Intergeneric Hybrids in the Bothriochloinae

I. *Bothriochloa intermedia* and *Dichanthium annulatum*¹

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The section Bothriochloinae of the subtribe Andropogoninae includes 6 apparently closely related genera, *Bothriochloa* O. Ktze., *Capillipedium* Stapf, *Dichanthium* Willemet, *Eremopogon* (Hack.) Stapf, *Euclasta* Franchet and *Pseudosorghum* (Hack.) Stapf. Morphological evidence suggests that hybridization and chromosome doubling played a major role in the evolution of these genera. This gave rise to species complexes characterized by sexual reproduction at the diploid level, whereas the higher polyploids are either facultative or obligate apomicts (Celarier and Harlan 1957). Indications of gene exchange between genera were pointed out by Celarier (1957), Celarier and Harlan (1955, 1957, 1958) Celarier, Mehra and Wulf (1958), Harlan, Celarier, Richardson, Brooks and Mehra (1958) and Mehra and Celarier (1958). A variable degree of chromosome irregularities in natural polyploids was demonstrated by Harlan et al (1958). In this and subsequent studies the morphology and cytology of artificially produced hybrids will be discussed.

Material and methods

Hybrids were produced as described by Richardson (1958). These were grown in a uniform nursery as discussed by Celarier and Harlan (1956). Morphological data are based on herbarium specimens filed with Oklahoma State University. Cytological observations were made from developing microsporocytes fixed in Carnoy's fluid and stained with aceto-carmine.

Results

Bothriochloa intermedia A. Camus and *D. annulatum* (Forsk.) Stapf are complex species. The plants of *B. intermedia* selected for this study differ from more typical members of this species in having solid pedicels and no indentations on the glumes. These are typical *Dichanthium* characters and appear to indicate that *B. intermedia* hybridizes with *Dichanthium* in nature. Other morphological characters such as the distribution of hairs on the first

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glume suggest that the species involved could be *D. annulatum*. The two collections of *B. intermedia* used in this study are both characterized by $2n=40$ chromosomes and differ from each other only in size of the inflorescence and consequently the length of the primary axis, number of inflorescence nodes and number of racemes. The two collections of *D. annulatum* are morphologically similar, but the one has $2n=20$ and the other $2n=40$ chromosomes. The morphological characters are presented in the form of a pictorialized scatter diagram in Fig. 1, following the technique described by Anderson (1949, 1957).

Fig. 1. Pictorialized scatter diagram indicating the morphological characteristics of the parents and hybrids.

D. annulatum (4099) ○; *D. annulatum* (3242) ⊕; *B. intermedia* (2655) ⊖; *B. intermedia* (5450) ⊖; Hybrids (2655 × 4099) ⊕; (4099 × 5450) ⊕; (2655 × 3242) ⊕; Backcross (5450 × (4099 × 2655)) ⊕.

Number inflorescence nodes:	<7	7-8	8.1-9	>9
	○	○	○	○
Number primary racemes:	<8	8-12	12.1-16	>16
	○	○	○	○
Number secondary racemes:	<1	1-4	4.1-8	>8
	○	○	○	○
Length-wide ratio of sessile spikelet:	3.5	3.5-4.5	4.5	
	○	○	○	
Chromosome number:	20	40	50	60
	○	○	○	○

The cytological data are listed in Table 1. The chromosomes of the diploid *D. annulatum* pair regularly to form 10 bivalents. The tetraploid *D. annulatum* and *B. intermedia* plants behave essentially similar in respect to chromosome pairing. The chromosomes usually associate into 20 pairs, but sometimes chromosomes fail to pair and occasionally a trivalent and 1 or 2 tetravalents are present.

Table 1. Cytological data of parents and hybrids

No.	Name	No. plants	Chromosome association								
			2n	I	Ave.	II	Ave.	III	Ave.	IV	Ave.
4099	<i>D. annulatum</i>		40	0-2	0.24	16-20	19.08	0-1	0.08	0-2	0.34
3242	<i>D. annulatum</i>		20			10	10				
2655	<i>B. intermedia</i>		40	0-4	1.21	18-20	19.30	0-1	0.01	0-1	0.04
5450	<i>B. intermedia</i>		40	0-3	1.30	18-20	19.24	0-1	0.02	0-1	0.04
Hybr.	2655 × 4099	7	40	2-12	8.13	12-19	14.72	0-1	0.13	0-1	0.51
	4099 × 5450	2	40	6-10	7.20	11-18	15.00	0-1	0.16	0-2	0.58
	2655 × 4099	10	60	0-4	2.92	25-30	27.48	0-1	0.08	0-2	0.47
	2655 × 3242	1	50	2-10	7.70	15-22	18.93	0-1	0.36	0-2	0.84
	5450 × (4099 × 2655)	1	40	4-12	6.27	14-18	16.70	0-1	0.03	0-1	0.06

The tetraploid *B. intermedia* was crossed with the diploid *D. annulatum* to produce a hybrid with $2n=50$ chromosomes. Morphological data suggest that an unreduced gamete of *B. intermedia* was fertilized by a normal 10 chromosome *D. annulatum* gamete. In a hybrid of this cytological constitution one would expect the chromosomes of *B. intermedia* to behave as in the original parent and the 10 *D. annulatum* chromosomes to remain unpaired during meiotic prophase. This is essentially what happens, except that the frequency of trivalents and tetravalents increased slightly. This seems to indicate that some degree of segmental homology exists between the chromosomes of *D. annulatum* and some chromosomes of *B. intermedia*.

Hybrids between tetraploid *B. intermedia* and tetraploid *D. annulatum* are of two morphological types. The one group is morphologically more or less intermediate between the two parents and characterized by $2n=40$ chromosomes. These represent hybrids which received 20 chromosomes from each parent. An average of 15 bivalents were observed in these hybrids, with the remaining 10 chromosomes present as univalents or associating into trivalents and quadrivalents. The second group resembles the female parent, *B. intermedia*, more closely in morphological characteristics and has $2n=60$ chromosomes. Morphological data suggest that a cytologically unreduced gamete was fertilized by a 20 chromosome gamete of *D. annulatum*. The 60 chromosomes of this hybrid are capable of associating in 30 pairs. One would expect the 40 chromosomes of *B. intermedia* to come together in 20 pairs and those from *D. annulatum* to be present as univalents or forming multivalents with some of the *B. intermedia* ones. The data, however, suggest that the *D. annulatum* chromosomes are able to form bivalents among themselves.

One plant was also obtained from crossing a 40 chromosome *B. intermedia*-*D. annulatum* hybrid back with *B. intermedia*. In this 40 chromosome plant trivalents and tetravalents are rare, but as many as 12 chromosomes fail to pair.

Discussion

The formation of cytologically reduced as well as unreduced gametes in *Dichanthium* and *Bothriochloa* was demonstrated by Brooks (1958) and Mehra (1960). By this means hybrids with variable numbers of chromosomes from different taxa may be obtained. In experimental hybrids with 3 or more identical sets of chromosomes the number of multivalents may differ in hybrids between different races of the same species as demonstrated by Muntzing and Prakken (1940) and Sparrow, Ruttle and Nebal (1942). True autopolyploids appear to be rare or absent in nature (Clausen, Keck and Hiesey 1945, Stebbins 1950). The tetraploid *D. annulatum* may be regarded as a sub-specific segmental allopolyploid. As is obvious from the hybrid cytology this tetraploid has two genomes which normally form bivalents, apparently within chromosome sets. When these two genomes are present together in the haploid condition, however, the chromosomes of the two sets are able to pair with each other.

Morphological data suggest that the *B. intermedia* plants studied have a number of *Dichanthium* genes incorporated into the basic *Bothriochloa* genome. The more typical *B. intermedia* tetraploids may be regarded as segmental allopolyploids as defined by Stebbins (1947). These tetraploids could have hybridized with a diploid *Dichanthium*, possibly *D. annulatum* and through continuous backcrossing gave rise to these abnormal plants. Mehlquist (1945) demonstrated experimentally how hybrids of this nature may resemble the female parent more closely and behave cytologically like a functional diploid.

The possible genome constitutions of the natural species are given in Fig. 2.

Fig. 2. Diagram showing the possible genome constitution of the parents and hybrids.

The 2n=40 chromosome *B. intermedia* was fertilized with pollen from a 2n=20 *D. annulatum* to produce a 2n=50 hybrid. The diploid genome of *B. intermedia* (BBB'B') and the haploid chromosome set (D) of *D. annulatum* are brought together. In this hybrid most of the D chromosomes

fail to pair. Some, however form multivalents with B' chromosomes, as could be expected, since B' chromosomes contain a considerable number of *Dichanthium* genomes.

Hybrids between tetraploid *B. intermedia* and tetraploid *D. annulatum*, as is evident from morphological data, originated from the fertilization of either the cytologically reduced or unreduced gamete of *B. intermedia* with a 20 chromosome *D. annulatum* pollen grain. The chromosomes of the $2n=60$ hybrid (BBB'B'DD') can associate in 30 pairs, although in most cells a few remain as univalents or form trivalents and tetravalents. This seems to indicate that chromosomes D and D' are sufficiently homologous to form pairs when their close homologous are not available. The $2n=40$ chromosome hybrid combines the haploid genomes from each parent (BB'DD'). Trivalents and tetravalents are present in some cells and 6-10 univalents in every cell studied. Most of the bivalents could be from pairing between D and D' chromosomes. The *Bothriochloa* chromosomes B and B' appear to associate to some extent but mostly remain unpaired.

Summary

A comparative study of cytology and morphology revealed that the cytologically reduced as well as unreduced gamete of *B. intermedia* can be fertilized by a normal gamete of *D. annulatum* to produce viable offspring. The $2n=40$ chromosome *D. annulatum* appears to be a segmental allopolyploid with the chromosomes of the two genomes sufficiently homologous to pair when present in the haploid condition. The $2n=40$ chromosome *B. intermedia* is regarded as a segmental allopolyploid with a number of *D. annulatum* genes incorporated in the one genome.

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