GENETIC ANALYSIS OF INTERSPECIFIC HYBRIDS IN THE GENUS BROMUS, SECTION CERATOCHLOA

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SPECIES of the *Ceratochloa* section of *Bromus*, with somatic chromosome numbers of 42 were hybridized by G. L. STEBBINS during the period 1943 to 1945. This was done to study the relationships between the species and to test the agricultural usefulness of the hybrids. In 1947 I began an investigation of these diploid and allopolyploid hybrids, attempting to explain the underlying cytological and genetic causes of their partial sterility. The parental material consisted of strains of three species described below:

Bromus catharticus Vahl.

B. catharticus is generally a large-leaved vigorous species with large spikelets and very short awns. The lemmas are 14 to 18 or 20 mm long and 11-nerved; they are heavily scabrous along the keel and along the smaller nerves but the surface of the lemma between the nerves is glabrous or nearly so. The leaves, about 30 cm long, are glabrous, but the basal and lower sheath in this strain is densely pubescent. The species is common in, and probably native to, southern Brazil, Uruguay and north-eastern Argentina, but it has been introduced as a forage grass wherever the climate is suitable. The strain used was from the Waite Research Institute, Australia.

Bromus haenkeanus Presl.

B. haenkeanus Presl. is less luxuriant than *B. catharticus*. Its leaves are narrower and approximately half the length, or about 12 cm. The spikelets are much narrower. The lemmas are from 9 to 14 mm long and are 7- to 9-nerved. The awns in the strains used were approximately 1 mm long. The lemma is uniformly, but not densely, scabrous. The species is typically an annual or a short-lived perennial like *B. catharticus*. It ranges through the Andes from Colombia to Patagonia. The two species occur together in several areas where they apparently remain quite distinct from each other. In North America *B. haenkeanus* has been introduced into California, Arizona, Nevada and Utah. I worked primarily with wild strains found near Carmel, California, and Sparks, Nevada.

Bromus stamineus Desv.

B. stamineus Desv. is a grass of moderate size, though slightly less vigorous and shorter-leaved than *B. catharticus*. The leaves are 12 cm, or more, in length, and both leaves and stems are rather densely pubescent. The lemmas are 12 to 15 mm long, 11-, 13-, or occasionally 15-nerved, and are frequently tinged with purple. The surface of the lemma is uniform in texture, but more densely scabrous than in *B. haenkeanus*. The awns are very long compared to those of the other two species, averaging

8 to 10 mm. This species is strongly perennial. In South America it is restricted to central Chile, but has been introduced in central California, where it has been found occasionally around Berkeley and in Sonoma County.

METHODS

Culture methods

The parental strains were grown side by side in the grass garden on the Oxford Tract at the University of California. The hybridizations were made in the field during the early summer of 1944. The F_1 seeds were germinated in a greenhouse and young plants were later transplanted to the field. These were allowed to flower and produce seed for the F_2 generation. The three species used in this work produce both chasmogamous and cleistogamous flowers; however, under the prevailing conditions of culture the flowers produced the first season were so regularly cleistogamous that no further treatment seemed necessary to insure self-fertilization. The F_2 plants were scored for fertility and the three most fertile individuals were selected. The seed from each was used to establish an F_3 population of approximately 20 plants. In a similar way, following selection for fertility, four new F_4 populations were propagated.

Other F_1 plants were divided, rooted in sand, and then treated with colchicine to induce polyploidy. From the tetraploid culms produced on the treated plants seed was collected to establish the " C_2 " generations. Cleistogamy was again relied on to effect self-fertilization. The six most fertile C_2 plants were selected, and from each a population of about 20 C_3 plants was established. Selection was repeated and the lines were continued as with the diploids.

Morphological analysis

Variation in the hybrid populations was described in terms of the two characters in which the parental varieties differed most widely. In *B. catharticus* x *haenkeanus* the useful characters were texture of the lemma and lemma length. The variability in texture ranged from a completely glabrous condition of the surface to a dense covering of scabrous hairs. Scores were assigned as follows:

- 0 Surface of lemma glabrous, i.e. not more than 6 to 10 hairs on the surface between the nerves. May be scabrous on the larger nerves. Typical of *Bromus catharticus*.

EXPLANATORY NOTE

Figures 1 and 2 show a phenotypic history of an interspecific hybridization from the parental species to the F_4 (diploid) and the C_4 (tetraploid). Variation is expressed in figure 1 in terms of lemma texture (nearly glabrous to densely pubescent) and of lemma length, and in figure 2 by nerve number and awn length. The intersection of horizontal and vertical lines represents the phenotypic mean of a population. In addition, the phenotype of each F_2 and C_2 individual is indicated. The seed fertility of the F_2 and C_2 plant is indicated by a diagonal line attached to the symbol representing the individual phenotype. The relative length of the diagonal represents the degree of fertility, the 100% point being marked by a short dash at 90 degrees to the fertility line. The dotted lines indicate the lines of descent.

FIGURE 1. *Bromus catharticus* x *haenkeanus*. Phenotypes of parental species and of hybrid populations.

FIGURE 2. *Bromus haenkeanus* x *stamineus*. Phenotypes of parental species and of hybrid populations.

- 1 Surface very sparsely scabrous, only occasional scattered hairs present. Sometimes found in *catharticus*.
- 2 Lemma sparsely scabrous, distribution of hairs not uniform. Hairy patches alternating with bare patches. Typical of F₁ hybrid between *catharticus* and *haenkeanus*.

FIGURE 3. *Bromus catharticus*, *haenkeanus*, and *stamineus*. Average seed fertility of parental species, F₁ hybrids, and diploid and tetraploid lines of descent.

3 Surface of lemma covered uniformly and moderately densely with short, scabrous hairs. Typical of *Bromus haenkeanus*.

Lemma length was measured from the point of the callus to the tip of the lemma (not the awn). In *B. stamineus* x *haenkeanus* awn length and number of nerves in the lemma were analyzed.

The essential morphological data is presented in figures 1 and 2, using the scatter diagram technique of ANDERSON (1949). Each diagram shows the complete history of an interspecific hybridization to the 4th generation. The parental species, the F₁, F₃, and F₄ hybrids are represented by population means. In the F₂ (and C₂) generations, the phenotype of each individual plant is shown. The lines of descent may be traced by means of dotted lines.

Fertility

Fertility was determined by counting the fertile florets in nearly mature panicles. Florets were considered fertile if the caryopsis had developed to full size or nearly so by the time the flowering panicle had begun to turn brown. Estimations were based on samples of 50 or more florets per plant. The seed fertility of each F₂ and C₂ plant is indicated on the scatter diagrams (figures 1 and 2) by a diagonal line attached to the symbol representing the individual phenotype. The relative length of the diagonal indicates the degree of fertility, the 100% point is marked by a short dash at 90 degrees to the fertility line. The fertility indicated for the hybrid populations (figure 3) are averages based on samples of approximately 20 plants in each population.

Cytological analysis

For cytological analysis immature spikelets were fixed in a fresh solution of absolute alcohol and glacial acetic acid (3:1) for 24 hours and then transferred to 70% alcohol for storage. Temporary smears of pollen mother cells were stained with aceto-carmine. An alcohol vapor chamber technique was used (BRADLEY 1948) to make temporary smears permanent.

Statistical study

Did selection for fertility have any effect on the genotypes of the hybrid populations? Inspection of the scatter diagrams suggested a non-random relationship. The fertility of a hybrid seemed to depend on the degree of resemblance between the hybrid individual and its parent. The following test was devised: The radial distances $D_1, D_2, \dots D_n$ were measured, on the scatter diagrams, from the mean phenotype of each hybrid population to the phenotype of each individual in the population. I assumed that the population mean is the best estimate of the genotype of the parent of the population. The quantities $S_1, S_2, \dots S_n$ represent the seed fertility of each individual in the population, transformed from percentage to angular units according to the relation: angle = arc sin $\sqrt{\text{percentage}}$ (SNEDECOR 1946, p. 447). The correlation between fertility and segregation for morphological characters was then computed, using the product moment formula:

$$r = \frac{\sum sd}{\sqrt{\sum s^2 \sum d^2}}$$

RESULTS

Morphological

All the morphological data is presented in figures 1 and 2 for *catharticus* x *haenkeanus* and *haenkeanus* x *stamineus* respectively. Comparison of the 2nd to the 4th generations shows certain trends to be evident. A net decline in quantitative characters is typical of both the diploid and the tetraploid lines of descent. Lemma length consistently declines from F_2 to F_4 as in the C_2 to C_4 lines (figure 1). Awn length clearly decreases in the tetraploid lines of descent and seems to in the diploid. The number of nerves declines in the diploid lines and from C_3 to C_4 in all the tetraploids (figure 2).

The genotypes of the tetraploid lines seem to be effectively "fixed" at the C_2 level. In spite of considerable random variation, the phenotypes of the three tetraploid lines of descent from each F_1 hybrid maintain their relative positions from the C_2 to the C_4 generation.

Fertility

Seed fertility of the parental species is in excess of 90%. The F_1 's *catharticus* x *haenkeanus* and *haenkeanus* x *stamineus* had a seed fertility of 4% and 15% respectively. This increased considerably in the later diploid generations; in the F_4 generation three out of the four diploid lines had approached the fertility of the original species. In the tetraploid material, the immediate effect of somatic doubling of the

TABLE 1

Correlation between segregation for length of lemma and texture of lemma, and segregation for fertility in the hybrid populations derived from the cross Bromus catharticus x haenkeanus

Population	N	r _{df}	Population	N	r _{df}
652B (F ₂)	8	.615*	652C (C ₂)	22	— .117
728 (F ₃)	21	.446*	764A (C ₃)	17	— .044
			764B (C ₃)	18	.445*
Summary	29	.488†	764C (C ₃)	25	.040
			809 (C ₄)	9	— .552
808A (F ₄)	16	.099	810ABC (C ₄)	11	.198
808B (F ₄)	14	— .42	811ABC (C ₄)	17	— .458*
All diploid	59	.258*	Tetraploid	119	— .124

* Significant.

† Highly significant.

TABLE 2

Correlation between segregation for length of awn and number of nerves in lemma, and segregation for fertility in hybrid populations derived from the cross Bromus haenkeanus x stamineus

Population	N	r _{df}	Population	N	r _{df}
654 (F ₂)	18	.235	655 (C ₂)	21	— .417*
730 (F ₃)	16	.155	718 (C ₃)	20	— .105
731 (F ₃)	13	.216	719 (C ₃)	11	— .785†
			720 (C ₃)	20	— .029
Summary	47	.188	818B (C ₄)	13	— .174
			819 (C ₄)	17	— .206
816A (F ₄)	20	.0005			
817A (F ₄)	17	.196			
All diploid	84	.144	Tetraploid	102	— .288†

* Significant.

† Highly significant.

F₁'s was a marked increase to approximately 80%. These tetraploid plants have been designated the C₁ generation. From the high point of 80% the fertility of all the tetraploid lines declined more or less rapidly. By the C₄ generation the average of the *haenkeanus* x *stamineus* populations had declined to about 50%; the *catharticus* x *haenkeanus* hybrids had dropped to an average of 25% (see figure 3).

Correlations

The results of the correlation computations are indicated in tables 1 and 2. In the majority of cases the correlations between segregation for morphological characters and segregation for fertility are positive in the diploid populations, while the corresponding figures for the tetraploid groups are negative. Exceptions to the tendencies indicated are seen in the highly fertile F₄ populations. Population 817A, with an average fertility of only 22% has a fairly high positive correlation, while the other

TABLE 3
Chromosome behavior at metaphase I

Population	No. cells	Chromosome associations average per cell				Bivalents paired one end only
		IV	III	II	I	
Part I. <i>Bromus catharticus</i> x <i>haenkeanus</i>						
525 (F ₁)	25	0.0	0.0	21.0	0.0	1.25
415 (F ₁)	20	0.0	0.0	21.0	0.0	.90
766A (C ₃)	19	7.84	0.58	24.95	0.79	—
766B (C ₃)	10	7.40	0.10	27.00	0.10	—
Part II. <i>Bromus haenkeanus</i> x <i>stamineus</i>						
412 (F ₁)	20	0.55	0.15	20.10	0.40	1.60
526 (F ₁)	26	0.0	0.0	21.0	0.0	2.60
655 (C ₂)	9	4.11	0.0	33.80	0.0	—
719 (C ₃)	20	5.00	0.15	31.70	0.15	—
719* (C ₃)	20	5.25	0.05	31.40	0.05	—

* Two different plants examined in this population.

F₄'s, all with fertilities of 72% or more, had correlations of nearly zero or were negative. In the tetraploids there were three exceptional populations, all in the *catharticus* x *haenkeanus* series. The two with positive correlations of about .2 or more were in the same line of descent (764B and 810).

Cytological analysis

STEBBINS and TOBGY (1944) demonstrated that the chromosomes of the parental species formed bivalents only at metaphase I and behaved regularly during the later stages of meiosis. Of the four different interspecific hybrids examined cytologically, three formed 21 bivalents regularly at metaphase I. The fourth formed 20 bivalents and a ring of four chromosomes in 70% of the cells examined, the rest being normal (table 3). The number of open bivalents was somewhat greater in the *haenkeanus* x *stamineus* F₁ than in the other. The same F₁ showed more irregularities in the later stages of meiosis. The most striking difference between the two interspecific hybrids was seen in the frequency of micronuclei. Note that the *catharticus* x *haenkeanus* F₁, with only about 1.5% of tetrads with micronuclei, was the more sterile, while the other F₁'s, with about 40% of the tetrads showing micronuclei, were fairly fertile.

The allotetraploids exhibit quadrivalent as well as bivalent chromosome association. Moreover, the frequency of micronuclei in the tetrad stage is higher than in the diploids. Average frequency of quadrivalents per cell varied from 4.1 to 7.8. The *catharticus* x *haenkeanus* plants produced, on the average, more quadrivalents than the *haenkeanus* x *stamineus* hybrids. The former tetraploids were fairly sterile while the latter were quite fertile. The cytological data is summarized in table 3 for metaphase I and table 4 for anaphases I and II and the tetrad stage. All the tetraploid plants analyzed cytologically at metaphase I had the expected chromosome number of 84.

TABLE 4
Chromosome behavior, anaphase I to tetrads

Population	Anaphase I			Anaphase II			Tetrads	
	No. cells	% with		No. cells	% with		No. cells	% with micro-nuclei
		Laggards	Bridges		Laggards	Bridges		
Part I. <i>Bromus calharticus</i> x <i>haenkeanus</i>								
525 (F ₁)	53	3.8	0.0	22	0.0	0.0	71	1.4
415 (F ₁)							65	1.5
766A (C ₃)	70	67.1	1.4				66	60.6
766B (C ₃)	50	76.0	0.0	30	46.7	40.0	63	33.3
Part II. <i>Bromus haenkeanus</i> x <i>stamineus</i>								
412 (F ₁)	63	33.3	3.2				64	39.1
526 (F ₁)	47	0.0	2.1	44	0.0	4.5	89	41.5
655 (C ₂)	83	16.9	5.0					
655* (C ₂)							27	7.4
719 (C ₃)							59	71.2
719* (C ₃)							67	58.2

* Two different plants examined in this population.

DISCUSSION

The principal facts established by the experiment were: (1) the sterility of the interspecific F₁ hybrids; (2) the increase in fertility of the diploid lines of descent; (3) the loss of fertility in the tetraploid lines of descent in spite of selection for fertility; (4) a correlation, positive in the diploid, negative in the tetraploid, between segregation for fertility and segregation for morphological characters.

Causes of sterility

DOBZHANSKY (1951) proposes two categories of hybrid sterility, genic and chromosomal. Genic sterility applies to the situation where the genetic constitution of the hybrid is such as to preclude its sexual maturity. Chromosomal sterility is sterility caused by lack of homology, or incomplete homology, between the chromosomes of the parents of a hybrid. This condition could give rise to inviable combinations following meiosis even though the hybrid constitution itself might be viable. The observed sterility in the F₁ hybrids described here apparently is not genic, the production of flowers and the cytological processes being normal up to the first metaphase of meiosis. Moreover, the fact that bivalent formation is regular excludes gross structural differences as the cause of sterility. Thus the sterility must be due to relatively slight differences between the parental sets of chromosomes. Since the two parents are different, although related, taxonomic species, the gene differences present in the hybrid may be sufficient to give rise to disharmonious and inviable recombinations. Likewise, structural differences may be present which are too small to affect the regularity of meiotic pairing but are large enough to give rise to lethal deficiencies in the gametophytes. The latter suggestion is recognized as the hypothesis of *cryptic*

structural hybridity (STEBBINS 1945). The criterion of somatic doubling (DOBZHANSKY 1951) has been offered as a means of distinguishing genic from chromosomal sterility: if the immediate effect of somatic doubling is a marked increase in fertility, then the sterility must have been due to differences in chromosome homology. Probable examples are *Primula kewensis* (NEWTON and PELLEW 1929), *Raphanobrassica* (KARPECHENKO 1928), *Tradescantia* (SKIRM 1942) and many others. On the other hand, if chromosome doubling fails to increase fertility, then sterility must have been at least partly genic. This situation has been observed in crosses between species of *Lycopersicum* (LESLEY and LESLEY 1943; McARTHUR and CHASSON 1947) and *Solanum* (PROPACH 1940; TATEBE 1936). The application of this criterion suggests that the sterility observed here was actually due to differences in homology between the chromosomes of the parents of the hybrid. It may not be possible to determine whether genic differences or a condition of structural hybridity causes the sterility of the F_1 hybrid. The following analysis will indicate that the observed results are consistent with the hypothesis of cryptic structural hybridity although they do not exclude the other possibility. The advantage of the structural hypothesis is that it readily lends itself to a quantitative analysis.

Diploid hybrids

Because of the indication that the sterility of the interspecific hybrids described here may be combinational in character, an analysis of hybrid meiosis from this point of view is appropriate. The diploid genetic complement of any two of the three parental species may be represented by the symbols AA and BB respectively; the complements of the respective gametophytes will then be A and B. The success of the parental species in the wild is evidence of the viability of the genetic combinations AA and BB. Likewise, the normally high seed and pollen fertility indicates viability of the haploid complements A and B. The vegetative vigor of the F_1 hybrids when grown in the field demonstrates that the new combination AB is also viable.

The gametophytes produced by the hybrid AB will contain various combinations of genetic material. Even in the event of normal bivalent formation in the F_1 hybrid, few, if any, of the gametophytes will contain material wholly from one or the other of the parental species.

Due to cytological crossing over, an A centromere may become associated with a mixture of A and B material. Assuming two chiasmata per bivalent (the minimum required to produce pairing at both ends of the bivalent) the probability of an A centromere with chromatin from the A parent only is $.5^2$ or $.25$. The probability of such an intact A chromatid segregating into any given spore is $.5$, and the probability, P , of the spore receiving any given number, N , of intact A chromatids is obtained from expanding $(p + q)^{21}$ where $p = .5^3 = .125$ and $q = 1 - p = .875$. Thus, for example, (table 5) the probability that a gametophyte would receive *any* six (out of 21 possible) intact A chromatids is 2.79% while the probability of receiving six or more is 3.91%. But the probability P' of the spore receiving a number, N , of *designated* intact A chromatids is simply p^N . Thus, (table 5), only $3.81 \times 10^{-4}\%$ of the gametophytes will have any particular combination of six A chromatids in the intact condition. Probabilities less than 10^{-6} are not listed.

TABLE 5

Chromatid combinations in the gametophytes produced by an interspecific hybrid with 21 pairs of chromosomes

N	P	ΣP	P'
13	—	—	—
12	.0000013	.0000014	—
11	.0000108	.0000121	—
10	.0000756	.0000877	—
9	.000441	.000529	—
8	.00214	.00267	—
7	.00855	.0112	—
6	.0279	.0391	.00000381
5	.0733	.112	.0000305
4	.151	.263	.000244
3	.235	.498	.00195
2	.258	.756	.0156
1	.182	.938	.125
0	.0606	.999	

N = Number of intact chromatids (not involved in chiasmata) from a designated parental species, out of a possible 21.

P = Probability that any N intact chromatids will go to a spore.

ΣP = Summation of P.

P' = Probability that N designated intact chromatids will go to a spore.

It is obvious that an F_1 hybrid is practically unable to produce a gametophyte with genetic material wholly from one or the other of the parental species. If viability of the gametophyte depends on the production of spores with a quantity of intact chromatin, then obviously a fairly small quantity of intact parental chromatin must be sufficient. The fertility, 4%, of the *catharticus-haenkeanus* hybrid corresponds to the frequency in table 5 of combinations containing about six or more intact chromatids of *catharticus* or *haenkeanus*. The *haenkeanus-stamineus* hybrid with a fertility of 15% must have had some viable gametophytes with as few as five intact chromatids.

The possibility of structural hybridity as a cause of F_1 sterility was mentioned earlier in this discussion. The concept of cryptic structural hybridity was first suggested by SAX (1933) and MÜNTZING (1938), while STEBBINS (1945) first proposed the term. A full discussion of this hypothesis of hybrid sterility is found in STEBBINS (1950). Briefly, the condition could originate through small segmental interchanges between non-homologous chromosomes or through overlapping inversions within a single chromosome. STEBBINS, VALENCIA and VALENCIA (1946) propose that the cytological effect of this condition would be lowered chiasma frequency and that the genetic effect would be sterility due to the deficiencies introduced into the gametophyte generation by crossing over between structurally different chromosomes. These writers suggest that the effect on fertility will be more pronounced than the change in chiasma frequency. They estimated that in diploid hybrids between grass species in the tribe Hordeae ($n = 7$) structural differences of as many as 30 small segments

may be present without altering the appearance of bivalent formation, but that more than four or five would be enough to produce complete sterility in the F_1 's. Regular pairing combined with F_1 sterility has been previously observed in the genus *Bromus* (STEBBINS and TOBGY 1944). STEBBINS (1950) lists two intergeneric hybrids and 24 genera in which one or more interspecific hybrids showing this same behavior have been reported. Of these, seven were reported to have been tested by the method of artificial allopolyploidy. In five of those tested chromosomal sterility was confirmed by the regaining of fertility; in the remaining two (the closely related *Solanum* and *Lycopersicum*), genic sterility was indicated.

The importance of deficiencies as gametophyte lethals has been evaluated by the work of MCCLINTOCK (1938, 1941, 1943, 1944), also by CLARK and ANDERSON (1933), STADLER (1943), and STADLER and ROMAN (1948). In maize deficiencies in chromosomes 3, 5, 9, and 10 have been studied at pachytene where accurate measurements are possible. Their size varied from a maximum of 2.8% of the gametic chromatin down to the limit of visibility (less than 0.1% of the chromatin). Practically all deficiencies large enough to be visible at pachytene were lethal to the male gametophyte; the female gametophyte was less sensitive. None of the homozygous deficiencies were completely viable in the diploid tissues, where they appeared as chimeras. Inasmuch as the loss of less than 1% of the chromatin complement in maize is usually lethal, it is suggested that other plants may be similarly affected, although polyploid species should be less sensitive than diploids. The species of *Bromus* in the section *Ceratochloa* with 21 pairs of chromosomes are presumably polyploid since the basic number in the genus is seven. Since there are no known living species in this section having a lower chromosome number, it is probable that these species have not arisen recently. It might be expected, then, that they would be sensitive to deficiencies, but that, due to higher chromosome number and probable polyploid origin, they would be less sensitive than maize.

It is possible to estimate the degree of structural hybridity in the F_1 hybrids. Chromosomal differences may be either inversions or translocations or a combination of the two. If the differences are the result of inversions, the production of deficient gametophytes will depend on the frequency and position of crossing over in the F_1 hybrid. A "cryptic" condition would result from a successive overlapping inversion and reversion (STEBBINS 1950, page 223). Designating the normal structure as ABCDEFGHIJ, an altered structure could be ABGCDEFHIJ. A chiasma between C and F in the F_1 hybrid would render both participating strands abnormal. The gametophyte receiving the strand deficient for G would presumably be abortive. The maximum abortion of gametophytes expected from this structural difference would thus be 25%, unless the distance between C and F were long enough for two or more chiasmata. Assuming that each structural difference behaved independently and that a gametophyte with one or more deficiencies is abortive, the minimum fertility in a hybrid with N structural differences of the kind described above would be $(.75)^N$.

If on the other hand the segmental differences should consist of small translocations, then sterility would be independent of chiasma frequency and position but would depend entirely on the distribution of chromosomes. Random distribution of two

TABLE 6
Estimation of number of structural differences in F₁ hybrids

Hybrid	Fertility	Number of segmental differences	
		Inversions	Trans-locations
<i>catharticus</i> x <i>haenkeanus</i>	4%	11 or more	5
<i>haenkeanus</i> x <i>stamineus</i>	15%	6 or more	3

pairs of chromosomes with a single reciprocal translocation would produce a maximum of 50% of abortive gametophytes. With N structural differences of this type segregating independently, the fertility would be equal to $(.5)^N$. In table 6 the number of cryptic structural differences in the F₁ hybrids is estimated according to these principles.

Selection for fertility in the diploids was highly effective in three generations (see figure 3). Since it seems likely that such sterility as appeared in the F₁ and subsequent generations was due to structural heterozygosity, the cause of increased fertility was apparently the selection of (structurally) homozygous lines.

In the relation between morphological variation and fertility in the diploid hybrids there is a tendency for the morphologically more extreme types to be more fertile. The hereditary cause of morphological variation in these hybrid populations must be segregation of the genes responsible for the differences between the parents. Therefore the observed relationship is probably due to linkage between factors determining morphological characters and those controlling the viability of the gametophyte. A model will clarify this point. Assume an interspecific hybrid with a basic chromosome number of $n = 2$. The partially homologous chromosomes A, a and B, b differ structurally as well as in genes affecting morphological characters. Thus the chromosomal constitution of the hybrid will be represented by the formula AaBb. With random distribution, the F₂ will contain the parental recombinations AABB and aabb. The F₁ combination will be present, together with new combinations such as AaBB, Aabb, aaBb, etc. The mean F₂ phenotype should approach that of the F₁. The structural heterozygote AaBb will be low in fertility and near the mean in phenotype. The parental types will have a maximum fertility and be most unlike the mean; the others should be intermediate both in fertility and morphological characters. Thus the hypothesis could readily account for the correlations observed.

A further point should be noted. After several generations of selfing the lines should approach homozygosity; as genetic variability decreases the correlations should also decline. Three of the F₄ populations (see table 1 and 2) exhibited correlations which were either negative or nearly zero. Inasmuch as the average fertility was very high, about 80%, the probable cause of the low correlation was that genetic variability had been nearly eliminated by selection. All the diploid populations of intermediate fertility showed the characteristic positive correlation.

Tetraploid hybrids

The behavior of the tetraploid derivatives of the colchicine-treated F₁ hybrids is more complex than that of the corresponding diploids. Somatic chromosome doubling

of the F_1 produces a C_1 plant which has two sets of chromosomes from each parental species. Since the sister sets derived by doubling are identical, pairing should take place between them. The partially homologous sets which pair in the diploid hybrid may also be expected to associate in the tetraploid. The fact that all the tetraploids show quadrivalent associations indicates that this is the case (table 3). According to WADDINGTON (1939), the pairing of the sister chromosomes produced by doubling would be termed homogenetic pairing, while the term heterogenetic pairing applies to the association of chromosomes of dissimilar origin as in the interspecific F_1 's. The prevalence of bivalent association in an allotetraploid would suggest that pairing was homogenetic. On the other hand, the presence of quadrivalents is indication of heterogenetic association. The critical evidence in regard to the type of association lies in the variability of the tetraploid populations. It is obvious that the sister genomes derived from each parent must be genetically identical. Therefore there is no potential for genetic variability unless heterogenetic pairing takes place. Conversely any genetic variability in the tetraploids is evidence per se of heterogenetic pairing.

The rate at which this potential variability is set free will depend on two factors: (1) the relative frequency of homogenetic as against heterogenetic association in the tetraploid and (2) the statistical limitations on gene segregation in the tetraploid conditions. Chromosome size and chiasma frequency should affect multivalent frequency; it is not likely that polyploid plants with small chromosomes and few chiasmata would form multivalents as frequently as polyploids with large chromosomes and many chiasmata. The low frequency of multivalents in *Primula kewensis* (UPCOTT 1939) and *Galeopsis tetrahit* (MÜNTZING 1932) is to be noted in this connection. The hypothesis of preferential pairing of completely homologous chromosomes has been introduced (SKIRM 1942; STEBBINS 1947) to explain the meiotic regularity of natural allopolyploids. Preferential pairing demands a sufficient degree of dissimilarity between the chromosomes derived from the parental species to prevent heterogenetic association. The experiments conducted by SKIRM (1942) with the natural hybrid between *Tradescantia canaliculata* and *T. humilis* suggest that the pairing mechanism may be rather sensitive to slight differences in chromosome homology. The tetraploid produced from this hybrid by somatic doubling displayed very few multivalents at metaphase I. Presumably the pairing of exactly similar chromosomes was responsible for this. When tetraploids were obtained from unreduced gametes the proportion of multivalents was high, suggesting that the basis of preferential pairing had been eliminated in meiosis by heterogenetic exchanges between the parental genomes.

A further consequence of random chromosome association in an allopolyploid will be tetrasomic segregation of the gene differences between the parental species. Homozygosity will be rapidly approached in a diploid line subject to self-fertilization, but much more slowly in a tetraploid. Five generations of selfing on the diploid level will reduce heterozygosity to 3.1% of the original; on the tetraploid level 47% will still remain (HALDANE 1936).

The occurrence of heterogenetic chromosome association in these experiments has been established by genetic as well as cytological evidence. With 84 chromosomes, the combinational situation becomes too complex for full exploration. However, a

TABLE 7

Frequency distribution of chromosomal types in a C_2 population derived by random chromosome association in an allotetraploid of the constitution 2/2 (chromosome formula AAaa BBbb)

Genetic index	Phenotype	Chromosomal types	No.	%
8 (0)	Parental	4/4 (0/0)	2	1.0
7 (1)	Nearly parental	4/3 3/4 (1/0 0/1)	16	8.2
6 (2)	Semi-parental	4/2 2/4 (2/0 0/2)	16	8.2
6 (2)	Semi-parental	3/3 1/1	32	16.3
5 (3)	Nearly intermediate	3/2 2/3 (1/2 2/1)	80	40.8
4	Intermediate	2/2	34	17.4
4	Intermediate	3/1 1/3	16	8.2
Total.....			196	

simplified model will show that the decline of fertility and the correlations between fertility and morphological variation are consistent with the hypothesis of cryptic structural hybridity. Specifications for the model are as follows: the parental species each have two pairs of chromosomes; they may thus be described by the formulae AA BB and aa bb. Chromosome A is homologous with a, likewise B is homologous with b, except for a small segment reciprocally translocated between a and b. The translocated segments are interstitial rather than terminal. A gametophyte or a zygote homozygous for a deficiency is assumed to be inviable. In the allotetraploid AAaa BBbb the chief variable to be considered is the degree of preferentiality in chromosome association. Preferentiality may vary between the limits of 0 (random association) and 100% (completely homogenetic association). With random association, chromosome A is as likely to pair with a as with A. With preferentiality of 50% A will pair with A 75% of the time and with a 25% of the time. Chromosome B will behave in a similar way.

Homogenetic association will produce a single class of gametes, AaBb; but if preferentiality is incomplete eight new combinations should be produced in varying proportions. The new combinations will be AABB, AaBB, AABb, Aabb, aaBb, aabb, AAbb, and aaBB. The last two are homozygous deficient for a small segment and therefore inviable. With random association in the allotetraploid AAaaBBbb, the viable gametes will be produced in the proportion 1AABB:2AABb:2AaBB:4AaBb:2aaBb:2Aabb:1aabb. If preferentiality were acting, the frequency of the AaBb gametes would increase at the expense of the other combinations. The C_2 generation will contain 19 combinations. In table 7 the C_2 genotypes have been represented by two digits, indicating the frequency of the "A" and the "B" chromosome respectively. Thus, the hybrid AAAa BBbb is abbreviated 3/2. The "genetic index" is the sum of the numerator and the denominator of the fraction; it is a measure of the amount of material from the AABB parent. I have assumed that phenotype will be a function of the genetic index.

Of the 19 possible C_2 types, the tetraploid parental combinations will be the least frequent (1%) and the nearly intermediate classes (3/2, 2/3, etc.) the most frequent

FIGURE 4. Model showing the effect of preferential chromosome association on fertility in allotetraploids with various chromosome combinations and with one, three, and five structural differences.

(41%). Fertility of each class has been computed on the assumption that fertility of the tetraploid is the frequency with which it will segregate gametes not of the constitution $AAbb$ or $aaBB$. Certain C_2 classes will lack opportunity for preferential pairing; these are $3/1$ ($1/3$), $3/3$ ($1/1$), and $4/3$ ($3/4$) etc. The $3/1$ combination has a fertility of 75%, the others will be 100% fertile. But in the majority of the combinations (67%) fertility will be related to the degree of preferentiality in chromosome pairing. A high frequency of homogenetic pairing will result in high fertility. Furthermore, there will be a tendency for the intermediate phenotypes (those with a genetic index of 4) to have a higher fertility than those with either higher or lower genetic indices. This tendency will be particularly pronounced with an intermediate degree or preferentiality. These considerations are summarized in the "A" set of curves in figure 4. In an allopolyploid with more chromosomes than the model just discussed, the effect of a larger number of cryptic structural differences may be estimated on the assumption that they are mutually independent. Thus, if a single cryptic structural difference were responsible for a fertility of $X\%$, then the effect of n structural differences would be a fertility of $X^n\%$. On this line of reasoning are based the "B" and "C" sets of curves in figure 4.

Does the model explain the behavior of the tetraploid hybrids? The most striking feature of the tetraploid lines of descent is the gradual loss of fertility. In the model it is clear (table 7) that the C_1 genotype and chromosome arrangement does not reproduce itself consistently in every C_2 individual unless chromosome association is strictly homogenetic. But since the C_1 type is unstable and since the fertility of most C_2 types is less than that of the C_1 , the decline of fertility with each succeeding generation of tetraploids (figure 3) seems inevitable unless selection for fertility can compensate for the instability. A further consideration is the physical basis of preferentiality, i.e. the interspecific differences between chromosomes. These differences are at a maximum in the C_1 type. Any heterogenetic association followed by crossing

over would reorganize the chromosomal material in such a way as to decrease preferentiality and hence fertility in the succeeding generations.

Another characteristic of the tetraploid population is the negative correlation between segregation for morphological characters and segregation for fertility. The properties of the model as shown in figure 4 are consistent with these observations. The C_1 constitution is phenotypically intermediate and highly fertile. Fertility in the morphologically more extreme types decreases in proportion to their resemblance to the C_1 chromosomal constitution. Linkage between factors determining hybrid morphology and those determining fertility should produce the observed correlation.

Although the evidence on this point is inconclusive, the results give some support to the theory of preferential affinity. The first point is that the number of quadrivalent associations (4 to 8 out of a possible 21) is lower than that observed in some natural or artificial autotetraploids. In *Arrhenatherum elatium* (MYERS and HILL 1940), *Dactylis glomerata* (MÜNTZING 1937), *Sorghum leiocladum* (GARBER 1950) the maximum possible number of quadrivalent associations is realized in at least some of the microsporocytes at metaphase I. Secondly, taking the view that quadrivalent associations indicate random chromosome association, the differences between the several tetraploid populations are in line with the theory that preferentiality influences allopolyploid fertility. Thus the populations representing the infertile *catharticus* x *haenkeanus* hybrids have the highest frequency of multivalents (table 3). In *haenkeanus* x *stamineus* the C_2 has a lower multivalent frequency than the C_3 , as well as a higher fertility. As to the degree of preferentiality, an estimate based on an average multivalent frequency of 5.0 and 7.5 per cell (table 3), assuming that all bivalents are homogenetic, gives a maximum preferentiality of 75% for the *haenkeanus* x *stamineus* hybrids and 60% in the *catharticus* x *haenkeanus* cross. Since some bivalents may be heterogenetic the actual values could be lower. A preferentiality of not more than 20 to 30% would be more consistent with the results should an estimate be based on the model described in figure 4 using the number of structural differences (table 6) and the fertility actually observed.

CONCLUSION

Association of chromosomes derived from different species (heterogenetic or illegitimate pairing) is established as a primary cause of genetic instability in these allotetraploid hybrids. The result of this instability is the segregating out of factors responsible for interspecific sterility. There is some evidence for preferential affinity; but, even if present, preferentiality is not very strong. Homogenetic association takes place not more than 20 to 30% more frequently than would be expected by chance alone.

The results were consistent with the hypothesis of cryptic structural hybridity; i.e. the theory that interspecific sterility is sometimes caused by structural differences too small to have a visible effect on chromosome pairing.

These hybrids belong to the category of segmental allopolyploids (CLAUSEN, KECK and HEISEY 1945). In plant breeding the usefulness of this category is limited by its instability which is mitigated, however, by the slow rate of segregation characteristic of the tetraploid condition, and by preferentiality. While a diploid interspecific

hybrid may be so sterile as to produce few progeny, the effect of the segregating out of sterility factors on the tetraploid level may be prolonged over several generations. Thus the use of unstable tetraploid lines of descent may be practical in the case of long-lived perennial plants.

SUMMARY

1. Diploid and tetraploid hybrids between three species of *Bromus* were studied to the fourth generation. All lines of descent were selected for fertility.

2. Cytological examinations of the F_1 's showed perfect chromosome pairing (21 bivalents). The allotetraploids averaged 6 quadrivalent associations, almost 30 bivalents, and less than 1 univalent per cell. All the 98 cells examined at metaphase I had the expected number of chromosomes (84).

3. The diploid lines, initially highly sterile, approached full fertility by the fourth generation. The allotetraploid lines, with an original fertility of approximately 80%, decreased at a more or less constant rate to about 45% during the experiment.

4. Both the diploid and the tetraploid lines segregated for morphological characters and for fertility. Segregation of morphological characters was correlated with segregation for fertility in such a way as to suggest linkage between the fertility factors and the genes determining the morphological differences between the species.

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