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CULM AND LEAF ANATOMY OF THE *BOTHRIOCHLOA INTERMEDIA* COMPLEX. *

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Abstract

Culm and leaf anatomy of the members belonging to the *Bothriochloa intermedia* complex were studied. The purpose of this study was twofold: first, to evaluate the usefulness of anatomy regarding the intergeneric and interspecific relationships among the members of this group: secondly, to check the validity of the anatomical features of these plants for the prediction of hybrids. For reconstruction, origin and interrelationships among the members of the genera *Bothriochloa, Capillipedium* and *Dichanthium* anatomy was found quite valuable when used in correlation to morphology, cytology and geographic distribution.

Introduction

During the past ten years intensive studies in morphology, cytogeography, cytogenetics, genetics, and embryology were made in the grass laboratory at Stillwater, Oklahoma, in an effort to understand the intergeneric and interspecific relationships of the genera *Bothriochloa A.* Camus, *Capillipedium* Stapf, and *Dichanthium* Willmet (Brooks, 1958, Celarier, 1957, Celarier and Harlan 1956, 1957, 1958, DeWet Harlan, 1968; DeWet *et al.* 1961, 1966; Faruqi, 1964, 1969; Harlan and Celarier, 1961). The members of these genera are distinct, but intergrade with each other wherever they overlap. The most active from this point of view are the members of the *B. intermedia* complex, which hybridize in nature with other taxa of the genus *Bothriochloa* as well as with some taxa of the other two genera. Morphological interpolations have been useful in establishing the relationships of the *B. intermedia* complex (Faruqi, 1969); however, difficulties are encountered in the classification due to intergradation of the various characters. Cytogenetical studies provide rather limited understanding of these relationships due to apomixis, polyploidy, and especially autosyndetic pairing of chromosomes (DeWet *et al.* 1961, Faruqi 1964).

Anatomical studies of the leaves and culm of a few species belonging to the genera *Bothriochloa, Capillipedium* and *Dichanthium* were made by Sabnis (1921), Vickery (1935), Brown (1958), and Metcalf (1960). Most of these studies, however, were restricted to a few species. The conclusions drawn by Brown (1958) regarding the similarities of leaf anatomy among the members of the tribe Andropogoneae cannot be applied very strictly. Brown (1958) looked for certain common characteristics that exist between the members of this group. Reynolds (1959), who studied leaf anatomy of Andropogoneae in far greater detail than any of the previous workers, pointed out significant differences at species level which could be helpful in the classification of Andropogoneae. This study of Reynolds, though lacking in the synthesis of demonstrating relationships, was a great step

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forward in pointing out the fact that interspecific anatomical differences in the leaf do exist among the members of the tribe Andropogoneae.

A limited value of culm anatomy in grasses due to the variation at different internode was pointed out by Metcalf (1960). DeWet (1960), on the other hand, showed that reliable information could be obtained if all the material was selected from comparable positions. Informations obtained from the study of leaf epidermis among the members of the genera *Bothriochloa*, *Capillipedium*, and *Dichanthium* were found valuable in demonstrating relationships between these genera. This study was found useful also in predicting natural hybrids (Faruqi, 1961). In the light of these early studies sections of culm and leaves of members of the *B. intermedia* complex were studied with an objective to correlate the anatomical characters with those of morphology for species differences, relationships, and also to check the validity of hybrids predicted on the basis of morphology.

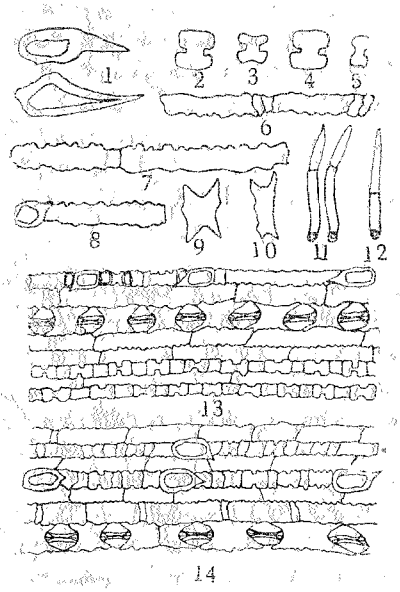
Materials and Methods

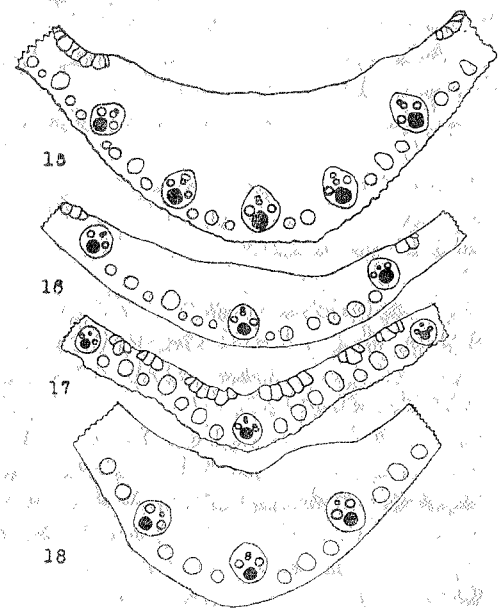
Members of the *Bothriochloa intermedia* complex and the diploid *B. longifolia* were collected from the Oklahoma State University Experiment Station's Uniform Nursery. Following DeWet's (1960) method, all the specimens of culm were collected from 1-2 mms below the basal node of the inflorescence. For the same reason all the leaf samples were selected from a flowering shoot. The third leaf in succession counting downward from the inflorescence, and only the region from 10-15 mms of the base were studied. In order to make the results comparable, sections were cut in a series and variations within the same material were noted.

All the materials were fixed in 70 of ethyl alcohol and after 24 hours or longer they were treated for 15 minutes with hydrofluoric acid for the removal of silica. Dehydration was done in tertiary-butyl alcohol.

Results and Conclusions

Bothriochloa intermedia as referred to in this discussion is recognized as orginally described by Robert Brown (1810). This species includes plants with the primary axis of the panicle subequal to or longer than the lower racemes. The panicle branches may be simple, sparsely divided, or strongly branched. Presence or absence of pits on the lower glumes of sessile spikelets is a variable character. The lower glumes of both sessile and pedicellate spikelets are oblong lanceolate with cilia confined usually on the lower half of the glume. The pedicels supporting one spikelet of each pair are distinctly grooved, and the node has a single row of hairs. Anatomically plants of this species are characterized by short cells of leaf epidermis single or paired (fig. 6), both 'A' (unicellular prickle hairs with swollen bases and curved tips originating over the veins from the rows of cork cells and silica cells of leaf epidermis), and 'B' (like 'A' but originate from short cells of leaf epidermis) type prickle hairs (Figs. 13 and 14), and variable interstomatal cells (Figs. 9-10) and siliceous cells (Figs. 2-5) are present (Faruqi, 1961.). The cross sectional





LEGEND TO FIGURES 1-18

Figs. 1-14. Leaf epidermis among the members of the *B. intermedia* complex. Magnifications for figures 1-12 X 275; and 13-14 X 180.

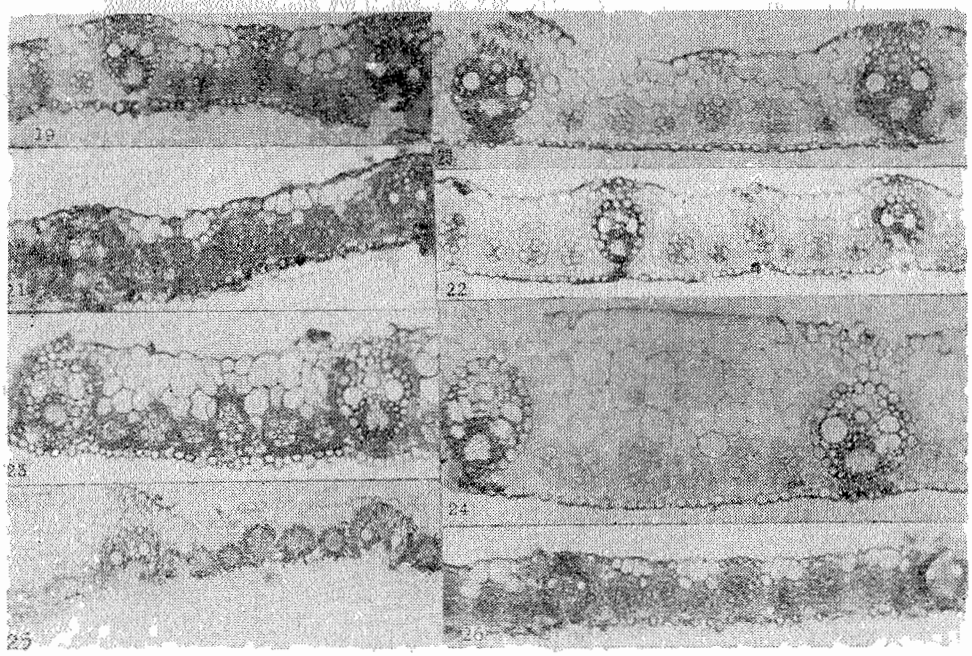
1. "A" type prickle hairs in *B. caucasica.*
2-3. Broad silica cells in *B. caucasica.*
4. Broad silica cell in *B. intermedia* X *D. annulatum.*
5. Narrow silica cell in *B. longifolia.*
6. Long cells and short cells in *B. longifolia.*
7. Long cell and short cell in *D. annulatum.*
8. Short cell modified in 'B' type prickle hair in *B. longifolia.*
9. Interstomatal cell in *B. longifolia.*
10. Interstomatal cell in *C. spicigerum.*
11. Bicellular microhairs in *D. annulatum.*
12. Bicellular microhair in *B. odorata.*
13-14. A portion of leaf epidermis in *B. ischaemum* and *B. odorata* respectively.

Figs 15-18. Cross sections midrib of leaf in the *B. intermedia* complex to note the thickness of keel and number of vascular bundles in the keel.

15. *B. longifolia.*
16. *B. intermedia* X *B. ewartiana.*
17. *B. ewartiana.*
18. *B. intermedia* X *D. annulatum.*

view of the leaf shows three primary bundles in the keel (Table 2, Fig. 18). The number of bulliform bands between two primary bundles of the lamina is usually two and the number of intercalary bundles is three to four (Table 2, figs. 19-21). Culm epidermis of *B. intermedia* in the cross sectional view is composed of equidimensional cells with moderate thickenings. In some of the collections, however, the equidimensional cells are interrupted by small bands of thin walled, radially elongated cells, but these radial cells are never as numerous as the equidimensional cells (Figs. 28 and 30). The vascular bundles in this species are grouped according to their size into three orders and are arranged in three rings. The internode is solid, except in the collection number 6511 where the internode is hollow.

Bothriochloa longifolia is a diploid that is endemic to the Bombay State of India (Faruqi, 1964). Morphologically the primary axis of the inflorescence in this species is somewhat equal to lower racemes. The glumes are pitted and in this respect it is similar to *B. intermedia*. Siliceous cells of leaf epidermis are quite narrow and uniform, prickle hairs are of 'B' type (Fig. 8). The keel is composed of five primary vascular bundles and a well developed mesophyll parenchyma (Figs. 15 and 19). In culm, the epidermis of *B. longiflolia* is composed of radially elongated thin walled cells, large vascular bundles and hollow internode (fig. 27). Since *B. intermedia* is a segmental allopolyploid, it should include the genomes of two related diploid ancestors. On the basis of anatomy, it seems that *B. longifolia* comes quite close to one of the putative parents. The most significant similarity between *B. intermedia* and *B. longifolia* is in the culm eqidermis. *Bothriochloa longifolia* is the only known species in this group that possesses thin walled radial epidermal cells of culm. Although this feature is not present in most of the collections of *B. intermedia*, yet certain collections of this species, as well as its probable hybrids, do show small bands of such cells on the culm (figs. 28 and 30). The other anatomical feature of *B. longifolia* is the hollow internode. The collection 6511 of *B. intermedia* is similar to *B. longifolia* in this respect. Morphological similarities between *B. longifolia* and *B. intermedia* are shared also in the pittedness as well as the pubescence on the lower half of the upper glume. The only difference in the pits present in the two species is that in *B. longifolia* these are uniform in size and are regularly present on each glume, but in *B. intermedia* the pits may be present on some of the glumes and absent on others, and those present may be highly variable. Faruqi and Holt (1966) through genetic analysis have demonstrated that pits in *B. intermedia* are controlled by more than one gene which do not behave regularly in the absence of a homozygous set. Thus the origin of tetraploid *B. intermedia* should involve one paren' with radial epidermal cells of culm epidermis, hollow internode and pits on the upper glume. *Bothriochloa longifolia* seems to be the plant with all these features.



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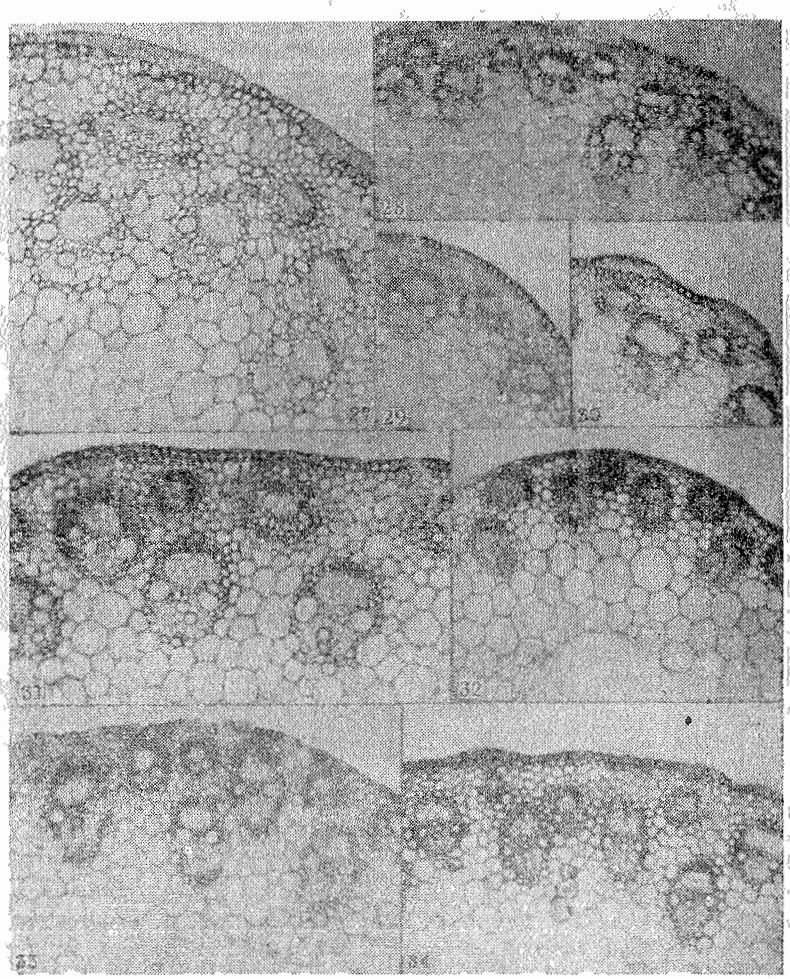
Figs. 19—26. Cross section of lamina in the members of the *B. intermedia* complex showing number of bulliform cell bands; presence, absence, and variation of sub-bulliform cells; number of vascular bundles of the second order and number of intercalary bundles. Magnification X 125.

19. *B. longifolia.*
20. *B. intermedia.*
21. *B. intermedia.*
22. *B. intermedia* X *D. annulatum.*
23. *B. odorata.*
24. *B. intermedia* X *C. parviflorum*
25. *B. caucasica.*
26. *B. intermedia* X *B. ewartiana.*



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Figs. 27—34. Culm in cross section to show types of epidermis, categories of vascular bundles according to size and shape and the number of rings the vascular bundles are arranged. Magnification X 125.

27. *B. longifolia.*
28. *B. intermedia* X *B. ischaemum.*
29. *B. ischaemum.*
30. *B. intermedia* X *B. ewartiana.*
31. *B. intermedia* X *C. parviflorum.*
32. *B. caucasica.*
33. *C. parviflorum.*
34. *D. annulatum.*

Table 1. An analysis of leaf epidermis within *Bothriochloa intermedia* Complex

Plant name	Short cells	Long cells	*Bicellular microhairs* Mean length (mm)	Ratio: basal/ distal cell	*Prickle hairs* 'A' type	'B' type	*Silica cells* Ratio : length/ breadth
Bothriochloa longifolia (Hack.) Bor ..	short 1—2	—	0.041	1.36	—	+	2.00
Bothriochloa ischaemum (L.) Keng ..	do	Pitted	0.055	0.77	—	++	2.00
Bothriochloa intermedia x *B. ischaemum* ..	do	—	0.050	0.78	+	++	1.22
Bothriochloa ewartiana (Domin) C.E. Hubbard	do	—	0.057	1.25	—	+	1.44
Bothriochloa intermedia (R. Br.) A. Camus	do	—	0.060	0.84	+	+	1.32
Capillipedium spicigerum S.T. Blake ..	do	Pitted	0.070	0.85	++	—	1.60
Bothriochloa intermedia x *C. parviflorum*	do	—	0.063	0.83	++	+	1.33
Bothriochloa caucasica (Trin.) C.E. Hubbard	do	Pitted	0.056	1.28	++	—	1.09
Bothriochloa odorata (Lisboa) A. Camus	do	do	0.060	0.79	++	+	1.48
Dichanthium annulatum (Forsk.) Stapf ..	short-long 4-5 in row	do	0.073	1.80	++	—	1.69
Bothriochloa intermedia x. *D. annulatum*	short-long 1-5 in row	do	0.058	1.20	++	+	1.33

—Absent.
+ Present.
++ Abundant.

Table 2. Anatomical features of leaf in cross section within *Bothriochloa intermedia* Complex.

Plant name	Sub-bulliform cell layers	Parenchyma in keel	Primary Vascular bundle in keel	Intercalary vascular bundle in keel	Bulliform bands between two primary bundles
Bothriochloa longifolia	1—3	+++	5	3	2
Bothriochloa ischaemum	1—2	+++	3	5	2
Bothriochloa intermedia x. *B. ischaemum* ..	1—3	+++	3	3	1—2
Bothriochloa ewartiana	1	+	1	7	3—4
Bothriochloa intermedia x *B. ewartiana* ..	1	++	1	5—6	3—4
Bothriochloa intermedia	1—2	+++	3	4	1—2
Capillipedium spicigerum	1—2	+++	3	3—4	1—2
Bothriochloa intermedia x *C. parviflorum* ..	1—3	+++	5	3	1—2
Bothriochloa caucasica	0	+++	1	5—6	2
Bothriochloa odorata	1—3	+++	5	3—4	1—2
Dichanthium annulatum	1—3	+++	3	3	1—2
Bothriochloa intermedia x *D. annulatum* ..	1—2	+++	3	3	1—2

\+ Low
++ Intermediate
+++ High

Bothriochloa ischaemum differs from *B. intermedia* that the inflorescence in the former has a much shorter primary axis as compared to primary racemes and the lower glume is not pitted. This species is distributed in Southern Europe, Middle East Asia, and extends upto North West Pakistan where it hybridizes with *B. intermedia* (Harlan, 1963; Faruqi, 1969). Leaf epidermis in *B. ischaemum* has exclusively 'B' type hairs and narrow siliceous cells (fig. 13). The keel has three primary vascular bundles. Number of intercalary bundles are five and number of bulliform bands are two. Culm epidermis is composed of equidimensional cells with moderate thickenings and the vascular bundles are in a single ring with two size categories (fig. 29).

The suspected natural hybrids are at variance from the *B. ischaemum* in a number of features. Number of intercalary bundles are only three and the frequency of 'B' type prickle hairs are more than in *B. intermedia*. Some of the hybrids also show bands of thin walled epidermal cells of culm epidermis (fig. 28).

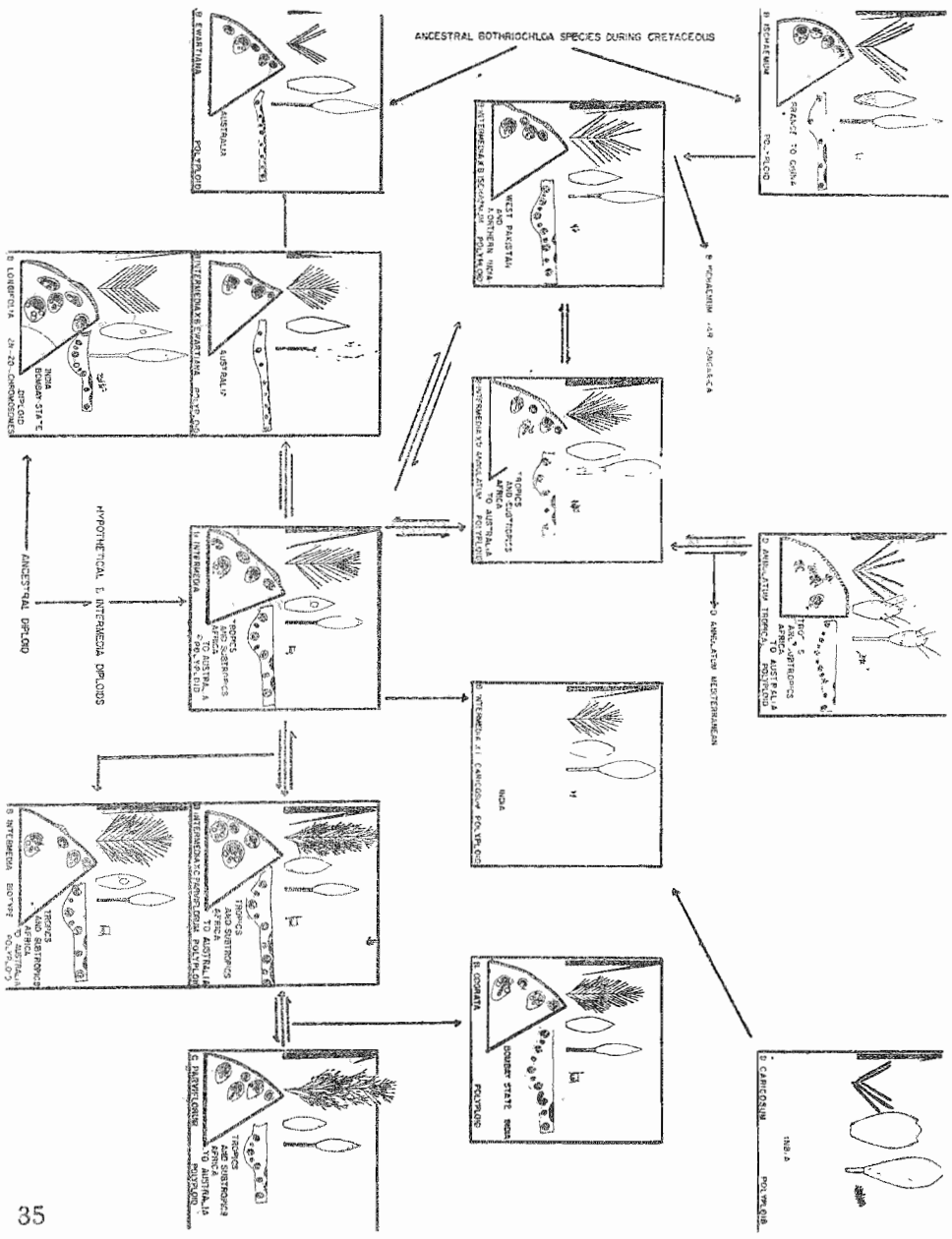
In morphology, the Australian endemic species *B. ewartiana* is quite similar to *B. ischaemum*. *Bothriochloa ewartiana*, however, is different from *B. ischaemum* in having its leaves mostly cauline instead of basal, as in the latter case. With respect to leaf epidermis *B. ewartiana* is similar to *B. intermedia*, and it is different only in the ratio of basal/distal cell of bicellular microhair (Faruqi, 1961). A flat keel with very little mesophyll tissue, 7-8 intercalary bundles, 3-4 bands of bulliform cells between two primary bundles, and usually three primary bundles of the second order characterize *B. ewartiana* (Fig. 17). With respect to stem it is again similar to *B. intermedia* and *B. ischaemum*.

The assumed natural hybrids of *B. intermedia* X *B. ewartiana* differ from *B. ewartiana* in having a somewhat developed keel with three vascular bundles of second order, and might possess also the thin wall radial cells of culm epidermis. In other respects the hybrids are similar to *B. ewartiana* (Figs. 16, 26 and 30).

The only anatomical difference between *B. odorata* and *B. intermedia* seems to be of leaf epidermis where in the former, 'A' type of epidermal hairs are in greater frequency (Figs. 14 and 23). This is a characteristic of *Capillipedium*. Morphologically also *B. odorata* is similar to *B. intermedia* (Faruqi, 1969), except that the former is characteristically aromatic, with unbranched culm, divided panicle branches and 15-17 spikelets per raceme. In the last two criteria it approaches *Capillipedium*. Presence of 'A' type prickle hair in greater frequency than the 'B' type in leaf epidermis is again intermediate between *B. intermedia* and the genus *Capillipedium* (Figs. 1 and 8). It seems that *B. odorata* has originated through hybridization between *B. intermedia* and some species of *Capillipedium*.

LEGEND TO FIGURE 35.

A diagramatic representation of the *B. intermedia* complex illustrating variation, range of hybridization and evolution on the basis of correlative evidence of morphology, anatomy, cytology and geographical distribution.


ANCESTRAL BOTHRIOCHLOA SPECIES DURING CRETACEOUS
B. EWARTIANA
AUSTRALIA
POLYPLOID
B. INTERMEDIA
WEST PAKISTAN
& NORTHERN INDIA
POLYPLOID
B. INTERMEDIA
TROPICS
AND SUBTROPICS
AFRICA
TO AUSTRALIA
POLYPLOID
B. INTERMEDIA
INDIA
DOUBLE STATE
POLYPLOID
B. PERTUSA
TROPICS
AND SUBTROPICS
AFRICA
TO AUSTRALIA
POLYPLOID
B. RESHAEMIUM
FRANCE TO CHINA
POLYPLOID
B. RESHAEMIUM
TROPICS
AND SUBTROPICS
AFRICA
TO AUSTRALIA
POLYPLOID
B. ANNULATUM
TROPICS
AND SUBTROPICS
AFRICA
TO AUSTRALIA
POLYPLOID
B. CARICINUM
INDIA
POLYPLOID
B. LONGIFOLIA
INDIA
BOMBAY STATE
DIPLOID
2N=20-CHROMOSOMES
B. INTERMEDIA BIOTYPE
TROPICS
AND SUBTROPICS
AFRICA
TO AUSTRALIA
POLYPLOID
ANCESTRAL DIPLOID
HYPOTHETICAL B. INTERMEDIA DIPLOIDS

The genus *Capillipedium* includes plants with strongly branched panicle and much longer primary axis than primary racemes. Moreover, it is characterized by 15 or fewer spikelet pairs per raceme, and the secondary and higher order branches of the panicle each disarticulate individually. Anatomically *Capillipedium* is quite similar to *B. intermedia*, however, the former has exclusively 'A' type of prickle hairs while the latter has both 'A' as well as 'B' types on their leaf epidermis.

Morphologically *B. caucasica* is similar to *Capillipedium* with respect to both morphology and leaf epidermis. Although in *B. caucasica* the spikelet pairs per raceme are 20 or less, it has an elongated primary axis with strong branching of racemes. Interestingly enough it possesses 'A' type of prickle hairs only (fig. 1). Although Chheda (1962) suggested the origin of *B. caucasica* through hybridization of *B. intermedia* with *Capillipedium*, neither morphology, nor anatomy substantiates this view. Other anatomical features characteristic to *B. causcasica* are small chlorenchyma cells in the leaf, absence of subbulliform cells, small vascular bundles and hollow pith of the culm. These characters of *B. caucasica* are shared neither with *Capillipedium* nor with *B. intermedia*. Hollow pith is the only character that *B. caucasica* shares with *B. intermedia*. This alone is not enough to prove hybridization in the absence of important characters of *B. intermedia* such as 'B' type prickle hairs of leaf epidermis, and 'B' type pubsescence on the lower glume. *B. caucasica* seems to be highly specialized species of *Capillipedium*..

The hybrids involving *B. intermedia* and *Capillipedium* demonstrate intermediate characters (Faruqi, 1969) (figs. 10, 24, 31 and 33). Anatomically these hybrids show 'A' type of prickle hairs with greater frequency than 'B' type.

Dichanthium annulatum is characterized by a very short primary axis, long hairs with bulbous bases arranged on the margin of a truncate lower glume, solid pedicel and lower 1-6 sessile spikelets male or neuter. The anatomy of leaf and culm in this species is not significantly different from that of *B. intermedia*. Leaf epidermis in *Dichanthium annulatum* is distinct from *B. intermedia* in having a high basal/distal cell ratio of bicellular microhairs, and 4-5 long short cells in a row. The assumed natural hybrid is intermediate (Table 1, figs. 7 and 11).

Although on the basis of morphology natural hybridizaion between *B. intermedia* and *D. annulatum* was proposed earlier (Faruqi, 1969) and the data on anatomy substantiated this view (figs. 4,7,11, 18, 22 and 34). However, these two species could not be crossed under laboratory conditions. Several thousand attempts to cross *B. intermedia* and *D. annulatum* ended in failure. In the opinion of the author the laboratory conditions put limitations with respect to the number of crosses that could be attempted artificially. Moreover, there are limitations also with respect to the parental combinations available and environmental fluctuations. On the contrary the nature offers a limitless scope for experimentation under diverse environmental conditions and varied genotypic

combinations. It is not possible, therefore, that all the successful experiments of the nature could be repeated under laboratory condition. In cases like the present one, only correlative evidences could be used, and here the data on anatomy is in accord with morphology to suggest that natural hybridization between *D. annulatum* and *B. intermedia* has taken place.

The data on anatomy in correlation to morphology (Faruqi, 1969), cytology and geographic distribution (Faruqi, 1964) has provided some idea regarding the extent of hybridization that is taking place among the various members of the genera *Bothriochloa*, *Capillipedium* and *Dichanthium*. On the basis of these evidences origin and relationships between the various members of this complex are proposed in a diagramatic scheme (fig. 35).

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