Fertility in Relation to Chromosomal Abnormalities in Some Hybrids with Bothriochloa intermedia (R. Br.) A. Camus

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The complex and highly polymorphic nature of *Bothriochloa intermedia* is in part the result of natural hybridization with at least five sympatric species belonging to three related genera, *Bothriochloa, Dichanthium* and *Capillipedium*. In addition, polyploidy and apomixis add to the complexity of the species (Harlan et al., 1958). The somatic chromosome number in this species varies from 40 to 80 in multiples of ten (Harlan *et al.*, 1958, 1961). No diploids ($2n = 20$) have yet been reported. Tetraploids which are usually facultative apomicts have been successfully hybridized with 15 species of the *Bothriochloa - Dichanthium - Capillipedium* agamic complex (Harlan *et al.*, 1961). The meiotic behavior of various species of *Bothriochloa* and occurrence of several meiotic irregularities in the hybrids were reported by Chheda *et al.* (1961 a & b). The hybrids and also their parents, for the most part, are characterized by irregularities apparent during meiosis in the form of univalents, multivalents and stickiness of chromosomes at metaphase, dividing and nondividing laggards, unequal chromosome distribution at anaphase and varying numbers of micronuclei at the telophase and tetrad stages. When certain combinations of parents are used, aneuploidy and/or desynapsis have often been observed in experimental hybrids even though in nature such plants have not been observed. This suggests some form of strong natural selective advantage for genomic balance.

Some of the possible factors responsible for maintaining euploidy in this species and several selection forces operating in this apomictic group of plants are elucidated in this study.

Materials and Methods

Four intraspecific, 11 interspecific and 11 intergeneric hybrids obtained by using a highly sexual and self sterile tetraploid synthetic plant *B. intermedia* (X-750) as the female parent and 1 diploid, 10 tetraploids, 2 pentaploids and 3 hexaploids as male parents were selected for this study. Four aneuploid hybrids obtained by growing the seed of open pollinated panicles of *B. intermedia* (X-750) were also used.

Open pollinated panicles of each of these hybrids were harvested while they were growing in the field. The hybrids were transplanted in a green house at the beginning of the fall season. Here, approximately 15 panicles were allowed to self pollinate under bags. Naked caryopses were extracted and estimates of seed-set on open and self pollinated heads were obtained on the basis of relative amounts of apparently good seeds obtained from each of the hybrids.

The seeds were germinated under conditions of controlled light, temperature, and humidity. The plants were grown in the Andropogoneae nursery under fairly uniform conditions. Detailed information of layout and maintainance of this nursery was presented by Celarier and Harlan (1956).

Bud material of hybrids was fixed in Carnoy's fluid and stored at 5°C. Smears were made in acetocarmine.

EXPERIMENTAL RESULTS

The data regarding 2*n* chromosome number, meiotic behavior, relative amount of seed-set and the F_2 progeny of the hybrids are summarized in Table I.

It becomes apparent, from the table, that while all the intraspecific hybrids were euploids the aneuploids arose either as a result of hybridization between distantly related species and/or when parents of different ploidy-levels were involved in the cross.

The meiotic behavior of the hybrids is, for the purpose of brevity, reported as slightly irregular, irregular, or desynaptic. The slightly irregular meiosis characterized 12 hybrids which exhibited on an average fewer than 5 univalents at metaphase I and a corresponding number of laggards at anaphase. These laggards were usually incorporated in the daughter nuclei and an average of not more than 3 micronuclei at the tetrad spore stage were seen. The 14 hybrids with irregular meiosis were characterized by an average of 5 to 15 univalents at metaphase I, an accordingly large number of dividing and nondividing laggards at anaphase and several micronuclei at the dyad and tetrad spore stage. A low frequency of multivalents at metaphase I and occasionally bridges and fragments were observed in the hybrids possessing slightly irregular and irregular meiosis.

Four hybrids with medium-strong type of desynapsis were also recorded. In these hybrids a few bivalents were seen at diakinesis and early metaphase I, but later most of the chromosomes appeared as univalents. At anaphase, unequal distribution of chromosomes with several dividing and nondividing laggards was common. The laggards formed micronuclei at the tetrad spore stage.

With reference to seed-set, on open and self pollination, in different hybrids, the data suggest that the hybrids differed conspicuously. In general, the seed-set was as good, or better, when the inflorescences were open pollinated than when selfed under bags. This difference, of course, could have been environmental, however one hybrid, 59 × 136-1, appeared to set good seeds when open pollinated, but was completely sterile when selfed. Two other hybrids, 59 × 160-1 and 59 × 177-3, were also highly self sterile. The hybrids with aneuploid chromosome number and/or desynaptic meiotic behavior were in most cases completely sterile and in some cases where a few seeds were produced, the majority of the plants died in early seedling stages. One hybrid, however, *B. intermedia* (×-750) O.P. 23 produced a fair amount of seed and F_2 progeny. The euploids even with irregular meiosis often produced good seed, particularly if the parents involved in the cross were closely related. Of particular interest is the intergeneric tetraploid hybrid, 59 × 169-4, obtained by hybridizing tetraploid *B. intermedia* with hexaploid *D. papilosum*. In this case, even though apparently a whole genome of 10 chromosomes is missing, the hybrid produced a fair amount of seed on self pollination. The hybrids, 59 × 173-1 and 59 × 173-2 resulting from the hybridization of two highly self sterile plants *B. intermedia* (×-750) and *D. annulatum* (×-98), were completely sterile.

The female parent *B. intermedia* (×-750) used to obtain the hybrids was highly sexual in its breeding behavior. All the male parents, with the exception of diploid *D. aristatum* 7199 were either completely or primarily apomictic. The morphological studies of the F_2 progenies revealed them to be very uniform. This indicates that the hybrids were highly apomictic. Two of the 8 segregating plants of 59 × 136-1 O.P. progeny were examined cytologically and found to be aneuploids.

TABLE I — CYTOLOGICAL, SEED SET AND F_2 PROGENY DATA OF SOME SELECTED HYBRIDS WITH *Bothriochloa intermedia* (×-750)

Male Parents*	Hybrid No.		Hybrid Chromosome No.	Meiosis	Seed Set	No. Seedlings	No. Planted	No. Survived	No. Segregating
B. int. 3965(*4n*)	59 × 128-1	S.**	40	Slightly	Good	81	75	72	0
		O.P.		Irregular	Good	138	67	62	4
B. int. 4394(*4n*)	59 × 130-1	S.	40	Desynaptic	None	0	0	0	0
		O.P.			Poor	10	1	0	0
	59 × 130-3	S.	40	Irregular	Good	71	71	65	0
		O.P.			Good	68	50	48	0
B. int. 6511 (*4n*)	59 × 136-1	S.	40	Slightly	None	0	0	0	0
		O.P.		Irregular	Good	81	53	52	8
B. pert. 5431(*4n*)	59 × 148-2	S. & O.P.	40	Slightly Irregular	None	0	0	0	0
	59 × 148-3	S.	42	Irregular	Poor	2	1	1	0
		O.P.			None	0	0	0	0
B. cauc. 4066(*4n*)	59 × 167-1	S. & O.P.	39	Irregular	None	0	0	0	0
	59 × 167-2	O.P.	40	Irregular	Poor	1	1	0	0
B. isch. 7498(*4n*)	59 × 160-1	S.	40	Slightly	Poor	4	4	4	0
		O.P.		Irregular	Fair	33	30	29	0
	59 × 160-2	O.P.	40	Slightly Irregular	Good	72	54	50	0
B. isch. 726(*5n*)	59 × 162-1	S. & O.P.	39	Slightly Irregular	None	0	0	0	0
	59 × 162-2	S. & O.P.	41	Slightly Irregular	None	0	0	0	0
	59 × 162-3	S. & O.P.	42	Slightly Irregular	None	0	0	0	0
	58 × 423-1	S. & O.P.	40	Desynaptic	None	0	0	0	0

Table I continued

Male Parents*	Hybrid No.	Hybrid Chromosome No.	Meiosis	Seed Set	No. Seedlings	No. Planted	No. Survived	No. Segregating
D. ann. 5430(4n)	59 × 172-1 O.P.	40	Irregular	Fair	35	(Not Grown)		
D. papi. 4063(6n)	59 × 170-1 S.	50	Irregular	Poor	7	7	7	1
	O.P.			Poor	4	3	3	0
	59 × 170-6 S. & O.P.	48	Irregular	None	0	0	0	0
D. papi. 4080(6n)	59 × 169-4 S.	40	Irregular	Fair	25	22	21	0
D. arist. 7199(2n)	59 × 175-1 S.	30	Irregular	None	0	0	0	0
	O.P.			None	0	0	0	0
D. cari. 7208(4n)	59 × 176-2 S. & O.P.	39	Desynaptic	None	0	0	0	0
D. fec. 6525(4n)	59 × 177-2 S. & O.P.	40	Irregular	Poor	5	0	0	0
	59 × 177-3 S.	40	Irregular	Poor	2	2	1	0
	O.P.			Fair	35	22	20	0
(×-750) Natural Hybrids	(×-750) O.P. 10							
	O.P.	41	Irregular	Poor			1	0
	(×-750) O.P. 21							
	S. & O.P.	39	Desynaptic	None			0	0
	(×-750) O.P. 23							
	S. & O.P.	38	Slightly Irregular	Fair			31	0
	(×-750) O.P. 29							
	O.P.	39	Irregular	Poor			2	0

* *B. int.* = *Bothriochloa intermedia; B. pert.* = *B. pertusa; B. cauc.* = *B. caucasica; B. isch.* = *B. ischaemum; D. ann.* = *Dichanthium annulatum; D. papi.* = *D. papilosum; D. arist.* = *D. aristatum; D. cari.* = *D. caricosum; D. fec.* = *D. fecundum*

** S. = Self pollinated and O. P. = Open pollinated

Discussion and Conclusions

In polyploid species with high chromosome numbers a certain degree of aneuploidy and meiotic irregularities may be expected in natural populations. When apomixis is involved along with polyploidy and natural hybridization, perpetuation of aneuploids and/or meiotically unbalanced forms would be expected, provided they did not possess any selective disadvantage. Thus workers in forage grasses have reported several cases of naturally occurring aneuploid series e. g., *Bouteloua curtipendula* $2n = 85\text{-}101$ (Harlan, 1949), *Pennisetum ciliare* $2n = 32\text{-}54$ (Fisher *et al.*, 1954), *P. dubium* $2n = 14\text{-}84$ (Gildenhuys and Brix, 1958) *Poa pratensis* $2n = 22\text{-}147$ (Grun, 1954) and *Themeda triandra* $2n = 20\text{-}80$ (Tateoka 1957 and Raman *et al.* 1959).

However, in *Bothriochloa intermedia* and other related species in the genus no aneuploid plants have been observed in nature (Chheda *et al.* 1961). The present study indicates that aneuploid hybrids and hybrids with desynaptic meiosis are in most cases highly sterile. Some may produce a few seed, but the progeny is weak and many die before flowering. Thus, in natural habitats where plant competition is greater, the subvital nature of progenies of such plants and their differential rates of reproduction would soon eliminate the occasional aneuploids that may have arisen. Chromosomal segregation in such progenies could also produce stable euploid plants. A somewhat similar situation is found in *Paspalum dilatatum* where plants with $2n = 40$ and 50 are observed in nature (Bashaw and Forbes, 1958) and the poor seed-set and high incidence of ergot are believed to be associated with meiotic irregularities (Hanson and Carnahan 1956).

Fertility in euploid plants apparently is governed by a complex of genetic factors. Thus different hybrids resulting from the same parents have shown different degrees of fertility ranging from completely fertile and good seed-set to self sterile and sometimes completely sterile. DeWald and Harlan (1961) and Harlan *et al.* (1961) have demonstrated that, in some members of the genera *Bothriochloa* and *Dichanthium* and their hybrids, seed-set is often influenced by the stimulating nature of the foreign pollen. They have reported that self sterility or low seed-set on selfing may be due to the failure of a plant's own pollen grains to germinate and grow into the ovary or very slow growth of the pollen tubes in the stigma. The self sterile nature of some of the hybrids here may also be due to similar causal factors.

The fact that all the hybrids which produced an F_2 progeny in this study were largely apomictic, even though the female parent was a sexual plant, indicates that apomixis in this group of plants is dominant to sexuality, although the number of genes involved is not known.

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