SOME BIOSYSTEMATIC CONSIDERATIONS

OF THE GENUS BOTHRIOCHLOA

By

GILBERT B. FLETCHER

Bachelor of Science

Centenary College

Shreveport, Louisiana

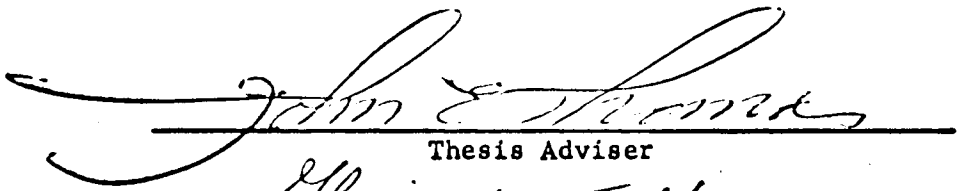
1956

Submitted to the Faculty of the Graduate College
of the Oklahoma State University
in partial fulfillment of the requirements
for the Degree of
MASTER OF SCIENCE
July, 1968

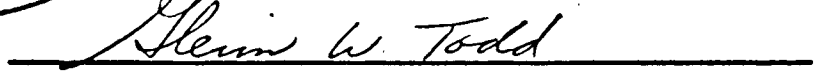
SOME BIOSYSTEMATIC CONSIDERATIONS

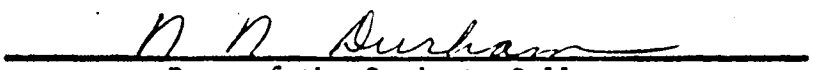
OF THE GENUS BOTHRIOCHLOA

Thesis Approved:



Thesis Adviser





Dean of the Graduate College

ACKNOWLEDGEMENTS

I wish to express my gratitude to my former principal advisor, Dr. J. M. J. de Wet, formerly Professor of Botany at Oklahoma State University, now Professor of Cytogenetics at the University of Illinois. Dr. de Wet suggested the problem and provided the materials and guidance essential to the successful conclusion of my research.

I also wish to express my appreciation to Dr. John E. Thomas, Head of the Botany and Plant Pathology Department, and my current principal advisor. Dr. Thomas has provided encouragement and assistance in this final year of my studies.

Dr. Sunil Saran, of the Botany Department, has provided many hours of assistance and guidance in surmounting many of the obstacles encountered during my studies, particularly in the cytological investigations. I am very grateful for his time and effort.

I would also like to express my gratitude to Dr. S. P. Sengupta for his assistance in the photographic portions of my studies. I would like to thank Mrs. Thomas W. Lee for her prompt and expert typing of the thesis.

Finally, I would like to thank my wife and children for their patience and encouragement during the many hours that were required to bring this study to its successful conclusion.

TABLE OF CONTENTS

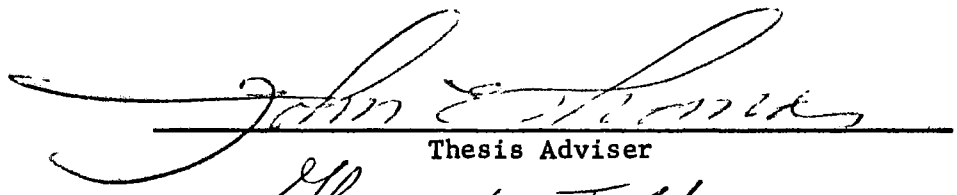
LIST OF TABLES

LIST OF PLATES

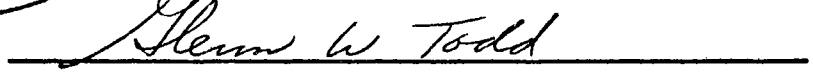
SOME BIOSYSTEMATIC CONSIDERATIONS

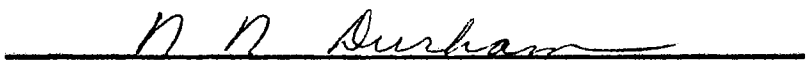
OF THE GENUS BOTHRIOCHLOA

Thesis Approved:



Thesis Adviser





Dean of the Graduate College

CHAPTER I

INTRODUCTION

For the past ten years, an extensive effort has been made at Oklahoma State University to better understand the evolutionary mechanisms operating in the tribe Andropogonae of the family Gramineae. Those genera that have been studied in some detail, so far, included the following: Capillipedium, Dichanthium, and Bothriochloa. This research project was initiated by the late Dr. R. P. Celarier, and continued by Dr. J. R. Harlan and Dr. J. M. J. de Wet. The basic purpose of these investigations was to obtain a thorough biosystematic knowledge of these genera. It was further desired to obtain an extensive morphological understanding of these three genera and an appreciation of their world-wide distribution. The data from these studies was complemented by various other biosystematic studies which involved gas chromatography, leaf anatomy, cytology and cytogenetics. Approximately three thousand collections of Old World representatives and several hundred collections of the New World representatives were grown in uniform nurseries and studied in biosystematic detail (42).

The vast economic importance of the Gramineae as well as some of their unique characteristics made them a logical choice for the biosystematists to use as a subject for their investigations. Recently Stebbins (63) stated:

> Thus plant evolutionists are gradually progressing toward their avowed goal; the understanding of the complex interrelationships between the evolutionary origin of the myriad diverse types of plants existing on the earth, and the establishment of the general principles which have governed this evolution.
>
> No group of plants has been more radically affected by this new approach than the grass family.

Grasses have always been difficult for the traditional taxonomists. The leaves of the grasses are remarkably similar on superficial examination, and the flowers have been reduced and simplified to such an extent that it is difficult to use them as a means of classification (63). To overcome these problems, the earlier grass taxonomists relied heavily on the gross morphology of the inflorescence. However, because of many cases of parallel evolution within the family this was not entirely satisfactory.

According to Stebbins (63):

> The near revolution which is taking place in grass taxonomy began when a few anatomists like Grob, Duval-Jouve, and Pee-Laby began to examine and compare the leaves of the grasses under the microscope.

These early efforts were substantially augmented when Avdulov (2), the Russian cytologist, discovered that the grasses could be grouped according to the number and size of their chromosomes. Significantly the arrangement of the grasses according to their chromosome size and number followed closely along the lines established by the anatomists. Additional work since the 1930's has tended to confirm these findings and the evolutionary picture is becoming less confused. The broad problems involved in the accurate delimination of tribes and subtribes are not the only ones that faced the grass taxonomists. As Stebbins (63) points out:

> Species delimination of the grasses in intrinsically difficult because interspecific boundaries have been blurred by hybridization and chromosome doubling or polyploidy.

It is to that specific question that the bulk of this study is devoted using the New World Bothriochloas as the specific subject.

Stebbins (63) has clearly set forth the basic concept of grass evolution in this paragraph:

> Hence, the following picture emerges of the broad outlines of the course of evolution in the grass family. The earliest grasses were herbs with stems having few to many nodes, relatively simple racemose or paniculate inflorescences, and spikelets with numerous florets, the bracts or glumes being undifferentiated like those of primitive bamboos. The flowers themselves were trimerous, with a perianth of three and perhaps six members, six stamens in two series, and an ovary with a single ovule and three styles and stigmas. They were probably already wind pollinated, but descended from ancestors with a well developed perianth and insect pollination. They probably existed in the middle of the Cretaceous period, judging from the scanty fossil evidence that is available, and inhabited regions with a tropical or subtropical climate.

Agnes Arber (1) has postulated that these primitive grasses had a leaf that was Commelinaceous in nature.

Stebbins (63) and deWet (23) have noted that the Arundinoid inflorescence may be similar to the most primitive type in the Gramineae.

Leaf Epidermis

The use of the anatomical features of the grass leaf as a means of making taxonomic distinctions was first initiated by Prat (56). Since that time several workers have developed particular specialties within this area. Tateoka (67) produced an elaborate system for characterizing the bicellular microhairs. He created a formula that would provide a numerical weight for the relative length of the basal and apical cell, the width of the microhair relative to its length, the thickness

of the basal cell wall compared to the apical cell wall, and finally the angle between the basal rim and the rim at the maximal width. Using his formula he was able to separate those grasses of the Panicoid type, which have a low total value from the formula, from the Eragrostoid grasses whose microhairs had a high total value.

Other anatomical features of the leaf epidermis which have been found to have taxonomic value include the following characters, as set forth by Metcalf (51) in his book on the anatomy of the Gramineae published in 1960. He recognized the short cells and silica bodies, the macrohairs, the microhairs, the prickle hairs, papillae, stomata, and long cells as having taxonomic significance. Macrohairs are large, usually over 100 microns, and are normally single celled. Microhairs normally are less than 100 microns in length and are composed of two cells designated as basal and apical. The prickle hairs are single celled resembling a rose prickle in outline. Papillae are evaginations of the cell walls of the long cells of the epidermis. The use of the stomata as a taxonomic character rests principally on the shape of the subsidiary cells. In the Panicoid group the subsidiary cells typically have a triangular shape. The use of the long cells in taxonomic work depends on their general length and shape as well as the thickness of the cell wall.

In 1954 de Wet (22) established in the genus Danthonia that the length of the guard cells provided a valid index of the polyploid condition. In 1958 R. P. Celarier (17) established that the same relationship existed in Bothriochloas of the Old World as de Wet had found in Danthonia. Additionally Celarier (17) and Gould (36) had also indicated that polyploidy in this group could be determined through

pollen grain size.

In 1961 Shamin A. Faruqi (33) studied the leaf epidermis in Bothriochloa, Capillipedium, and Dichanthium. He found that all three genera had more or less typical panicoid features and were in general similar. He indicated that there were some differences which might serve to separate certain elements taxonomically. Faruqi (33) recognized an "A" and a "B" type of prickle, the former found in the silica cell rows and the latter found in the long cell rows. He also recognized the pitting (papillae) but did not discuss it further. None of the Bothriochloas of the western hemisphere were examined in this study.

The Glume Pit of Bothriochloa

Many species of Bothriochloa possess a pit, of a glandular appearance, located normally in about the center of the epidermis of the glume of the sessile, fertile spikelet. Several investigators have drawn attention to this pit in the last ten years.

Gould (39) in 1959 noted the occurrence of this pit in several species of the New World Bothriochloas, and while not speculating on the function of the pit, he noted that it forms an interesting taxonomic feature and links the New World species of this genus with the Old World species. In other species of the genus which he examined, none of the glumes had pits. In a third group of species there were some, usually the glumes of the lower racemes, that possessed the pits, while the glumes of the upper racemes were pitless. Gould (39) also noted that in the B. barbinoidis species, which has a range throughout Mexico and the southwestern United States, all areas except southern California included both the pitted and the non-pitted types. He also noted:

All five New World taxa with irregularly pitted glumes are highly polyploid (2n=120 or 2n=180) and presumably derived through hybridization of pitted and non-pitted types.

Plants of Andropogon (Bothriochloa) barbinoidis with regularly or irregularly pitted glumes occur almost throughout the range of this species. Pitted types are most frequent in central Texas, where A. edwardsianus with deep, regular pits and a relatively low chromosome number occupies a restricted range.

In 1961, Heslop-Harrison (47), working with Bothriochloa decipiens (Hack.) C. E. Hubbard, which is a pitted species of Bothriochloa endemic to Australia, attempted to investigate a possible function of the pit in encouraging cleistogamy. He noted that in this species the functioning of the glume pit probably occurred through a complex mechanism such that, under long day length, the inflorescence axis elongates beyond the subtending leaf sheath prior to flowering. In this case the glumes open and normal chasmogamy can occur. In the short day length situation, the inflorescence axis does not elongate beyond the subtending leaf sheath and in this case the glume remains appressed and the pit, which internally forms a boss, prevents the exertion of the stigma and the anther, and thereby insures cleistogamy.

Faruqi (34) in 1963 investigated the structure of pits in B. intermedia and certain hybrids. He concluded that because of the dense cytoplasm, thin walls, and prominent nuclei, the pits might function as hydathodes or fluid vesicles. He also noted that in most crosses of pitted species with non-pitted species no pits were present in the progeny. For this reason, he assumed that pitting is controlled by a number of recessive genes.

It may then be concluded that the glume pit is a phenomenon that has had some selective value under certain conditions for the Bothriochloas and that it forms a unique taxonomic tool in many more

cases. It might well be a relict from an ancestoral type that was insect pollinated. The glume pit, in some cases, is good evidence of introgression.

The New World Bothriochloas

In 1957 Gould (38) examined the nomenclature of the species that are now considered as New World Bothriochloas in the following manner:

> The Old and New World andropogons of the subgenus Amphilophis comprise a relatively distinct group, recognized as a separate genus by many systematists including O. Stapf, A. Camus, J. T. Henrard, and S. T. Blake. Both Amphilophis Nash and Bothriochloa Kuntze have been proposed as generic names for the species comprising this subgenus, with Bothriochloa (1891) antedating Amphilosphis (1901). . .
>
> The Amphilophis andropogons are distinguished primarily on the basis of inflorescence characters. The pedicels, and at least the terminal rachis joints, have thickened margins and a medial groove or a broad thin membranous central area. The inflorescence characteristically is a leafless terminal panicle, with several to numerous racemose branches. In a few species there are as few as two or three branches per inflorescence.

At that time Gould recognized the following species as native within the group: (1) A. exaristatus (Nash) Hitchc., (2) A. saccharoides Swartz, var. torreyanus (Steud) Hack. and A. saccharoides var. longipaniculata Gould, (3) A. edwardsianus Gould, (4) A. hybridus, Gould, (5) A. altus Hitchc., (6) A. springfieldii Gould, (7) A. wrightii Hack., (8) A. barbinoidis Lag. var. barbinoidis and A. barbinoidis Lag. var. perforatus (Trin) Gould.

The main features that Gould has used to separate the various species include the presence or absence of the glume pit, the relative size and width of the sessile and pedicellate spikelets, the length of the panicle axis in relation to the length of the longest raceme, the pubescence of the culm nodes, and many other less significant features.

Gould's study was principally concerned with the members of the Andropogonae that were found in the southwestern United States and northern Mexico and with herbarium specimens chiefly from this same region.

The Compilospecies Concept

J. R. Harlan and J. M. J. de Wet (44) in 1963 published a paper which served to clarify the confusing picture which had arisen concerning the species Bothriochloa intermedia (R. Br.) A. Camus, in the Old World. The two authors along with R. P. Celarier and their students had been investigating the biosystematic relationships of Bothriochloa, Dichanthium and Capillipedium, based on their world-wide populations. From these studies an introgressive pattern which had not previously been described became apparent. Bothriochloa intermedia was found to inhabit almost the entire range of their investigations. In the Gangetic Plain of India B. intermedia introgressed with D. annulatum. In the foothills of West Pakistan B. intermedia introgressed with B. ischaemum. In northern Australia B. intermedia introgressed with C. parviflorum. Again in Australia B. intermedia introgressed with B. edwartiana. There was also evidence that B. intermedia and B. pertusa in India and B. intermedia and B. insculpta in East Africa also were forming introgressive progeny. In order to confirm these suspicions extensive crosses were made and in many cases the results of these hybrid crosses were morphologically similar to the introgressive hybrid population represented in the world-wide collections. This ability to readily form interspecific hybrids and to absorb the germ plasm of distinctive populations was termed the compilospecies concept. Other

examples of similar germ plasm robbing species were cited as Poa pratensis, Dactylis glomerata, and Bouteloua curtipendula. Since this record of extensive introgression had occurred in the Bothriochloa, Dichanthium: Capillipedium complex, it would of course be important to observe if similar indications of introgression were obvious in the New World representatives of this complex.

Apomixis

Apomixis is a general term including vegetative reproduction and agamospermy. Vegetative reproduction includes the use of bulbils, tubers, rhizomes, stolons, etc. Agamospermy designates those types of reproduction which result in the formation of seeds and embryos by means of non-sexual processes (5).

There are two types of agamospermy (62). The first is adventitious embryony, as in the citrus fruits, where the embryo develops directly from diploid tissue, i.e., the nucellus or the ovule integument. The second form is found when the diploid gametophyte is formed without fertilization. This occurs in either apospory, in which embryo sacs are formed directly from a cell of the nucellus or inner integument, or it occurs in diplospory, in which the embryo sacs arise from the archesporium but meiosis is modified or omitted so that chromosome number reduction does not occur. These processes may occur autonomously or, as is common in the Dichanthium, Bothriochloa, Capillipedium complex, it occurs only in response to pollination without fertilization (5). This process is called pseudogamous apospory.

Apomixis is quite common in the plant kingdom and relatively very common in the Gramineae. Borgaonkar (5) reported thirty-nine genera

in the grass family in which apomixis of one type or another had been reported in the literature.

Stebbins (62) had recognized the existence of agamic complexes. These consist of several basic diploid species with a superstructure of interrelated polyploid apomictic species.

Early in the studies of the Dichanthium, Bothriochloa, Capilliped-ium complex it had become obvious that in many of the crossing experiments an unusual prevalence of maternal characters was encountered. Apomixis is a rather extensive phenomenon, as noted in Stebbins (62) and Carnahan and Hill (11).

Celarier and Harlan (15) decided to determine the extent of occurrence of apomixis within the genera they were investigating. To demonstrate that apomixis did occur in this generic complex, Celarier and Harlan felt three criteria had to be met which were as follows:

(a) Progenies of distinguishable parents should be of the maternal type.

(b) This maternal inheritance must not be due to self-fertilization.

(c) Nor can it be due to complete genetic dominance of one set of characters over the other.

A system of emasculation and pollinations was developed by W. L. Richardson (58) at this University in conjunction with the apomixis experiments. To demonstrate the absence of self-fertilization, four thousand four hundred and fourteen florets of one diploid, D. annulatum, were emasculated and only four seeds were produced. From these four seeds, one plant survived to maturity and it was not of the maternal type. Reciprocal crosses were made to insure that the maternal nature of the progeny could not be caused by genetic dominance. In their conclusions Celarier and Harlan (15) found the following:

> Both Bothriochloa and Dichanthium are strongly apomictic, but there is a real difference between the two. Dichanthium is considerably more sexual than Bothriochloa at the tetraploid level and faculative apomicts are common.

And later:

> Thus we see the Amphilophiastrae as a polyploid complex with both sexuality and apomixis and the later both facultative and obligatory. However, even with the obligatory apomixis there is still a possibility of genetic exchange since these materials are pseudogamous and pollen in all cases studied seemed to be functional.

Apomixis had previously been thought of as a process which stops evolutionary progress. Clausen (19) indicated that this concept was not always valid because apomictic groups that retained a degree of sexuality would really possess an evolutionary advantage, being able to expand in a restricted environment through apomixis and at the same time accumulating recessive mutations which would be combined through sexual reproduction to facilitate colonization of new environments. The effectiveness of this type of dual system is apparent in the widespread success of Bothriochloa intermedia from Africa through all of Asia and even into Australia (42).

Since it had been clearly established that apomixis was widespread throughout the genera principally involved in these biosystematic studies, several important areas warranted further investigation. It became important to know what mechanism controlled the alternatives of sexual or apomictic reproduction. Several workers contributed to this field. In 1963 Harlan and de Wet (45) published findings that indicated that sexual reproduction and agamospermous apomixis were neither genetical nor operational alternatives. In other words within the same apomictic plant it was possible to find both sexual and apomictic embryo sacs and in some cases both might be successful. They also

concluded that the success of either of the two alternatives was governed by the interaction of several mechanisms associated with reproduction. These mechanisms were probably governed by environmental factors.

Several workers in the field have provided insight into the workings of some of the controlling phenomena. Knox and Heslop-Harrison (50) conducted experiments with Dichanthium aristatum to demonstrate the effect of light regime on the number of reduced (sexual) embryo sacs as opposed to the number of unreduced (aposporous) embryo sacs. Using controlled conditions they exposed some plants to 16-hour day length and others to 8-hour day length and grew the plants for 135 days under these conditions. They found that plants exposed to a continuous regime of short days produced 68.5% to 79.0% aposporous sacs while plants exposed to 40 days of short daylight followed by long daylight produced only 27.0% to 46.5% of the aposporous sacs. Knox (49) conducted a similar set of experiments using agricultural stations at various latitudes in Australia. Using the same species he found that where the day length exceeded 14 hours the incidence of apomictic sacs was low, 54.82%, 60.69%, and 63.08%, while at the northern stations with short day lengths the percentages of aposporous sacs were 92.96%, 87.45%, and 91.49%.

Nygren (53, 54) in 1949 and 1951 in observations of the reproductive phenomena in the species Calamagrostis purpurea, found that the age of the inflorescence of the plants was the most important influence on whether the plant was apomictic or sexual. He found that when the panicles first developed the pollen mother cells underwent normal meiotic division but as the panicle aged the pollen mother cells

underwent mitotic division rather than meiotic.

Zatyko (69) established that gibberellic acid and B. indoleacetic acid or a mixture of the two inhibited fruit drop, and allowed the development of apomictic seeds.

Saran (59) used Dichanthium intermedium (R. Br.) de Wet et Harlan, which is the compilospecies mentioned earlier, as the subject of his investigation into the phenomena of light regime influence on apomixis. He found that if these plants were clipped back and allowed to grow out under a condition of 14-hour day length, there was an average of 1.2 embryo sacs per ovule. During the early stages of megagametogenesis, two developing embryo sacs were present. One was the normal, sexual sac, the other was an aposporous sac, developing in the nucellar tissue. The aposporous sac degenerated early, never growing beyond the two nuclear state. Only the sexual sac matured.

Under the 12-hour day length the percentage of apomictic sacs went up from 21.6% that had been found in the 14-hour regime, to 63.4%. Of the 634 apomictic sacs observed 434 were in the mature four nucleate stage.

All of the foregoing evidence illustrates the fact that in Gramineae and more specifically within the Dichanthium, Bothriochloa, Capillipedium complex, there is a high incidence of apomixis, which is more prevalent in the higher levels of polyploidy and is often controlled by photoperiod and other environmental and genetic factors to a lesser degree. Any thorough understanding of the biosystematics of these genera must include this knowledge of the apomictic phenomena.

Cytogeography

In 1958 Harlan and Celarier et al. (42) were able to discern the over-all pattern that eventually gave rise to the compilospecies concept. In the initial studies of the Old World bluestem grasses certain populations which they described as "Centers of Evolutionary Activity" were revealed. In eastern Africa Bothriochloa intermedia and B. pertusa were found to be sympatric. In western Pakistan B. intermedia and B. ischaemum were sympatric. On the Gangetic Plain of India and in eastern Pakistan B. intermedia and D. annulatum were found. In eastern China B. intermedia and B. ischaemum were sympatric and finally in Australia B. intermedia and C. parviflorum were found to be sympatric. This created an almost world-wide distribution of the genera under investigation. Further work on the compilospecies and its parental generic complex revealed that B. intermedia was the thread that sewed this complex together, but the comparable picture of the Bothriochloas of the New World had not been examined in detail.

Two other concepts which are of value in understanding the development of the New World Bothriochloas were set forth by Harlan (40) on the types of gene centers that he had encountered in the Old World. He has summarized the features of these two types of centers in the following way:

Endemic Center	Microcenter
Species narrowly endemic	Species cosmopolitan
Species narrowly restricted ecologically	Species generally ruderal
Habitats old and stable	Habitats new and disturbed
Mostly basic diploids	Segmental alloploids 4n,5n,6n

Plants mostly sexual	Plants mostly apomictic, but with a low frequency of sexual reproduction
No evident introgression	Introgression conspicuous

In the case of the endemic center, we have a group of old relect, basic diploid species that have become highly adapted to a few special stable environments. The species have endured for geologic time because the habitats have endured for geologic time. In the case of the microcenters, we have extreme polymorphism due to active hybridization of cosmopolitan, ruderal species under the influence of relatively recent human disturbances.

Throughout the range of the three contributing genera to the Bothriochloa, Dichanthium, Capillipedium complex in the Old World a cytogenetic pattern has been clearly established (16, 13, 18). The basic number of the complex is $n=10$, chromosomes. Many areas have been found that have tetraploid and pentaploid races; many of these are apomictic in nature, and represent introgressive hybridization of the basic species where two or more of the species are sympatric (42).

The principal chromosome numbers for the native New World Bothriochloas are $2n=60$, $2n=120$, $2n=180$, and $2n=220$, so that the lowest chromosome number would be equivalent to the hexaploids of the Old World. Earlier work on the New World Bothriochloas had indicated that they had a higher incidence of sexual reproduction at the higher ploidy levels than had been encountered in the Old World species (6).

Conclusions

Within the Gramineae tribe Andropogonae, extensive research in the biosystematics of the generic complex involving Bothriochloa, Dichanthium and Capillipedium has been accomplished. Significant results of this research include extensive evidence of intergeneric introgression, principally centered on the compilospecies B. intermedia. Evidence of

widespread pseudogamous apospory, as an apomictic evolutionary device which combined with a propensity for increase in polyploidy, on the basic chromosome number of n=10, has enabled the generic complex to occupy much of the tropical, sub-tropical, and temperate grasslands of the world.

Recent developments in the understanding of the significance of the anatomical features of the leaf epidermis, will be combined with traditional morphological studies, cytology and cytogeography, in an attempt to unravel the biosystematics of the New World Bothriochloas.

CHAPTER II

MORPHOLOGICAL STUDY

The purpose of this study was to determine the most logical divisions of certain collections of the various grass plants that occur in Mexico and South America that have historically been included within the genera Bothriochloa or Andropogon. The need for such a study resulted from the efforts of several workers with closely allied plants on a world-wide basis. Among these workers, the most prominent have been Dr. J. R. Harlan, Dr. J. M. J. de Wet, and the late Dr. R. P. Celarier. Their findings indicated that there was a world-wide distribution of these plants forming a polyploid complex perhaps more extensive and important than any of the other plant complexes previously studied biosystematically. While the cytological and morphological study of these plants had been rather thoroughly accomplished for Asia, Africa, Europe, and Australia, the only significant recent studies in this complex were the investigations of Dr. F. W. Gould (38) on the North American Bothriochloas, with principal emphasis on those Bothriochloa species in the United States and northern Mexico. Therefore, in an attempt to widen the understanding of this complex, as it is found throughout the North and South American continents, the following studies were initiated.

Materials and Methods

Dr. J. M. J. de Wet made extensive collections of these plants through Texas and Mexico in 1966 and through southern Brazil, Uruguay, Paraguay and Argentina in the spring of 1967. These collections furnished seed which was used in the cultivation of all of these plants under very nearly identical conditions at Oklahoma State University. This cultivation system has been described in "An Oklahoma Andropogon Garden" (14). The plant material for the study was collected in September of 1967 and prepared as herbarium specimens and then examined for the morphological features that will be described below. At least ten mature specimens for each of the accession numbers being studied were prepared in this manner and identical material has been placed in the herbarium at the University of Illinois in Urbana.

The features examined on each of the specimens were as follows:

A. Quantitative features.

1. The number of nodes in the inflorescence.
2. The length of the inflorescence in milimeters.
3. The length in milimeters of the longest raceme, often the lowest, but not always.
4. The length of the main axis of the inflorescence in milimeters from the attachment of the lowest raceme to the base of the apical raceme.
5. The length in milimeters of the sessile fertile spikelet.
6. The length in milimeters of the sterile pedicellate spikelet including the pedicel.
7. The length of the awn in milimeters from its point of emergence above the glume.
8. The number of primary branches in the inflorescence.
9. The number of primary branches arising from the lowest

node.

10. The average number of secondaries found on the primary branches of the lowest two nodes.

B. Qualitative characters.

1. Bearding on the nodes of the culm.

2. Gross pubescence of the leaves.

3. Description of the shape of the leaves.

4. Spikelet pubescence.

5. Glume pit. (The glume of some of the taxa within the Andopogonae possess a distinctive singular glandular circular pit in the lower glume of the sessile spikelet.)

6. The general description of the shape and compactness of the inflorescence.

The above listed features were examined and data collected for thirty-eight accessions. Normally ten inflorescences were examined for each accession number, although in some cases only eight were found suitable for examination. Observations were made with the aid of a dissecting microscope. On the completion of the data collection the results were averaged for each accession number and then these average values were compared with the data for all other accessions to find general similarities. It was found that this rather clearly produced distinctive groups. The data for each accession, which obviously was included in each group, was averaged with the other members of the group and a group mean determined. Then using this group mean all data for each inflorescence was used to calculate the standard deviation for each character for each group. This data was then plotted on a polygonal graph similar to the one used by Davidson (21) and Gould (38). A feature added to these graphs was the presentation of one standard deviation above and below the mean so that a concept of the

PLATE I

INFLORESCENCE MORPHOLOGY OF SELECTED ACCESSIONS

In each picture a culm node, a typical inflorescence, and a sessile spikelet are shown. The size of the materials in the photograph can be ascertained by comparison with the 10 cm. scale at the same magnification.

Legend:

Figure 1: B. wrightii, Complex I, Accession 11470, from Mexico.

Figure 2: B. alta, Complex II, Accession 11493, from Mexico.

Figure 3: B. exaristata, Complex II, Accession 11345, from Brazil.

Figure 4: B. saccharoides var. saccharoides, Complex II, Accession 11567, from Uruguay.

Figure 5: B. saccharoides var. longipaniculata, Complex II, Accession 11584, from Uruguay.

Figure 6: B. barbinoidis var. schlumbergeri, Complex III, Accession 11445, from Mexico.

PLATE I

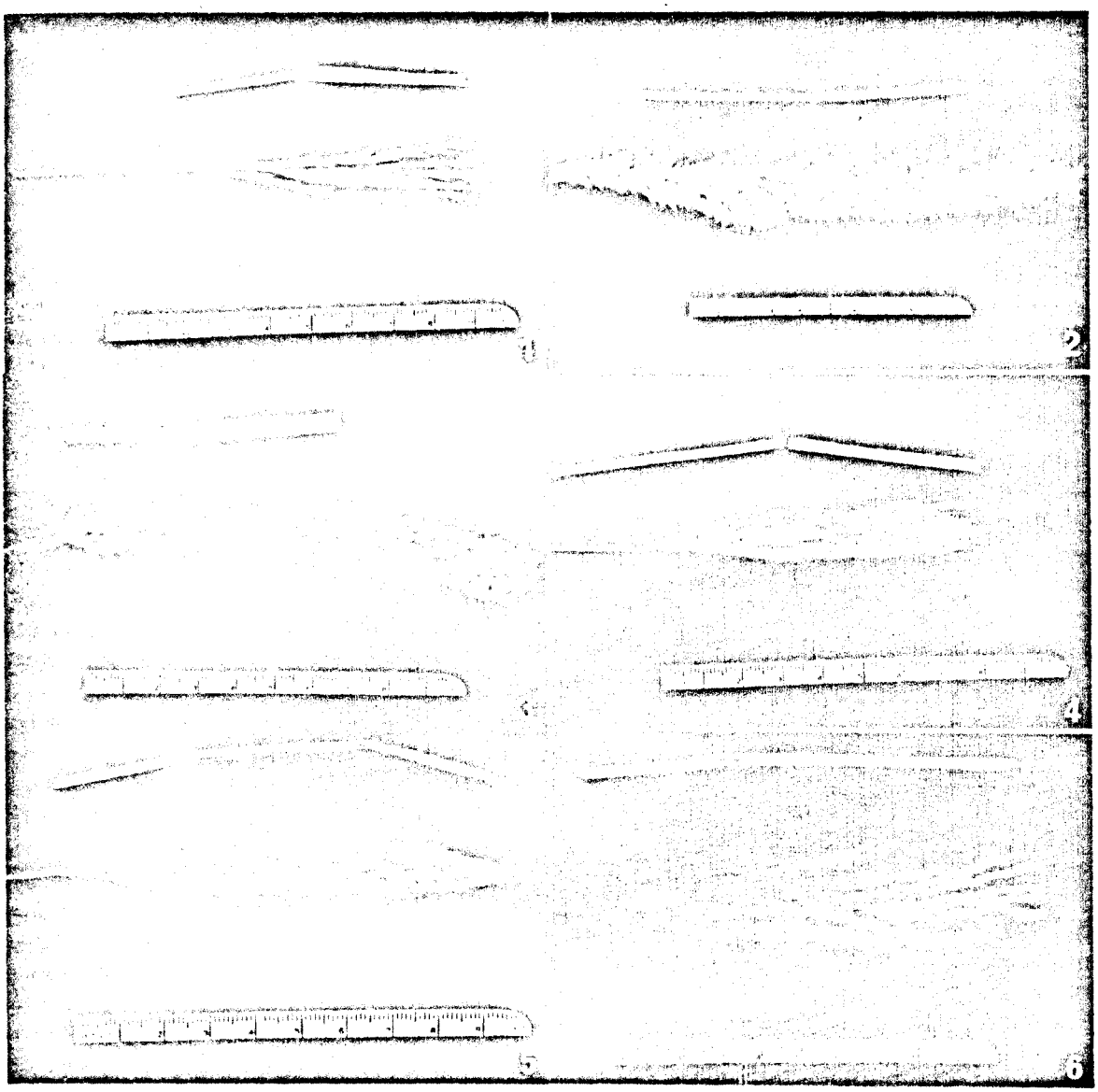


PLATE II

INFLORESCENCE MORPHOLOGY OF SELECTED ACCESSIONS

In each picture a culm node, a typical inflorescence, and a sessile spikelet are shown. The size of the materials in the photography can be ascertained by comparison with the 10 cm. scale at the same magnification.

Legend:

Figure 7: B. barbinoidis var. barbinoidis (villous form), Complex III, Accession 11406, from Mexico.

Figure 8: B. barbinoidis var. barbinoidis (less villous form), Complex III, Accession 11449, from Mexico.

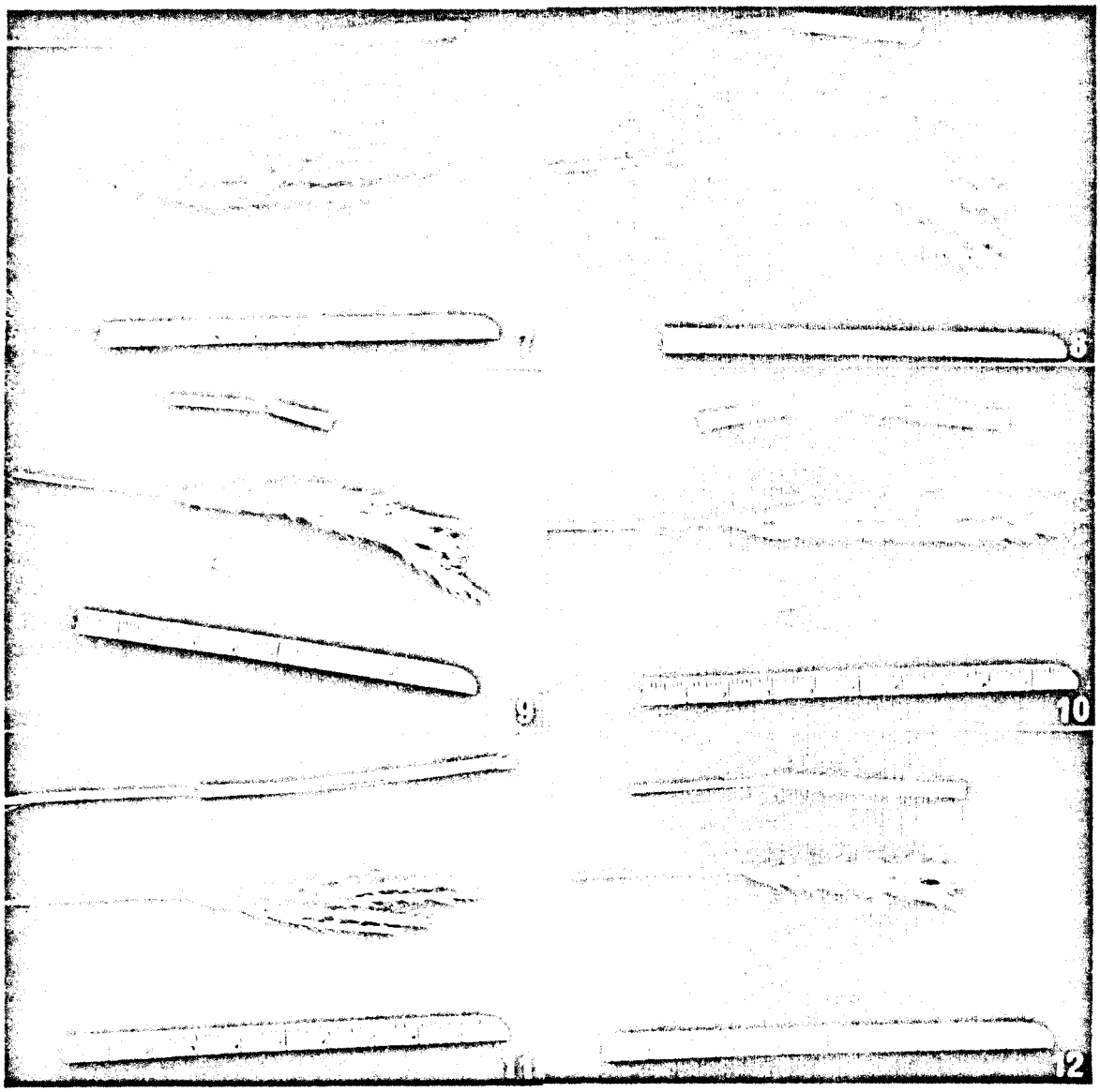
Figure 9: B. springfieldii var. springfieldii, Complex IV, Accession 11441, from Mexico.

Figure 10: B. springfieldii var. australis, Complex IV, Accession 11592, from Uruguay.

Figure 11: B. hybrida, Complex V, Accession 11378, from Mexico.

Figure 12: B. edwardsiana, Complex V, Accession 11458, from Mexico.

PLATE II



variation within the population is also conveyed. The qualitative characters were not similarly portrayed but have been indicated as comments in the keys and in the description of the species complexes.

In the accessions studied there are certain key characters which seem to bind the species and their varieties together well enough so that it is advisable to form these species together into complexes or sections within the genus. Hybridization studies have not yet been completed on the accessions, and the results of such studies by revealing the effectiveness of interspecific gene barriers may well indicate that certain of the taxa that at present appear to be good species are so closely allied that they should be considered properly as varieties of the same species. Augmenting information concerning inflorescences unavailable at this location was provided by de Wet (26).

Key to the species of New World *Bothriochloas*

Based on Key Morphological Characters

1. Pedicellate spikelets about as broad and as long as the sessile ones, neuter or rarely male.

    2. Inflorescence with 3-10 branches arranged along a 2-5 cm. long primary axis; branches usually not divided, rarely with the lower ones bearing 2-3 racemes.

        3. Leaves narrowly linear-lanceolate, 2-5 mm. wide, glabrous to sparsely hairy near the base; sterile zone below raceme usually 1-2 cm. long; sessile spikelets 4-6 mm. long, often pitted; $2n=120$ *B. wrightii* (Hack.) Henr.

        3. Leaves broadly linear-lanceolate, 3-10 mm. wide, glabrous to strongly pilose; sterile zone below raceme usually more than 2 cm. long; sessile spikelets 5-7 mm. long, often pitted; $2n=60$ *B. hirtifolia* (Presl.) Henr.

    2. Inflorescence with 5-20 branches arranged along a 4-10 cm. long primary axis; lower branches divided about 2-3 cm. from the base; sessile spikelets 4-6 mm. long, usually pitted; leaves 3-10 mm. wide, glabrous except near the base; $2n=120$ *B. campii* (Swallen) de Wet

1. Pedicellate spikelets often involute, usually shorter than the sessile ones, neuter.

    4. Inflorescence more or less cylindrical, with numerous divided branches arranged on a primary axis that exceeds the lower branches in length; sessile spikelets 2-5 mm. long.

        5. Inflorescence 20-25 cm. long; sessile spikelets awned; culms robust; 2n=120. B. alta (Hitchc.) Henr.

        5. Inflorescence 5-20 cm. long; culms slender to robust.

            6. Sessile spikelets awned; 2n=60, 120
            B. saccharoides (Swartz) Rydb.

            6. Sessile spikelets awnless, or at most 2-5 mm.; 2n=60
            B. exaristata (Nash) Henr.

    4. Inflorescence obtriangulate to ovate, with few to numerous branches that are at most as long as the primary axis; sessile spikelets 4-8 mm. long, pitted or not pitted often on the same raceme.

        7. Inflorescence usually with numerous, strongly divided branches, when few, racemes moderately hairy and up to 12 cm. long; sessile spikelets 4-5 mm. long; culms rather robust, with well developed cauline leaves 5-10 mm. wide 2n=180. B. barbinoidis (Lag.) Herter

        7. Inflorescence with 5-15, often undivided branches, sometimes with the lower ones bearing 2-3 racemes; sessile spikelets 4-6 mm. long; culms usually less robust.

            8. Leaves primarily in a dense basal tuft; blades rarely exceeding 3 mm. in width, racemes moderately pilose; 2n=60 B. edwardsiana (Gould) Parodi.

            8. Leaves not primarily in a basal tuft, cauline leaves well developed, blades usually at least 5 mm. wide.

                9. Racemes densely white villous, sessile spikelets usually densely pilose on the back below the middle; culm-nodes densely bearded when young; 2n=60, 120
                B. springfieldii (Gould) Parodi.

                9. Racemes moderately hairy; sessile spikelets at most with a few coarse hairs at back below the middle; culm nodes glabrous or shortly hairy.

                    10. Sessile spikelets 4-5 mm. long; awn 10-15 mm. long; pedicellate spikelet longer than the pedicel; 2n=120
                    B. reevesii (Gould) Gould.

PLATE III

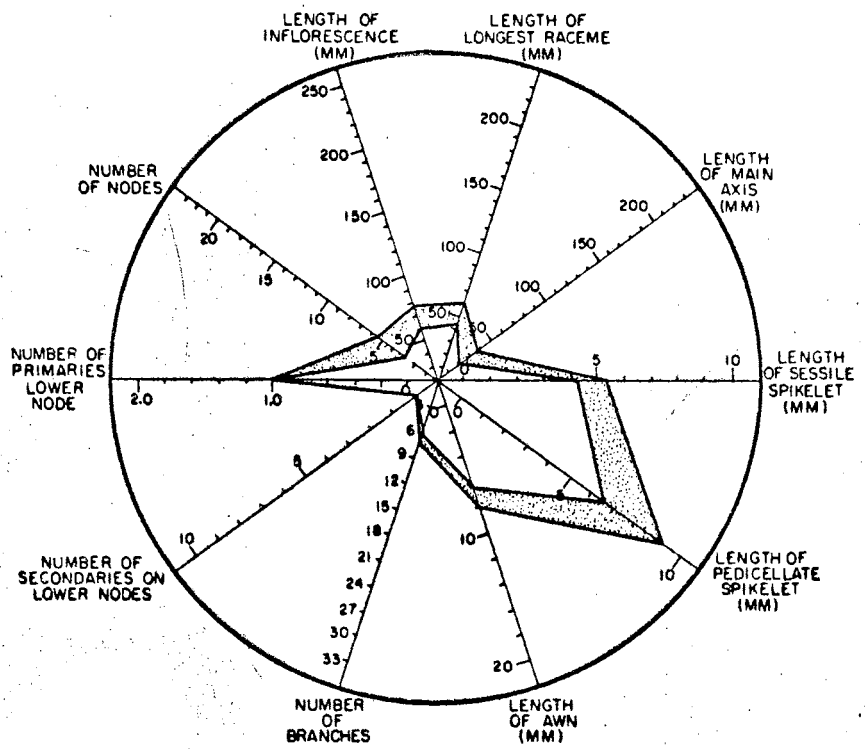
POLYGONAL GRAPHS OF MORPHOLOGICAL DATA FOR *B. WRIGHTII* AND *B. ALTA*

The stippled area enclosed in the solid lines on each graph represents the variation included in one standard deviation above and below the mean value.

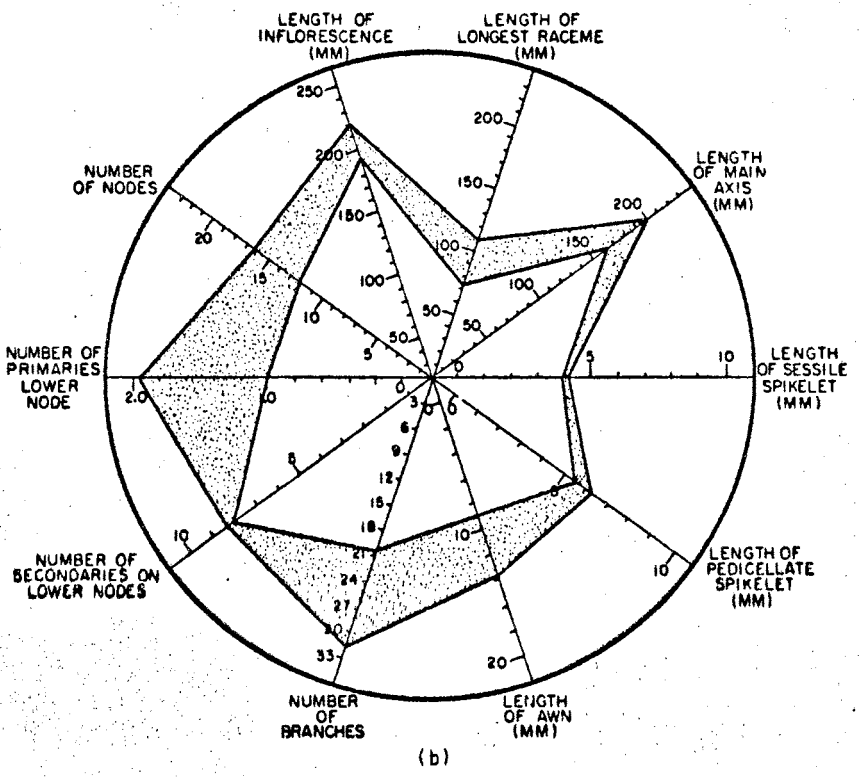
Legend:

Figure 13a: *B. wrightii*, Complex I.

Figure 13b: *B. alta*, Complex II.



(a)



(b)

Figure 13

PLATE III

PLATE IV

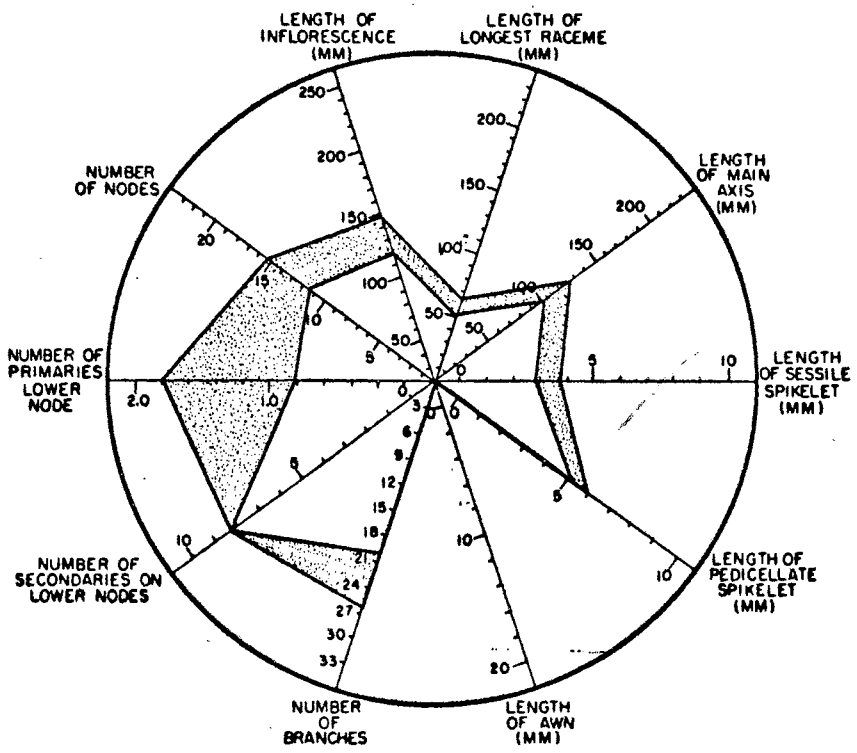
POLYGONAL GRAPHS OF MORPHOLOGICAL DATA FOR *B. EXARISTATA* AND *B. SACCHAROIDES* VAR. *SACCHAROIDES*

The stippled area enclosed in the solid lines on each graph represents the variation included in one standard deviation above and below the mean value.

Legend:

Figure 14a: *B. exaristata*, Complex II.

Figure 14b: *B. saccharoides* var. *saccharoides*, Complex II.



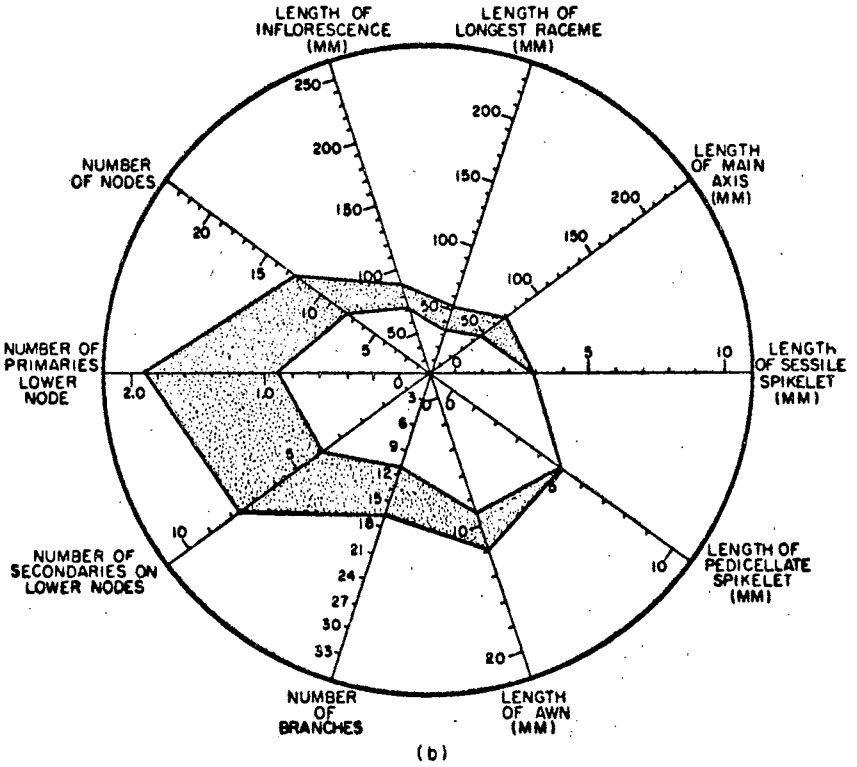


Figure 14

PLATE IV

PLATE V

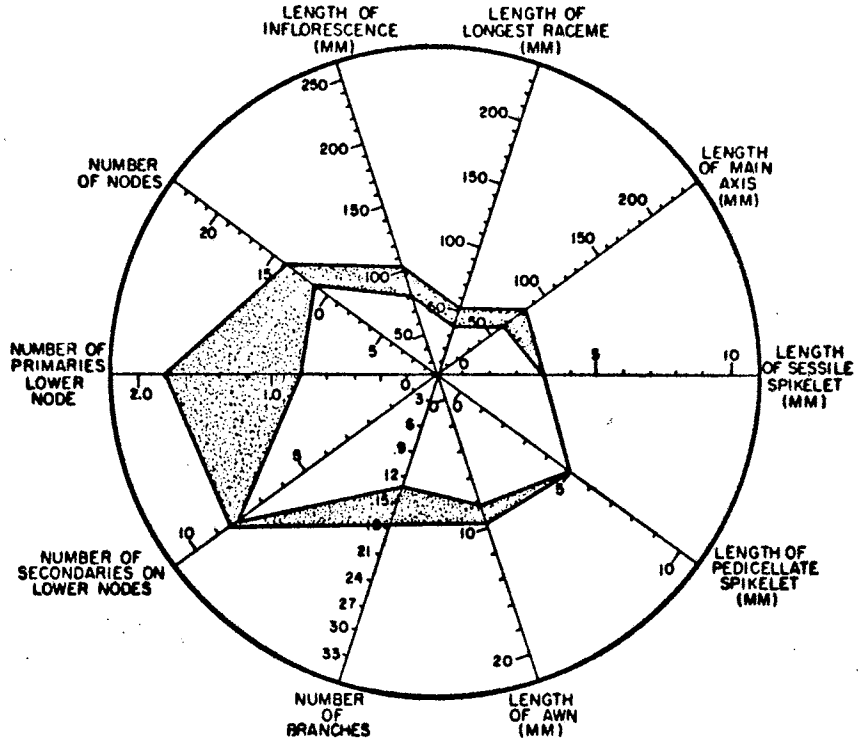
POLYGONAL GRAPHS OF MORPHOLOGICAL DATA FOR
B. SACCHAROIDES VAR. *LONGIPANICULATA*
AND *B. BARBINOIDIS* VAR. *BARBINOIDIS*

The stippled area enclosed in the solid lines of each graph represents the variation included in one standard deviation above and below the mean value.

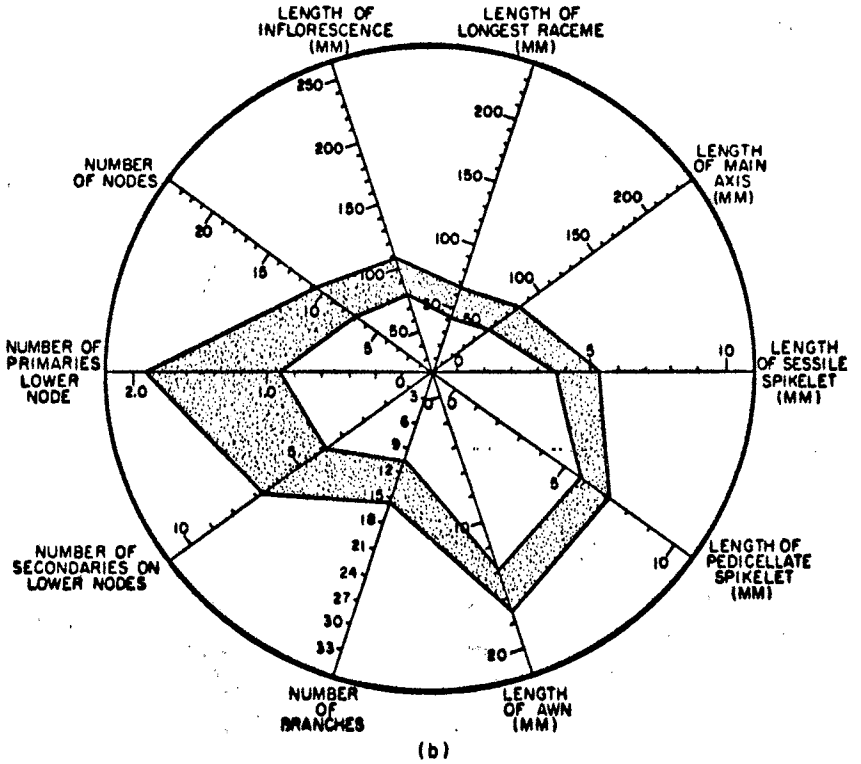
Legend:

Figure 15a: *B. saccharoides* var. *longipaniculata*, Complex II.

Figure 15b: *B. barbinoidis* var. *barbinoidis*, Complex III.



(a)



(b)

Figure 15

PLATE V

PLATE VI

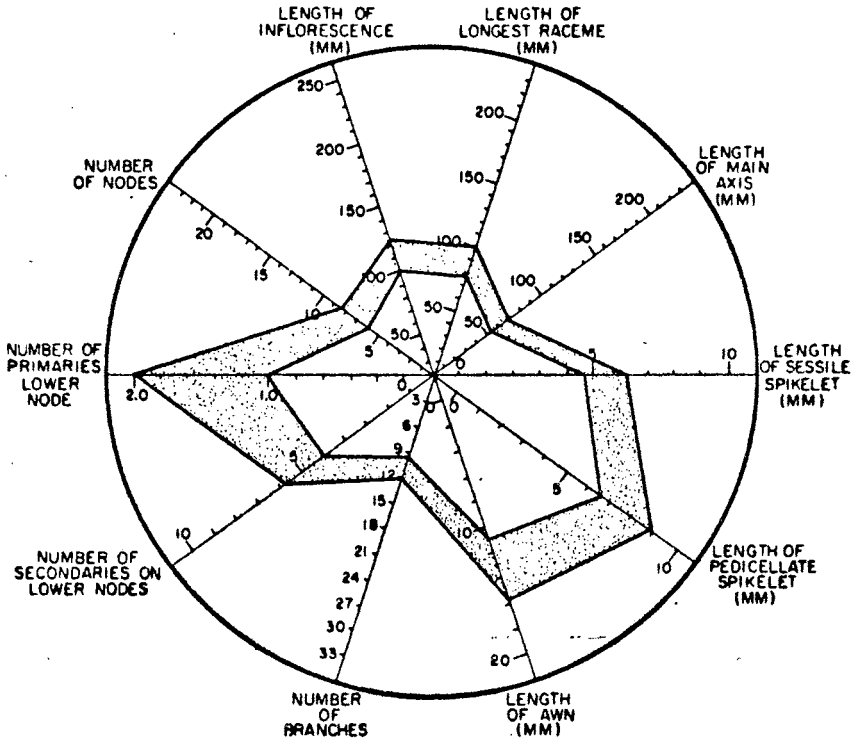
POLYGONAL GRAPHS OF MORPHOLOGICAL DATA FOR
B. BARBINOIDIS VAR. *SCHLUMBERGERI* AND
B. SPRINGFIELDII VAR. *SPRINGFIELDII*

The stippled area enclosed in the solid lines on each graph represents the variation included in one standard deviation above and below the mean value.

Legend:

Figure 16a: *B. barbinoidis* var. *schlumbergeri*, Complex III.

Figure 16b: *B. springfieldii* var. *springfieldii*, Complex IV.



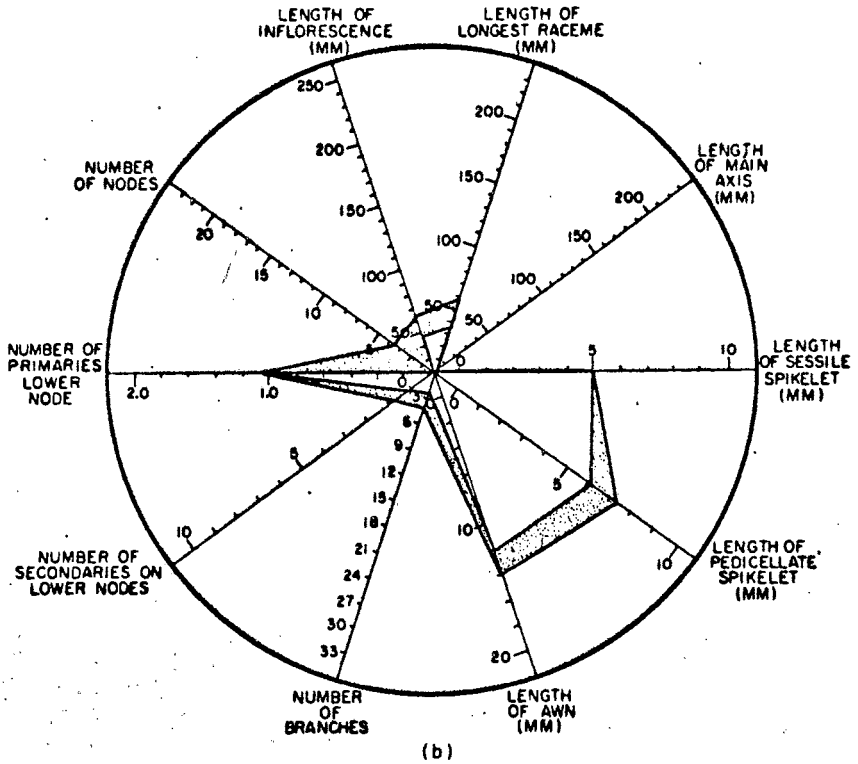


Figure 16

PLATE VI

PLATE VII

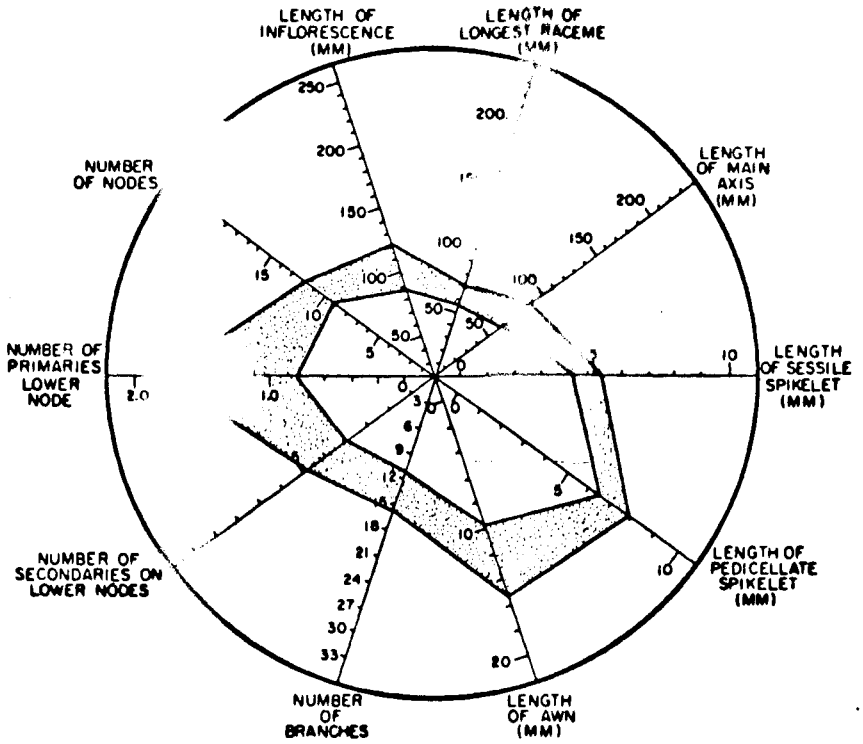
POLYGONAL GRAPHS OF MORPHOLOGICAL DATA FOR
B. SPRINGFIELDII VAR. *AUSTRALIS*
AND *B. EDWARDSIANA*

The stippled area enclosed in the solid lines on each graph represents the variation included in one standard deviation above and below the mean value.

Legend:

Figure 17a: *B. springfieldii* var. *australis*, Complex IV.

Figure 17b: *B. edwardsiana*, Complex V.



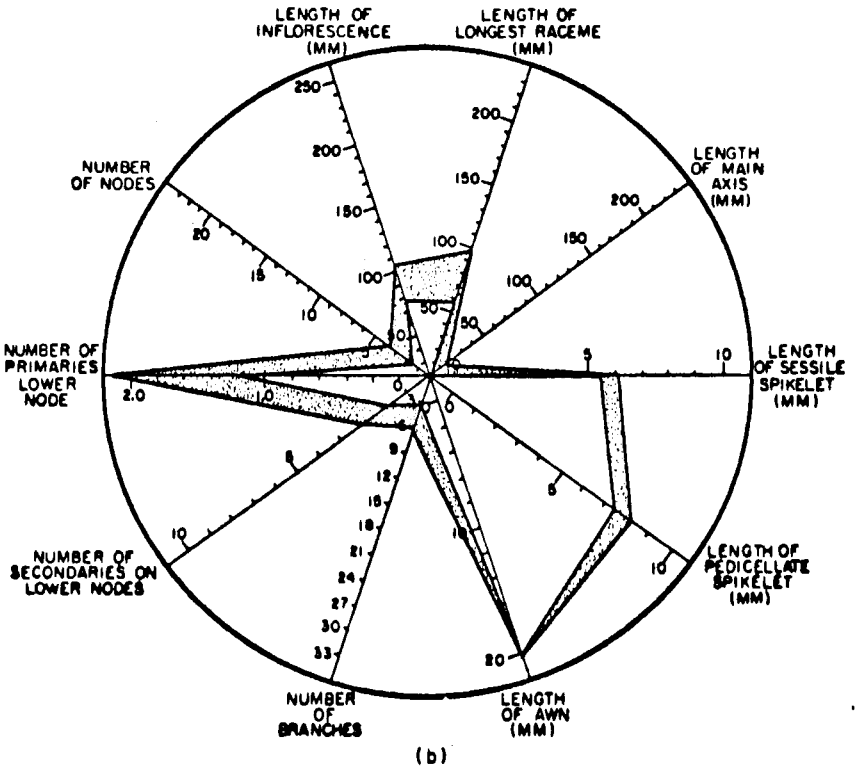


Figure 17

PLATE VII

10. Sessile spikelets 4-6 mm. long; awn 15-20 mm. long; pedicellate spikelet at most as long as the pedicel; 2n=120
B. hybrida (Gould) Gould.

Species Complexes

The key presented above permits the separation of all the species which were encountered in this study. To present a more informative and accurate picture of the affinities which exist in the New World *Bothriochloas*, it is necessary to establish certain species complexes. The following discussion is designed to clarify the basic factors which group these species into the complexes.

The morphological feature common to all species in the first complex concerns the pedicellate spikelets. All species belonging to the first complex possess pedicellate spikelets which are as long and as broad as the sessile ones. All accessions examined, that belong to this complex, were collected from the Mexican region. The significant features and those features which serve to distinguish the various species within the complex are as follows:

a. *B. wrightii*: inflorescence 3-10 branches, leaves 2-5 mm. wide, glabrous to sparsely hairy, 2n=120, stomata 45-49 microns, bicellular hairs 59-70 microns.

b. *B. hirtifolia*: inflorescence 3-10 branches, leaves 3-10 mm. wide, leaves generally strongly pilose, 2n=60, stomata 30-36 microns, bicellular hairs 43-50 microns, leaf abaxial surface possesses macrohairs.

c. *B. campi*: inflorescence with 5-20 branches, leaves 3-10 mm. wide, leaves glabrous except near the base, no data available on leaf epidermis.

The data on *B. hirtifolia* and *B. campi* morphology was provided by de Wet (26). Another significant feature common to all members of this complex is the general possession of glume pits. Generally within this

complex the inflorescences have a subdigitate character; however, this is less pronounced in the B. campi species.

The data on the stomata and bicellular hair length was derived from the leaf epidermal studies and it can be seen in this and in the other complexes the lengths vary in accordance with the chromosome number rather than as a feature of the complex. The qualitative features of the leaf epidermis do represent the complexes. In this complex the most significant qualitative feature of the epidermis occurs in the papillae. In this complex the papillae do not significantly extend over the adjoining stomata in any of the species. A detailed discussion of this phenomenon is contained in the chapter on the leaf epidermis.

All of the other complexes can be distinguished from the first complex by their possession of pedicellate spikelets that are always narrower than the sessile ones.

The second complex has the principal characteristic of possessing an essentially cylindrical inflorescence, with the lower branches of the inflorescence shorter than the elongated primary axis. The four taxa that belong to this complex and their distinguishing characters are as follows:

a. B. alta: inflorescence 20-25 cm. long, culms very robust, 2n=120, stomata 34-39 microns, bicellular hairs 56-71 microns.

b. B. exaristata: awns absent or minute, inflorescence 12-16 cm. 2n=60, stomata 29-31 microns, bicellular hairs 49-52 microns.

c. B. saccharoides var. saccharoides: awns present, inflorescence 5-15 cm., 2n=60, stomata 32-34 microns, bicellular hairs 46-53 microns.

d. B. saccharoides var. longipaniculata: awns present, inflorescence 14-20 cm. long, 2n=120, stomata 34 microns, bicellular hairs 50 microns.

It is quite apparent that the normal pattern of difference in the length of stomata and bicellular hairs as correlated with a difference in ploidy level is not found in the two varieties of B. saccharoides. This peculiarity will be discussed in much more detail in the chapter on the leaf epidermis and a possible cause set forth. Otherwise there is a good consistency in the characteristics within the complex. This complex includes accessions from the Mexican region and the South American region.

The third complex is the most varied of all the complexes. The unifying characteristics are the presence of reduced pedicellate spikelets, inflorescences ovate to obtriangulate, lower inflorescence branches at least as long as the primary axis, usually longer. This complex includes one species with two varieties; one of the varieties occurs in two forms. The distinguishing features, chromosome numbers and anatomical measurements are as follows:

a. B. barbinoidis var. schlumbergii: Inflorescence obtriangulate, long sterile zone below the racemes on the inflorescence branches, 2n=180, somata length 36 microns, bicellular hair length 62-65 microns.

b. B. barbinoidis var. barbinoidis: Inflorescence ovate or subdigitate with few racemes, 2n=180, stomata length 33-40 microns, bicellular hair length 63-78 microns.

   1. Villous form: Relatively few racemes that are long and white villous.

   2. Less villous form: Numerous racemes that are less pilose.

The presence of pitting of the glume in this group is evidence of introgression between this species and B. hybrida. B. hybrida in turn has probably picked up the genetic determinants for glume pit formation from B. edwardsiana as postulated by Gould (39). All members of this complex were from the Mexican region.

The fourth complex contains one species with two varieties which have different chromosome numbers. The variety springfieldii is found only in the Mexican region and has a chromosome number of 2n=120. The variety australis has a chromosome number of 2n=60, and is probably the most uniform of any of the taxa examined. It is found only in the South American region. The characters which unify the complex are the presence of digitate inflorescences, small pedicellate spikelet, white villous racemes, with the hairs on the raceme long, and finally the culm nodes are densely bearded when young.

The varieties and their distinctive characters are as follows:

a. B. springfieldii var. springfieldii: primary axis of inflorescence short far exceeded by the inflorescence branches; 2n=120, stomata length 37-40 microns, bicellular hair length 70-71 microns.

b. B. springfieldii var. australis: primary axis as long as, or slightly longer than, the lower branches of the inflorescence, 2n=60, stomata length 29 microns, bicellular hair length 55-59 microns.

The fifth and final complex contains three species, with all of the accessions coming from the Mexican region. The unifying characters within the complex are the digitate inflorescence, small pedicellate spikelets, and the only moderately hairy racemes. The member species and their distinguishing characteristics are as follows:

a. B. edwardsiana: leaf blades narrowly linear lanceolate, rarely more than 3 mm. wide, 2n=60, stomata length 35 microns, bicellular hair length 51 microns.

b. B. reevesii: leaf blades broader, 5-8 mm., pedicellate spikelet longer than pedicel, 2n=120, no data on stomata and bicellular hair length, no glume pit.

c. B. hybrida: leaf blades 5-8 mm., pedicellate spikelet at most as long as the pedicel, 2n=120, stomata length 36-37 microns, bicellular hair length 68-74 microns, glumes pitted.

TABLE I

QUANTITATIVE MORPHOLOGICAL DATA

		Complex I	Complex II				Complex III		Complex IV		Complex V	
		B. wrightii	*B. alta*	*B. exaristata*	*B. saccharoides*	*B. saccharoides longipaniculata*	*B. barbinoidis*	*B. barbinoidis* var. *schlumberg.*	*B. springfieldii*	*B. springfieldii* var. *australis*	*B. edwardsiana*	*B. hybrida*
1. Number of Nodes	High	7(5.6)	16(16.2)	16(15.3)	15(12.1)	14(13.9)	13(10.3)	9(8.2)	4(3.8)	13(11.8)	4(3.8)	8(6.9)
	Mean	4.5	14.3	13.4	9.9	12.6	8.8	7.1	1.9	10.5	2.7	5.5
	Low	(3.4)	(12.4)9	(11.5)10	(7.7)7	(11.3)11	(7.3)6	(6.0)6	(0.0)1	(9.2)8	(1.6)1	(4.1)3
2. Length of Inflores-cence	High	85(77.1)	230(219.9)	160(146.7)	95(89.6)	105(100.9)	120(107.0)	130(124.2)	70(64.8)	150(122.3)	105(104.6)	85(76.6)
	Mean	68.8	207	134	80.0	92.2	94	112.2	56.5	106.0	87.1	65.0
	Low	(60.5)55	(194.1)180	(121.3)110	(70.4)60	(83.5)80	(81.0)70	(100.2)90	(48.2)45	(89.7)75	(69.6)65	(53.4)45
3. Length of Longest Branch	High	60(59.6)	120(109.7)	75(66.9)	60(51.7)	55(51.4)	75(66.6)	110(98.3)	70(59.7)	85(73.4)	100(97.0)	70(57.7)
	Mean	52.5	93	59	43.0	45.6	55	86.9	49.0	65.0	78.3	48
	Low	(45.4)45	(76.3)70	(51.1)45	(34.3)20	(39.8)35	(43.4)35	(75.5)70	(38.3)35	(56.6)50	(59.6)50	(38.3)35
4. Length of Main Axis	High	45(38.0)	215(202.8)	140(127.2)	80(69.6)	80(80.5)	90(80.1)	80(69.4)	8(6.6)	100(86.4)	11(11.1)	50(39.5)
	Mean	30	186	114	59.0	72.2	67	61.9	3.6	74	7.1	29
	Low	(22.0)20	(169.2)150	(110.8)90	(48.4)35	(63.9)55	(53.9)40	(54.4)50	(.7)1	(61.6)45	(3.1)2	(18.5)15
5. Length of Sessile Spikelet	High	5(5.3)	4(4.0)	4(3.8)	3(3.0)	3(3.0)	5(5.1)	7(6.4)	5(5.0)	6(5.2)	6(6.3)	5(5.1)
	Mean	4.8	4	3.3	3	3.0	4.4	5.7	5	4.7	5.9	4.5
	Low	(4.3)4	(4.0)4	(2.8)3	(3.0)3	(3.0)3	(3.7)3	(4.9)5	(5.0)5	(4.2)4	(5.5)5	(3.9)3
6. Length of Pedicellate Spikelet	High	10(9.2)	7(6.3)	6(6.0)	5(5.0)	5(5.0)	7(7.0)	10(8.9)	7(7.2)	8(7.9)	8(8.3)	8(6.7)
	Mean	7.9	6.0	5.5	5.0	5.0	6.4	7.8	6.7	7.2	7.9	6.0
	Low	(6.6)6	(5.7)6	(5.0)5	(5.0)4	(5.0)5	(5.8)5	(6.7)6	(6.2)6	(6.5)6	(7.5)7	(5.3)5
7. Length of Awn	High	8(7.9)	15(13.9)	None	12(11.7)	10(10.0)	18(16.9)	17(15.8)	14(13.8)	17(15.3)	20(20)	16(15.6)
	Mean	7	11.4	None	10.2	9.3	15.2	13.4	13	12.5	20	14.1
	Low	(6.1)5	(8.9)8	None	(8.7)8	(8.6)8	(13.5)10	(11.0)10	(12.2)12	(9.7)6	(20)20	(12.6)10
8. Number of Branches	High	7(6.7)	32(32.3)	28(25.9)	19(16.7)	18(17.5)	19(15.2)	13(12.0)	4(3.8)	20(15.5)	6(6.1)	13(10.8)
	Mean	6.5	26.6	23	13.9	15.4	12.9	10.9	3	13.3	4.7	9
	Low	(6.3)6	(20.9)16	(20.1)18	(11.1)8	(13.3)13	(10.6)9	(9.8)9	(2.2)2	(11.1)9	(3.3)3	(7.2)6
9. Average Number of Secondaries	High	0(0.0)	8+(8+)	8+(8+)	8+(7.6)	8+(8+)	9(6.8)	6(5.7)	1(1.0)	7(4.8)	2(1.9)	3(2.8)
	Mean	0.0	8+	8+	5.7	8+	5.3	4.9	1.0	3.9	1.4	1.5
	Low	(0.0)0	(8+)8+	(8+)8+	(3.8)2	(8+)6	(3.8)3	(4.1)4	(1.0)1	(3.0)3	(.9)1	(.2)0
10. Primary Branches of Lower Node	High	1(1.0)	2(2.0)	2(1.8)	2(1.9)	2(1.8)	2(1.9)	2(20)	1(1.0)	2(1.8)	2(2.2)	2(1.9)
	Mean	1	1.5	1.3	1.4	1.3	1.4	1.5	1	1.3	1.7	1.4
	Low	(1.0)1	(1.0)1	(.8)1	(.9)1	(.8)1	(.9)1	(1.0)1	(1.0)1	(.8)1	(1.2)1	(.9)1

NOTE: The figures shown in the table represent the following:
1. For the high line, the first figure is the highest value observed followed by the value in parentheses for one standard deviation above the mean.
2. The mean line contains the mean value only.
3. For the low line, the value, in parentheses, represents one standard deviation below the mean. This is followed by the lowest value observed.

B. reevesii and B. hybrida are very similar and on further investigation may well need to be placed within a single species.

Conclusions

The general morphological nature of over two hundred accessions has been examined, with a detailed morphological study made of thirty-eight accessions which typified the variation found within the total collection. A key is provided which permits the identification of the species of the New World Bothriochloas based on morphological features. Five species complexes have been established within the genus. These complexes are based on affinities revealed through comparison of morphology, chromosome number, and leaf epidermal anatomy. Two of the complexes have taxa which occur both in the Mexican region and in the South American region. Three of the complexes are composed of taxa found in the Mexican region.

CHAPTER III

LEAF ANATOMY

Since 1936, it has been considered that the epidermal composition in the Gramineae has phylogenetic and taxonomic significance (56). In Stebbins' (62) classical work on the evolution of the plants, he touched lightly on this subject. Renewed emphasis in this area was generated by de Wet's (22) work on Danthonia in 1954 in which he established that the size of the stomates could be significant cytological criteria. In 1958 Celarier and Mehra (17) made the first biosystematic epidermal investigation in the genera which are now considered in the Bothriochloa, Capillipedium, Dichanthium complex. They showed that there was a correlation between the degree of polyploidy and the length of the stomates in what was then considered Dichanthium annulatum, Bothriochloa ischaemum, Bothriochloa intermedia, and Bothriochloa pertusa. None of the specimens investigated were from the New World and none of them had a ploidy level greater than 2n=60. Stebbins (63) had shown that the epidermal features of the Gramineae were of significant assistance in recognizing the major subdivisions within the Gramineae. Tateoka (67) in 1959 had shown that there were consistent patterns in the formation of the bicellular hairs in regard to such features as the relative thickness of the cell wall of the basal cells and the apical cells, the relative length of the basal and apical cells, the width of the base and apex of the basal cell, and many other

features. In 1961, S. A. Faruqi (33) examined the general epidermal features of some specimens drawn from the members of the Bothriochloa, Capillipedium, Dichanthium complex, and he recognized the existence of an "A" type of prickle found in the silica rows and a "B" type of prickle found in the long cell rows. He also observed some occasional pitting in the long cells; these pits were termed papillae by Metcalf (51) in his work on the monocots. Metcalf also presented numerous drawings illustrating the various features as they occur in many grass genera.

Using all of these previous studies as a basis, it was decided to investigate the occurrence of the anatomical features of the epidermis of the accessions which were available for the members of genus Bothriochloa that occur in Mexico and South America. The general objectives of this study were the following:

1. To determine if the size of the stomates varies according to the degree of polyploidy as earlier studies of related groups had indicated.

2. To determine if a similar correlation exists in either the length of the bicellular hairs or the proportion of the length of the basal cell to the apical cell length.

3. To determine if there are qualitative features which are characteristic of one taxa that are not found in the other taxa within this genus.

Materials and Methods

In July and August of 1967, the leaves of the Mexican and South American Bothriochloas, which were being grown in the standard garden,

were removed for study. Normally a section of leaf about 5 cms. in length was removed from the middle portion of an average-sized leaf approximately half-way up the leafy portion of the plant. The leaf sections were then placed in a solution of equal parts glycerol, 95% alcohol, and water, and allowed to remain for 2-3 days to soften the tissues (22). The sections were then placed on a slide abaxial surface down and, while being constantly irrigated with the same glycerol, water, alcohol solution, an extremely sharp razor blade was rubbed across the upper surface until all of the tissue above the abaxial epidermis had been removed. This left a single transparent layer of epidermis. A piece about 1 cm. square was then removed and mounted in glycerol on a slide abaxial surface up. Sealing the slide in wax created a permanent slide. The section was then studied at both 150x and 440x magnifications. A sketch was made of a typical section at 440x. Selected representative segments of each of the diverse groups which resulted from the epidermal and morphological studies were photomicrographed and are shown in the accompanying plates.

During the course of the investigation the following characters seemed most worthy of study:

1. Presence or absence of macrohairs, their length, and basal construction.

2. Presence or absence of prickles, specifically their occurrence in the silica cell rows ("A" type) or in the long cell rows ("B" type), or their occurrence in both.

3. The presence or absence of "donut like" papillae in either the stomata rows or in the long cell rows or in both.

4. The presence or absence of an extension of the papillae

tissue so that it covers a portion of the adjacent stomate. This has been designated as overlap.

5. The length in microns of the bicellular hairs and the length of the basal cell and apical cell in microns.

6. The presence of a distinctive "fish-shaped" long cell in the stomata rows.

7. The length of the stomatal guard cells in microns.

For each accession, at least twenty stomata were measured and the mean value and maximum and minimum were recorded. At least ten bicellular hairs were measured and the mean was calculated. Macrohairs, where present, were measured and their length recorded. A tabular presentation of the results is presented in Table II. In those cases where more than three accessions of a particular taxa were investigated only typical examples were selected to illustrate the entire taxa.

Experimental Results

In answer to the specific objectives of this study the following results were obtained. There is a correlation between the lengths of the stomata, in the accessions studied, and their chromosome number. The rule can be created that if the length of the stomatal guard cells are on an average 35 microns or less in length then the Bothriochloa plant under investigation belongs to a group that has a chromosome number of $2n=60$. If the length on the average is greater than 35 microns or more, then the plant will have a chromosome number of $2n=120$ or $2n=180$. There were some few exceptions to this rule. In the B. alta group, 11493 had a stomata length of only 34.45 microns; in B. barbinoidis, 11449 had a stomatal length of only 33.23; in the B.

PLATE VIII

LEAF EPIDERMAL PHOTOMICROGRAPHS

Each picture shows the characteristic epidermal features. All figures ca. 350X.

Legend:

Figure 18: B. hirtifolia, Complex I, from Mexico, showing characteristic basal construction for a macrohair in the lower right corner.

Figure 19: B. wrightii, Complex I, from Mexico, showing absence of papillae.

Figure 20: B. alta, Complex II, from Mexico, showing characteristic papilla overlap.

Figure 21: B. exaristata, Complex II, from Brazil, showing extreme overlap.

Figure 22: B. saccharoides var. saccharoides, Complex II, from Uruguay, showing long stomata in upper row and short stomata in lower row in Accession 11567.

Figure 23: B. saccharoides var. saccharoides, Complex II, from Mexico, showing the consistency of features in the two geographic regions.

PLATE VIII

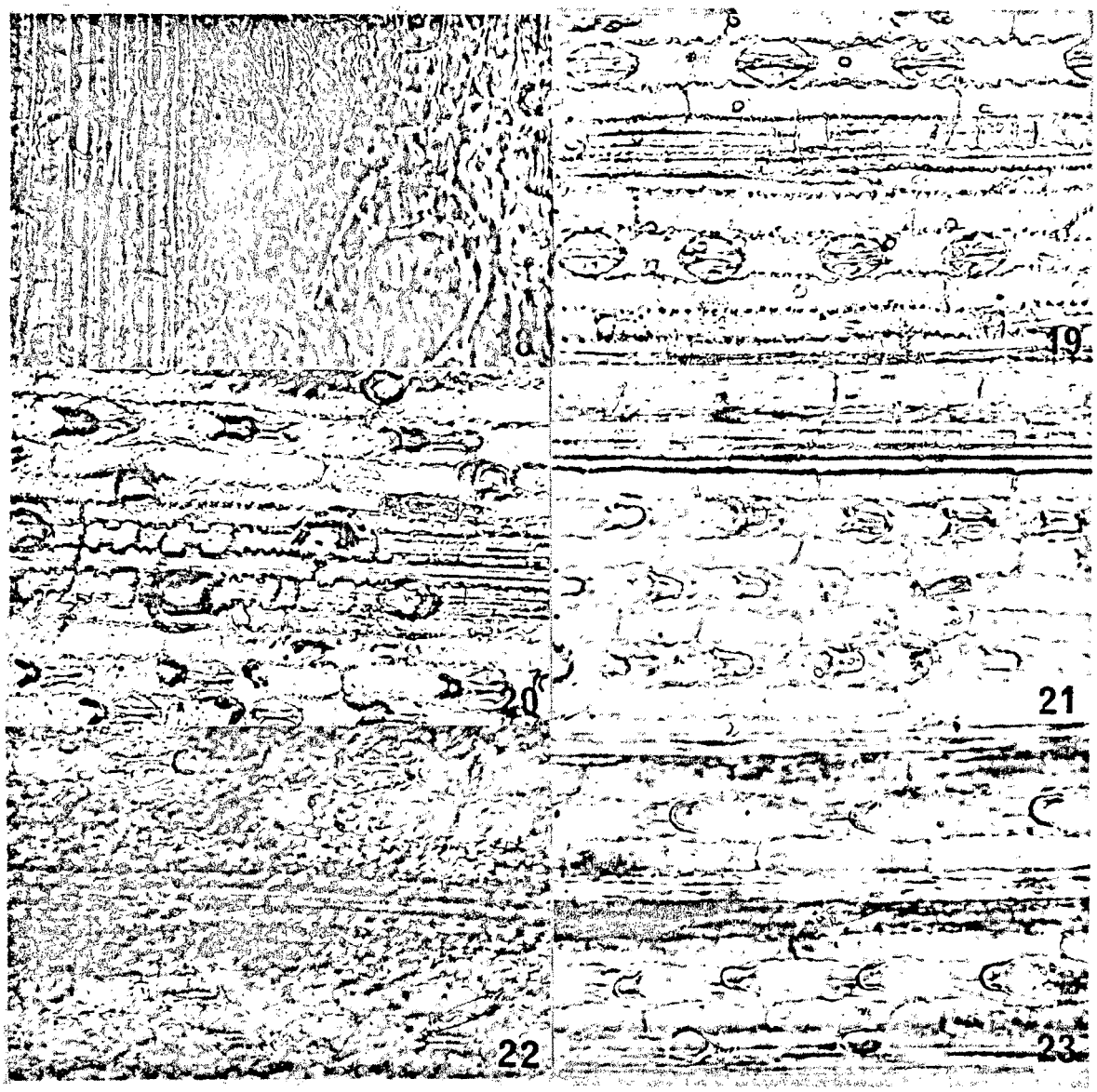


PLATE IX

LEAF EPIDERMAL PHOTOMICROGRAPHS

Each picture shows the characteristic epidermal features. All figures ca. 350X.

Legend:

Figure 24: B. barbinoidis var. barbinoidis, Complex III, from Mexico.

Figure 25: B. barbinoidis var. schlumbergeri, Complex III, from Mexico.

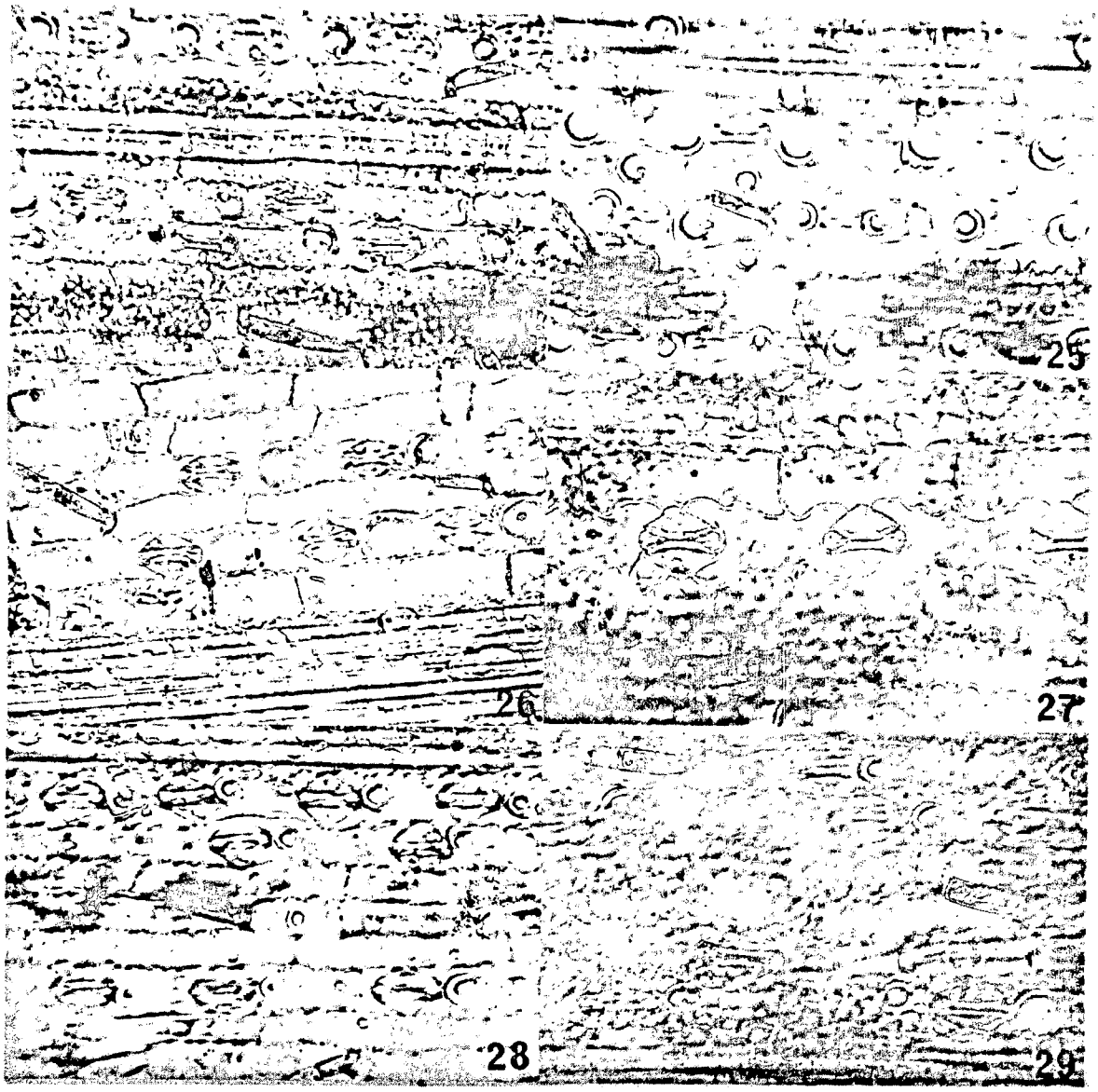
Figure 26: B. springfieldii var. springfieldii, Complex IV, from Mexico.

Figure 27: B. springfieldii var. australis, Complex IV, from Uruguay.

Figure 28: B. hybrida, Complex V, from Mexico.

Figure 29: B. edwardsiana, Complex V, from Mexico.

PLATE IX



saccharoides var. longipaniculata group, 11584 had a stomata length of only 34.52 even though all of these accessions have chromosome numbers of 2n=120. In the B. hirtifolia group, 11465 had a stomatal length of 36.36 although it has only 60 chromosomes. In all other cases the rule stated above is valid. These results seem in general agreement with Celarier's (17) work in which he found in Bothriochloa ischaemum, a related group, the stomata mean lengths were 30.7 microns, and 31.8 microns in two hexaploid accessions. In B. intermedia the hexaploids had a mean stomata length of 29.1 and 32.8 microns, and in B. pertusa the hexaploids had mean stomata lengths of 30.0 microns and 34.9 microns. However, in two accessions of D. annulatum from Africa, Celarier found that the hexaploid mean stomata length was 36.8 microns and 45.9, so clearly this rule cannot be applied in all cases in the Bothriochloa, Capillipedium, Dichanthium complex, but it seems to have widespread application in the complex.

In the study of the bicellular hairs it was found that they all had the features that Tateoka (67) had established as the Panicoid type. These accessions had bicellular hairs in which the basal cell had a clearly thicker cell wall than the apical cell. The bicellular hairs of all accessions had generally near equal lengths of basal and apical cells. These hairs all were of a long, thin appearance rather than the rather rounded appearance of the bicellular hairs of other groups. Other Andropogons studied concurrently with the basic investigation all were shorter and rounder than the Bothriochloa bicellular hairs. The bicellular hair total length was found to have a similar relationship to the chromosome number as had been encountered in the stomata length. A length of 60 microns or more indicates a chromosome

TABLE II

LEAF EPIDERMIS DATA

	Complex I		Complex II										Complex III					Complex IV				Complex V					
	B. wrightii		B. alta	B. exaristata				B. saccharoides				B. sacc. longi-paniculata	B. barbinoidis var. barbinoidis			B. barbinoidis var. schlumberg.		B. spring-fieldii	B. springfieldii var. australis			B. Edward-siana	B. hybrida			B. hirtifolia		
1. Accession Nr.	470	393	517	493	304	344	345	459	567	572	611	584	401	413	425	440	445	441	577	594	586	458	379	381	383	465	466	467
2. Chrom. Nr. 2n	120	120	120	120	60	60	60	60	60	60	60	120	180	180	180	180	180	120	60	60	60	60	120	120	120	60	60	60
3. Macrohairs	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	2	0	0	0	0	1	1	1
4. Bic. Length	59.3	70.4	71.8	65.3	49.0	52.2	52.4	46.2	52.9	51.0	48.8	50.1	71.3	66.3	63.0	62.3	65.3	68.8	46.4	55.5	59.8	50.6	68.5	73.8	69.7	43.0	49.9	44.9
a. Base	28.7	31.5	38.2	35.4	22.5	26.7	24.8	23.5	24.8	25.7	25.3	26.0	37.3	33.4	32.0	31.7	35.6	29.0	23.0	27.6	29.9	25.8	34.5	35.0	32.2	20.7	25.1	23.2
b. Apical	30.6	38.9	33.6	29.9	26.5	25.5	27.6	22.7	28.1	25.3	23.5	24.1	34.0	32.9	31.0	30.6	29.7	39.8	23.4	27.9	29.9	24.8	34.0	38.8	37.5	22.3	24.8	21.7
c. Ratios	.94	.81	1.14	1.18	.85	1.04	.90	1.03	.89	1.02	1.07	1.08	1.09	1.03	1.03	1.03	1.20	.73	.98	.99	1.00	1.04	1.01	.89	.86	.93	1.01	1.07
5. Prickles																												
a. In Silica Cell Row, "A"	0	0	1	2	1	1	1	1	1	1	1	1	1	1	1	2	2	1	1	1	1	1	1	0	0	2	2	2
b. In Long Cell Row, "B"	0	1-	1	0	0	1	1	1	0	0	1	0	1	1	0	1	1	0	0	0	0	0	1	0	0	0	2	2
6. Overlap	0	0											T	0	0	T	T		0	0	0	0	0	0	0	0	0	0
a. to 1/3			1						1	1	1	1						1										
b. 1/3-1/2								1																				
c. More than 1/2				1	1	1	1																					
7. "Bosses"	0		0	0	0	0	0	0	0	0	0	0								0	0			1	1			
a. In Stom. Row		1											1	1	1	1	1	1	1			1	1			1	1	1
b. In LC														1		1	1	1					1			1	1	1
8. Stomata Length	45.56	49.03	39.17	45.56	31.35	29.98	29.26	32.36	33.35	33.58	31.05	34.52	37.44	40.14	35.58	36.06	36.36	39.81	29.53	29.37	29.28	34.85	36.69	37.24	38.89	36.36	30.48	33.28
9. Fish Shape Cells	0	0	0	0	0	0	0	T	0	0	0	0	0	0	0	0	0	0	0	0	0	0	T	0	0	0	0	0
10. Geographic Location	Mex.	Mex.	Mex.	Mex.	Bra.	Bra.	Bra.	Mex.	Urug.	Urug.	Arg.	Urug.	Mex.	Mex.	Mex.	Mex.	Mex.	Mex.	Urug.	Urug.	Urug.	Mex.	Tex.	Tex.	Tex.	Mex.	Mex.	Mex.
11. Total Bic. & Stom.	106.86	119.43	110.97	99.75	80.35	81.18	81.66	78.56	86.25	84.58	79.85	84.62	108.74	106.44	98.58	98.36	101.66	108.61	75.93	84.87	89.08	85.45	105.19	111.04	108.59	79.36	80.38	78.18

1. 0=none; 1 = less than 300 units; 2= more than 300 units.

2. T indicates transitional forms are observed.

3. In all others number 1 indicates normal occurrence of the feature and 2 indicates greater than normal occurrence.

number of either 2n=120 or 2n=180. Conversely a plant with bicellular hairs less than 60 microns long will have 60 chromosomes. Only two exceptions were found to this rule 11470 with 2n=120 chromosomes had a mean bicellular hair length of only 59.3 microns, and 11584, the B. saccharoides var. longipaniculatum accession, had bicellular hairs of only 50.1 microns. In all other cases the division into the ploidy groups was accurate. The ratio between the length of the basal cell and the apical cell does not serve as an effective method for separating the various polyploid levels. Certain ratios are associated with certain groups of the various taxa found in the accessions studied. A table of the various ratios along with the high mean value for a single accession within the group and a similar low value as well as the mean value for all accessions within the group is shown in Table III.

The general observations that can be drawn from this table are rather limited. If the ratio is greater than 1.14 the plant is likely to be B. alta or B. barbinoidis. If the ratio is less than .94 the plant could be either B. exaristata, B. hirtifolia, B. hybrida, B. saccharoides, B. springfieldii or B. wrightii. As a broad generalization, it has been observed that the accessions with a chromosome number of 2n=60 seem to have ratios around 1.00 while the accessions with chromosome numbers of 180 or 120 may have either higher or lower values. This is merely a trend, however, with many exceptions.

The most effective indicator of chromosome ploidy level from epidermal features for the accessions studied is to combine the mean length of the stomatal guard cells with the mean length of the bicellular hairs. If these two dimensions total more than 95 microns the

TABLE III

RATIOS OF LENGTH OF BASAL CELL TO LENGTH OF APICAL CELL IN BICELLULAR HAIRS

	High	Low	Mean	Chromosome Number
Complex I				
1. *B. hirtifolia*	1.07	.93	1.00	60
2. *B. wrightii*	.94	.94	.94	120
Complex II				
3. *B. alta*	1.18	1.14	1.16	120
4. *B. exaristata*	1.04	.85	.93	60
5. *B. saccharoides*	1.07	.89	1.00	60
6. *B. saccharoides* var. *longipaniculatum*	1.08	1.08	1.08	120
Complex III				
7. *B. barbinoidis* var. *barbinoidis*	1.15	.97	1.06	180
8. *B. barbinoidis* var. *schlumbergeri*	1.20	1.03	1.12	180
Complex IV				
9. *B. springfieldii* var. *springfieldii*	.73	.73	.73	120
10. *B. springfieldii* var. *australis*	1.02	.98	1.00	60
Complex V				
11. *B. edwardsiana*	1.04	1.04	1.04	60
12. *B. hybrida*	1.07	.86	.95	120

chromosome number of the accession should be either 120 or 180. If the total of these two lengths is less than 95 microns then the accession should have a chromosome number of 2n=60. Only one exception to this rule was found in the more than eighty accessions studied. In 11584 the B. saccharoides var. longipaniculatum accession the sum of the lengths is only 84.62 even though it has 120 chromosomes. This accession has the same erratic stomata lengths as the B. saccharoides group which will be discussed in more detail. This may be an explanation of the difficulties with the B. saccharoides var. longipaniculatum accession as well. A listing of the combined lengths of stomata and bicellular hairs is shown in Table II.

Macrohairs were found on the abaxial surface in only two groups, B. hirtifolia and B. springfieldii var. australis. There was a clear difference in the total length of the macrohairs in these two groups; however, the basal construction in both groups was the same. The macrohairs offer an easy method of separating the groups. They are of limited application, unfortunately.

The prickles in the accessions studied were found to occur in both the silica and the long cell rows, but their occurence was of a more erratic nature and they therefore are not as good a feature for taxonomic or systematic study as the other features discussed earlier.

The pappilae form an interesting element of this study. The existence of transitional forms in some accessions indicates that the "donuts" and the overlapping form of the papillae are variations of the same structure. The evidence examined is not conclusive as to which form is more advanced. It could be that the extreme overlapping forms of B. exaristata are the most primitive and the forms like B. wrightii

that often lack even donuts are advanced through reduction or the reverse trend could be in effect with some unknown selective force favoring the development of the overlapping form for either protection or water loss reduction.

Variation in Stomate Size

During the course of this study it was observed that accession 11567, which from all other data appeared to belong to the B. saccharoides group, had what appeared to be an excessively large average value for the length of the stomata guard cells. Its value initially computed was 36.59 microns. The slide was rechecked to preclude an administrative error and it was found that it appeared to have stomata of two rather distinct sizes, one size around 33.50 microns and the other greater than 37.95 microns. Since this type of variation had not been encountered a more extensive survey was made involving 105 stomata measurements. The results of these measurements and their statistical analysis is given in Table IV. It can be seen that the data seems to present the results of two nearly completely separated populations of stomata sizes, rather than normal variation within a single length.

In order to correlate this information more exactly similar statistical analyses were made on other accessions of the B. saccharoides type numbered 11576 and 11611. It was found that they had a standard deviation of 1.56 and 1.50 respectively. This compares closely with the standard deviation of 1.56 which was computed for the shorter group of the 11567 stomata. The data for the longer group of 11567 stomates had a standard deviation of 1.84. The mean value of the longer group was 38.41 microns which is comparable to the mean value

for several of the American Bothriochloas with 120 or 180 chromosomes.

TABLE IV

STATISTICAL DATA ON B. SACCHAROIDES STOMATA LENGTH

	Accession Number	Mean	2SD*	SD**
1.	11567 short stomata	33.35	30.22 - 36.48	1.56
2.	11567 long stomata	38.41	34.73 - 42.09	1.84
3.	11576	31.51	28.39 - 34.63	1.56
4.	11611	31.05	28.06 - 34.04	1.50

*2SD equals 2 standard deviations above and below the mean which should include 90% of the variation in stomata length if normal distribution is observed.

**SD equals the value of 1 standard deviation for the accession indicated.

An additional data search was made of the stomata lengths of all twenty-eight of the B. saccharoides accessions which had been studied epidermally and it was found that fourteen of these had at least one, of the twenty or more stomata which had been measured, with a length greater than 36.34 which would place it in the category of the longer stomata. A similar study was conducted on all of the B. springfieldii var. australis accessions which had been investigated, in this case nine accessions, and while the mean stomata length of the two groups is not very different, there was not a single stomate in the B. springfieldii var. australis group which would exceed the 36.34 micron

length. All of the data in support of this discussion are presented in Tables IV, V, and VI. This evidence seems to substantiate the fact there does exist within the B. saccharoides group an unusual factor which produces stomates of two almost completely different size groups and that this factor is not present in all accessions in this variety.

According to the work of Stebbins and Kush (65) and Stebbins and Shah (66) in 1961, the stomata in a single row of epidermal cells arise from the same initial in a linear fashion. There should be a linear arrangement of the long or short stomates if they are due to nuclear control rather than regional enzymatic influence. In order to initially investigate this type of relationship the 105 stomates were studied by moving, as nearly as possible, down the rows of stomates rather than randomly sampling the entire leaf surface. The measurements from this study are presented in Table VII. It can be seen that there is a linear relationship rather than a random occurrence of the long stomata. It is not a perfect relationship, however. In Table VII, it can be seen that rows 7, 4, 1, and 2 present the clearest linear relationship. The dashed lines in the rows indicate some type of disruption in the normal sequence of stomata and long cells. This disturbance might be a bicellular hair or some malformed cell walls.

Earlier work (68) has shown that in some plants regions of polyploid cells have been found in a basically diploid plant. Obviously a much more extensive investigation would have to be conducted before it could be claimed that this is the mechanism that causes the formation of the long stomates in this case. No record of a similar phenomenon in the Gramineae has been mentioned in Metcalf's (51) work.

TABLE V

STOMATA VARIATION IN THE B. SACCHAROIDES TAXA

	Accession Number	Mean	Number of Large Stomata/ Number Measured
1.	11566	34.68	8/22
2. a.	11567 (short)	33.35	30/105*
b.	11567 (long)	38.41	
3.	11572	33.71	1/20
4.	11574	31.79	0/22
5.	11576	31.46	0/22
6.	11581	32.09	2/20
7.	11582	37.88	17/20
8.	11587	30.88	0/21
9.	11596	31.50	0/22
10.	11597	28.91	0/22
11.	11599	30.15	0/23
12.	11600	30.62	7/22
13.	11601	36.85	14/22
14.	11603	28.24	0/22
15.	11604	33.07	2/22
16.	11605	20.57	0/23
17.	11606	28.08	0/22
18.	11607	30.94	0/22
19.	11608	32.38	1/22
20.	11609	31.44	0/22
21.	11610	32.68	3/22
22.	11611	31.05	0/22
23.	11612	29.14	0/22
24.	11613	35.60	9/22
25.	11614	30.54	0/22
26.	11615	29.76	1/22
27.	11624	33.26	1/22
28.	11625	35.44	9/23

*Total for all 11567 stomata.

TABLE VI

STOMATA VARIATION IN THE B. SPRINGFIELDII VAR. AUSTRALIS TAXA

	Accession Number	Mean	Number of Large Stomata/ Number Measured
1.	11577	29.53	0/21
2.	11589	29.44	0/21
3.	11594	29.37	0/22
4.	11586	29.28	0/22
5.	11592	32.27	0/23
6.	11595	29.87	0/22
7.	11583	29.60	0/21
8.	11585	31.88	0/22
9.	11591	29.97	0/22

TABLE VII

SEQUENTIAL MEASUREMENTS OF STOMATA OF 11567

Row	1	2	3	4	5	6	7
1.	31.74	32.30	31.74	*40.94	*36.80	34.50	32.30
2.	33.35	34.50	32.20	*40.02	*37.95	34.04	32.20
3.	32.20	34.96	31.74	*41.86	30.36	32.66	32.66
4.	34.50	*36.34	30.36	*40.48	*37.72	30.82	31.28
5.	34.04	*36.80	31.74	*41.40	*38.64	31.74	30.36
6.	34.96	*37.26	34.50	*41.40	*43.70	*37.26	30.36
7.	*38.18	*36.80	34.50	*37.26	34.96	*38.64	32.66
8.	32.66	35.42	31.74	*37.26	34.50	33.35	31.74
9.	32.66	*37.95	*39.10	*36.80	*38.18	33.58	30.82
10.	31.74	*36.34	30.36	33.35	*36.80	34.04	29.90
11.	32.66	34.04	34.04	34.50	34.04	36.34	34.04
12.	31.74	34.50	*39.10	34.04	*36.34	*37.26	32.20
13.	33.12	*37.95	34.04	30.82	32.20	*36.80	30.36
14.	34.50	*39.10	35.42	35.42	34.96	34.04	31.74
15.	32.20	*39.10	34.50	*37.26	32.20	34.04	30.36

* 36.34 microns or longer is considered in the long stomata category.

_ _ _ _ Indicates a break in the normal sequence of stomates and long cells.

Conclusions

The abaxial leaf epidermis of 83 accessions of New World *Bothriochloas* from Mexico, Texas, and eastern South America were examined. These accessions were found to be separable into groups principally on the basis of the leaf epidermis study but supplemented in some cases by chromosomal counts and morphological data which have been more fully discussed in earlier chapters of this thesis.

Certain features were found to be characteristic of various taxa studied. These were macrohairs, prickles, papillae, stomata guard cell length, bicellular hair length, and to a less apparent degree the ratio of basal cell length compared to apical cell length in the bicellular hairs.

An unusual feature was observed in the accession 11567 of the *B. saccharoides* group. It appears to have stomata of two almost entirely separate size groups. The smaller stomata had a mean length normal for *Bothriochloas* with $2n=60$. The larger had a mean length equal to many of the *Bothriochloas* with chromosome numbers of $2n=120$ or $2n=180$. Data is presented that indicates the rather widespread occurrence within the *B. saccharoides* group of the two sizes of stomata. No similar phenomenon is encountered in the *B. springfieldii* var. *australis* group, although it also has 60 chromosomes. It is recommended that a much more extensive study be conducted to observe the extent of occurrence of this phenomenon, its probable cause and mechanism of action.

Although some difficulties were experienced with the *B. saccharoides* group, discussed above, it was found that either the length of the stomata or the length of the bicellular hairs could be used as a feature to separate the Bothriochloa's $2n=60$ from those with $2n=120$ or

2n=180 chromosomes. An even more clear cut separation is shown by adding the lengths of these two features. It is not possible to separate the 2n=180 Bothriochloas from the 2n=120 Bothriochloas by a similar system.

CHAPTER IV

CYTOGEOGRAPHY

Materials and Methods

Two types of cytological studies were conducted to determine the chromosome numbers, general nature of the karyotype, and the pairing at meiosis in the American Bothriochloa accessions studied. In those cases where the pollen mother cells were investigated the following procedure was used. The inflorescences were gathered at the proper stage of development and were fixed in a modified Carnoy's fluid (absolute alcohol, glacial acetic acid, and chloroform in a 6:3:1 proportion). Fixation was done between 9:30 and 11:00 a.m. The fixed material was stored in a refrigerator until squash preparations were made of the anthers using aceto-carmine stain according to Belling's (3) method. Pairing was scored on ten cells for each accession number studied in this manner. The meiotic metaphase I cells were studied using an oil immersion lens and a magnification of 1425x. Photomicrographs of selected cells were taken at 1350x magnification.

In those cases where root tip squash preparations were made the following procedure was used. The root tips were collected from the accessions growing in the uniform garden between 10:00 a.m. and 2:00 p.m. throughout the growing season. The material was pretreated in a saturated solution of alpha-bromo napthalene for forty-five minutes and then transferred after several washings. The storage solution

consisted of absolute alcohol and acetic acid (3:1 proportion) plus two drops of ferric chloride. To prepare the root tips to make slides, they were placed in acetocarmine stain and heated to 60 degrees centigrade for twenty minutes. The tips were then removed and squashed between two slides. One drop of acetocarmine was then added and the root tip debris material was removed and pressed between forceps to concentrate the quantity of suitable cells and remove obstructions which would prevent later chromosome spreading under pressure. The root tip cells were studied and photographed in the same manner as the pollen mother cells. In certain cases camera lucida drawings were felt to more accurately represent the chromosomes because they were not all in the same plane.

Discussion

The results of the cytological studies of twenty-seven accessions of the New World Bothriochloas using the methods established above lead to the following conclusions. The New World Bothriochloas have three principal chromosome numbers $2n=60$, $2n=120$ and $2n=180$. When this is correlated with earlier cytological work in the Bothriochloa, Dichanthium, Capillipedium complex, it is clear that these chromosome numbers represent hexaploids, dodecaploids, and octadecaploids of the basic $n=10$ chromosome number found within the complex. These are the highest values found throughout the world-wide distribution. All chromosomes examined in a metaphase condition exhibited the small size characteristic of the Panicoid group (63). All of the meiotic chromosomes examined within this group exhibited complete bivalent formation. This is probably due to genetic control (31).

PLATE X

CYTOLOGY OF SELECTED *BOTHRIOCHLOA* SPECIES

All figures ca. 1350X.

Legend:

Figure 30: *B. barbinoidis* var. *barbinoidis*, meiotic metaphase, showing 90 bivalents, from anther squash.

Figure 31: *B. hirtifolia*, mitotic early metaphase, showing $2n=60$ chromosomes, from root tip squash.

Figure 32: *B. exaristata*, diakinesis in meiosis, showing 30 bivalents, from anther squash.

Figure 33: *B. springfieldii* var. *springfieldii*, mitotic metaphase, showing $2n=120$ chromosomes from root tip squash.

PLATE X

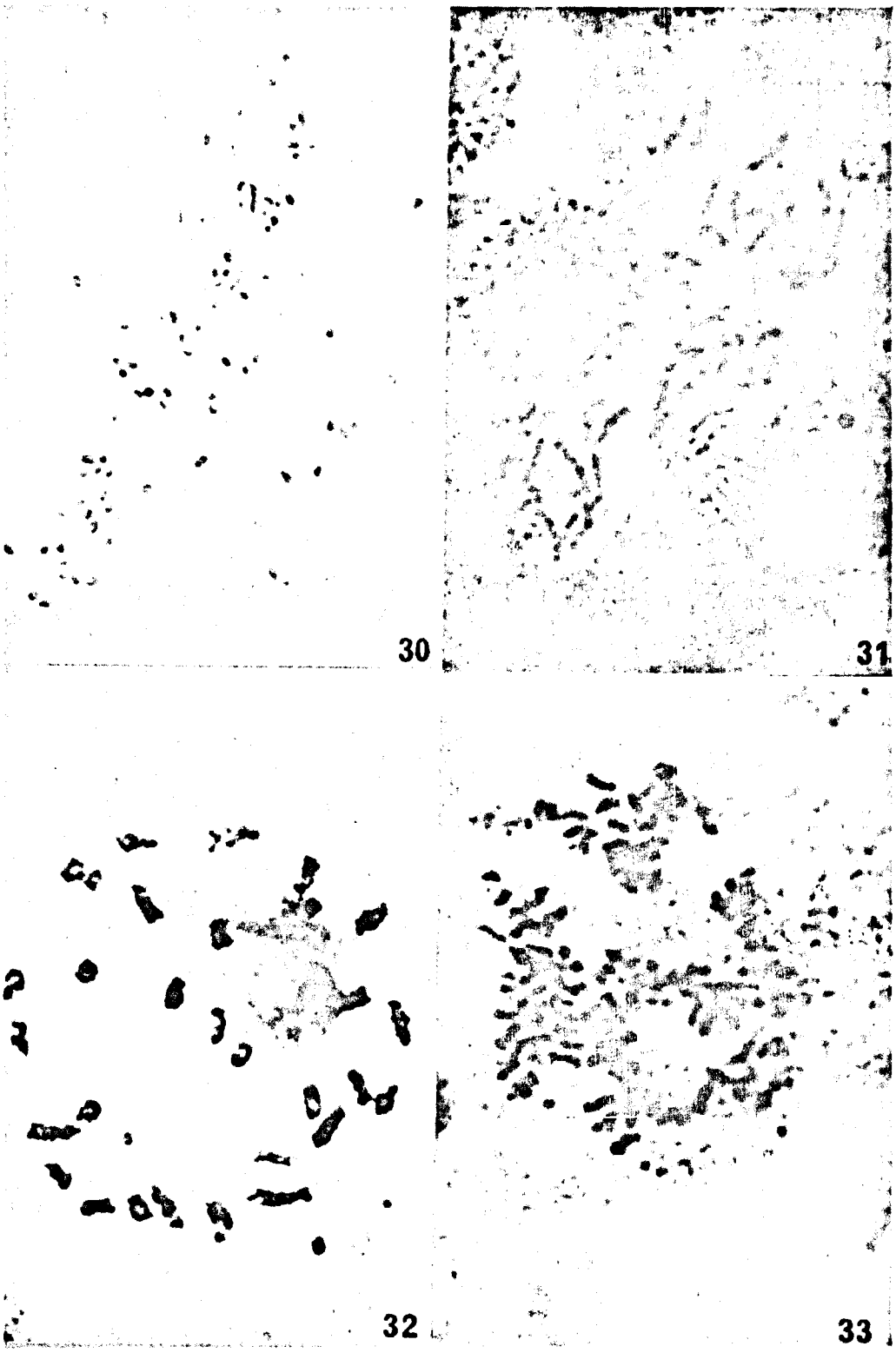


PLATE XI

CYTOLOGY OF SELECTED *BOTHRIOCHLOA* SPECIES

Camera lucida drawings of metaphase chromosomes from root tip squash preparations. All figures at ca. 1350X.

Legend:

Figure 34: *B. springfieldii* var. *australis*, 2n=60, Complex IV.

Figure 35: *B. saccharoides* var. *saccharoides*, 2n=60, Complex II.

Figure 36: *B. springfieldii* var. *australis*, 2n=60, Complex IV.

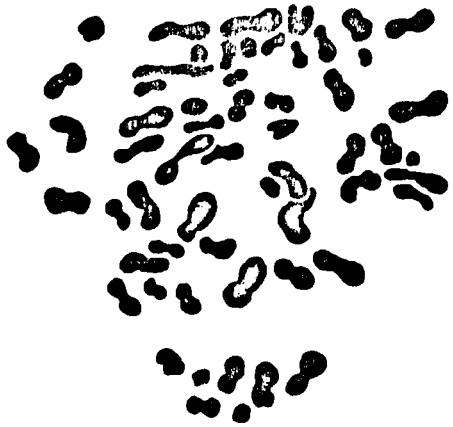
Figure 37: *B. saccharoides* var. *saccharoides*, 2n=60, Complex II.

Figure 38: *B. alta*, 2n=120, Complex II.

Figure 39: *B. barbinoidis* var. *barbinoidis* (villous form), 2n=180, Complex III.

PLATE XI

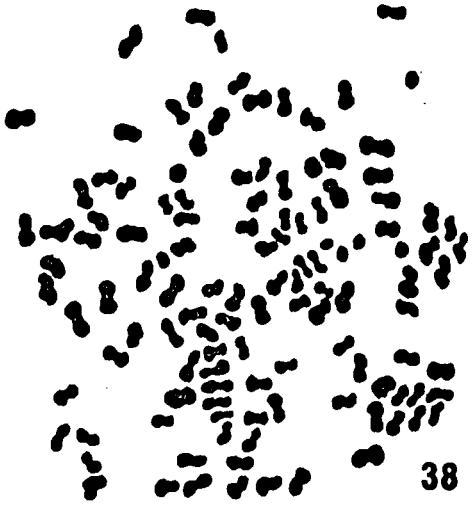
34



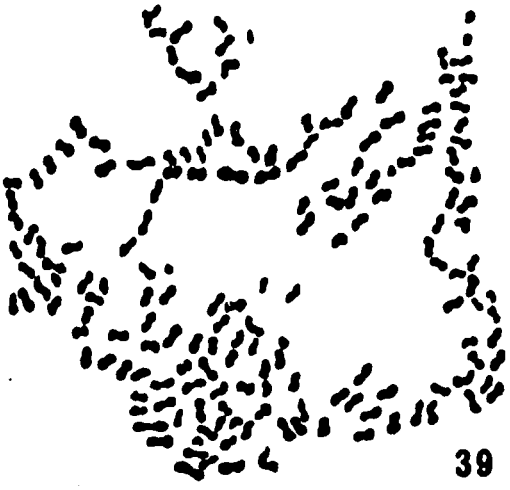
35

36

37



38



39

When the chromosome numbers of the various accessions are compared with the species complexes that were developed on the basis of morphological affinities it can be seen that only in the B. barbinoidis complex is the 2n=180 chromosome number found. All four of the other complexes have species or varieties with 2n=120 and 2n=60 chromosome numbers. The correlation between the chromosome numbers and the epidermal features of stomata length and bicellular hair length has been clearly set forth in the chapter on the leaf anatomy.

Migration

Hurley (48) and others have recently brought increasing support to the continental drift concept. Using evidence from Glossopteris and Gangamopteris beds as well as other information such as the modifications of the earth's magnetic fields, the disproportionately thin levels of silt on the western coast of Africa, the unusual geological patterns in the Atlantic Ocean floor, and the distribution of the extremely ancient rock formations, these geologists conclude that the major continents that currently exist must have been joined during the Mesozoic era.

If Hartley's (46) distribution of the Andropogonae is superimposed over the Gondwana land mass, as presented in Hurley's paper, an interesting insight into the distribution of these plants is immediately apparent. It can be seen that a likely center of origin for the Andropogonae would be found in India, where there is obviously a center of diversity. It can also be seen that a likely chain of migration exists from this center in several directions. One arm of this migration would come from the Indian center and pass across northern

PLATE XII

CYTOGEOGRAPHY OF THE MEXICAN REGION

Each symbol represents the site of collection of one of the accessions. The square symbol indicates a chromosome number of 2n=60. The triangular symbol indicates 2n=120 and the circular symbol indicates 2n=180. The number within the symbol represents the species collected.

Legend:

Figure 40:

1. = *B. alta*
2. = *B. barbinoidis* var. *barbinoidis*
3. = *B. barbinoidis* var. *schlumbergeri*
4. = *B. edwardsiana*
5. = *B. exaristata*
6. = *B. hirtifolia*
7. = *B. hybrida*
8. = *B. saccharoides* var. *saccharoides*
9. = *B. saccharoides* var. *longipaniculata*
10. = *B. springfieldii* var. *springfieldii*
11. = *B. springfieldii* var. *australis*
12. = *B. wrightii*

PLATE XII

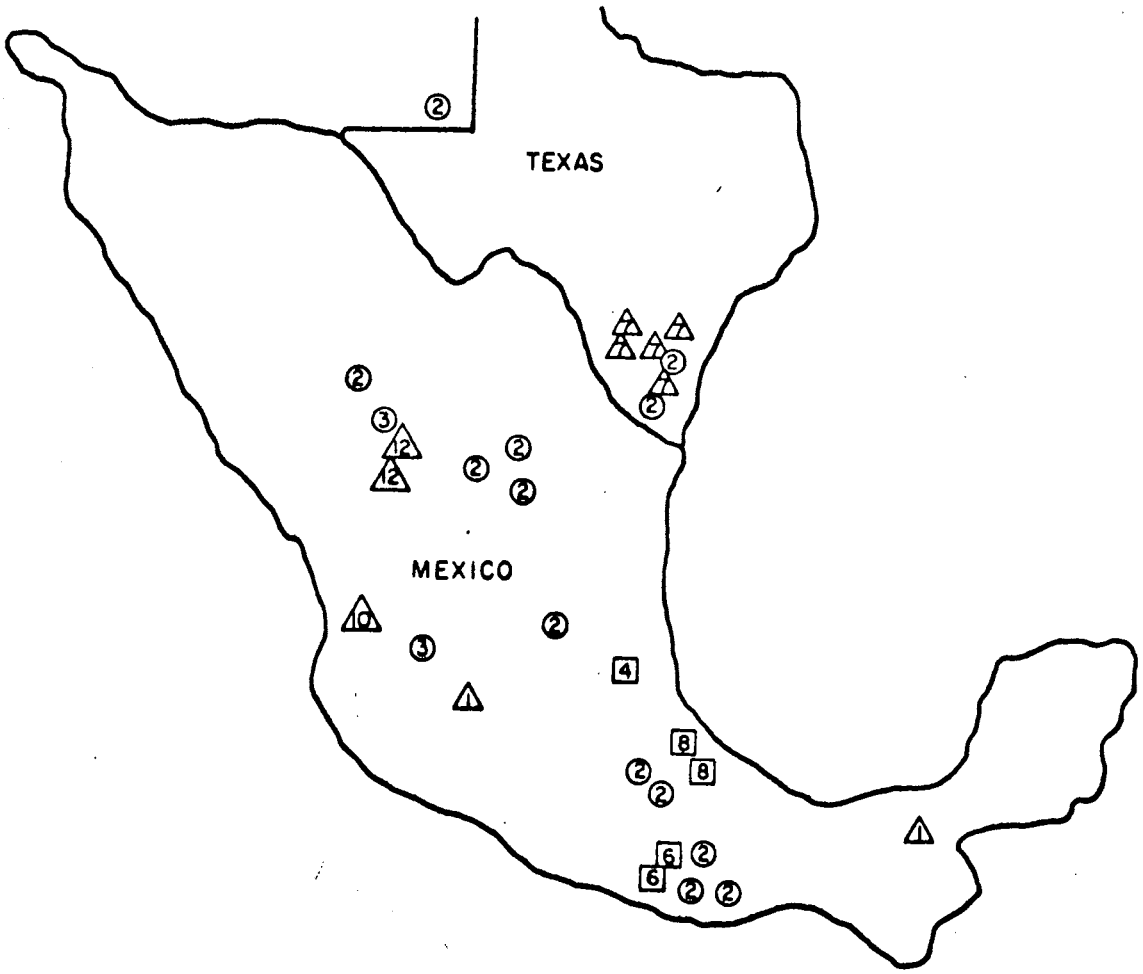


Figure 40

PLATE XIII

CYTOGEOGRAPHY OF THE SOUTH AMERICAN REGION

Each symbol represents the site of collection of one of the accessions. The square symbol indicates a chromosome number of 2n=60. The triangular symbol indicates 2n=120 and the circular symbol indicates 2n=180. The number within the symbol represents the species collected.

Legend:

Figure 41:

1. = B. alta
2. = B. barbinoidis var. barbinoidis
3. = B. barbinoidis var. schlumbergeri
4. = B. edwardsiana
5. = B. exaristata
6. = B. hirtifolia
7. = B. hybrida
8. = B. saccharoides var. saccharoides
9. = B. saccharoides var. longipaniculata
10. = B. springfieldii var. springfieldii
11. = B. springfieldii var. australis
12. = B. wrightii

PLATE XIII

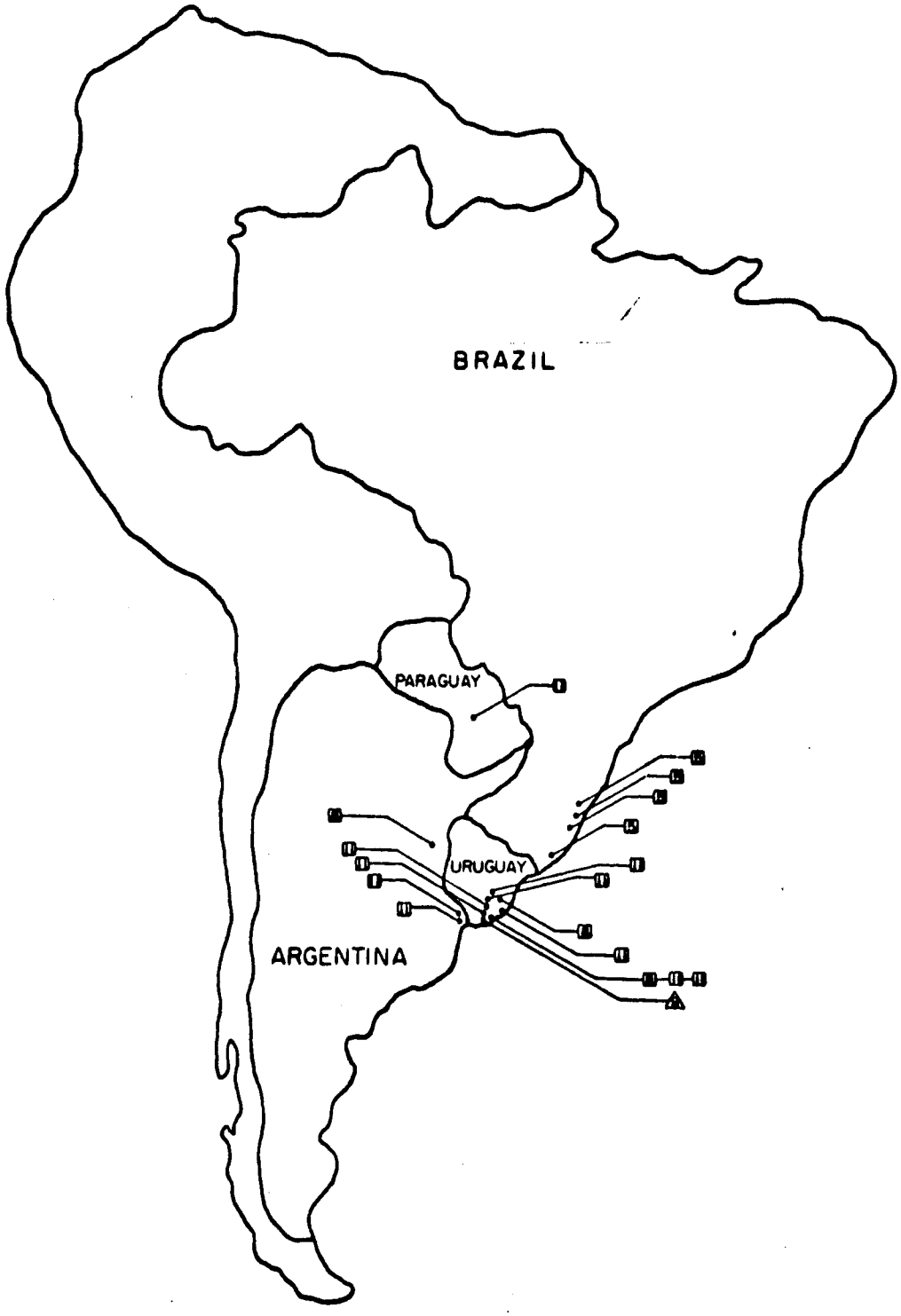
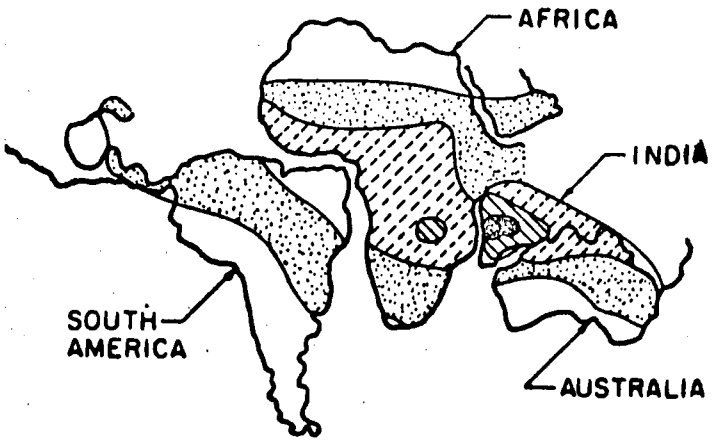


Figure 41

PLATE XIV

DISTRIBUTION OF ANDROPOGONAE



40% of the Known Andropogonae in Floras of This Region

30% of the Known Andropogonae in Floras of This Region

20% of the Known Andropogonae in Floras of This Region

10% of the Known Andropogonae in Floras of This Region

1. Arrangement of land masses (After Hurley, 1968).
2. Information on flora data occurrence of Andropogonae (After Hartley, 1958).

Figure 42

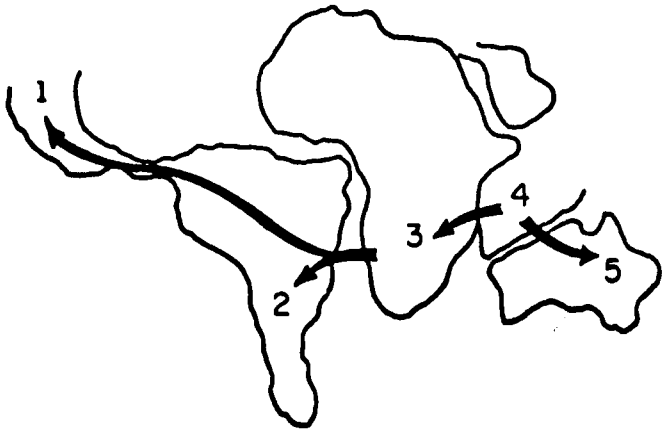
Australia and spread along the eastern portion of Australia. A second migratory arm would move north, with the movement of the Indian land mass, and under favorable climatic conditions, these Andropogonae could spread to the east to establish themselves on the Chinese and Indochinese land areas. A third migratory arm of this grass tribe would take the Andropogonae across the southern part of Africa and into the New World land mass. The probable entry point would be in the region of present-day Uruguay, Argentina and southern Brazil. This migratory arm could then have swung north to give rise to the present North American representatives of the Andropogonae tribe or a second migratory arm could have passed from Africa at a more northerly point and in this manner given rise to the North American Andropogonae.

In considering the Bothriochloa, Capillipedium and Dichanthium generic complex within the Andropogonae, an interesting pattern involving the polyploid levels can be seen. The diploids ($2n$-20) and tetraploids ($2n$=40) are relatively more common in India. The tetraploids and hexaploids are relatively more common in Africa and in Australia. An almost complete dominance of the hexaploids is found in the South American region, while the Mexican region has some hexaploids and a high percentage of dodecaploids and octadecaploids, which are not found in any other locality.

It appears that the Bothriochloas entered the New World at the hexaploid level from Africa. Their increase in ploidy level as they encountered the more variable ecological situations as they spread northward is in accordance with Stebbins' (62) concepts for this type of migration. A similar migration can be postulated for the Dioscoreas (57). The Dioscoreas of South America occur in a polyploid

PLATE XV

DISTRIBUTION OF POLYPLOIDY



	Percentages				
	2n	4n	6n	12n	18n
1. Mexican Region	0	0	17	33	50
2. South American Region	0	0	94	6	0
3. African Region	0	85	15	0	0
4. Indian Region	7	78	15	0	0
5. Australian Region	0	72	28	0	0

Figure 43

series with a basic chromosome number of nine, while the African members of this group occur in two polyploid series, one with a basic chromosome number of nine and the other with a basic chromosome number of ten. The Asian Dioscoreas all have a basic chromosome number of ten.

Within the New World Bothriochloas certain other distributional patterns have been observed. B. exaristata in the collections investigated was narrowly endemic to the coastal regions of Brazil, while Gould (35) has reported its occurrence in the coastal areas of Texas and Louisiana. The species B. edwardsiana or one similar to it must have brought the glume pit character into the Mexican region where it has now become established in B. barbinoidis, B. wrightii and B. hybrida. The species B. saccharoides was found, in the collections investigated, to occur in both the Mexican region and in the South American region.

Conclusions

The cytological examination of twenty-seven accessions using the procedures of anther and root tip squashes provided data on the chromosome numbers and karyotype. These studies in general confirmed the concept that the New World Bothriochloas consist of a polyploid series with a basic chromosome number of $n=10$. The New World representatives occur predominantly at $2n=60$, $2n=120$, and $2n=180$. The geographic distribution of the different polyploid levels seems to indicate that the New World Bothriochloas have been derived from a $2n=60$ ancestoral type that occurred in Africa in Cretaceous times.

CHAPTER V

CONCLUSIONS

From the study of the morphology, leaf anatomy, cytology and geographic distribution of the New World Bothriochloa accessions conducted during the past two years, it is possible to reach the following principal conclusions.

The New World representatives of the genus Bothriochloa can be divided into five species complexes primarily on morphological grounds but substantiated by the other means of investigation. The first complex contains the species B. campii (Swallen) de Wet, B. hirtifolia (Presl.) Henr., B. wrightii (Hack) Henr. and is characterized by the possession of pedicellate spikelets as long and as broad as the sessile ones. Some members of this complex have the glume pit character and the distribution of the complex is restricted to the Mexican region. The chromosome numbers of the members of this complex include both $2n=60$ and $2n=120$. The epidermal features of most significance include the presence of short macrohairs on B. hirtifolia and the absence of significant papillae overlap over the stomates throughout the complex.

The second species complex includes B. alta (Hitchc.) Henr., B. exaristata (Nash.) Henr., and B. saccharoides (Swartz.) Rydb. with two varieties var. saccharoides and var. longipaniculata (Gould) Gould. This complex is chiefly characterized by their cylindrical inflorescence, narrow pedicellate spikelets, and long main axis relative to the

length of the lower racemes. Its members were collected both in the Mexican and the South American regions. None of the species in this complex possess the glume pit character. The chromosome numbers include 2n=60 and 2n=120. No species within this complex possesses macrohairs. All of the species within the complex have the epidermal feature of papillae which overlap their stomata at least to one-third the length of the stomate.

The third complex is morphologically the most variable. The members are B. barbinoidis (Lag.) Herter with three varieties var. barbinoidis, var. palmeri (Hack.) de Wet, and var. schlumbergeri (Fourn.) de Wet (26). This complex is characterized by pedicellate spikelets that are reduced, ovate to obtriangulate inflorescences, and the lower inflorescence branches at least as long as the main axis of the inflorescence. This complex was found exclusively in the Mexican region and has the chromosome number of 2n=180 for all of its members. The glume pit character is found in some but not in all members of this complex. Epidermally the members of this complex possess no macrohairs and generally the papillae do not overlap the stomata to a significant extent.

The fourth complex contains the single species B. springfieldii (Gould) Parodi with two varieties var. springfieldii found only in the Mexican region and var. australis de Wet found only in the South American region. Morphologically the complex is characterized by its digitate inflorescence, small pedicellate spikelet and white villous racemes. None of the members of this complex possess the glume pit character. The variety australis has a chromosome number of 2n=60 and the variety springfieldii has a chromosome number of 2n=120. The

significant epidermal features of the complex include the occurrence of long macrohairs in some members of the australis variety, the absence of prickles in the long cell rows, and the general absence of significant papillae overlap over the stomata.

The last complex includes B. edwardsiana (Gould) Parodi, B. hybrida (Gould) Gould, and B. reevesii (Gould) Gould. This complex is morphologically characterized by pronounced digitate inflorescence, small pedicellate spikelets, and less hairy racemes. B. reevesii and B. hybrida are extremely similar differing only in the fact that B. hybrida possesses the glume pit character while it is not found in B. reevesii. B. edwardsiana also possesses the glume pit character distinctly. All members of this complex are found in the Mexican region. B. edwardsiana has a chromosome number of $2n=60$ while both the other species have a chromosome number of $2n=120$. Epidermally the complex is characterized by the absence of macrohairs and the general absence of papillae overlap over the stomata.

Other conclusions produced from these studies include the confirmation that the New World Bothriochloas form a polyploid complex with the basic chromosome number of $n=10$. These investigations also revealed that in the South American region the common chromosome number was $2n=60$, while in the Mexican region $2n=120$ and $2n=180$ very commonly occurred.

The investigations into the leaf anatomy established that it was possible to distinguish specimens with chromosome numbers of $2n=120$ and $2n=180$ from those with a chromosome number of $2n=60$ by measuring either the length of the stomata guard cells or the length of the bicellular hairs. An even more complete distinction can be obtained by combining

these two measurements. A significant abnormality was observed in the measurements of the B. saccharoides stomata guard cells. It was observed that in several accessions belonging to this species the stomata appeared to occur in two distinct sizes rather than the normal variation that is found in other species. Because of the essentially linear pattern of distribution that was found in the different sizes of B. saccharoides stomata, it has been postulated that this may be caused by regions of cells possessing a doubled chromosome number (a mosaic pattern).

It was also observed that the many features of the leaf epidermis, i.e., macrohairs, bicellular hairs, prickles, papillae, etc., provide consistent patterns of occurrence which can be used to discriminate, in many cases, between the various species that belong to the New World Bothriochloa.

Finally, based on all of the studies contained in this thesis and the literature search that has accompanied them plus a review of current geological concepts, it has been postulated that the New World Bothriochloas probably arose from hexaploid ancestors in the African land mass prior to the occurrence of the continental drift in the Cretaceous period. A portion of this ancient migration carried the glume pit character to the Mexican region.

BIBLIOGRAPHY

1. Arber, A. 1965. The Gramineae. Stechert-Hafner Service Agency, Inc., pp. 1-480.

2. Avdulov, N. 1931. "Karyosystematische Untersuchung der Familie Gramineen." Bull. Appl. Bot., Genet., Pl. Breed., Suppl., Vol. 44, pp. 1-428.

3. Belling, J. 1926. "The Iron-Acetocarmine Method of Fixing and Staining Chromosomes." Biol. Bul., Vol. 50, pp. 160-162.

4. Bor, N. L. 1960. The Grasses of Burma, Ceylon, India and Pakistan. Pergamon Press.

5. Borgaonkar, D. S. 1963. "Apomixis and Speciation in the Bothriochloinae." (Unpublished Ph.D. thesis, Oklahoma State University, Stillwater).

6. _____ and J. M. J. de Wet. 1961. "Interspecific Hybrids in Bothriochloa III. Relationships of Some American Species." Proc. Okla. Acad. Sci., Vol. 41, pp. 10-13.

7. _____ and _____. 1960. "A Cytogenetical Study of Hybrids Between Dichanthium annulatum and Dichanthium fecundum." Phyton, Vol. 15(2), pp. 137-144.

8. _____ and _____. 1963. "Cytological Studies in Some Segregating Populations of Hybrids Within the Bothriochloinae." Cytologia, Vol. 28(1), pp. 54-67.

9. Brown, W. V. 1958. "Leaf Anatomy in Grass Systematics." Bot. Gaz., Vol. 119, pp. 170-178.

10. _____. 1950. "A Cytological Study of Some Texas Gramineae." Bul. Torrey Bot. Club, Vol. 77, pp. 63-76.

11. Carnahan, H. L., and H. D. Hill. 1961. "Cytology and Genetics of Forage Grasses." Bot. Rev., Vol. 27, pp. 1-162.

12. Celarier, R. P. 1957. "The Cyto-Geography of the Bothriochloa ischaemum Complex II. Chromosome Behavior." Amer. Jour. Bot., Vol. 44(9), pp. 729-738.

13. _____. 1959. "Cytogeography of the Old World Bothriochloinae." Proc. IXth Int. Bot. Cong., Vol. 2, p. 62 (abstr.).

14. Celarier, R. P. and J. R. Harlan. 1956. "An Andropogoneae Garden in Oklahoma" Taxon, Vol. 5, pp. 183-186.

15. _____ and _____. 1958. "Apomixis in Bothriochloa, Dichanthium and Capillipedium." Phytomorph, Vol. 7, pp. 93-102.

16. _____ and _____. 1958. "The Cytogeography of the Bothriochloa ischaemum complex. Gramineae. I. Taxonomy, and Geographic Distribution." J. Linn. Soc. (Bot.), Vol. 55, pp. 755-760.

17. _____ and K. L. Mehra. 1958. "Determination of Polyploidy From Herbarium Specimens." Rhodora, Vol. 60(712), pp. 89-97.

18. _____, _____ and M. L. Wulf. 1958. "Cytogeography of the Dichanthium annulatum complex." Brittonia, Vol. 10, pp. 59-72.

19. Clausen, J. 1961. "Introgression Facilitated by Apomixis in Polyploid Poas." Euphytica, Vol. 10, pp. 87-94.

20. Davis, D. H. and V. H. Heywood. 1965. Principles of Angiosperm Taxonomy. New York, D. Van Nostrand Co., Inc.

21. Davidson, J. F. 1947. "The Polygonal Graph for Simultaneous Portrayal of Several Variables in Population Analysis." Madrono, Vol. 9, pp. 105-110.

22. de Wet, J. M. J. 1954. "Stomatal Size as a Cytological Criteria in Danthonia." Cytologia, Vol. 19, pp. 176-181.

23. _____. 1954. "The Genus Danthonia in Grass Phylogeny." Am. Jour. Bot., Vol. 41, pp. 204-211.

24. _____. 1965. "Diploid Races of Tetraploid Dichanthium Species." American Naturalist, Vol. 99(906), pp. 167-172.

25. _____. 1963. "Aneuploidy and Apomixis in Bothriochloa and Dichanthium." Bot. Gaz., Vol. 124(6), pp. 437-439.

26. _____. 1968. "Biosystematics of Bothriochloa barbinoidis (Gramineae)." Amer. Jour. Bot. (in press).

27. _____ and D. S. Borgaonkar. 1963. "Aneuploidy and Apomixis in Bothriochloa and Dichanthium." Bot. Gaz., Vol. 124(6), pp. 437-440.

28. _____, _____ and H. R. Chheda. 1961. "Intergeneric Hybrids in Bothriochloinae. II. Bothriochloa and Capillipedium." Cytologia, Vol. 26, pp. 268-273.

29. de Wet, J. M. J., D. S. Borgaonkar and W. L. Richardson. 1963. "Chromosome Number and Mode of Reproduction in Bothriochloinae." Caryologia, Vol. 16, pp. 47-55.

30. _____ and J. R. Harlan. 1966. "Morphology of the Compilospecies Bothriochloa intermedia." Am. Jour. Bot., Vol. 52 (53), pp. 94-98.

31. _____, _____ and W. L. Richardson. 1965. "Biosystematics of the Bothriochloininae (Andropogoneae, Gramineae) A Report of Progress, 1960-65." Stillwater: Oklahoma State University.

32. _____ and B. D. Scott. 1965. "Essential Oils as Taxonomic Criteria in Bothriochloa." Bot. Gaz., Vol. 126(3), pp. 209-214.

33. Faruqi, S. A. 1961. "Leaf Epidermis in Bothriochloa." Proc. Okla. Acad. Sci., Vol. 42, pp. 26-30.

34. _____. 1963. "Cytogeography of the Bothriochloa intermedia Complex." (Unpublished Ph.D. thesis, Oklahoma State University, Stillwater).

35. Gould, F. W. 1953. "A Cytotaxonomic Study of the Genus Andropogon." Am. Jour. Bot., Vol. 40, pp. 297-306.

36. _____. 1957. "Pollen Size as Related to Polyploidy and Speciation in the A. saccharoides - A. barbinoidis Complex." Brittonia, Vol. 9, pp. 71-75.

37. _____. 1956. "Chromosome Counts and Cytotaxonomic Notes on the Grasses of the Tribe Andropogonae." Am. Jour. Bot., Vol. 43, pp. 395-404.

38. _____. 1957. "New North American Andropogons of the Subgenus Amphilophis and a Key to Those Species Occurring in the U. S." Madrono, Vol. 14, pp. 18-29.

39. _____. 1959. "The Glume Pit of Andropogon barbinoidis." Brittonia II, pp. 182-187.

40. Harlan, J. R. 1963. "Two Kinds of Gene Centers in Bothriochloininae." The American Naturalist, Vol. 97(893), pp. 91-97.

41. _____, M. H. Brooks, D. S. Borgaonkar, and J. M. J. de Wet. 1964. "Nature of Inheritance of Apomixis in Bothriochloa and Dichanthium." Bot. Gaz., Vol. 125, pp. 41-46.

42. _____, R. P. Celarier, W. L. Richardson, M. H. Brooks, and K. L. Mehra. 1958. "Studies on Old World Bluestems, II." Okla. Agric. Exp. Sta. Tech. Bull. T-72.

43. Harlan, J. R., J. M. J. de Wet, W. L. Richardson and H. R. Chheda. 1961. "Studies on Old World Bluestems. III." Okla. Agric. Exp. Sta. Tech. Bull., T-92.

44. _____ and _____. 1963. "The Compilospecies Concept." Evolution, Vol. 17(4), pp. 497-501.

45. _____ and _____. 1963. "Role of Apomixis in the Evolution of the Bothriochloa - Dichanthium Complex." Crop Sci., Vol. 3, pp. 314-316.

46. Hartley, W. 1958. "Studies on the Origin, Evolution, and Development of the Gramineae I, The Tribe Andropogoneae." Austr. J. Bot., Vol. 6, pp. 116-128.

47. Heslop-Harrison, J. 1961. "The Function of the Glume Pit and the Control of Cleistogamy in Bothriochloa decipiens (Hack.) C. E. Hubbard." Phytomorph., Vol. 10, pp. 378-383.

48. Hurley, P. M. 1968. "The Confirmation of Continental Drift." Sci. Amer., Vol. 218, pp. 53-65.

49. Knox, R. B. 1967. "Apomixis: Seasonal and Population Differences in a Grass." Science, Vol. 157, pp. 325-326.

50. _____ and J. Heslop-Harrison. 1963. "Experimental Control of Aposporous Apomixis in a Grass of the Andropogoneae." Botaniska Notiser, Vol. 116, pp. 127-141.

51. Metcalfe, C. R. 1960. Anatomy of the Monocotyledons, I. Gramineae. London: Oxford University Press.

52. Myers, W. M. 1947. "Cytology and Genetics of Forage Grasses." Bot. Rev., Vol. 13, pp. 318-421.

53. Nygren, A. 1949. "Apomictic and Sexual Reproduction in Calamagrostis purpurea." Hereditas, Vol. 35, pp. 285-300.

54. _____. 1951. "Embryology of Poa." Carnegie Inst. Washington Year Book, Vol. 50, pp. 113-115.

55. Parodi, L. R. 1958. Gramineas Bonaerenses (Quinta Edicion). Buenos Aires.

56. Prat, H. L. 1936. "La Systematique des Graminees." Ann. Sci. Nat., 10e. Ser., Vol. 18, pp. 165-258.

57. Ramachandran, K. 1962. "Dioscorea Chromosome Numbers." Journal of the Indian Botanical Society, Vol. 41, pp. 93-98.

58. Richardson, W. L. 1958. "A Technique of Emasculating Small Grass Florets." Indian J. Genet. Plt. Breed., Vol. 18, pp. 69-73.

59. Saran, S. 1968. "Mode of Reproduction in the Compilospecies Dichanthium intermedium. (R. Br.) de Wet et Harlan." (Unpublished Ph.D. thesis, Oklahoma State University, Stillwater).

60. Singh, A. P. and J. M. J. de Wet. 1960. "Interspecific Hybrids in Bothriochloa. I. Relationships Between B. ambigua and B. ischaemum." Phyton, Vol. 15(2), pp. 159-162.

61. _____ and _____. 1961. "Interspecific Hybrids in Bothriochloa II. Relationship Between Some American and Australian Species." Proc. of the Okla. Acad. of Sci., Vol. 41, pp. 35-38.

62. Stebbins, G. L. 1950. Variation and Evolution in Plants. New York: Columbia Univ. Press.

63. _____. 1956. "Cytogenetics and Evolution of Grasses." Am. Jour. Bot., Vol. 43, pp. 890-905.

64. _____ and B. Crampton. 1959. "A Suggested Revision of the Grass Genera of Temperate North America." Recent Advances in Botany, pp. 133-145.

65. _____ and G. S. Khush. 1961. "Variation in the Organization of the Stomatal Complex in the Leaf Epidermis of Monocotyledons and Its Bearing on Their Phylogeny." Am. Jour. Bot., Vol. 48(1), pp. 51-59.

66. _____ and S. S. Shah. 1960. "Developmental Studies of Cell Differentiation in the Epidermis of Monocotyledons." Dev. Bio., Vol. 2(6), pp. 477-500.

67. Tateoka, T. 1959. "Notes on Some Grasses IX. Systematic Significance of Bicellular Microhairs of Leaf Epidermis." Bot. Gaz., Vol. 121, pp. 80-91.

68. Wardlaw, C. W. 1952. Phylogeny and Morphogenesis. London: Macmillan and Co., Ltd.

69. Zatyko, J. M. 1963. "Parthenocarpy Induced in Sterile Ribes Species by Gibberellic Acid." Naturwissenschaften, Vol. 50, pp. 230-231.

VITA

Gilbert Boughton Fletcher

Candidate for the Degree of

Master of Science

Thesis: SOME BIOSYSTEMATIC CONSIDERATIONS OF THE GENUS BOTHRIOCHLOA

Major Field: Botany and Plant Pathology

Biographical:

Personal Data: Born in Boston, Massachusetts, April 20, 1935, the son of Mr. and Mrs. Gilbert Fletcher.

Education: Graduated from Freeport High School, Freeport, New York, in June, 1953; received the Bachelor of Science degree from Centenary College of Shreveport, Louisiana, in 1956, with a major in biology. Completed requirements for the Master of Science degree at Oklahoma State University in July, 1968.

Professional Experience: Officer in the Chemical Corps of the U. S. Army for the past 11 years.