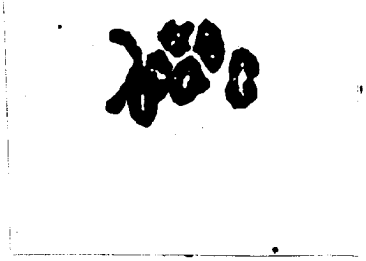

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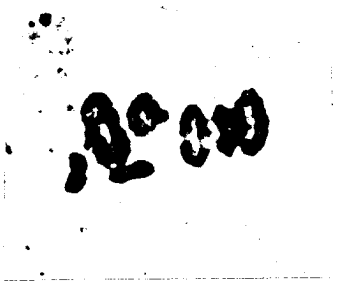

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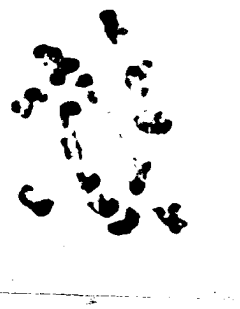

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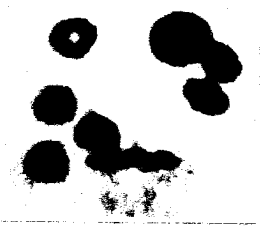

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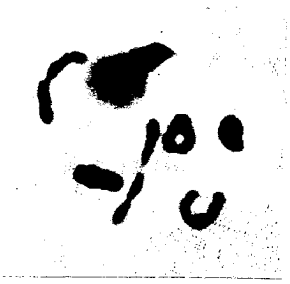

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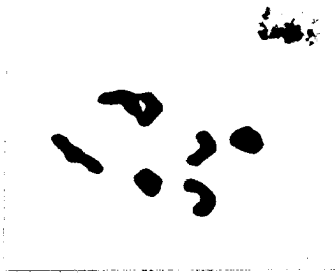

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Fig. 25. Early metaphase I showing major spiralling in B. texensis pair. 1200X ca.

Fig. 26. Early metaphase I in B. texensis. Note spiralling among the bivalent associations. 1200X ca.

Fig. 27. Late diakinesis in B. texensis showing presence of one open bivalent. 1200X ca.

Fig. 28. Diakinesis in tetraploid B. ciliatus with 14 bivalents. Note association of two bivalents with the nucleolus. 1200X ca.

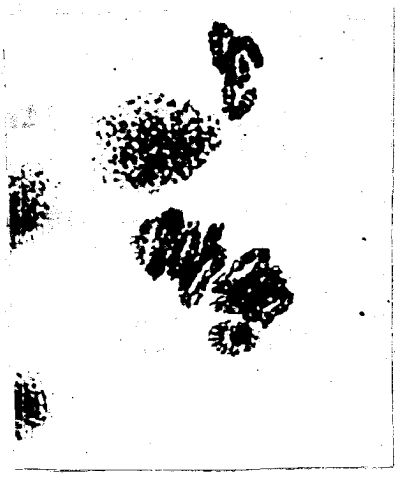
Fig. 29. Diakinesis in tetraploid B. ciliatus with 10 bivalents and 2 IV. 1200X ca.

Fig. 30. Diakinesis in B. auleticus with 19 closed bivalents and 2 open bivalents. 820X.

Fig. 31. Metaphase I in B. purgans (F-354). 820X.

Fig. 32. Diakinesis in B. purgans with 12 closed bivalents and 2 open bivalents. 820X.

Fig. 33. Diakinesis in tetraploid B. ciliatus with 1 accessory chromosome in addition to the regular complement.



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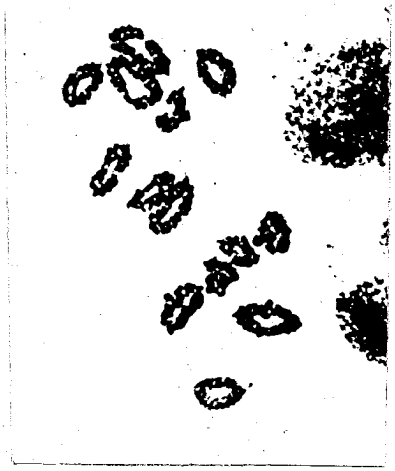

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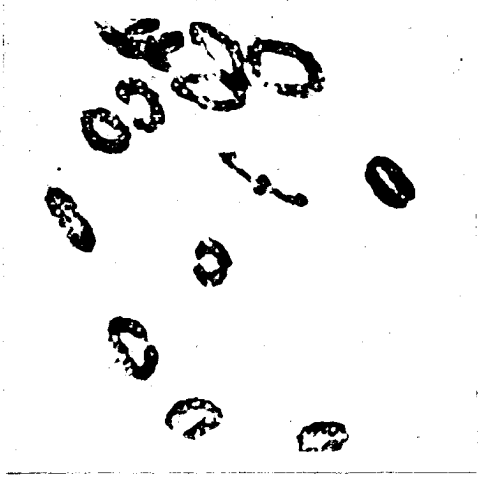

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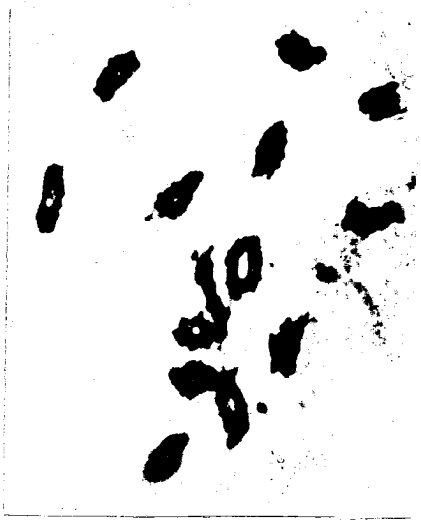

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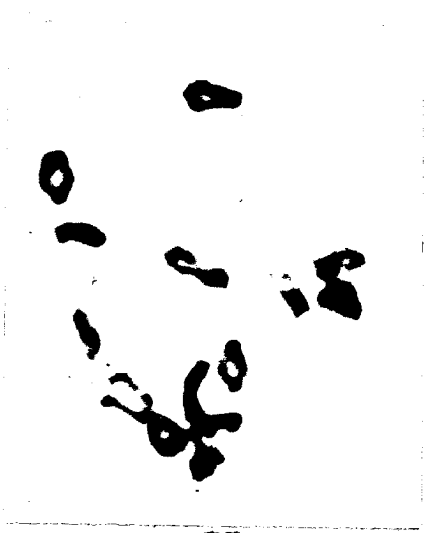

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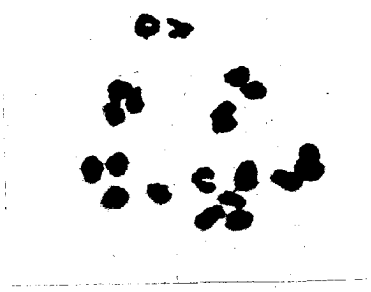

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Table 1

Mean chromosome associations per sporocyte of diploid and tetraploid Bromopsis species examined

Species	No. of plants	No. of cells	Mean chromosome associations per cell				2n=	Source
			I	II	II open	II closed	IV	
B. anomalous	2	27	.15	1.0	5.9	0	14	Pullman, Wash.
B. ciliatus	5	58	.21	1.07	5.8	.02	14	Calif.
B. ciliatus	5	41	.59	.97	12.73	0	28	Colorado
B. laevipes	2	12	.40	.45	6.45	0	14	Calif.
B. texensis	3	8	0	.90	12.6	.25	28	Texas
B. purgans	3	38	.26	.74	12.9	.05	28	F354 USDA
B. purgans	1	5	0	0	7.0	0	14	Missouri

As mentioned previously, chromosome association analyses in complex polyploids of this section was rendered difficult by the nature of the material. B. auleticus from Uruguay, South America, was the only exception. Analyses of a number of pollen mother cells from several plants revealed a 2n=42 chromosome number. The chromosomes were largely associated as bivalents, although there were occasional quadrivalents.

The average chromosome associations among clones of B. inermis and B. pumpellianus are summarized in Table 2 (see Figs. 34-49). For a more complete analysis of the associations by clones the reader is referred to Appendix Tables 10 and 11. The most striking feature of these associations is the extreme range possible. No doubt there were differences in proportions of the various associations from clone to clone although the biased sampling does not permit valid inferences concerning the

probability of significant differences. The extreme ranges possible within the clones tends to obscure whatever real differences might have been present between clones.

Table 2

Mean chromosome associations among clones of B. inermis and B. pumpellianus

		B. inermis	B. pumpellianus
No. of clones examined		37	11
No. of cells analyzed		163	40
Type of association			
I	range	0-20	0-4
	mean	1.8	0.8
II	range	0-14	0-13
open	mean	5.3	3.92
II	range	1-23	6-21
closed	mean	13.32	14.64
III	range	0-4	0-2
	mean	0.6	0.5
IV	range	0-7	0-6
	mean	2.2	2.5
V	range	0-3	0-2
	mean	0.1	0.05
VI	range	0-3	0-2
	mean	0.6	0.6
VII	range	0-2	0
	mean	0.03	
VIII	range	0-3	0-2
	mean	0.2	0.23
X	mean	0	0.003

Fig. 34. Diakinesis in Alberta clone with 5 open bivalents, 16 closed bivalents, 1 association of III, 1 V, and 1 VI. Note heteromorphic association of pair at top. 1200X ca.

Fig. 35. Diakinesis in Alberta clone with 1 open bivalent, 16 closed bivalents, 2 associations of IV, 1 VI, and 1 VIII. 1200X ca.

Fig. 36. Mitosis in anther smear of a South Park clone with a 2n=56 plus 4 or 5 accessory chromosomes. 1200X ca.

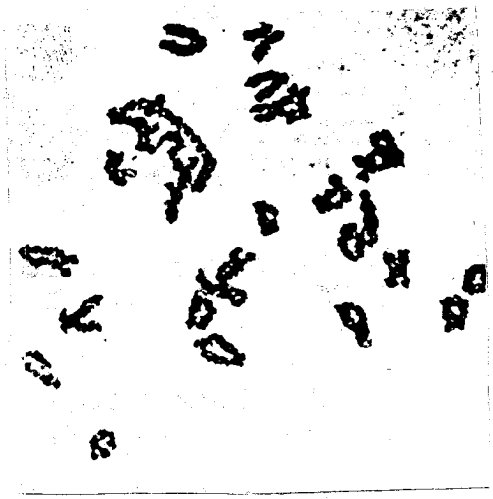
Fig. 37. Diakinesis in Alberta clone with 3 univalents, 2 open bivalents, 21 closed bivalents, 1 association of III, and 1 IV. 1200X ca.

Fig. 38. Diakinesis in Alberta clone with 1 open bivalent, 20 closed bivalents, 2 associations of IV, and 1 VI. 1200X ca.

Fig. 39. Early anaphase I in Pullman, Washington, clone showing common early anaphase appearance. 1100X ca.

Fig. 40. Diakinesis in Alberta clone with 1 univalent, 14 bivalents, 1 association of III, and 6 IV. 1200X ca.

Fig. 41. Diakinesis in Alberta clone with 1 univalent, 3 open bivalents, 18 closed bivalents, 2 associations of IV, and 1 V. 1200X ca.



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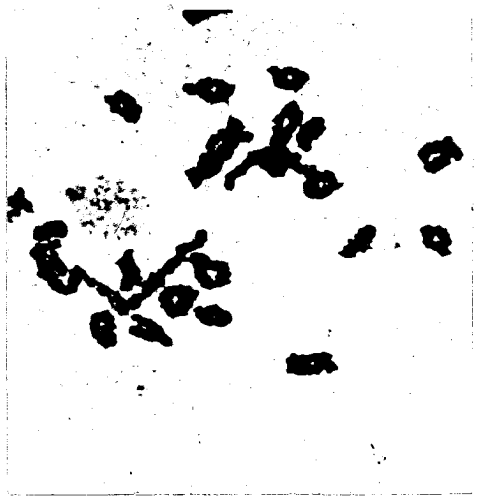

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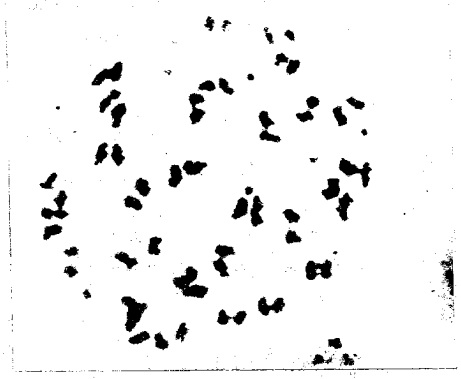

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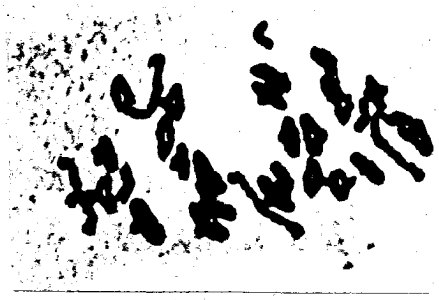

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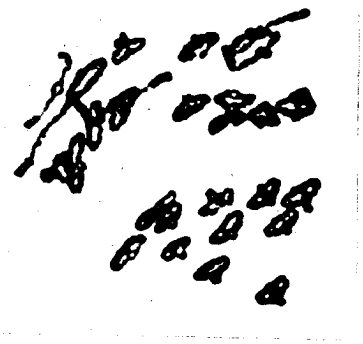

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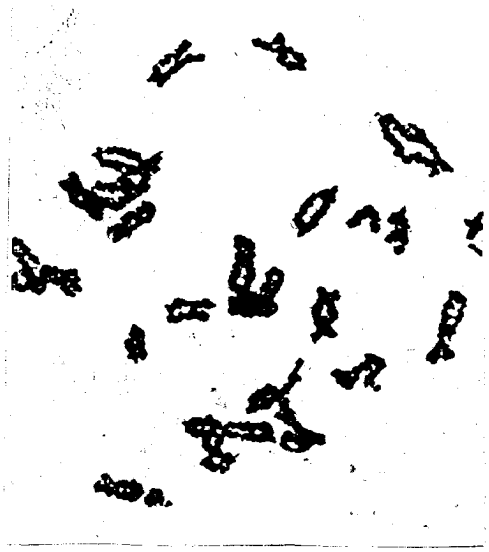

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Fig. 42. Diakinesis in clone 314-8 with 1 univalent, 10 open bivalents, 9 closed bivalents, 1 association of III, 2 IV, and 1 VI. Note the loose bivalent associations and apparent chiasma between pairs in upper right hand corner. 1200X ca.

Fig. 43. Diakinesis in clone 298-30-10-4 with 1 open bivalent, 15 closed bivalents, 4 associations of IV, and 1 VIII. Note the attraction of at least 8 chromosomes to the nucleolus. 1200X ca.

Fig. 44. Anaphase I in clone 460-1 showing equational division and disjunction of 5 univalents which lagged. 540X.

Fig. 45. Anaphase I in clone 460-1 with bridge in addition to 3 univalents. 540X.

Fig. 46. Common sticky appearance of metaphase I stages in clone 2-5.

Fig. 47. Second meiotic division in Fischer 100 indicating a bridge and fragments resulting from crossing-over in a heterozygous inverted segment and in segment between inversion and the centromers. 820X.

Fig. 48. Quartet stage in Lincoln 11 showing the presence of two micronuclei. 820X.

Fig. 49. An association of VIII in 255-44 x 554-22 #7.

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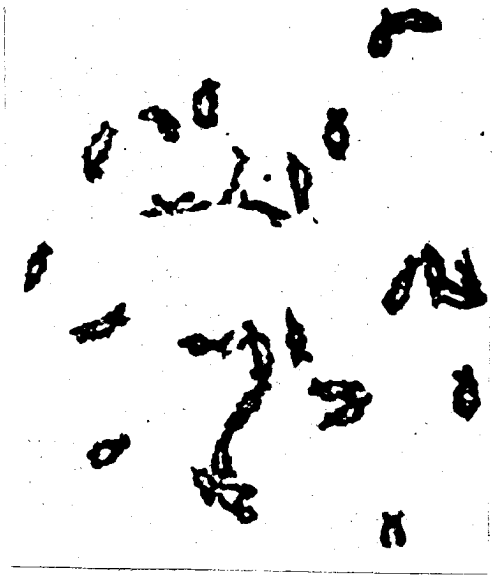

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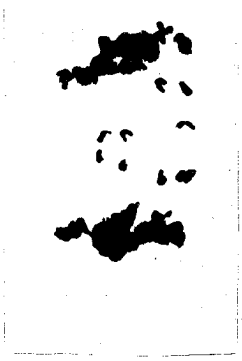

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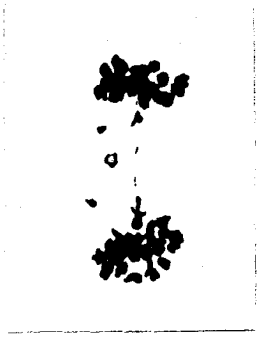

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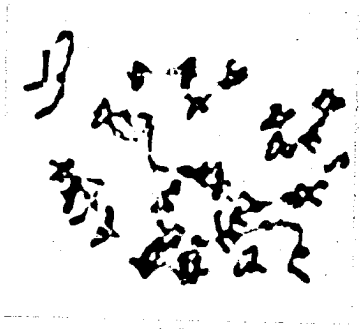

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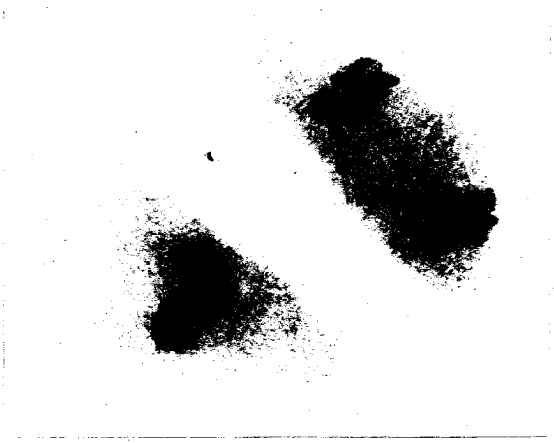

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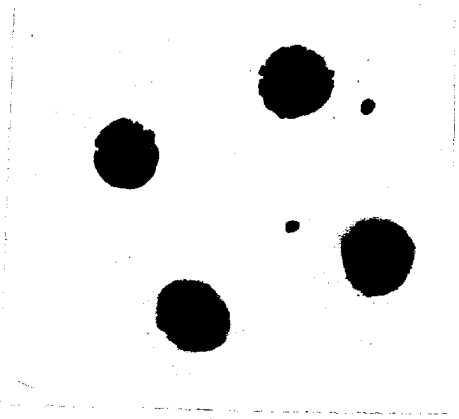

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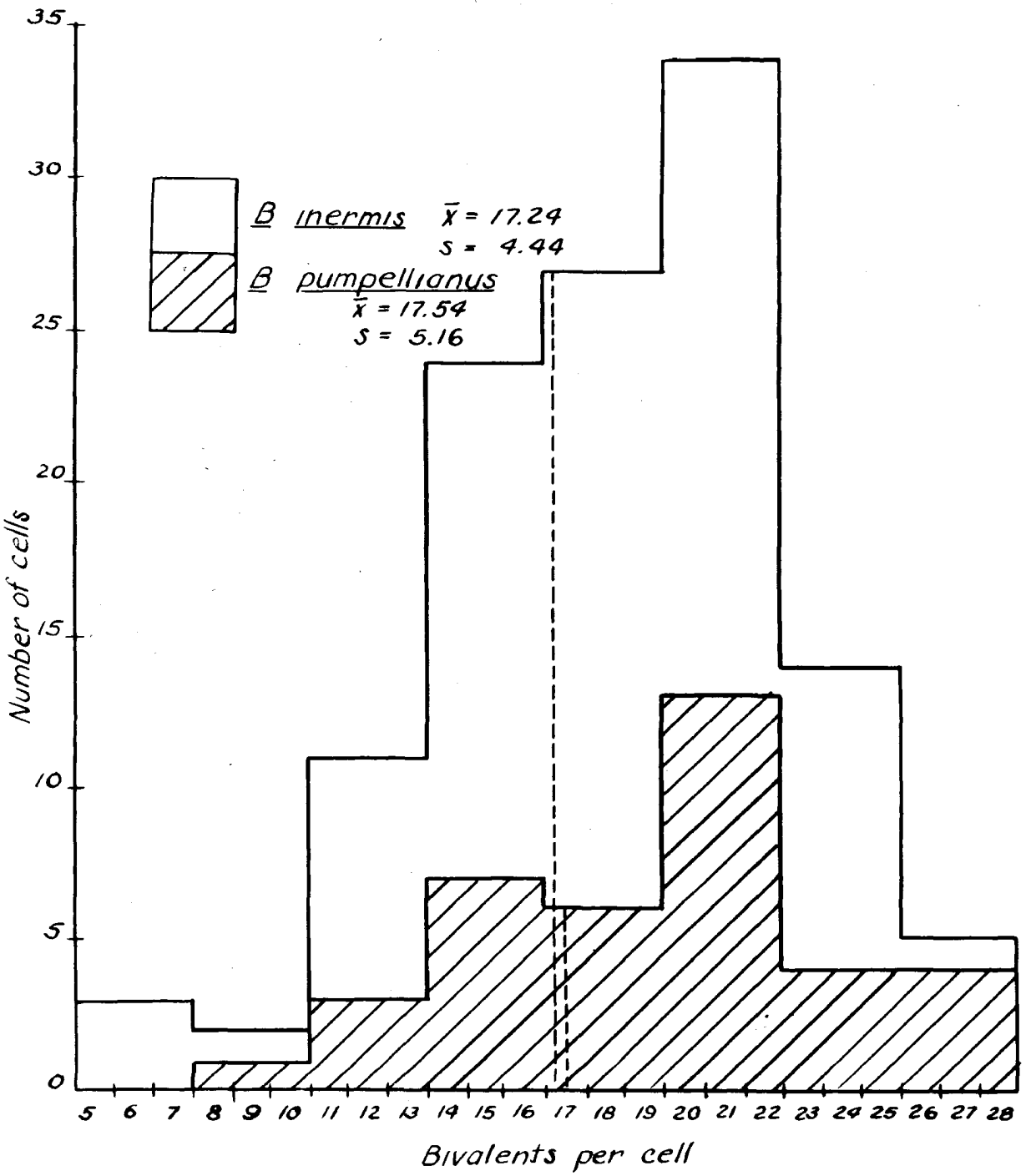
As indicated earlier by Elliott and Love (9), B. inermis cannot be considered a functional diploid in view of the extreme multivalent associations possible. The complex genetic segregation to be expected from such associations will be considered later. Other features of breeding behavior will be complicated as a result of this departure from functional diploidy. The range encountered in the various chromosome associations from cell to cell seem impossible to account for on the basis of chiasma failure among autosyndetic associations. The various multivalent associations might have appeared more uniformly on this assumption. Segmental interchanges among structurally similar chromosomes and proximal preferential pairing in prophase would more satisfactorily account for the varying associations and also the loose heterogenetic bivalent associations observed in both species. There was an apparent tendency for odd-numbered multivalents to occur at a lower frequency than even-numbered associations of 4 and above. The similarity of associations obtained in pollen mother cells of both species was rather striking. In view of the artificial selection practiced within B. inermis one might expect more nearly functional diploid relations.

The bivalent distributions among sporocytes of both species are summarized in Fig. 50. The means of both species are essentially the same. Whatever factors are responsible for bivalent association appear independent of artificial selection

Fig. 50

Comparative bivalent distributions in sporocytes of B. inermis and B. pumpellianus



or other factors attendant to the culture and distribution of B. inermis by seed over a short span of time. As will be mentioned later, these two members probably have been isolated since the Pleistocene. The similarity of the chromosome associations observed is evidence of a more stable meiotic mechanism than some of the cytological irregularities might seem to indicate.

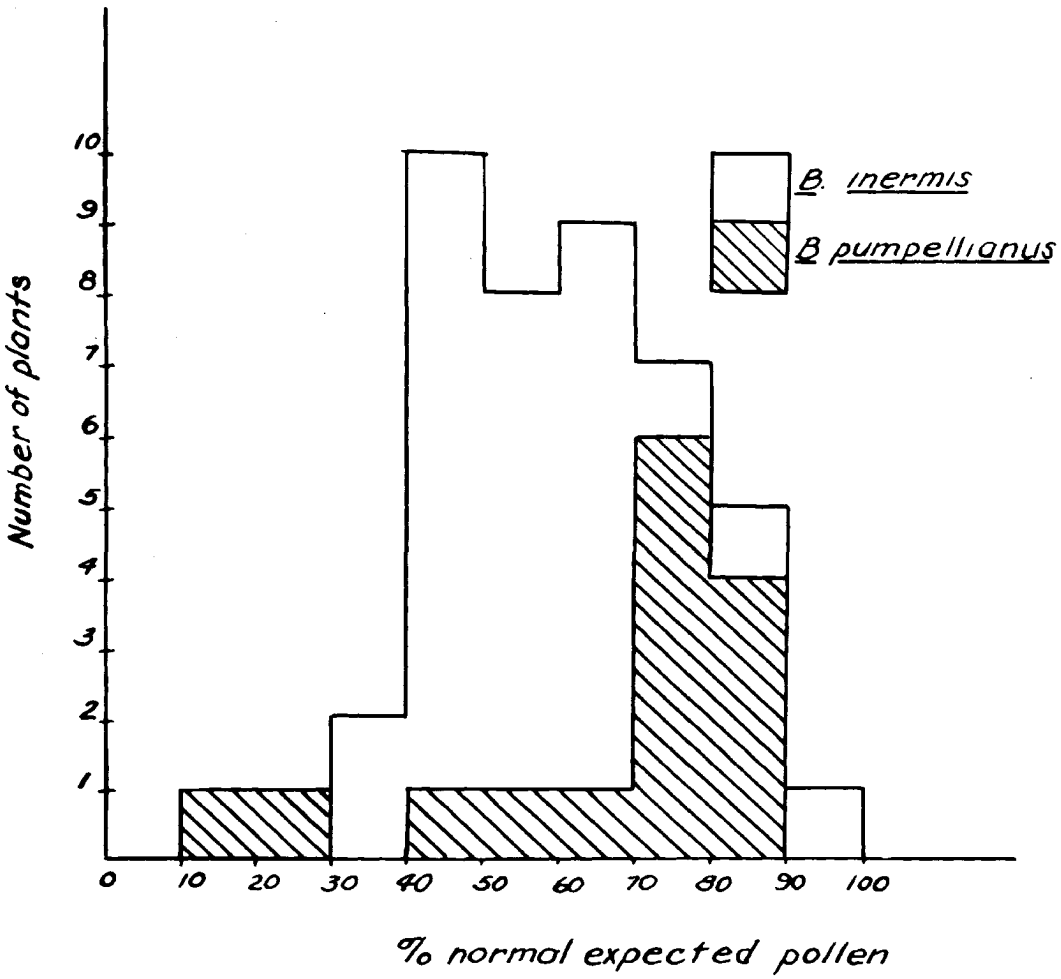
Analyses of micronuclei at the quartet stage were relatively simple and a number of clones of each species were examined at this stage. The summary of results follow in Fig. 51.

Since such an analysis was so readily obtainable it seemed desirable to correlate these data with other fertility measures. On the basis of the quartet analyses a number of crosses within B. inermis were made between clones with relatively few micronuclei per 100 quartets (H X H), between clones with intermediate numbers of micronuclei (HM x HM), and between clones with large numbers of micronuclei per 100 quartets (L x L). The quartet analyses of the resulting heterozygous hybrids yielded variable results. In general, hybrids resulting from the (L x L) combination were like the parents with some exceptions. Likewise, those resulting from the (H X H) combination gave fewer numbers of micronuclei like their parents.

Random cross-pollination was simulated among these hybrids placed in a group on the greenhouse bench at flowering. When the resulting seed was mature the panicles were harvested

Fig. 51

Comparative distributions of percentage normal expected pollen from clones of B. inermis and B. pumpellianus from micronuclei analyses at quartet stages



individually and the number of flowers and seeds were counted, and per cent seed set determined. The analysis of variance of seed-setting of the various clones and combinations is presented in Table 3.

Table 3

Analysis of variance of greenhouse seed-setting under open-pollination of selected B. inermis clones

Source of variation		df	MS	F
Panicles		2	8.35	
Clones		12	334.95	5.26**
H x H	5		292.66	4.60**
L x L	2		787.55	12.37**
Backcrosses	1		99.23	
Other comb.	1		260.04	
Between groups	3		207.30	3.26*
H x H vs. L x L	1		518.90	8.15**
Remainder	2		51.45	
Error		24	63.66	
Total		38		

* Exceeds 5% level of significance
** Exceeds 1% level of significance

The comparison of H x H vs L x L combinations was highly significant indicative of a real difference in seed-setting between the two series.

In another greenhouse experiment designed to measure the seed-setting ability of several superior B. inermis clones used in the thesis study by Hawk (15) the analysis presented in Table 4 was obtained.

Table 4

Analysis of variance of greenhouse seed-setting of selected B. inermis clones

Source of variation	df	MS	F
Panicles	4	97.25	
Clones	7	1392.00	16.85 **
Error	(27)	82.6	
Total	(39)		

Highly significant differences were obtained in seed-setting ability as expressed by percent seed set per flower subjected to cross-pollination in the greenhouse. One should not necessarily infer, however, that the same results would be obtained under field conditions. The data suggest that the various clones do not set seed with equal facility. This is significant information for the breeder. This feature may impose additional restrictions upon the randomness of pollination necessary in various testing procedures designed to sample gamete populations validly.

From a quartet analysis it has been possible to predict a theoretically expected percentage of normal eggs subjected to pollination in the greenhouse experiment. Assuming that micronuclei arise as a result of irregularities at the first meiotic division and that they result in a deficiency in the nucleus as well as a departure from normality when present in the cytoplasm the following has been suggested: Those quartets with no micronuclei may be considered as comparable to 100%

normal eggs and those with 1 micronucleus as 37.5% normal. Under such a hypothesis the seed set of the 19 clones obtained under open-pollination in the greenhouse and the expected percentage of predicted normal eggs gave an r value of+ .77. This highly significant correlation coefficient suggests the predictive value of micronuclei analyses in estimating the ability of clones to set seed under open-pollination at least in the greenhouse.

Considerable importance often has been attached to the percentage of stainable pollen as indicative of meiotic normality. It was desired in this study to determine the association of stainable pollen under field conditions with the percentage of normal pollen expected from the micronuclei analyses of quartet stages. The correlation of these values in 30 B. inermis clones from the nursery gave an r of -.1. A negative correlation of this magnitude might tend to discourage the use of pollen stainability as a measure of fertility relations. The whole field of gametophyte ontogeny needs serious study. No doubt significant changes occur in the population products which enter this ontogeny and those which emerge as functional pollen grains. Male gametes floating in tapetal fluid must compete directly for nourishment required in their growth and metabolism. Deficient gametes would not be expected to compete favorably with those more properly balanced genetically. Whether nutrition is the critical factor or not, it is safe to postulate that rather critical selection occurs during gametophyte

development.

Very few diakinesis or metaphase stages of B. riparius plants could be analyzed. A considerable number were observed in which the chromosome number was approximately 2n=70. The associations in 5 cells which could be accurately determined are summarized in Table 5 to follow.

Table 5

Clone	I	II open	II closed	III	IV	V	VI	VII	VIII	2n=
E-2	6	18	12	0	1					70
E-3	2	1	25	0	4					70
	3	5	13	1	2	1		1		70
	0	4	16	0	4		1		1	70
	1	3	15	2	2		2	1		70

The associations observed were indicative of a structural hybrid chromosome complement similar to that observed in B. inermis and B. pumpellianus. The percentages of quartets free of micronuclei among certain clones of B. riparius examined ranged from 33 percent to 74 percent.

B. erectus material from Lund, Sweden, shown in several figures, was not available for earlier crossing experiments. Root-tip mitoses in seedlings of this material revealed a 2n=56 chromosome number. The rather extensive introgression of this species with B. inermis reported by Zherebrina (49) already has been cited in the literature review of this study.

Photomicrographs of diakinesis and other meiotic stages in these species are presented to indicate the irregularities present (see Figs. 51-55).

Controlled crosses

A total of 7,621 flowers were artificially pollinated during the course of the study to ascertain the existing fertility relations within and between certain individuals of the Bromopsis section. Some open-pollination under greenhouse and field conditions was observed and the various percentages of seed set tabulated.

As might be expected, the results obtained from crossing various diploid members of the section with the higher polyploids were of a negative nature. As is indicated in summary form in Appendix Table 8 the plants obtained from crossing B. inermis, B. riparius, or B. pumpellianus with diploid members proved to be selfs without exception. There were obtained, however, a number of plants from crossing diploid and tetraploid members among themselves which appeared to be of hybrid origin. Under Iowa field conditions the diploids of this section from the Pacific coast were unable to survive the summer growing seasons. Potential hybrids among these members were, as a result, eliminated before flowering stages were attained. Likewise, a number of potential hybrids between

Fig. 51. Spikelet of B. riparius clone E-3. 3x ca.

Fig. 52. Lemma characters of B. erectus (2n=56) from Lund, Sweden. 7.5X ca.

Fig. 53. Lemma of B. riparius clone E-3. 7.5X ca.

Fig. 54. Lemma of B. riparius clone E-9. 7.5X ca.

Fig. 55. Diakinesis in B. riparius clone E-3 (2n=70) 820X.

52

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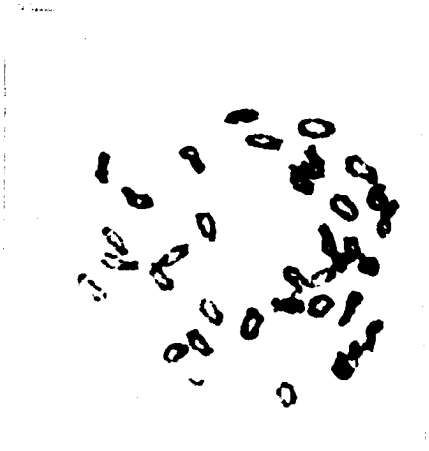


55

tetraploid B. purgans F-354 and diploid B. orcuttianus failed to survive to flowering. None of the potential hybrids obtained from crossing B. texensis with either B. inermis or B. pumpellianus survived beyond the first seedling leaf stage.

The summary of fertility relations obtained from crossing various plants within and between B. inermis and B. pumpellianus are presented in Table 5. For a more complete summary by clones the reader is referred to Appendix Tables 1, 2, 5. The similarity of average seed set is apparent. There were some individual instances of much higher fertility between B. inermis and B. pumpellianus from Pullman, Washington, than the average indicated in Table 5. The same might be said of particular combinations from the other sources of B. pumpellianus. The Pullman, Washington collection of the latter species contained some introgressive types between B. inermis and B. pumpellianus. This phenomenon will be enlarged upon in later paragraphs. Fertility values as high as some obtained in crosses between clones of B. inermis and of B. pumpellianus from various sources suggest a rather close relation of the two. It is suggested further that B. pumpellianus germplasm and the introgressive products in the original distribution of the latter deserve additional study.

The high standard deviations obtained both within and between these species suggest the importance of specific factors conditioning compatability. They are, of course, confounded

with the physical manipulations attendant to crossing in addition to climatic (temperature, light intensity, etc.) differences when the crosses were made, and, perhaps, other factors. They do suggest, however, that the crossing taking place may not be entirely at random.

Table 5

Seed set from crosses within and between B. inermis and B. pumpellianus

Crosses	No. of flowers crossed	Total seed set	Ave. seed set	s
Within B. inermis	1010	450	44.55	23.0%
Within B. pumpellianus	304	142	46.71	29.7%
Between B. inermis and B. pumpellianus from Pullman, Washington	610	149	24.43	25.1%
Between B. inermis and B. pumpellianus from Alberta, Canada	415	129	31.08	26.9%
Between B. inermis and B. pumpellianus from Bodenburg Butte, Palmer, Alaska	229	79	34.50	25.1%

The seed set obtained from crossing B. inermis and B. riparius is summarized in Table 6.

Table 6

Seed set from crosses among B. pumpellianus, B. inermis, and B. riparius

Crosses between	No. of flowers crossed	Total seed set	Ave.% seed set	s
B. inermis and B. riparius	380	131	34.47	18.9%
B. inermis as female 48.18%				
B. riparius as female 28.89%				
B. riparius and B. pumpellianus	996	265	26.61	21.8%
B. pumpellianus as female 26.43%				
B. riparius as female 26.83%				

The relative ease with which members of either 2n= 56 species could be crossed to B. riparius 2n=70 suggested a rather close relation among the three. There was an apparent increase in seed set in crosses involving B. inermis as the female parent. No differences were obtained in reciprocal crosses between B. pumpellianus and B. riparius.

The results of these crosses in general served to emphasize the fact that some plants were better parents than others in the various species crosses. Some crosses in which a considerable number of flowers were involved failed completely. Reciprocal crosses did not always yield the same seed set although they were essentially alike in a number of instances.

Natural crosses in the field

Data on seed-setting from a number of B. inermis, B. riparius, and F_1 clones under open-pollination in the nursery are presented in Table 7. The results appear similar to those obtained in the greenhouse experiments. The rather high seed-set of the B. riparius x B. inermis hybrids suggested the possibility of selfed plants being involved. In the reciprocal cross, represented by (278-18-15 x E-9) plants 3, 6, and 8, where morphological characters of the male parent were expressed in the F_1 hybrids there can be no doubt as to the validity of the seed-set obtained. The B. inermis parent 454-10 used in the former cross proved to cross readily with a number of B. riparius clones and the high seed set may have been due to specific compatability factors.

Evidence for the introgression of B. inermis and B. pumpellianus in North America

In view of the sympatric distributions and artificial fertility relations reported for B. inermis and B. pumpellianus it would seem possible to expect some hybridization in nature.

In an Aspen parkland just off the campus of the University of Alberta, Edmonton, Alberta, which has probably been broken at least once (30), the two species occur together. Among the clones of B. pumpellianus obtained from the boundary of an Aspen clump were typical members of the species. A number of

Table 7

Seed-set under open-pollination in the field of representative clones of *B. inermis*, *B. riparius*, and F_1 hybrids

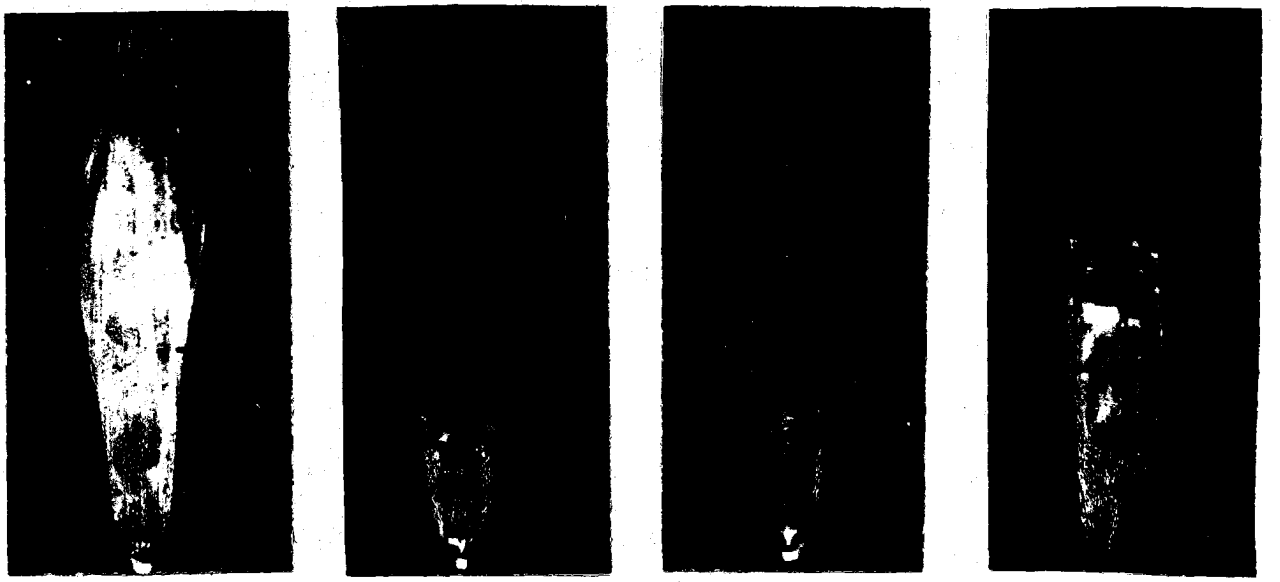
Cross	Plant	Total number of flowers	Total seed set	Ave. % seed set
B. inermis				
298-30 x 291-15	5	1311	830	69.3
255-44 x 554-22	2	1560	327	20.9
" "	6	1105	687	62.2
" "	9	1567	1056	67.4
B. riparius				
E-2		318	141	44.3
E-1326		462	242	52.4
E-1327		1188	328	27.6
B. riparius x *B. inermis*				
E-3 x 454-10	3	894	565	63.2
" "	5	696	531	76.3
" "	8	654	452	69.1
B. inermis x *B. riparius*				
278-18-15 x E-9	3	1662	402	24.2
	6	705	131	18.4
	8	264	3	1.1
B. inermis				
268-44		1427	746	55.1
278-18-15		907	597	65.8
298-30		955	629	65.8

panicles collected from individual clones in several areas throughout the parkland (30), however, revealed a series of forms intermediate between the two species. It was possible in fact to select a series of mature lemmas from plants in the area which intergraded from the more or less glabrous condition found in B. inermis to dense marginal pubescence typical of B. pumpellianus. On the basis of this material a series of model lemmas were selected and a pubescence value assigned to each (see Fig. 56). The limit of typical B. inermis pubescence was arbitrarily set at class 2 while class 6 represented the lemma pubescence of type B. pumpellianus specimens examined. Forms of B. pumpellianus from Alaska, Yukon, and the Northwest Territories were found in the herbarium specimens examined with more extremes of pubescence which were assigned values of 7 and 8.

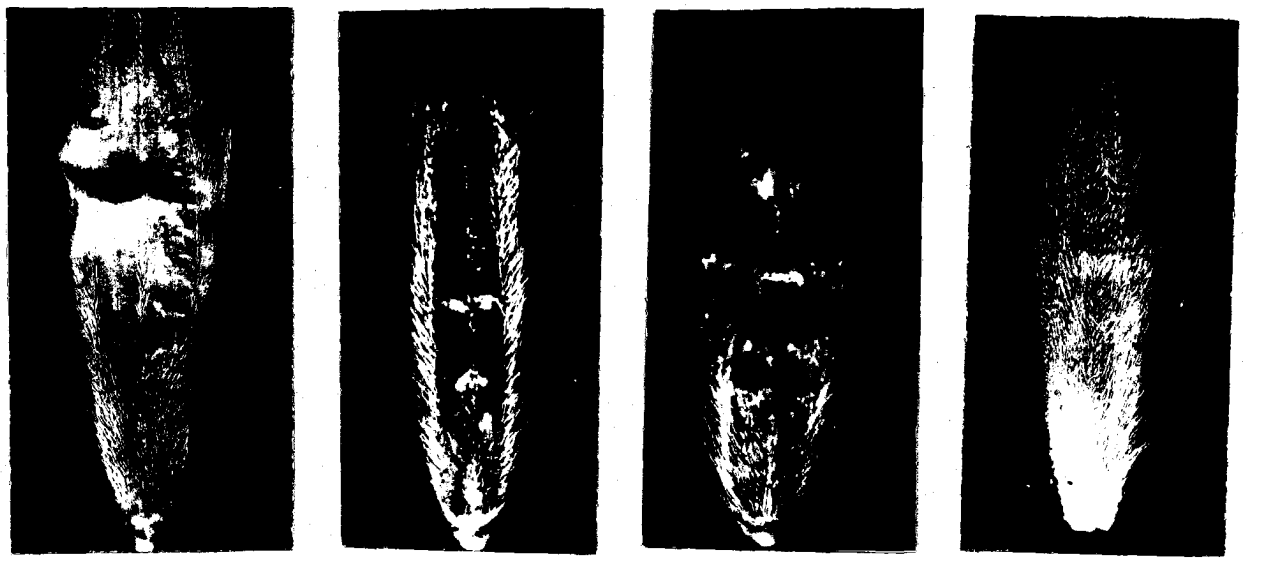
The distribution of lemma pubescence scores of several hundred B. inermis and B. pumpellianus specimens collected in various localities of North America over a 90 year period are presented in Fig. 57. A considerable number of intermediates were encountered. From purely a priori considerations the pubescence classes 3 and 4 should perhaps contain the greatest number of potential hybrids. Their geographical distribution, as presented in Fig. 58, would suggest that hybridization occurred in separate places before the turn of the present century. Between 1895 and 1900 intermediates were collected

Fig. 56

Lemma pubescence classes



1 2 3 4



5 6 7 8

Fig. 57

Distribution of lemma pubescence among 501 specimens of *B. inermis* and *B. pumpellianus* collected in the period 1860-1947

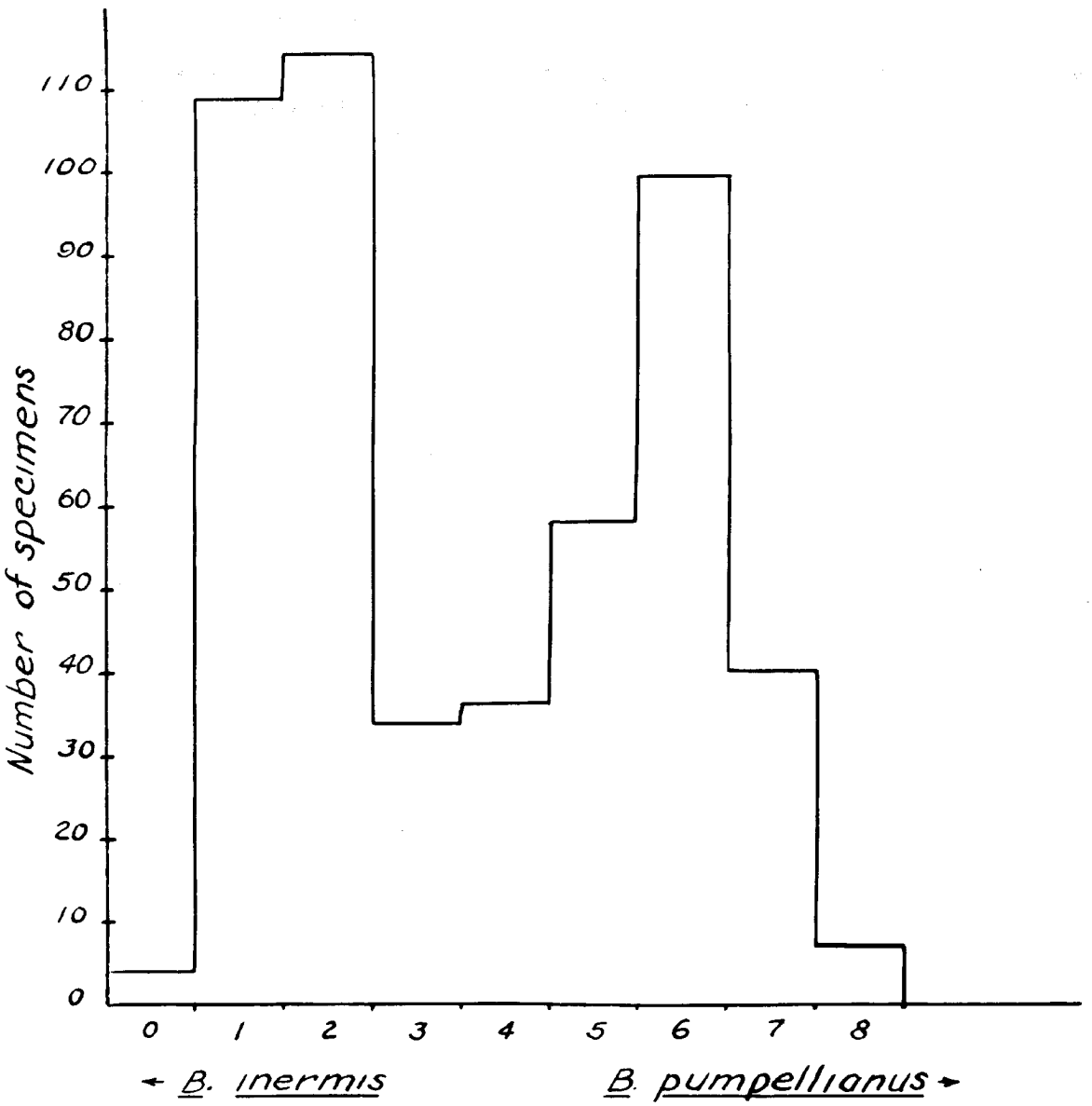
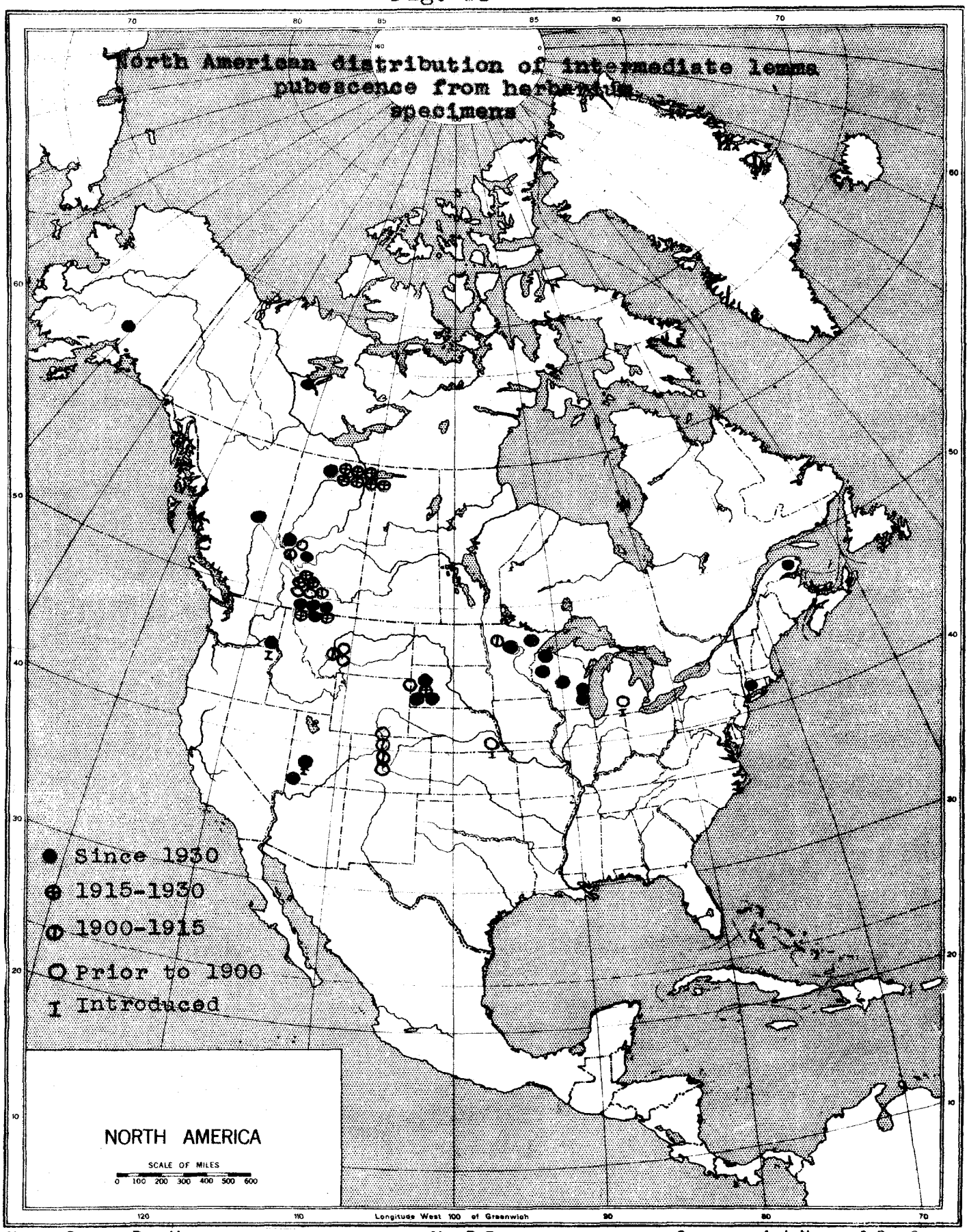


Fig. 58



NYSTROM SERIES OF DESK MAPS No. D 5

in Colorado, Montana, and Alberta. During this same period B. inermis was rapidly being increased in hay and pasture plantings throughout the Intermountain region. Two class 4 specimens were collected prior to the introduction of B. inermis, one from the Rocky Mountains (E. Bourgeau 1858-Gray Herb.), and one from South Park, near Gray's Peak, Colorado (Wheeler Expedition. 393, 1873-Gray Herb.). Neither of these were sufficiently mature to allow accurate scoring of their potential pubescence, but their inclusion seemed worthwhile from the historical point of view. Intermediates also were found among specimens from the Black Hills, South Dakota, Minnesota, Wisconsin, Connecticut, and Quebec as shown in Fig. 58.

B. pumpellianus was introduced into various gardens, nurseries, and observational plots from 1880 to the present, some plantings of which are no doubt responsible for the intermediates listed as introduced in Fig. 58. The specimen from the Experiment Station, Lincoln, Nebraska was collected in 1890, (Mo. Bot. Gard. 216672), the one from the Michigan Agricultural College grass gardens (C. F. Wheeler-Gray Herb.) was taken in 1895, and the one from the Ephraim Branch, Great Basin Experiment Station, Utah, (B. L. Richards Jr. 5138-Gray Herb.) in 1934. Several plants grown from B. pumpellianus seed obtained from the Soil Conservation Service nursery at Pullman, Washington, were previously mentioned in this same category.

There is considerable discontinuity in the distribution of B. pumpellianus in the United States as indicated in Fig. 2. Typical specimens were noted from the Intermountain region, the Black Hills, the Cheyboygan area in Michigan, and near Ottawa, Canada. Hitchcock (19) considered the Michigan B. pumpellianus an introduction. This interpretation is questioned in view of the Canadian and American distributions of the intermediates as well as typical B. pumpellianus. Very similar North American distributions have been reported by Fassett (11), Munns (34), Hopkins (20), Hitchcock (19), and Marie-Victorin (32) for the following species of flowering plants:

Rubus parviflorus, Populus balsamifera, Picea glauca, Arabis divaricarpa, Scirpus pumilus, Listera borealis, Antennaria pulcherrima, Festuca scabrella, Poa interior, Poa alpina, Poa canbyi, Melica smithii, Agropyron bakeri, Agropyron spicatum, Deschampsia atropurpurea, Danthonia intermedia, Calamagrostis neglecta, Phleum alpinum, and Orysopsis asperifolia.

The B. inermis specimens examined from Central Europe did not exceed class 2 lemma pubescence. Within the Lincoln variety of B. inermis the lemmas examined were largely class 1. In 300 lemmas taken from a seed sample of the Fischer variety three were scored in class 3. This seed was produced in the Soil Conservation Service nurseries near Ames, Iowa in 1944 from seed taken from an old stand in a southern Iowa field

which had persisted more than 20 years. The seed from which this old stand was planted had been obtained from a Mechanicsburg, Ohio, source. The previous history of this seed lot is unknown. The closely related pubescent B. riparius also was being produced in the same nursery as the Lincoln and Fischer varieties sampled in this study. The Lincoln variety, grown from an unknown seed source, did not exhibit any pubescence exceeding class 2, however. The original foreign source of this variety may have been quite different from that of the Fischer variety, although the two crossed readily and set an abundance of seed under artificial conditions in the greenhouse.

Various features of the artificial hybrids between B. inermis and rather typical B. pumpellianus will be discussed at some length later. It would be well to point out that they are intermediate in lemma pubescence, although more nearly approaching the pubescent condition (class 5) than a true intermediate con ition. It is entirely possible, of course, that the intermediates sampled under natural conditions contained backcrosses and other complex segregation products between the two species. Inasmuch as both parents and hybrids are largely cross-pollinated it is reasonable to assume that most of the natural crossing would have been to the aggressive parent B. inermis since its introduction and increase in these areas.

In the Intermountain region, the intermediates have persisted most often in altered environments. Roadsides, highway

shoulders, and railway embankments seem to have common environments in which the hybrids flourished. These disturbed habitats may have served a two-fold function: in addition to providing a habitat for the hybrids they also served as an avenue by which B. inermis was able to escape and invade the restricted range of B. pumpellianus. B. inermis was, however, carried into and planted widely in the range of B. pumpellianus, thus allowing additional opportunity for hybridization.

On the basis of specimens examined it can be reasonably stated that hybridization between the two species is currently under way in the following:

1. The area just west of the campus of the University of Alberta, Edmonton, Alberta.
2. Waterton Lakes parklands, Alberta.
3. In the Black Hills, South Dakota, in the vicinity of the junction of U. S. highway 85 and Camp Este road.

There are evidences of many other isolated areas within the possible range of B. pumpellianus where hybridization has probably occurred within the past 20 years. The most interesting, however, is the intermediate condition found in the specimens from Minnesota, Wisconsin, Connecticut, and Quebec which are, supposedly, outside the known range of the species.

An explanation for the presence of these intermediates may be found in one or more of the following postulates:

1. They may bear some relation to the now relic populations of B. pumpellianus along the Michigan lake shores.
2. They may represent introductions of closely related material from Asiatic sources carried in by early immigrants.
3. They might conceivably represent extreme variants within B. inermis.
4. They may have resulted from hybridization in these areas with B. pumpellianus carried in from other American or Canadian ranges of this species.

Immigrant floras of introduced weedy species in particular may be cited as an example favoring the latter. The pattern of this distribution, however, does not favor such a hypothesis. The possibility of extreme variants is discounted since the pubescence should also have shown up in various other localities of comparable latitude and soil conditions which were sampled equally well. There is a possibility that introduction of closely related pubescent material from European or Asiatic sources is involved. It was reported by Pieters (41), for instance, that much of the B. inermis originally introduced from Europe was adulterated with seed of B. erectus as well as other contaminants. In view of the introgression of these species observed by Zherebrina (49) and the fertility of B. inermis, B. pumpellianus, and B. riparius crosses it seems reasonable to expect some complication of the pubescence patterns of B. inermis in this country. The lemma pubescence of typical B. erectus is fairly

uniform over the back while that of typical B. pumpellianus is more nearly restricted to veins and margins. Among the specimens examined were curious patterns which were difficult to delimit under the arbitrary arrangement adopted.

One of the complicating factors in any analysis of B. inermis is the fact that different importations have been made from widely separated localities in Central Europe and Russia. Indeed, it may well be possible that a considerable part of the morphological, as well as cytological variability now found in B. inermis is due to the mixture - in North American areas - of germplasm from widely differing sources.

In the writer's opinion the most plausible explanation of the intermediate pubescence types found around the Great Lakes lies in the possibility of relation to the remnant lake shore distribution of B. pumpellianus. Since no specimens of B. pumpellianus were reported from eastern United States and Canada, excepting the collection from the vicinity of the Great Lakes and one specimen from Ottawa, Canada, the presence of these intermediates is accepted as evidence of relic distributions in the particular areas. The very nature of these habitats bespeaks of endemism. The distributions of the intermediates in eastern United States and Canada are, without exception, in terminal moraine areas where stony, isolated habitats presented a minimum of floral competition. B. pumpellianus and certain introgressive products are, perhaps, peculiarly adapted to these stony habitats.

In the Intermountain region B. pumpellianus has persisted in cool, rocky, montane habitats where floral competition was reduced to a minimum and has in no way been able to compete with B. inermis in the more fertile valleys in these mountain areas where it once was, apparently, much more plentiful. Plants of typical B. pumpellianus, in spite of morphological similarity, are not comparable in vigor to those of B. inermis. Artificial hybrids between the two species have, in the main, been as vigorous as average B. inermis plants and some have exhibited some heterosis.

The fact that any recombination products with B. pumpellianus-like characters appeared in the restricted sampling of Minnesota, Wisconsin, Connecticut, and Quebec seems quite remarkable. In the terminal moraines or higher, rocky habitats chance recombination products with B. pumpellianus characters would not have been critically selected against. It is expected further that the reduced fertility of the hybrids would not have been selected against critically as long as effective asexual propagative characteristics were maintained.

Controlled hybrids between B. inermis and B. pumpellianus

The relative ease with which hybrids were obtained from certain combinations of these two species was emphasized in earlier paragraphs. Evidence for the introgression of the two in nature since the introduction of B. inermis also has been presented. Should the transfer of specific characters from

B. pumpellianus to B. inermis become desirable, certain features of the cytological and genetic behavior of these hybrids should be kept in mind.

Hybrids obtained between typical appearing B. pumpellianus and B. inermis largely were intermediate in morphological characters. Certain of these characters are shown in Figs. 59 - 63. It was impossible to set definite limits on the extent of this intermediacy since the parents themselves were of such a highly variable nature. Where plants of B. pumpellianus with typical lemma pubescence were used as parents with B. inermis clones from the nursery, the F_1 hy rids obtained were of class 4 or 5 lemma pubescence as shown in Figs. 61 and 62. Culm nodes of the hybrids were also intermediate in pubescence when the parents represented extremes of this character. However, this character difference has proven so variable among clones of B. pumpellianus as to render it useless as a constant species difference.

The meiotic analyses of the hybrids made with the Edmonton, Alberta, source of B. pumpellianus are presented in Table 8. Chromosome associations observed were essentially similar to those of both parent species. Univalents were quite common. In the hybrids examined the number of quartets free of micronuclei per 100 ranged from 12 to 41. This was considerably lower than the average of the parents indicating that certain meiotic factors were contributing to this instability.

Fig. 59. Spikelet of hybrid 7-7. 3x ca.

Fig. 60. Spikelet of hybrid 8-3. 3x ca.

Fig. 61. Lemmas of hybrids 7-7 and 7-2 exhibiting class 4 pubescence. 7.5X ca.

Fig. 62. Lemmas of hybrids 7-4 and 8-3 exhibiting class 5 pubescence. 7.5X ca.

Fig. 63. Node and culm pubescence in hybrid 7-2. 1X ca.

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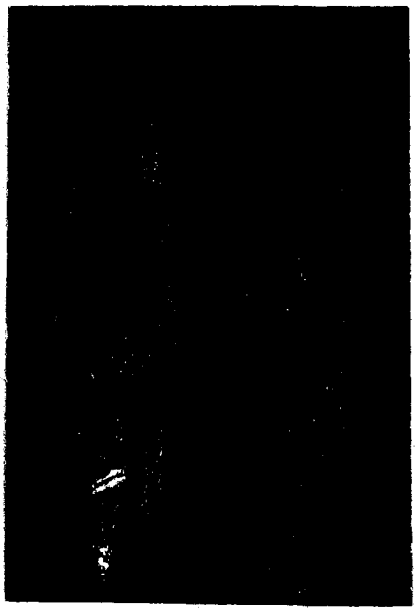

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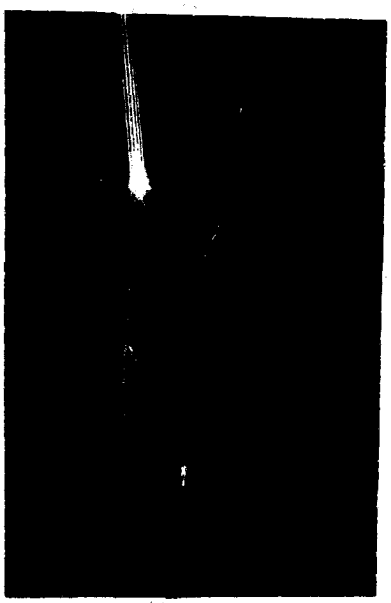

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A number of these hybrids were allowed to open-pollinate at random with a group of B. inermis clones in a greenhouse experiment previously mentioned in Table 3. The seed-setting of these hybrids resulting from this procedure is presented in Table 9 to follow. The seed set among these hybrids ranged from 13.6 percent to 33.3 percent per flower subject to pollination. Typical B. inermis clones in the same experiment ranged from 13.0 per cent to 58.8 percent. While the seed-set among the hybrids failed to approach the high level possible in B. inermis it was sufficiently higher than the low fertility clones of B. inermis to indicate a close relation of the two.

A considerable number of hybrids between the B. pumpellianus and B. inermis were grown and analyzed for various features. Characteristic meiotic stages among these hybrids are shown in Figs. 64-71 . The B. pumpellianus parent (P-8) in these cases did not exhibit a typical B. pumpellianus lemma pubescence. This clone has a glaucous appearance with short pubescence over the leaves and culms quite unlike typical B. inermis. The summary of meiotic chromosome associations observed in a number of these clones is presented in Table 10. Hybrids 12 and 18 were somewhat more irregular than the others as evidenced by large numbers of univalents and open bivalents. Details of this are presented in Figs. 64 and 69.

A number of hybrids were obtained from crossing B. riparius with B. inermis and analyses of these are included

Table 8

Meiotic chromosome associations observed in B. inermis x B. pumpellianus F_1 hybrid 8-2

Cell No.	I	II open	II closed	III	IV	V	VI	VII	VIII
1	4	4	17	2	1				
2	2	5	15	0	0	0	1	0	1
3	2	8	14	0	1	0	1		

Table 9

Seed-setting under open-pollination in the greenhouse of certain B. inermis x B. pumpellianus F_1 hybrids

Clone Number	% seed set
7-2	13.6
7-4	21.9
7-7	23.9
8-2	33.3
8-3	25.5

Fig. 64. Metaphase I in P-8 x 255-44 #12 with 10 univalents including 2 ring univalents, 2 open bivalents, 14 closed bivalents, 3 association of III, and 1 V. The ring univalents are evidence of chromosome duplication on either side of the centromers. Note fragment in lower right corner. 820X.

Fig. 65. Metaphase in P-8 x 255-44 #8 with 2 univalents, 5 open bivalents, 18 closed bivalents, and 2 associations of IV. 820X.

Fig. 66. Diakinesis in P-8 x 255-44 #16. Note the multivalent associations with chiasma held to other associations. 875X ca.

Fig. 67. Early anaphase I in P-8 x 255-44 #12. 820X.

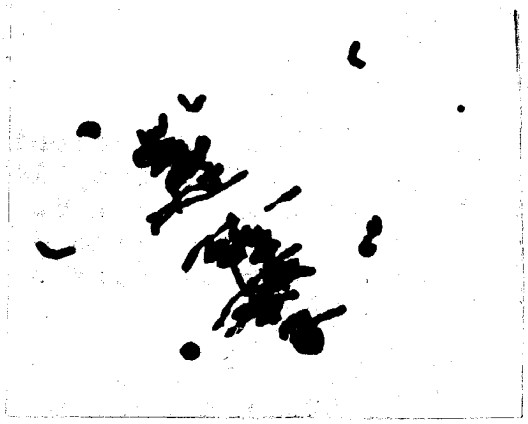
Fig. 68. Anaphase in #12 showing equational division of 5 lagging univalents. 820X.

Fig. 69. Anaphase I of P-8 x 255-44 #18 showing extreme irregularity of lagging and bridges. 820X.

Fig. 70. First metaphase orientation of an association of V in P-8 x 255-44 #6. 1200X ca.

Fig. 71. Late diakinesis association of VI in P-8 x 255-44 #15. 1200X ca.

65



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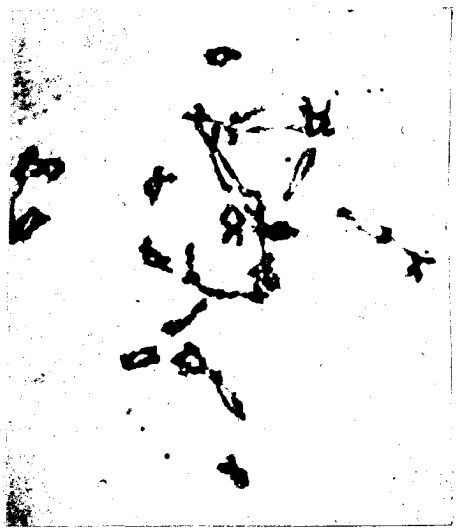

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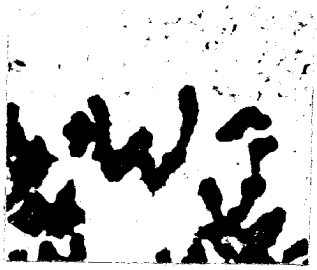

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Table 10

Summary of meiotic chromosome association in F_1 hybrids of P-8 x 255-44

		#5	#6	#8	#10	#12	#13	#15	#16	#18
No. of cells		1	10	11	3	6	6	4	3	3
Type of association										
I	mean	5.0	1.6	2.2	1.7	8.8	1.2	0.8	1.7	3.3
	range		0-5	0-4	0-4	6-12	0-3	0-2	1-2	2-6
open II	mean	3.0	3.2	3.9	3.3	6.3	3.3	1.8	3.0	6.7
	range		1-6	0-6	1-6	3-9	1-5	1-3	0-5	5-8
closed II	mean	16.0	11.9	16.9	11.7	12.7	14.8	13.8	10.7	15.0
	range		5-17	14-22	6-16	9-19	7-19	11-17	7-13	12-17
III	mean	1.0	0.8	0.6	0.3	1.3	0.2	0	0.3	0
	range		0-3	0-2	0-1	0-3	0-1	0	0-1	0
IV	mean	1.0	3.5	1.7	3.7	0.2	3.5	3.8	2.3	0.7
	range		1-6	0-4	3-4	0-1	1-4	3-4	1-3	1-2
V	mean	0	0	0.5	0	0.7	0	0.3	0	0
	range		0	0-2	0	0-1	0	0-1	0	0
VI	mean	1.0	0.9	1.8	1.0	0.2	0.7	1.0	0	0
	range		0-3	0-1	0-2	0-1	0-2	0-2	0	0
VII	mean		0	0	0	0	0	0	0.7	0
	range		0	0	0	0	0	0	0-2	0
VIII	mean		0.3	0	0.3	0	0.2	0.3	0.3	0.3
	range		0-2	0	0-1	0	0-1	0-1	0-1	0-1

in the study. They were generally intermediate in morphological characters between the parents. A few of these details are shown in Figs. 72-76. The typical leaf and culm pubescence of B. riparius is an objectionable feature from a forage standpoint although the non-spreading habit of growth which it has may be desirable in some instances. The figures presented show the intermediate expression of these characters in F_1 hybrids. There were plants among the first backcross progeny to B. inermis which were as pubescent, or more so, than the F_1 hybrids. There also were some which appeared intermediate between the F_1's and the recurrent parent, B. inermis. The plants of the second backcross were, for the most part, like the recurrent parent in this character although a few still possessed some short pubescence not typical of the B. inermis parent. The differences in chromosome numbers in these hybrids and backcrosses may have been responsible for part of the observed complexity.

Similar results were obtained for spikelet characters in these complex hybrids as shown in Figs. 77 - 79. The lemma pubescence of the F_1's, likewise, was generally intermediate although variable from plant to plant.

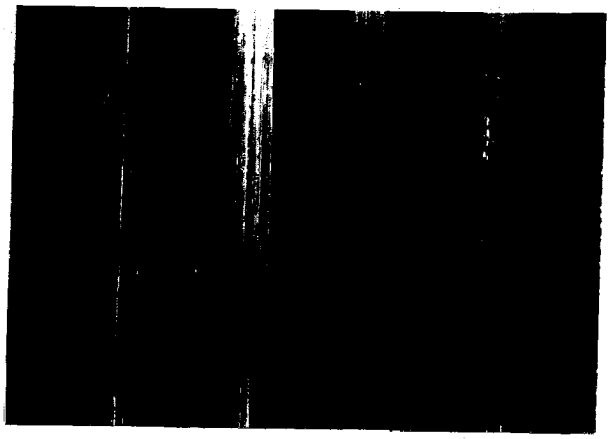
Mitoses in root-tip cells of second backcross plants are shown in Figs. 81 and 82. The somatic number shown in Fig. 81 is 54 while that of Fig. 82 is 56. The possibility of chromosome loss during smearing was recognized and discretion was employed

Fig. 72. Left to right: B. inermis, three F_1 hybrids, and B. riparius. 1X ca.

Figs. 73-74-75. Backcrosses of inermis-riparius F_1 to B. inermis. 1X ca.

Fig. 76. Second backcrosses to B. inermis. 1X ca.

72



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in the analyses of cells where error seemed at all possible. In spite of these precautions numerous cells in these hybrids and backcrosses were observed to be deficient in somatic chromosomes. In root-tip cells of an inermis-riparius F_1, for instance, the chromosome numbers in the cells observed ranged from 46 to 63. At least two pairs and apparently 5 chromosomes in Fig. 81 are satellited. Terminal associations of certain chromosomes were observed frequently in the root-tip cells examined.

Table 11 summarized the meiotic chromosome analyses of a number of hybrids and backcrosses examined in this group. Associations similar to those reported for the parent species were observed for the most part. A few more complex multivalents (IX and X) were observed here than in the parents, indicative of additional interchanges since divergence. There were variations in the 2n number of chromosomes observed in various cells, the ranges of which are listed in Table 11. These differences are confounded, however, with the possible misinterpretation of extremely complex associations.

There may be barriers involved in these hybrids other than the whole chromosome meiotic irregularities already noted. The summary of quartet analyses of a number of these hybrids is presented in Table 12. The very low number of quartets free of micronuclei in any of the hybrids or backcrosses is apparent. Some of these micronuclei have,

Fig. 77. Spikelet of hybrid #8. 3x ca.

Fig. 78. Spikelet of a first backcross hybrid. 3X ca.

Fig. 79. Spikelet of a second backcross hybrid. 3X ca.

Fig. 80. Lemmas of 3 *inermis-riparius* F_1 hybrids. 1X ca.

Fig. 81. Root-tip mitosis in second backcross hybrid in 12-4 showing 54 chromosomes in this cell. 1160X ca.

Fig. 82. Root-tip mitosis in second backcross hybrid 12-9 showing 56 chromosomes. 1190X ca.

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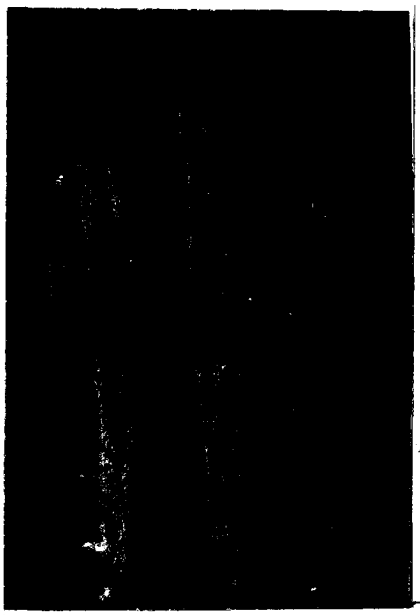

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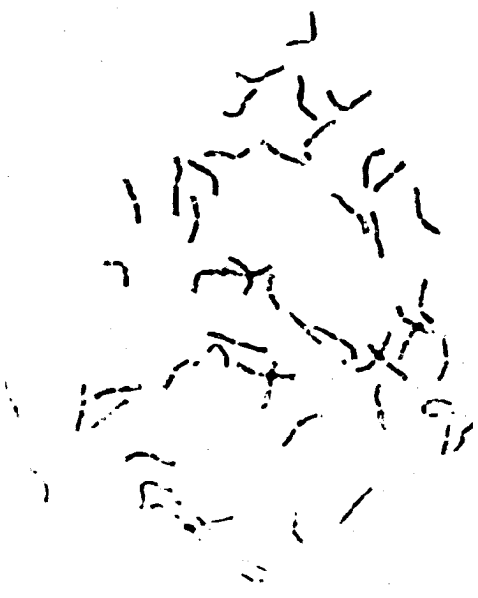
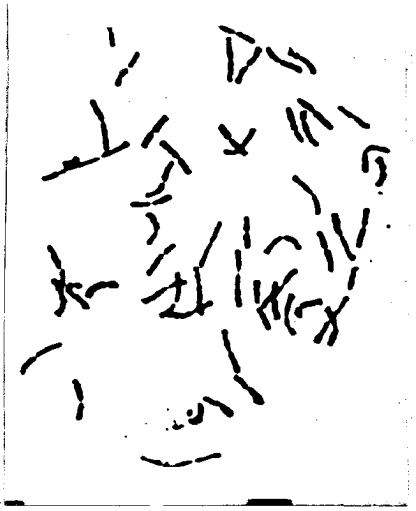


81



82

Table 11

Meiotic chromosome analyses of hybrids and back-crosses involving B. inermis, B. riparius, and B. pumpellianus

	278-18-15 x E-9	454-10 x E-4	E-4 x 454-10	BC_1 to inermis	BC_2 to inermis	pump.-rip. BC to inermis
No. of plants	3	1	1	3	1	1
No. of cells	18	7	6	9	5	6
Type of association						
I mean	4.1	3.3	.7	2.1	2.2	2.8
range	0-8	0-7	0-1	0-6	2-3	1-7
open mean	1.6	5.6	1.2	3.1	3.2	4.5
II range	0-4	0-10	0-3	0-7	0-7	2-6
closed mean	19.7	13.7	18.0	15.6	14.6	13.7
II range	13-24	9-19	13-25	7-25	11-19	9-18
III mean	1.3	.7	.3	.7	1.4	.7
range	0-3	0-3	0-2	0-3	0-3	0-2
IV mean	1.5	2.0	3.2	1.9	2.4	2.7
range	0-4	0-5	0-5	0-4	1-4	1-5
V mean	.7	.6	0	0	0	0
range	0-3	0-2	0	0	0	0
VI mean	.2	.3	.8	.8	.8	.3
range	0-2	0-2	0-3	0-4	0-2	0-1
VII mean	.1	0	0	0	0	0
range	0-1	0	0	0	0	0
VIII mean	0	.6	.5	.7	0	0
range	0	0-2	0-1	0-3	0	0
IX mean	.6	0	0	0	0	.2
range	0-1	0	0	0	0	0-1
X mean	0	0	.2	0	0	.2
range	0	0	0-1	0	0	0-1
Range in 2n chromosome no.	59-63	58-63	63-73	56-62	51-61	56-60

Table 12

Summary of quartets free of micronuclei in analyses of various *inermis* *riparius* hybrids and backcrosses

Combination	Cross	Plant no.	% quartets free of micronuclei
F_1	278-18-15 x E-9	8	12
F_1	454-10 x E-4	1	2
F_1	E-4 x 454-10	1	13
F_1	E-3 x 454-10	7	0 2% bridges at Ana.I
BC_1	$\frac{\text{I-E}}{\text{I}}$ 3-17	17	8 5% bridges at Ana.I
BC_1	$\frac{\text{I-E}}{\text{I}}$ 6-11	11	5 2% bridges Ana.I
BC_1	C-10	10	23
BC_1	C-5	5	2 1% bridges at Ana.I
BC_2	12-2	2	0
BC_2	12-3	3	15

no doubt, arisen from the random distribution of unequal chromosome numbers in some of these hybrids. The rather low percentages of quartets free of micronuclei in the BC_2 clones examined leads one to conclude that other factors also were involved.

Examination of Figs. 83 - 97 depicting meiotic stages in certain of these complex hybrids may reveal several interesting features of this irregularity. Heteromorphic bivalents are apparent in Figs. 84 and 85. The association of satellited members with those without satellites seems to indicate further the pairing complexity possible. This will have a profound effect upon the concept of chromosome duplication, pairing, and recombination. Only on rare occasions was it possible to achieve effective staining of satellited chromosomes at meioses.

Giant spores similar to the one shown in Fig. 96 were observed on occasion among certain of the species hybrids. They may have arisen from failure of cell wall formation at the two or four nucleate stage. This failure may have resulted from the loss of genetic materials from the main nucleus controlling this process or from other factors controlling the growth and development of the young spores.

Fig. 83. Diakinesis in F_1 454-10 x E-4 #1 showing an association of eight chromosomes in upper right hand corner. 1225X ca.

Fig. 84. Diakinesis in first backcross 3-17 with 2 univalents, 4 open bivalents, 20 closed bivalents, 1 association of IV, and 1 VI. 1000X ca.

Fig. 85. Diakinesis in 3-17 showing 1 univalent, 3 open bivalents, 19 closed bivalents, 1 association of III, and 3 IV. 1000X ca.

Fig. 86. Diakinesis in first backcross C-10 showing a rather loose association of 8 chromosomes in upper right corner. 1190X ca.

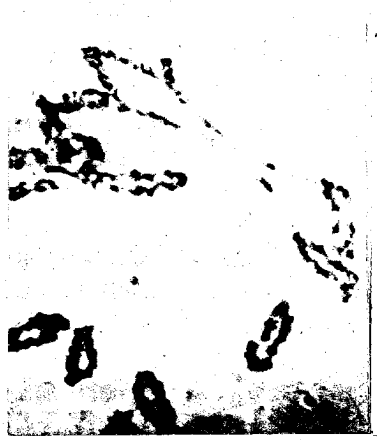
Fig. 87. Diakinesis in C-10 showing 2 univalents, 7 open bivalents, 13 closed bivalents, 2 associations of III, and 2 IV. 1125X ca.

Fig. 88. Diakinesis in second backcross 12-2 with 2 univalents, 4 open bivalents, 15 closed bivalents, and 4 associations of IV. 1190X ca.

Fig. 89. Polar view of anaphase I in C-10. 800X ca.

Fig. 90. Metaphase II in backcross 6-11 showing an anaphase I bridge still intact. 1225X ca.

Fig. 91. Quartet with six micronuclei in second backcross 12-3. 1190X ca.



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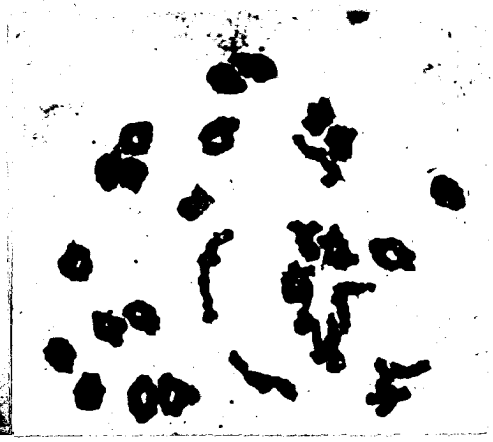

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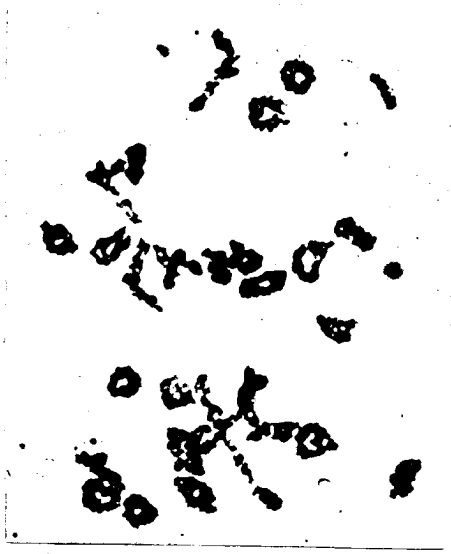

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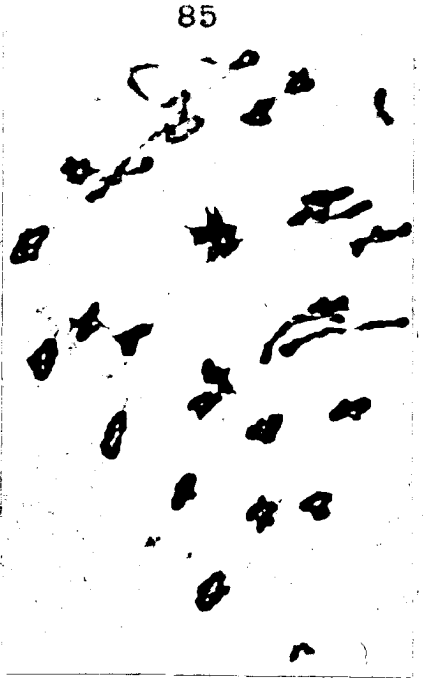

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Fig. 92. Spikelet of pumpellianus-riparius F_1 backcrossed to B. inermis.

Fig. 93. Diakinesis in 307-35-3 x (P-10 x E-4) shown in 3 univalents, 6 open bivalents, 9 closed bivalents, 1 association of IX, and 1 X. 1188X ca.

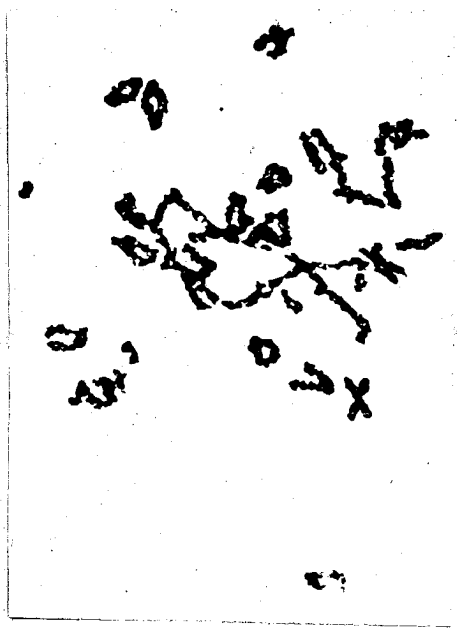
Fig. 94. Diakinesis in same clone with 2 univalents, 6 open bivalents, 11 closed bivalents, 2 associations of III, 2 IV, and 1 VI. 1188X ca.

Fig. 95. Spikelet of riparius-pumpellianus F_1 20-2. 3X ca.

Fig. 96. Spores of riparius-pumpellianus F_1 20-9 showing the result of wall failure. 940X ca.

Fig. 97. Leaf sections from top to bottom: B. pumpellianus P-8, B. riparius E-3, B. erectus from Lund, and second backcross of riparius-inermis back to B. riparius.

92



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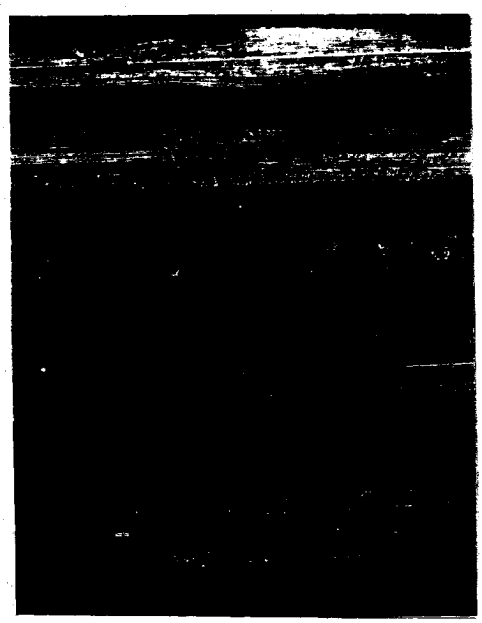


97

The polyhaploid B. inermis clone 554-39

During the course of the study a considerable number of clones of various species were brought in from the nursery for greenhouse analyses of various kinds. At the suggestion of Knobloch (28) an aberrant clone (554-39) was investigated. This clone proved to be a polyhaploid.

In root-tip cells of this clone the haploid chromosome complement was observed. Under an artificially extended photoperiod the clone came into flowering along with a normal sister clone brought into the greenhouse at the same time. The haploid failed to grow as rapidly as the sister clone and the foliage and flower parts were somewhat reduced in size.

A series of photomicrographs showing details of this clone are presented in Figs. 98 - 108. The reduced size of the spikelet shown in Fig. 98 and the comparatively small pollen grains in Fig. 100 and 101 are apparent. At least two chromosomes of the somatic complement shown in Fig. 104 show terminal constrictions. From a superficial examination of pachytene pairing (shown in Figs. 102 and 103) one would conclude that rather normal synapsis had occurred although there are unpaired strands in certain sections. Significant features of the chromosome make-up are revealed in the diakinetic stage shown in Fig. 105. A rather loose association of six chromosomes and an association of four indicates a high degree of internal pairing possible. The multivalent associations may be due in

Fig. 98. Spikelet at anthesis showing a slightly reduced number of flowers. 3X ca.

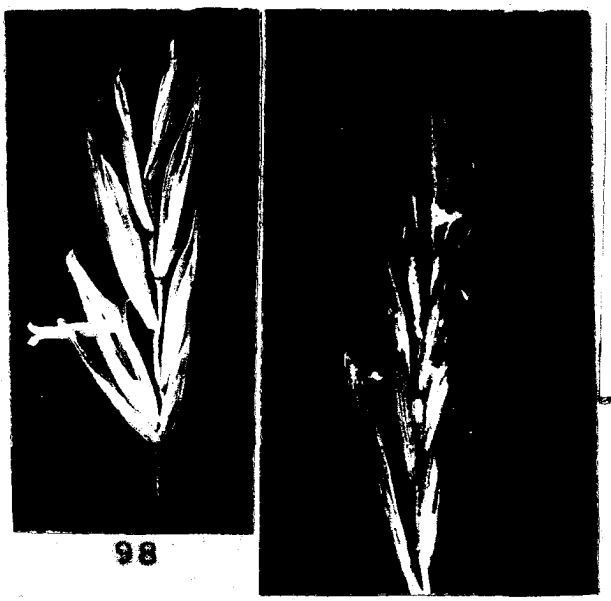
Fig. 99. Spikelet of clone 314-8 for comparison. 3X ca.

Figs. 100-101. Pollen grains of polyhaploid stained with aceto-carmine in comparison with pollen (Fig. d) from a normal sister clone 554-49. 790X ca.

Figs. 102-103. Pachytene stages indicating the synapsis attained within the polyhaploid. 1125X ca.

Fig. 104. Root-tip mitosis showing the complement of 28 chromosomes. 1125X ca.

Fig. 105. Diakinesis showing one association of six chromosomes, one of four, and nine bivalents. 1175X ca.



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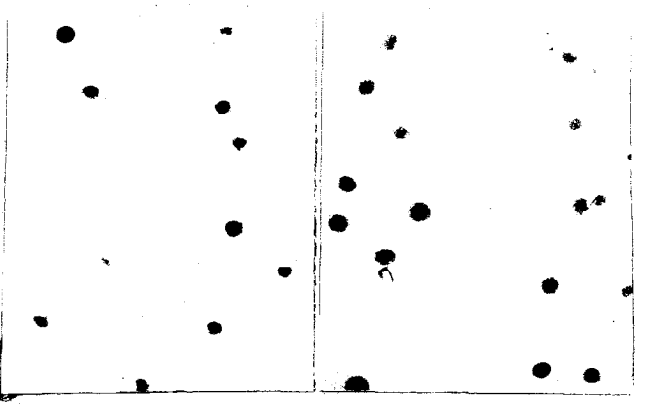

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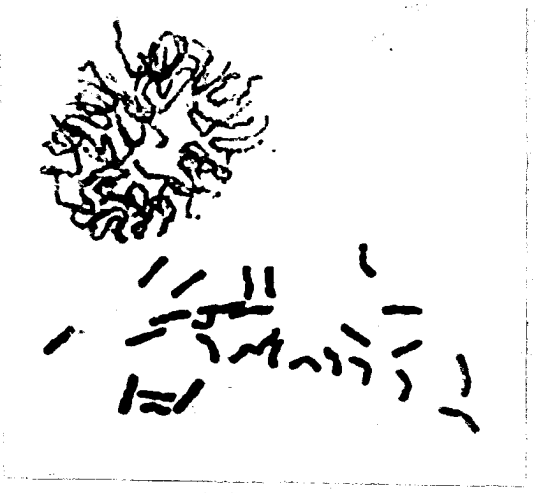

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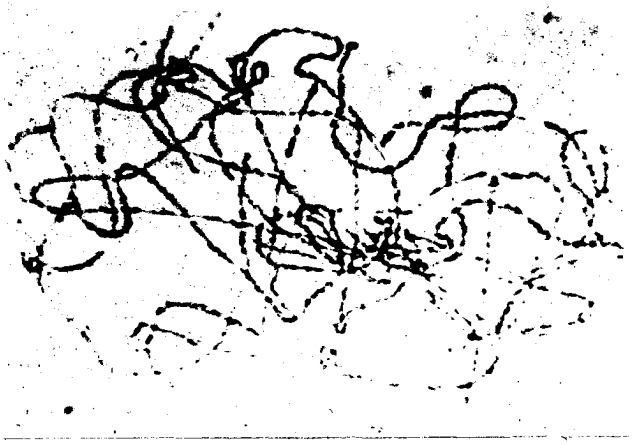

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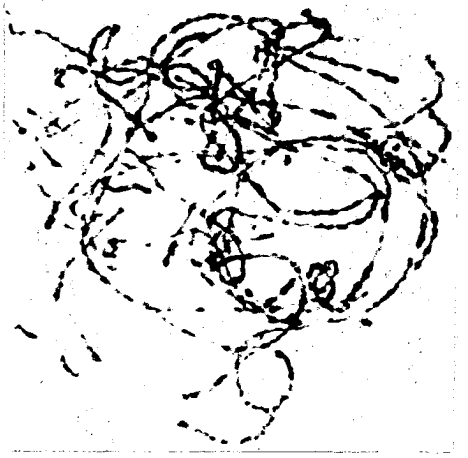


103

Fig. 106. Diakinesis showing association of two pairs with the nucleolus. 1000X ca.

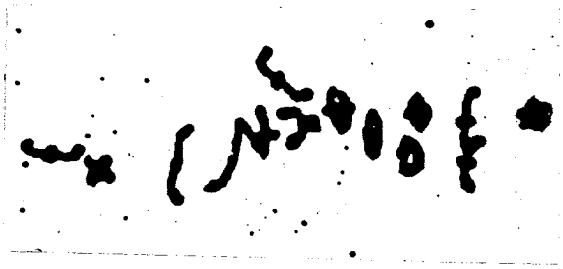
Fig. 107. Metaphase I showing one association of IV, 6 open bivalents, and 6 closed bivalents. 1175X ca.

Fig. 108. Metaphase I showing 7 open bivalents and 7 closed bivalents. 1188X ca.

Fig. 109. Metaphase I showing 7 open bivalents, 6 closed bivalents, and two univalents. 1186X ca.

Fig. 110. Anaphase I showing a 14-14 distribution of the chromosome complement. 1175X ca.

106



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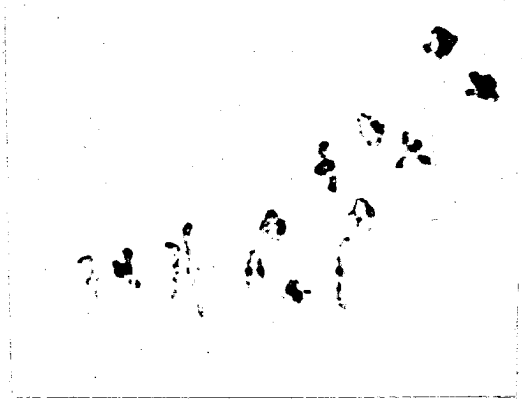

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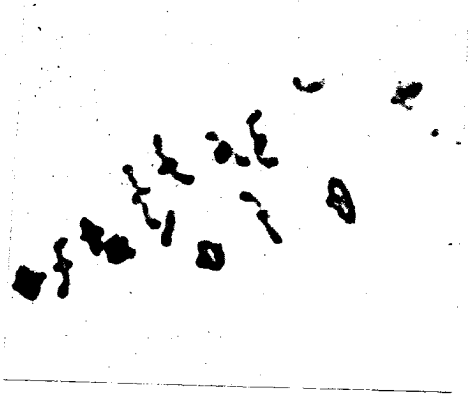

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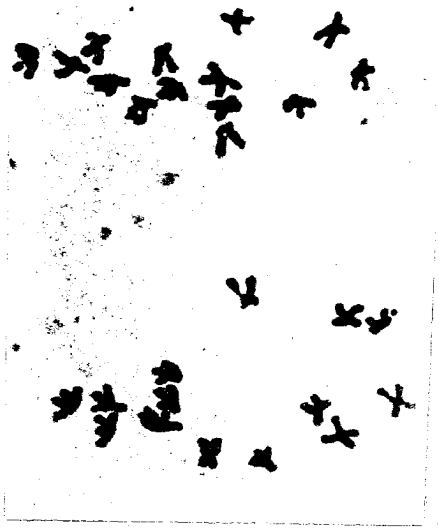

110

part to the polyploid nature of the species and to segmental interchanges which have occurred during its phylogeny. The series of diakinetic and metaphase I stages shown in Figs. 106 - 110 provide additional evidence of possible internal pairing. A summary of associations observed at diakinesis and metaphase I are present in Table 13 below.

Table 13

Meiotic chromosome associations in polyhaploid *B. inermis* clone 554-39

	No. of cells	Mean chromosome associations per cell				
		I	II open	II closed	IV	VI
Diakinesis	11	.2	2.6	8.8	1.1	.09
Metaphase I	51	.12	7.2	6.5	.06	0

The normal disjunction of the haploid complement at anaphase I is shown in Fig. 110. The quartet stages examined were 87% free of micronuclei and, as was shown in Fig. 100, a high proportion of stainable pollen was present. No seed-set was obtained upon selfing or outcrossing this clone in the greenhouse. It reached flowering stage very late in the spring when greenhouse temperatures were high and conditions generally unfavorable for seed-setting.

Because of the presence of many closed bivalents in the polyhaploid one might conclude that the species was nearly an autopolyploid which arose by doubling from a hybrid between two closely related allotetraploids (45). The fact that a predominant number of bivalents still appear at the octoploid level leads one to suspect forces in addition to homology as being responsible for the pairing. A supplementary explanation must, therefore, be sought to account for the observed associations.

DISCUSSION

The analyses of the various hybrid combinations among B. inermis, B. riparius, and B. pumpellianus have indicated rather close relationships among these species. The results indicate that seed-setting ability is not necessarily an accurate measure of the extent of recombination possible among them. Cytological analyses reveal rather serious limitations to the expectation of normal recombination among these closely related members. The transfer of characters from one to the other may be attended with many difficulties, particularly if the inheritance is of a quantitative nature. To be at all reasonably sure of success in the transfer of characters from one entity to another large populations would be extremely desirable.

In the case of B. pumpellianus and B. inermis, where a certain degree of introgression and segregation has apparently been under way since the introduction of B. inermis, effective transfers may already have been executed in nature. The survey of plants within the original distribution of B. pumpellianus should prove valuable should a situation arise in which the desired character was not readily forthcoming in available material of B. inermis.

One need be concerned, however, with the implications of an apparently irregular meioses within the species B. inermis

itself. One might also be critical of speculative ventures but, nevertheless, great significance must be attached to the maintenance of the apparent irregularity of the meiotic mechanism found in these species. The occurrence of meiotic irregularity among the long-lived perennial grasses with means of asexual propagation has been emphasized in the literature cited in this study.

In this regard it seems advisable to point out the differences between meiotic mechanisms in annual grasses and cereals and that of long-lived perennials. In the annual, natural selection operates rather critically upon sexual reproductive capacity. The successful spread of a superior genotype is dependent, to a considerable degree, upon the ability to set the greatest possible amount of viable seed. As a result, the meiotic mechanism is under critical selection pressure for factors promoting seed set and viability. Normal meioses is the rule, for example, in diploid barley as well as in polyploid wheat and oats where chromosome members are typically associated as bivalents. Offtypes, such as speltoids in wheat and fatuoids in oats, have been ascribed to occasional aberrant chromosome behavior (23). In the application of present-day breeding methods, evolved largely around studies with annuals, notably corn and small grains, testing programs exploit this feature of normal meiosis.

In a predominantly vegetatively reproduced long-lived perennial, natural selection must act primarily upon the asexual

stages of the life cycle. The clones, or sectors thereof, which have been unable to compete under varying extremes of environment will be eliminated. Since an individual of this type need only replace itself occasionally through sexual reproduction in nature, the selection pressure affecting the two reproductive cycles is not comparable. Later it will be postulated that there is a selective value attached to a certain minimum level of sexual reproduction to hold the species together and to allow a minimum level of recombination with other clones of the species.

Considerable artificial selection has been practiced in B. inermis since it was first cultivated as a forage crop. One must conclude, however, that selection, artificial or otherwise, has done little to reduce cytological irregularity. The chromosome complement, in reality, appears much more stable than might be assumed. The conclusion that some selective value is attached to the maintenance of this irregularity seems inescapable. Excepting, for the moment, the benefits accruing from heterosis, maintenance of irregularity is evidence that suppression of random recombination was selected for rather than against.

In a long-lived sod population of largely asexually reproduced cross-pollinated plants a relatively few vigorous heterozygotes are likely to predominate. Only a few zygotes at a time can enter and compete for establishment in the population. In an annual, however, relatively large numbers begin growth anew each year in a particular season affording

optimum conditions for the establishment of large numbers.

As far as the perennial, cross-pollinated species is concerned, a stabilizing selective force which would enable a plant to produce a few parental type gametes in contrast to all possible recombination products would constitute an immediate selective advantage since the effective breeding population is a small highly selected one. To achieve this end, long-lived grasses can sacrifice the regularity of meiotic mechanism so necessary in annuals for quantity production of gametes. Of the cytological mechanisms known to date, the heterozygous inversion system and segmental translocation achieve the desired effect--that of suppressing recombination. Crossing-over in heterozygous inverted segments, in most cases, leads to the establishment of dicentric chromatids which result in bridges at anaphase in addition to acentric fragments. The resulting deletions may lead to inviability or to unbalance and only those gametes receiving the inverted segment intact may prove balanced. Segmental translocations, particularly in these members, may lead to recombination suppression in a number of ways. Multivalent associations achieve a reduction in the number of linkage groups which may result in fewer kinds of recombination products. Secondly, chiasma formation may be suppressed in the vicinity of the insertion region. Thirdly, the variable multivalent associations from cell to cell may have led to heterogenetic pairing as evidenced by observed heteromorphic bivalents. In

these, relatively little recombination would be expected. Under such a hypothesis zygotene pairing would take place on a segmental rather than whole chromosome basis. Preferential pairing would take place between homologous segments lying in close proximity as meiosis got under way. This would result in non-homologous segmental associations in which crossing-over would be suppressed. Synapsis of homologous segments of a certain magnitude may be sufficient to satisfy the pairing impulses of the whole chromosome. Unless the association or synapsis of segments occurred in several placed along the chromosome simultaneously it is doubtful if opportunity for recombination in more than a few segments would become possible.

Since the asexual stages of these species are dominant in the life cycle it would seem possible that the genetic system under which they reproduce might be peculiarly adapted to the emphasis of somatic mechanisms. Since some evidence of somatic pairing was observed and since it may possibly take place between only partially homologous chromosomes the following hypothesis is suggested:

Crossing-over in somatic cells as a result of pairing of non-homologous, or partially homologous, chromosomes with only duplicated segments may tend to scatter segments of the original chromosomes over the entire complement. Certain pairs of homologous chromosomes would, on such an assumption, lose their identity and further emphasis would be on individual

chromosomes. Assuming that the loci are distributed more or less at random over the whole complement some degree of higher polyploidy would seem necessary in order to obtain balanced gametes. With polyploidy and dominance all but one of the duplicated original loci would then be released for evolutionary purposes.

We may, for example, assume that somatic pairing and recombination occurred in mitosis of a mother cell. Clonal sectors arising from mitotic divisions of a daughter cell in which a more favorable recombination was obtained than that present in the mother cell or other daughter cell might easily possess a selective advantage. The process might be considered a very conservative stabilizing mechanism in that the original somatic condition would not be relinquished unless the new recombination proved more favorable. Thus, any favorable somatic recombination or mutation would be saved and unfavorable ones would be discarded.

Under such a hypothesis of somatic interchange and mutation, recombination could occur only among the loci present. A certain minimum level of sexual reproduction would have a selective value in that it would hold the species together and permit some sexual recombination between selected parental clones. From this standpoint various forms of apomixis would appear more desirable than exclusive vegetative reproduction since they would maintain the floral structure and still allow

some sexual reproduction leading to the interchange of germplasm throughout the species. Such a hypothesis would explain aneuploidy in certain grass genera where chromosome numbers are in multiples of other than two chromosomes. It might also account for the similarity of certain individuals of widely differing chromosome number in species like Poa pratensis, for example. The lower chromosome plants may merely have fewer of the same loci that are contained in the higher chromosome plants.

The biased sampling of the cytological data presented in studies of this nature needs emphasis. Highly selected samples of units only vaguely definable are not adapted to treatment with refined statistical techniques. Sampling of meiotic stages, in addition to the selective restrictions previously alluded to, is difficult because the stages are arbitrarily selected steps in a dynamic process. Regularly dividing cells tend to be slightly ahead of those with irregularities in these stages. It is difficult, therefore, to compare normal and irregular cells at any given time since they are at different stages. The piling up in certain stages of a large number of pollen mother cells and the rapid consumation of other stages is indicative of the intricate energy relations involved. The desirable diakinesis stage for analyses of these complex members which immediately precedes first metaphase is of such short duration that one only rarely encounters it.

A sizeable proportion of irregularities observed cytologically are of whole chromosome magnitude. There are, however, evidences of many small irregularities leading to inviability which may or may not be detectable. In anaphases and quartets, numerous instances of chromosome fragmentation have been observed. Micronuclei, from those of apparently several chromosomes on down to the limits of microscopic visibility, have been detected.

The significance of irregular meiosis in these complex polyploid species should not be underestimated in breeding programs. Certain features of breeding methods applicable to annuals, particularly as regards the potentiality of random recombination, may greatly reduce their effectiveness in these species. It has been shown, for instance, that selected clones of B. inermis do not set seed with equal facility under artificially controlled conditions of open-pollination. It has also been shown cytologically that the numerous chromosome irregularities may not be formed entirely at random in gamete production.

Recurrent selection (21) recently has received the enthusiastic acclaim of corn breeders. In the utilization of this breeding method it has been assumed that recombinations occur randomly and with equal facility among all members of the selected population. There is, admittedly, little direct evidence to the contrary in corn. The extension of the concept to include any cross-pollinated crop, as advocated by some breeders, may not be entirely justified. Breeding techniques

developed for and used successfully with annual crops may not prove equally suitable for some of the long-lived, rhizomatous, polyploid grasses. The phylogenetic forces leading to normal bivalent chromosome association and random recombination in the functionally diploid annual need not have had comparable effects on the meiotic mechanism of the polyploid perennial possessing extensive asexual propagative features.

The available cytological evidence in B. inermis indicates that barriers to free recombination during gamete formation may be common. The heterozygous inversion system and segmental translocation, both capable of recombination suppression, have been exploited in varying degrees in the species. As a result, blocks of genes with selective survival values may be held intact and transmitted, to a large degree, as units to the resulting viable gametes. In this species, then, the effectiveness of recurrent selection may be in proportion to the failure of the cytological mechanisms present to suppress recombination.

It would seem desirable in the breeding program to exploit vegetative propagation wherever possible. Two by two combinations of highly selected clones might reveal certain combinations which unite well to produce a desirable progeny in spite of meiotic irregularity.

The reasonable degree of F_1 fertility displayed by artificial hybrids between B. inermis and B. pumpellianus

is suggestive of a rather recent migration, as regards geological time, from a geographical center which was a common source of germplasm for the group. It seems logical that this migration took place from the direction of Asia. This is not a unique situation, however, since the early studies of Gray (14) considerable interest has centered about the floristic affinities of Eastern Asia and North America. In the Gramineae and within the genera Festuca, Poa, Agropyron, Calamagrostis, and Phleum there are, no doubt, additional examples of the same relation existing in Bromus.

It is expected further that this extensive migration most likely occurred in a Pleistocene era when cool moist conditions prevailed over a large area of North America. The now widely separated endemic distribution of B. pumpellianus, for example, is confined to reasonably cool and moist habitats. It would appear that some isolating mechanism, geographical or otherwise, was operative in restricting the variability of the population of this complex gaining access to the North American continent. Such a hypothesis does conveniently explain the apparent failure of B. pumpellianus to maintain confluency of distribution over an area where B. inermis is particularly well adapted.

Certainly significant changes have occurred in populations of these members since the introduction of B. inermis. The alterations of natural conditions that may have been imposed by the planting of increasing acreages of B. inermis are difficult

to enumerate. Hybridization of the two species has surely occurred in nature, but since the two species are neither comparable from the standpoint of population frequency nor competitive ability, any analysis of this introgression is complex.

The question of specific validity has unavoidably arisen in the study of B. inermis and B. pumpellianus. It is not the purpose of this discussion to defend or deny various concepts involving this rather unwieldy unit in nature. In the sense of Dobzhansky (7) the environmental barrier between North America and Asia provides a convenient definitive criterion to reduce B. pumpellianus at least to a race (subspecies) of B. inermis.

The Clausen, Keck, and Hiesey (4) concept of internal degree of separation does not allow for varying degrees of fertility in defining the ecotype as the applicable systematic unit based on experimental evidence in this instance. It does become applicable, however, upon consideration of the additional criterion of a vigorous second generation. From the standpoint of nomenclature the distinct sub-species as diagrammed by these authors (4) is the applicable homologue of B. pumpellianus.

SUMMARY AND CONCLUSIONS

1. Meiotic analyses of several Bromopsis section members revealed a euploid series of chromosome numbers from n=7 to n=35. A diploid race of B. anomalous and diploid and tetraploid races of B. ciliatus and B. purgans were observed. A 2n=28 chromosome count was confirmed for B. texensis and 2n=42 for B. auleticus, from Uruguay, South America.
2. No hexaploid forms of either B. pumpellianus or B. inermis were observed although a polyhaploid clone of B. inermis with a somatic complement of 28 chromosomes was discovered. The clones of B. inermis and B. pumpellianus from Washington, Alberta, and Alaska all proved to be 2n=56.
3. Root-tip mitoses of B. erectus from Lund, Sweden, revealed a 2n=56 chromosome number.
4. B. riparius, originally collected in European Russia, was observed to be 2n=70.
5. Certain irregular features of meioses were pointed out in these complex polyploids. The number of bivalents per cell varied widely. Univalents and multivalent associations as high as 8 chromosomes were fre uently observed. At anaphases lagging univalents and bridges were common. Micronuclei were often present in the quartet stages.

6. Meioses in the polyhaploid B. inermis clone revealed a high degree of internal pairing which suggested that this species may be nearly autopolyploid in its constitution.
7. Crossing of the diploids, tetraploids, and the hexaploid, B. auleticus with B. inermis, B. pumpellianus, or B. riparius failed. The hexaploid, tetraploids, and diploids used would be of doubtful value, therefore, as sources of germplasm in the improvement of B. inermis.
8. Crosses among B. inermis, B. pumpellianus, and B. riparius indicated a considerable degree of relationship. In most cases the F_1 hybrids were at least partially fertile as expressed in their ability to set seed under open-pollination. Cytological examination of these hybrids revealed many irregularities. As a result, the presence of barriers to complete random recombination among them would be expected. Root-tip mitoses in the same hybrids revealed varying numbers of chromosomes present from cell to cell.
9. The introgression of B. pumpellianus and B. inermis in the North American range of B. pumpellianus since the introduction of B. inermis was pointed out. It was suggested further that certain recombinations might be obtainable in these areas which possessed certain characters not found in ordinary B. inermis.

10. The irregularity of the meiotic mechanism in B. inermis revealed by cytological analyses indicated the failure of random recombination to occur. Breeding programs dependent upon the validity of random sampling among gamete populations on this basis may not prove successful. It was shown that selected plants of B. inermis may not set seed with equal facility under rather uniform artificial conditions of open-pollination.

11. Breeding programs emphasizing exploitation of all possible means of vegetative reproduction are suggested. Two by two combinations of highly selected clones might reveal certain combinations which would unite well sexually to form a desirable progeny in spite of cytological irregularity.

12. The persistence of these irregularities in B. inermis even under artificial sexual selection over a considerable period has led to the postulate that some selective value must be attached to their maintenance. Since the chromosome complement appears stable in spite of these difficulties and the asexual stage is so dominant in nature the possibility of a genetic system peculiarly adapted to this vegetative propagation may be involved. A system in which somatic pairing and recombination among only partially homologous chromosomes at various levels of polyploidy is suggested.

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APPENDIX

Appendix Table 1

Summary of artificial crosses within B. inermis

Parents	Fertility level	Flowers crossed	Seed set	% Seed set	Plants reaching flowering stage
255-44 x 554-22	H x M	65	39	60.0	17
298-30 x 291-15	HM x L	35	7	20.0	4
291-15 x 298-30		29	1	3.5	1
298-30 x 255-44	H x H	43	13	30.3	
255-44 x 298-30		29	14	48.4	
298-30 x 278-18-15	HM x HM	70	40	57.2	
278-18-15 x 298-30		69	15	21.8	
291-15 x 268-44	L x L	94	15	16.0	
268-44 x 291-15		34	14	41.2	
269-44 x 562-49		45	35	77.8	
276-1 x 562-49		37	27	73.0	
279-20 x 562-49		45	19	42.2	
279-41 x 562-49		40	28	70.0	
543-32 x 562-49		36	12	33.3	
548-26 x 314-8		27	11	47.4	
314-8 x 562-49		26	0	0	
542-37 x 562-49		38	25	66.0	
Lincoln #235 x 307-35-3		32	17	53.1	
Fisher x Lincoln *		216	118	54.7	
	Totals	1010	450	44.55	
				s = 23.0%	

*Unnumbered seedlings

Appendix Table 2

Summary of artificial crosses between B. inermis and B. pumpellianus

Parents	Flowers crossed	Seed set	% Seed set	Plants reaching flowering stage
B. pumpellianus from Pullman, Wash. (p-3054)				
255-44 x P-8	42	1	2.4	1
P-8 x 255-44	73	32	43.8	19
P-8 x 298-45	64	27	42.2	13
298-45 x P-8	19	0		
554-22 x P-8	49	0		
P-9 x 255-44	30	5	16.7	4
P-9 x 298-30	29	4	13.8	1
P-6 x 554-22	15	1	6.7	0
P-2 x 291-15	31	14	45.2	4
P-2 x Lincoln 1	29	17	58.7	12
P-2 x 307-35	25	14	56.1	
307-35 x P-2	50	8	16.0	
P-3 x 554-22	26	1	3.9	1
P-13 x B. inermis 77	33	1	3.0	0
291-15 x P-19	39	3	7.7	
454-10 x B. pump. *	56	21	37.5	20
Totals	610	149	24.43	
			s = 24.6%	
B. pumpellianus from Alberta, Canada				
298-30 x TP 5073-1	22	15	68.2	
255-44 x TP 5077-7	64	6	9.4	
307-3533 x KP 1-1	19	14	74.0	
298-45-9 x KP 1-1	60	9	15.0	
554-22 x TP 5077	38	9	23.7	
454-10 x TP 5073-1	22	11	50.0	
KP 1-1 x 298-45-9	22	2	9.1	
KP 1-1 x 454-10	28	20	71.5	
TP 5073-1 x 454-10	28	10	35.8	
TP 5073-1 x 255-44	60	22	36.6	
TP 5077-7 x 255-44	20	0		
KP 1-2 x Fisher 101	32	11	34.4	
Totals	415	129	31.08	
			s = 26.9%	

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Appendix Table 2 (con'd)

Parents	Flowers crossed	Seed set	% Seed set	Plants reaching flowering stage
B. pumpellianus from Bodenburg Butte, Palmer, Alaska				
314-8 x 25-1	25	5	20.0	
548-26 x 25-1	32	2	6.3	
562-49 x 25-15	45	29	64.4	
562-49 x 28-4	36	17	47.2	
1-6 x 25-1	66	24	36.3	
25-10 x 562-49	25	2	8.0	
Totals	229	79	34.5	
			s = 23.1%	
Combined totals	1254	357	28.47	
			s = 25.1%	

*Bulk pollen

Appendix Table 3

Summary of artificial crosses between *B. inermis* and *B. riparius*

Parents	Flowers crossed	Seed set	% Seed set	Plants reaching flowering stage
554-22 x E-2	14	0		
E-4 x 454-10	14	4	28.6	4
454-10 x E-4	25	8	32.0	1
E-1 x 454-10	102	43	42.1	12
E-3 x 454-10	53	14	26.5	12
278-18-15 x E-9	71	45	63.5	4
E-1333-3 x Fisher 100	62	9	14.5	
E-4 x *B. inermis* *	39	8	20.6	7
Totals	380	131	34.47	
			s = 18.9%	

Average % seed set with *B. inermis* as female parent 48.18%

Average % seed set with *B. riparius* as female parent 28.89%

*Bulk pollen

Appendix Table 4

Summary of artificial crosses between *B. riparius* and *B. pumpellianus*

Parents	Flowers crossed	Seed set	% Seed set	Plants reaching flowering stage
E-1 x *B. pumpellianus* *	24	11	46.0	
E-3 x "	64	29	45.3	
E-4 x P-2	19	4	21.1	
E-4 x P-3	20	0		
E-4 x *B. pumpellianus* *	124	21	16.9	
E-9 x "	43	16	37.3	
P-1 x E-4	24	8	33.3	
P-1 x *B. riparius* *	19	10	52.7	
P-2 x "	16	7	43.7	
P-3 x "	90	17	18.9	
P-10 x E-4	142	12	84.5	
P-10 x *B. riparius* *	40	3	7.5	
P-25 x "	58	30	51.7	
P-8 x "	103	25	24.2	
KP 1-3 x E-1	40	16	40.0	
TP 5073-8 x E-9	28	20	71.5	
E-9 x TP 5073-8	52	23	44.1	
E-1333-1 x KP 1-2	90	13	14.5	
Totals	996	265	26.6	

s = 21.8%

Average % seed set with B. pumpellianus as female 26.43%

Average % seed set with B. riparius as female parent 26.83%

*Bulk pollen sources

Appendix Table 5

Summary of artificial crosses within *B. pumpellianus*

Parents	Flowers crossed	Seed set	% Seed set	Plants reaching flowering stage
P-7 x P-9	63	20	31.8	
P-8 x P-19	62	35	56.5	
KP 1-3 x TP 5073	26	19	73.2	
P-10 x TP 5077-7	40	0		
26-16 x 25-15	45	40	88.9	
25-15 x 26-16	18	11	61.2	
25-1 x 26-16	50	17	34.0	
Totals	304	142	46.71	
			s = 29.7%	

Appendix Table 6

Summary of artificial crosses between *B. inermis* and *B. texensis*

Parents	Flowers crossed	Seed set	% Seed set	Plants reaching flowering stage
554-22 x *B. texensis*	29	0		
255-44 x "	40	0		
298-45 x "	61	2		1 (self)
B. texensis x 298-45	34	10		0
" x *B. inermis*	36	0		
B. inermis x *B. texensis*	52	0		
Totals	252	10	2.5	

Average % seed set with *B. inermis* as female parent .6%

Average % seed set with *B. texensis* as female parent 14.3%

Appendix Table 7

Summary of artificial crosses between *B. pumpellianus* and *B. texensis*

Parents	Flowers crossed	Seed set	Plants reaching flowering stage
B. texensis 34 x 28-3	50	0	
P-1 x B. texensis	25	0	
P-2 x "	12	0	
P-3 x "	26	0	
P-6 x "	22	0	
P-7 x "	45	0	
B. texensis x P-2	91	12	0
" x P-3	18	2	0
Totals	289	14	4.8% seed set

Average % seed set with B. pumpellianus as female 0

Average % seed set with B. texensis as female parent 8.8%

Appendix Table 8

Summary of miscellaneous artificial crosses

Parents	Flowers crossed	Seed set	Plants obtained	Plants reaching flowering stage
B. inermis x B. orcuttianus	95	1	1 self	
B. riparius x "	298	8	8 selfs	
" x B. purgans P354	126	7	7 selfs	
B. inermis x "	18	0		
B. riparius x B. laevipes	347	9	9 selfs	
" x B. vulgaris	43	1	1	0
" x B. grandis	22	1	1	0
" x B. auleticus	80	0		
" x " 271-11	58	0		
B. auleticus x B. riparius	87	0		
" x " 269-1	88	0		
" x B. inermis	48	0		
B. inermis x B. auleticus	124	13	13 selfs	
B. pumpellianus x orcuttianus	93	0		
" x B. purgans 354	123	0		
" x B. laevipes	82	0		
" x B. vulgaris	36	0		
" K1-3 x B. purgans 33	42	0		
B. texensis x B. ciliatus	24	3	0	
B. ciliatus x B. texensis	14	0		
B. laevipes x B. orcuttianus	25	13	12	0
B. orcuttianus x B. laevipes	23	11	4	0
B. purgans 354 x B. orcuttianus	31	12	8	0
B. inermis x B. anomalous 8025	44	0		
Total	1990			

Appendix Table 9

Summary of artificial backcrosses made in the study

Parents	Flowers crossed	Seed set	% Seed set
(*B. inermis* x *B. riparius*) x *B. inermis*			
(278-18-15 x E-9) #1,3,8 x (255-44 x 554-22) #1,11,14	126	45	35.7
(278-18-15 x E-9) #1 x (307-35 x P-2) #2	47	13	27.7
(454-10 x E-4) x (255-44 x 554-22) #11	41	0	
(454-10 x E-4) x (P-8 x 255-44) #18	17	5	34.0
(*B. riparius* x *B. inermis*) x *B. inermis*			
(E-3 x 454-10) #3 x (P-2 x 291-15) #3	56	18	32.2
(E-3 x 454-10) #7 x (255-44 x 554-22) #14	27	0	
(E-3 x 454-10) #9 x (P-9 x 255-44) #5	17	1	5.9
(E-3 x 454-10) #11 x (255-44 x 554-22) #11	13	5	38.5
(E-3 x 454-10) #11 x (255-44 x 554-22)#15	19	0	
Totals of first backcross	363	87	24.0
B. inermis x (first backcrosses)			
268-44 x $\frac{\text{I-E}}{\text{I}}$ 3-17	44	21	47.6
Fisher #100 x $\frac{\text{E-I}}{\text{I}}$ 6-10	30	2	6.7
$\frac{\text{I-E}}{\text{I}}$ 3-17 x Lincoln 234	38	15	39.5
$\frac{\text{I-E}}{\text{I}}$ 3-17 x Fisher 125	64	51	80.0
Totals of second backcross	176	89	50.6
B. inermis x (*B. pumpellianus* x *B. riparius*)			
307-35-3 x (P-10 x E-4)	54	35	65.0
(P-10 x E-4) x 307-35-3	42	25	59.4
Lincoln 1 x (P-10 x E-4)	64	6	9.4
Total	160	66	41.2
B. riparius x (*B. pumpellianus* x *B. riparius*)			
E-4 x (P-10 x E-4)	22	1	4.5
B. pumpellianus x (*B. inermis* x *B. pumpellianus*)			
(P-8 x 255-44) #18 x P-1	34	6	17.6
P-25 x (307-35 x P-2) #1	32	4	12.5
Total	66	10	15.2

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Appendix Table 9 (con'd)

Parents	Flowers crossed	Seed set	% Seed set
(*B. riparius* x *B. inermis*) x *B. riparius*			
(E-3 x 454-10) #6 x E-1333-1	24	11	46.0
(E-3 x 454-10) #7 x E-9	54	9	16.7
E-7 x (E-3 x 454-10) #9	50	6	12.0
E-2 x (E-3 x 454-10) #10	24	8	33.0
Total	152	34	22.4
(*B. pumpellianus* x *B. inermis*) x (*B. pumpellianus* x *B. inermis*)			
(P-8 x 255-44) #6 x (307-35 x P-2) #1	30	14	46.7
B. inermis x (Knowles *pumpellianus* x Turner *pumpellianus*)			
562-49 x 28-4	36	17	47.2
(307-35 x P-2) #1 x (255-44 x 554-22) #11	99	1	1.0
(E-3 x 454-10) #10 x Fisher 100	42	3	7.1
Grand total	1146		

Appendix Table 10

Chromosome association summary in B. inermis sporocytes

Clone	No. of cells	I	II open	II closed	III	IV	V	VI	VII	VIII
291-15	10	0.1	3.4	14.6	0.1	2.3	0	0.5	0	0.8
554-22	10	0.9	7.1	11.7	0.4	2.1	0.3	0.8	0	0.2
MP 16	9	1.2	2.9	17.9	0.9	0.9	0.7	0.3	0.1	0.1
298-45-9	8	2.5	5.3	10.3	0.5	3.3	0	1.0	0	0.3
P-8 x 255-44 Plant 8	8	2.5	13.5	17.5	0.6	1.8				
255-44 x 554-22 Plant 11	7	1.7	7.1	15.6	0.9	1.1				
460-1	6	0.7	5.2	8.7	0.3	4.5	0.3	0.3	0	0.8
BM 7 Plant 7	1	1.0	4.0	17.0	1.0	1.0	0	1.0		
278-18-15	5	1.8	7.2	9.0	1.2	2.4	0	1.2	0	0.2
298-30	9	0.8	5.6	10.7	0.7	2.8	0.1	1.2	0	0.2
Fisher 125	3	1.3	9.0	15.3	0.7	1.0				
Fisher 124	2	2.0	11.5	9.0		1.0	0	1.3		
Lincoln 1	3	1.7	11.0	10.3	1.0	1.3	0	0.3		
307-35	1	0	7.0	17.0	0	2.0				
255-44	2	3.5	5.0	10.5	0.5	3.5	0	0.5	0	0.5
307-35-3	7	1.9	9.6	7.9	0.9	3.4	0.1	0.4		
268-44	4	2.8	6.8	13.8	0.8	1.3	0	0.5		
298-45	3	0.7	9.0	8.0	1.7	1.3	0.3	1.3		
298-30-10-4	5	0.8	2.0	12.6	0.2	0.4	0	0.6	0	0.6
298-30 x 291-15 Plant 2	3	1.7	4.7	16.7	0	1.7	1.0			
255-44 x 554-22 Plant 13	2	2.0	4.0	17.5	0.5	1.5	0	0.5		
255-44 x 554-22 Plant 7	1	0	0	18.0	0	3.0	0	0	0	1.0
255-44 x 554-22 Plant 14	5	3.6	3.6	15.8	0.4	1.6	0	0.2	0	0.6
P-8 x 255-44 Plant 5	1	5	3	16	1	1	0	1		
P-8 x 255-44 Plant 6	10	1.6	3.2	11.9	0.8	3.5	0	0.9	0	0.3
P-8 x 255-44 Plant 10	3	1.7	3.3	11.7	0.3	3.7	0	1.0	0	0.3
P-8 x 255-44 Plant 12	6	8.8	6.3	12.7	1.3	0.2	0.7	0.2		

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Appendix Table 10 (con'd)

Clone	No. of cells	I	II open	II closed	III	IV	V	VI	VII	VIII
P-8 x 255-44 Plant 13	6	1.2	3.3	14.8	0.2	3.5	0	0.7	0	0.2
P-8 x 255-44 Plant 15	4	0.8	1.8	13.8	0	3.8	0.3	1.0	0	0.3
P-8 x 255-44 Plant 16	3	1.7	3.0	10.7	3.3	2.3	0	0	0.7	0.3
P-8 x 255-44 Plant 18	3	3.3	6.7	15.0	0	1.7	0	0	0.7	0.3
255-44 x P-8	1	3.0	4.0	10.0	1.0	4.0	0	1.0	0	0
Fisher 123	2	1.0	0	14.5	0	3.5	0	2.0		
Lincoln 11	5	1.0	3.8	13.8	0	3.0	0	0.8	0.2	0.2
278-18-15 x 298-30(4-7)	2	1.0	9.0	15.0	0	1.0	0	0.5		
268-44 x 291-15 (6-8)	2	4.0	2.5	18.5	0	2.5				
314-8	1	1.0	10.0	9.0	1.0	2.0		1.0		
Total 37	163	65.8	194.4	492.8	22.2	80.9	3.8	22.0	1.0	7.2
$\bar{x}$		1.8	5.3	13.32	0.6	2.2	0.1	0.6	0.03	0.2
Range per cell		0-20	0.14	1-23	0-4	0-7	0-3	0-3	0-2	0-3

Appendix Table 11

Chromosome summary in B. pumpellianus sporocytes

Clone	No. of cells	Mean association per cell									
		I	II open	II closed	III	IV	V	VI	VII	VIII	Other
KP 1-1	5	.8	9.4	17.0	0	.6					
KP 1-2	2	0	3.0	12.0	0	3.5	0	2.0			
TP 5077-23	12	1.2	3.3	14.8	0.5	2.5	0.3	0.4			
TP 5077-1	4	1.5	4.3	8.8	1.5	3.8	0.3	0.5	0	.3	
TP 5073 (26-1)	3	1.0	2.0	20.7	0.3	1.7	0.0	0.3			
KP 1-3 x TP 5073	1	0	5.0	11.0	0	2.0	0	0	0	2.0	
TP 5073 (26-16)	3	0.3	1.3	20.3	0.3	2.3	0	0.3			
Palmer #1440 (25-1)	1	0	5	12	0	4	0	1			
Palmer #1440 (25-10)	3	0.3	0	16.0	0.3	2.7	0	0.7	0	0.3	0.3X
Palmer #1440 (25-18)	5	0.6	4.8	15.4	1.4	2.4	0	0.2			
P-3	1	3.0	5.0	13.0	1.0	2.0	0	1.0			
Total	40	8.7	43.1	161.0	5.3	27.5	0.6	6.4	0	2.6	
$\bar{x}$		0.8	3.92	14.64	.5	2.5	0.05	0.6	0	0.23	
Species range per cell		0.4	0-13	6-21	0-2	0-6	0-2	0-2	0	0-2	