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Haploidy from *Hordeum* Interspecific Crosses

I. Polyhaploids of *H. parodii* and *H. procerum*

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Summary. Interspecific crosses of *Hordeum parodii* (2n = 42) with *H. bulbosum* (2n = 14 or 28) and *H. vulgare* (2n = 14), and of *H. procerum* (2n = 42) with *H. bulbosum*, *H. vulgare* and *H. parodii* were made. Crosses between *parodii* and diploid *bulbosum* resulted in haploids (2n = 21) of *parodii*, whilst the crosses of *parodii* by tetraploid *bulbosum* or diploid *vulgare* gave hybrid progeny. The *procerum* by diploid *bulbosum* cross invariably produced haploids (2n = 21) of *procerum*, whereas *procerum* by tetraploid *bulbosum* or diploid *vulgare* crosses resulted in both hybrids and haploids of *procerum*. The cross between *procerum* and *parodii* gave hybrid progeny which did not reach maturity.

Cytological observations on two-week-old embryos obtained from reciprocal crosses revealed chromosome variability (not less than 21 in any cell) in haploid producing crosses. This shows that chromosome elimination leads to haploid formation irrespective of which species was used as female parent.

The results indicate that the ratio of the parental genomes in the zygote determines whether predominantly haploids or hybrids will be produced in any cross combination. Furthermore, *procerum* appears to be not only more efficient in eliminating *bulbosum* chromosomes in comparison with *parodii*, but also capable of eliminating *vulgare* chromosomes. The possibility of 'stability factors' in overcoming chromosome elimination, a hierarchy of chromosome elimination and the general existence of genome balance for chromosome stability in interspecific crosses, are discussed.

Introduction

Selective elimination of *Horderum bulbosum* chromosomes following hybridization with *H. vulgare* results in haploids of *vulgare* (Subrahmanyam and Kasha 1973a). Is selective chromosome elimination a widespread phenomenon in *Hordeum* and other groups of plants? The elimination of apparently whole genomes was reported in a cross of *Hordeum leporinum*[1] (2n = 4x = 28) × *H. vulgare* (2x) by Hamilton et al. (1955) and in a cross of *H. bulbosum* (4x) × *H. secalinum* by Cauderon and Cauderon (1956). The importance of such a phenomenon as a potential tool for producing haploids has only recently been realised (Kao and Kasha 1969; Kasha and Kao 1970; Lange 1971a, b; Subrahmanyam and Kasha 1973a, b; Symko 1969). A high frequency of haploid formation from interspecific crosses of *H. lechleri* (6x) × *H. vulgare* (2x) and *H. jubatum* (4x) × *H. bulbosum* (2x) (Rajhathy and Symko 1974), and from intergeneric crosses of *Triticum aestivum* (6x) × *Hordeum bulbosum* (Barclay 1975), has since been reported.

The main objective of this study was to examine the distribution of chromosome elimination leading to haploid formation among *Hordeum* interspecific hybrids and to find a general basis for such a phenomenon. This paper reports seven additional interspecific crosses, four of which produced polyhaploids or polyhaploids and hybrids.

Materials and Methods

The species listed in Table 1 were used. *Hordeum vulgare* plants were maintained throughout their growth period in a glasshouse (25° ± 2° C). As *H. bulbosum*, *H. parodii* and *H. procerum* are perennial with a winter growth habit, the clones of individual species were placed in a cold chamber (10° C with 10 h. day length) for vernalization. After a period of 2 months, plants were removed from the cold chamber and repotted into fresh soil mixture. The plants of *parodii* and *procerum* were transferred to a glasshouse, whereas the *bulbosum* plants were transferred to a growth chamber. Diploid and tetraploid cytotypes of *bulbosum* were maintained in two different compartments to avoid admixture of pollen.

For controlled pollinations, florets in spikes of seed parents were emasculated 1-2 days prior to anthesis. Emasculated spikes were enclosed in cello-

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[1] *leporinum* later confirmed as *murinum* (Rajhathy et al. 1963).

Table 1. Species of *Hordeum* used in the study

Species	Line designation	Ploidy (x = 7)	Source
H. bulbosum L.	ANU 1	2x	Dr. D.H.B. Sparrow, Waite Agric. Res. Inst. University of Adelaide Australia
H. bulbosum L.	ANU 2	2x	Dr. R. Oram, Div. of Plant Industry, CSIRO Canberra, Australia
H. bulbosum L.	ANU 3	4x	"
H. vulgare L.	Clipper	2x	"
H. parodii Covas	ANU 76	6x	Dr. J.H. Hunziker, Argentina. Obtained through Dr. D.R. Dewey, Utah State University, Logan, U.S.A.
H. procerum Nevski	ANU 81	6x	"

Table 2. Seed set induced, embryos cultured and the progeny plants obtained from the interspecific crosses

Parental species and ploidy level (X = 7)	No. of florets pollinated	Percentage of seed set		No. of embryos cultured	Percentage of embryos giving plants
		Average	Range		
H. parodii 6x by *H. bulbosum* 2x	1164	8.1	0-96.5	70	50.0
H. parodii 6x by *H. bulbosum* 4x	422	6.9	0-38.9	24	54.2
H. parodii 6x by *H. vulgare* 2x	172	11.1	0-28.4	11	90.9
H. procerum 6x by *H. bulbosum* 2x	447	12.3	0-35.6	26	57.7
H. procerum 6x by *H. bulbosum* 4x	784	11.2	0-56.1	69	36.2
H. procerum 6x by *H. vulgare* 2x	893	6.9	0-33.9	52	51.9
H. procerum 6x by *H. parodii* 6x	511	1.0	0- 4.1	5	80.0

phane bags to prevent stray pollination. One to three days after each emasculation, the bag was removed, while the stigmas were hand-pollinated with fresh pollen, and the bag immediately replaced. Pollen of only one species and cytotype was used on any one day, to avoid the complication of pollen mixtures.

A day after pollination, tillers were cut 1¼ - 2½ cm above the second node from the top and transferred to nutrient solution (Subrahmanyam and Kasha 1973c). Gibberellic acid (GA_3) at a concentration of 75 ppm was added to the florets, 1 drop per day for two successive days after pollination (Subrahmanyam and Kasha 1971).

The induced seeds 14-15 days after pollination were surface sterilized with 5 % sodium hypochlorite and rinsed with sterile water. The seeds were dissected and the embryos placed onto the culture medium described by Gamborg, Miller and Ojima (1968), but omitting 2, 4-dichlorophenoxyacetic acid and sequestrene and adding 8g of bacto-agar, 28 mg $FeSO_4 \cdot 7H_2O$ and 37 mg Na_2-EDTA per litre of solution.

The embryos were incubated at 22° C in the dark. When root/shoot initiation occurred, they were transferred to a light chamber at 22° C. The seedlings at the 2-3 leaf stage were removed from the vials and potted in the soil mixture.

Chromosome numbers of parental and progeny plants were determined by collecting and pretreating root tips in cold water (0-2° C) for 20 hours and fixing in acetic-ethanol (1:3) for 60 minutes. The root-tips were then rinsed in distilled water, hydrolysed in 1N HCl at 60° C for 15 minutes, treated with 2 % cellulysin (Calbiochem, San Diego, California) for 15 minutes, and stained for 1 hour in leucobasic fuchsin. Slides were prepared by the squash technique in 45 % acetic acid. Two-week-old embryos from reciprocal crosses were cytologically examined following an earlier technique (Subrahmanyam and Kasha 1973a).

Chromosomes of different genomes and species of *Hordeum* are indistinguishable. Thus, the progeny from each cross were classified as either hybrids or haploids, depending on their morphology and chromosome number.

Results

The results presented in Tables 2 and 3 reveal that four out of the seven crosses gave rise to haploids of one of the parents.

The data on the florets pollinated, the seeds induced, the embryos cultured and the seedlings obtained from various crosses are presented in Table

Table 3. Morphological characteristics, chromosome numbers and types of progeny plants from the interspecific crosses

Parental species and ploidy level (x = 7)	No. of plants	Morphology	Chromo-some No.	Type
H. parodii 6x by *H. bulbosum* 2x	35	parodii like	21	haploids
H. parodii 6x by *H. bulbosum* 4x	13	bulbosum like	35	hybrids
H. parodii 6x by *H. vulgare* 2x	10	intermediate	28	hybrids
H. procerum 6x by *H. bulbosum* 2x	15	procerum like	21	haploids
H. procerum 6x by *H. bulbosum* 4x	5	procerum like	21	haploids
	18	bulbosum like	35	hybrids
H. procerum 6x by *H. vulgare* 2x	8	procerum like	21	haploids
	11	mostly procerum like with intermediate spikes	28	hybrids
H. procerum 6x by *H. parodii* 6x	4*	procerum like	42	hybrids

* Two plants died prior to chromosome number determination and the remainder died before reaching flowering.

2. The percentage of seed set in each cross was variable, depending on the vigour of the parents and the environmental conditions at the time of pollination. Thus, it was difficult to detect differences in seed setting ability among different crosses. However, the highest percentage of seed set in each cross represents the potential level of success. A very high proportion of embryos gave rise to full seedlings (36.2% in *procerum* x *bulbosum* 4x cross to 90.9% in *parodii* x *vulgare* cross). Irrespective of the cross combination, an additional 25% of the total embryos (except in the *parodii-vulgare* cross) produced either roots and/or shoots but did not pass the culture-vial stage.

Chromosome numbers and morphological features of different progenies from various crosses are summarised in Table 3 and individual crosses are dealt with separately.

Hordeum parodii x *H. bulbosum* cross

All the plants obtained from the cross involving diploid *bulbosum* had a chromosome number of 2n = 21 (Fig.1a) rather than 2n = 28, which would be expected in hybrids derived from a hexaploid (2n = 6x = 42, Fig.1b) and a diploid (2n = 2x = 14, Fig.1c). Moreover, the spikes (Fig.2b) and morphology of these plants were similar to those of *H. parodii* (Fig.2c).

The cross involving tetraploid *bulbosum* resulted in hybrid plants with the expected chromosome number (2n = 5x = 35, Fig.1d). These hybrids were *bulbosum*-like or intermediate in vegetative characteristics and had not reached flowering even after 6 months, while the 21 chromosome plants of comparable age reached flowering within 3 months of potting.

Hordeum parodii x *H. vulgare* cross

All the progeny from these crosses were found to be hybrid with the expected hybrid chromosome complement (2n = 4x = 28, Fig.1e) derived from a hexaploid (2n = 6x = 42) and a diploid (2n = 2x = 14, Fig.1f). The spike (Fig.2d) and vegetative characters of these hybrids were intermediate between *parodii* (Fig.2c) and *vulgare* (Fig.2e). These hybrids required 90-100 days to reach maturity.

Hordeum procerum x *H. bulbosum* cross

The progeny from the crosses involving diploid *bulbosum* invariably had a chromosome number of 2n = 21 (Fig.3a) instead of a hybrid number (2n = 4x = 28) expected between a hexaploid (2n = 6x = 42, Fig.3b) and a diploid (2n = 2x = 14, Fig.1c). On the other hand, the crosses with tetraploid *bulbosum* resulted in a total of 23 mature plants of which five had 21 chromosome complement (2n = 5x = 35, Fig.3c). The 21

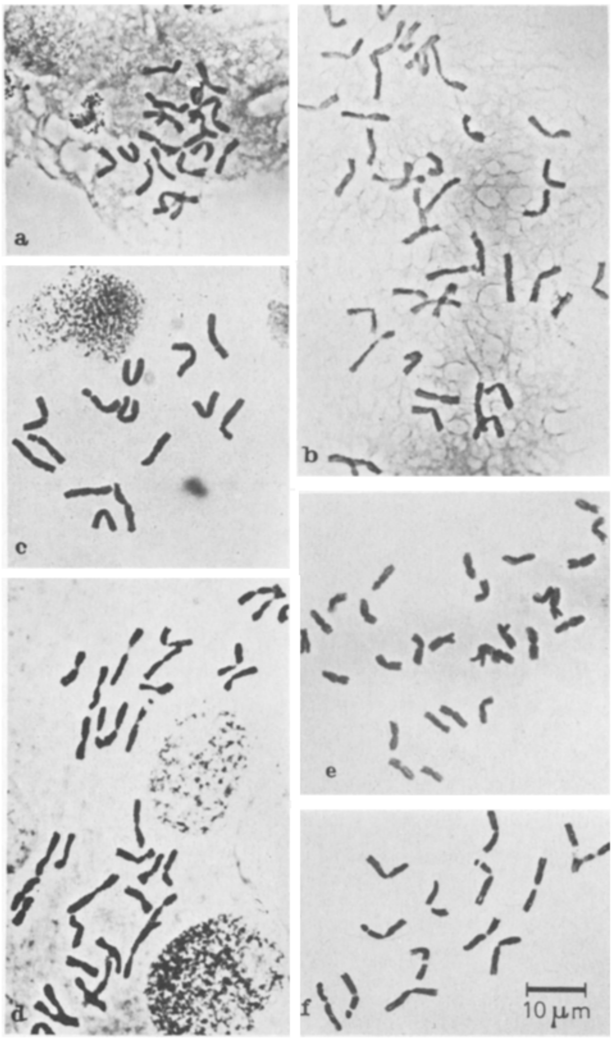

Fig. 1a-f. Somatic chromosomes of parents and progeny for crosses involving *H. parodii*. (a) Haploid *parodii* (21) from *H. parodii* by diploid *bulbosum* cross. (b) *H. parodii* (42). (c) *H. bulbosum* (14). (d) Hybrid (35) between *parodii* and tetraploid *bulbosum*. (e) Hybrid (28) between *parodii* and *vulgare*. (f) *H. vulgare* (14)

chromosome plants resembled *procerum* (Fig. 4c, d), whereas the hybrids were more *bulbosum*-like vegetatively, with a spike morphology (Fig. 4b) intermediate between *procerum* (Fig. 4d) and *bulbosum* (Fig. 4a).

Hordeum procerum × *H. vulgare* cross

Two kinds of progeny were obtained from this cross. Eight plants contained 21 chromosomes (Fig. 3e) and were morphologically similar to *procerum* (Fig. 4d, e). Eleven plants were vegetatively very similar to *procerum* with an intermediate spike morphology (Fig. 4f) and the chromosome number of 2n = 4x = 28 (Fig. 3d) expected of a hybrid between a hexaploid and a diploid. The plants with 21 chromosomes reached flowering in 3 months. The hybrids required a minimum of 4 months to reach flowering. One hybrid plant showed 21 chromosomes in the roots and produced a mixture of *procerum*-like and hybrid tillers.

Hordeum procerum × *H. parodii* cross

It was relatively difficult to induce seeds from this cross. Among the four seedlings obtained, two died before the chromosome number could be determined and the remainder, which had 2n = 42 chromosomes, died after 2 months in pots.

All the F_1 plants from different crosses exhibited ovule sterility (i.e. no seed set). Studies on the meiotic chromosome behaviour in different progenies and their consequences are underway.

Chromosome variation in hybrid embryos

A valid estimation of the proportions of cells with different chromosome numbers in 2-week-old embryos from reciprocal crosses was difficult because of the problems in getting chromosome spreading. Nevertheless, the observations on the few countable cells in various crosses revealed the following:

(1) Haploid (21) to hyperhaploid complement in the crosses between diploid *bulbosum* and *parodii* or *procerum*; (2) haploid to hyperhaploid, and near hybrid to hybrid complement (28 or 35) in crosses between *procerum* and *vulgare* or tetraploid *bulbosum*; (3) only hybrid chromosome number (28 or 35) in crosses between *parodii* and *vulgare* or tetraploid *bulbosum*; and (4) micronuclei in interphase cells and occasional chromosome fragments in dividing cells in embryos from the crosses between diploid *bulbosum* and *parodii* and between *procerum* and *bulbosum* or *vulgare*.

Fig.2a-e. Spikes of parents and progeny from crosses involving *H. parodii* embedded in plastic resin prior to photographing. (a) *H. bulbosum*. (b) Haploid *parodii*. (c) *H. parodii*. (d) *H. parodii-vulgare* hybrid. (e) *H. vulgare*

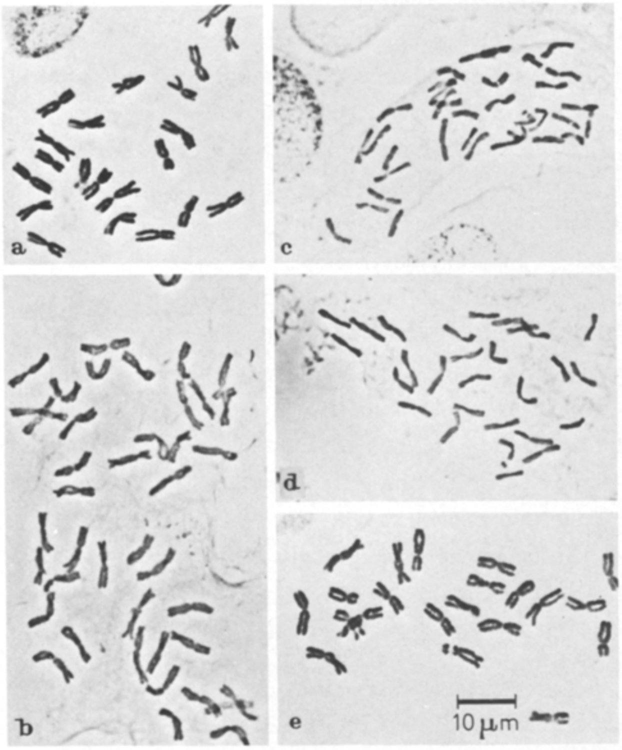

Fig.3a-e. Somatic chromosomes of progeny for crosses involving *H. procerum*. (a) Haploid *procerum* (21) from *procerum-bulbosum* cross. (b) *H. procerum* (42). (c) Hybrid (35) between *procerum* and tetraploid *bulbosum*. (d) *H. procerum-vulgare* hybrid (28). (e) Haploid *procerum* (21) from *procerum-vulgare* cross

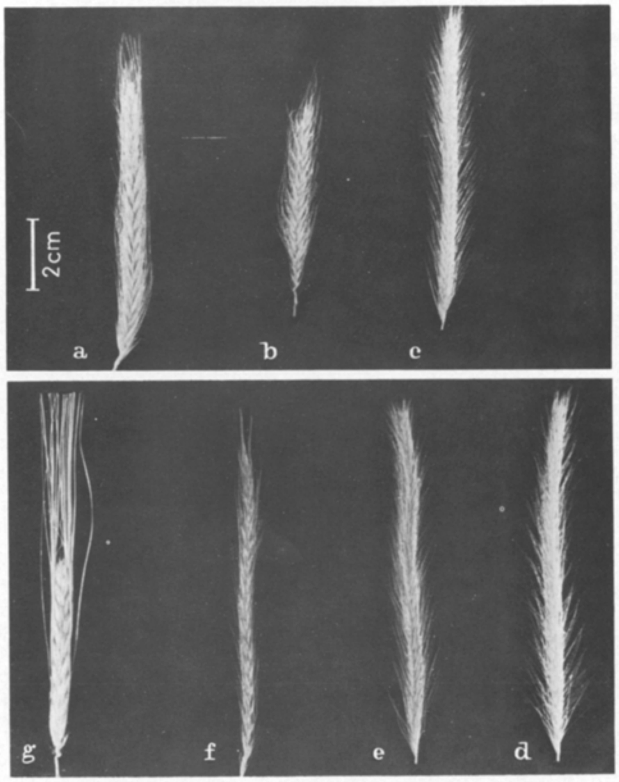

Fig.4a-g. Spikes of parents and progeny from crosses involving *H. procerum* (embedded in plastic resin prior to photographing). (a) *H. bulbosum*. (b) Hybrid between *procerum* and tetraploid *bulbosum*. (c) Haploid *procerum* from *procerum-bulbosum* cross. (d) *H. procerum*. (e) Haploid *procerum* from *procerum-vulgare* cross. (f) *H. procerum-vulgare* hybrid. (g) *H. vulgare*

Discussion

The main objective of this study was to elucidate the phenomenon of chromosome elimination leading to haploid formation among *Hordeum* interspecific hybrids and to find a general basis for such a phenomenon. The results, in that 4 out of 7 crosses gave rise to haploids of one of the parents, are consistent with the observations on chromosome variability in the embryos for the corresponding crosses. From the *parodii* (6x) by *bulbosum* (2x) and *procerum* (6x) by *bulbosum* (2x) crosses, the presence of chromosome numbers varying down to 21 in the embryonic cells expected to have 28 chromosomes, the absence of cells with less than 21 chromosomes and the morphological resemblances of the progeny to one of the parents (*parodii* or *procerum*), are indicative of selective elimination of *bulbosum* chromosomes leading to haploid formation. Production of haploids resembling *procerum* and hybrids from the crosses *procerum* by tetraploid *bulbosum* and *procerum* by diploid *vulgare*, and the apparent chromosome variability in the hybrid embryos from the same crosses, also suggest that elimination of *bulbosum* and *vulgare* chromosomes must have resulted in the haploids from the respective crosses. Selective elimination of *bulbosum* chromosomes from *vulgare-bulbosum* hybrids has been extensively demonstrated (Subrahmanyam and Kasha 1973a, b; Bennett et al. 1976). The phenomenon of chromosome elimination is known in six other *Hordeum* interspecific combinations (Kasha 1974). Preliminary results (Subrahmanyam 1976 and unpublished), indicate chromosome elimination in at least four more *Hordeum* crosses. It is probable that selective elimination of a single genome is occurring in such crosses, from the fact that the minimum chromosome number has always been 7 between diploid by diploid crosses. Thus a total of fifteen *Hordeum* interspecific cross combinations exhibit chromosome elimination.

Genome Balance

Formation of haploids from *parodii* (6x) by *bulbosum* (2x) cross, hybrids from *parodii* (6x) by *bulbosum* (4x) and similarly haploids from *procerum* (6x) by *bulbosum* (2x), hybrids (and a few haploids) from *procerum* (6x) by *bulbosum* (4x) strongly suggest that a balance between the ratio of the parental genomes in each hybrid determines whether predominantly haploid or hybrid progeny are produced. For instance, a ratio of 3 *parodii* (par) genomes to 1 *bulbosum* (blb) genome leads to the elimination of *bulbosum* chromosomes and subsequent formation of *parodii* haploids, whilst the 3 par:2 blb ratio results in stable hybrids. Similarly a ratio of 3 *procerum* (prc):1 blb genomes gives haploids, while the 3 prc:2 blb ratio results mostly in hybrids. It is well documented in *vulgare-bulbosum* crosses (Kasha and Sadasivaiah 1971; Subrahmanyam and Kasha 1973a, b) that a 1 *vulgare* :1 *bulbosum* genome ratio results in *vulgare* haploids, while 1 *vulgare* :2 *bulbosum* genomic ratio results in stable hybrids. Furthermore, the genomic combination of 1 *vulgare* :4 *bulbosum* is more stable than that of 1 *vulgare* :2 *bulbosum* in hybrid endosperm tissues (Subrahmanyam and Kasha 1973a). Hybrids from *jubatum* (4x) by *bulbosum* (4x) cross (Wagenaar 1960) and haploids of *jubatum* by crossing with diploid *bulbosum* (Rajhathy and Symko 1974) were obtained. Thus a ratio of 2 *jubatum* to 2 *bulbosum* genomes results in hybrids, whereas a 2 *jubatum* :1 *bulbosum* genome ratio results in haploids through elimination of *bulbosum* chromosomes. Results (unpublished) from the crosses of *brachyantherum* (4x) with diploid and tetraploid *bulbosum* indicate genome balance as with *jubatum.* The successful production of *Hordeum* interspecific hybrids at specific ploidy levels (Rajhathy et al. 1963) is consistent with the suggestion that the genome balance is important to obtain stable hybrids. Thus, one can propose that crosses involving other species in a specific genome balance may also exhibit chromosome elimination.

Genetic Control of Chromosome Elimination and Stability

It has been proposed that specific chromosomes carry factors which are critical for chromosome elimination in interspecific hybrids between *H. vulgare* and *H. bulbosum* (Kasha et al. 1970). Subsequently, it was demonstrated that chromosomes 2 and 3 of *vulgare* carry genetic factors controlling elimination of *bulbosum* chromosomes (Barclay et al. 1972; Kasha et al. 1972; Ho and Kasha 1975). Furthermore, it has been shown that at least two, and possibly three, such factors are involved in control (Ho and Kasha 1975).

From the observations on genome balance (Kasha and Sadasivaiah 1971) and the evidence for its genetic control by specific chromosomes (Ho and Kasha 1975), it is clear that the factors causing the elimination of the *bulbosum* chromosomes (hence 'eliminating factors') are located on the *vulgare* chromosomes. Possibly such factors are widespread among *Hordeum* species. Results of Subrahmanyam and Kasha (1973a) suggest that *bulbosum* chromosomes also carry genetic factor(s) to overcome or offset the factors on the *vulgare* chromosomes governing chromosome elimination. The relatively stable triploid hybrids and the greater stability in the endosperm with 4 *bulbosum* : 1 *vulgare* genomes provide further evidence of 'stability factors' on *bulbosum* chromosomes. As genetic and cytogenetic knowledge on *bulbosum* is obscure, it has not been possible to locate 'stabilizing factor(s)' on any specific chromosome(s) of *bulbosum*. In the present study, it is important that the *procerum* (6x) by *vulgare* (2x) cross resulted in hybrids as well as haploids of *procerum*. This suggests that a *procerum* (6x) by *vulgare* (4x) cross should result in stable hybrids. To test this possibility, autotetraploid *vulgare* stocks are being produced or acquired. Furthermore, the problem of which chromosome(s) of *vulgare* carry 'stabilizing factor(s)' in crosses between *procerum* and *vulgare* remains to be solved.

Hierarchy of Chromosome Elimination in *Hordeum*

With the increasing awareness of selective chromosome elimination in somatic cell hybrids of mammals (Weiss and Ephrussi 1966; Weiss and Green 1967; Handmaker 1971; Westerveld et al. 1971; Rao and Johnson 1972) a hierarchy in chromosome elimination (hamster > mouse > rat > man chromosome) is apparent. Based on the present study and on the information discussed, (page 6), the following order of chromosome elimination in interspecific hybrids of *Hordeum* is evident:

$$\left.\begin{array}{l}\textit{arizonicum}\ (6x)\\ \textit{procerum}\ (6x)\end{array}\right] > \textit{vulgare}\ (2x) > \textit{bulbosum}\ (2x)$$

lechleri (6x) > *vulgare* (2x)

$$\left.\begin{array}{l}\textit{parodii}\ (6x)\\ \textit{jubatum}\ (4x)\\ \textit{brachyantherum}\ (4x)\end{array}\right] > \textit{bulbosum}\ (2x)$$

vulgare (4x) > *bulbosum* (4x or 2x)

bulbosum (4x) > *secalinum* (4x)

In the haploid producing crosses of *procerum* (6x), *lechleri* (6x) and *arizonicum* (6x) with *vulgare* (2x), it appears that the 'eliminating factor(s)' are more or less of the same strength in eliminating *vulgare* chromosomes. The results from the crosses with diploid and tetraploid *bulbosum* indicate that *procerum* (6x) has a stronger genetic mechanism in eliminating *bulbosum* chromosomes than does *parodii* (6x), *jubatum* (4x) or *brachyantherum* (4x), since only the *procerum* (6x) by *bulbosum* (4x) cross results in both haploids and hybrids, although no differences are apparent in crosses with diploid *bulbosum*. Further experimentation is needed to determine whether chromosome elimination occurs in crosses between species of similar 'genetic potential' in eliminating *bulbosum* chromosomes.

Mechanism of Chromosome Elimination

The widespread occurrence of selective chromosome elimination and a common genetic basis (genome balance) for chromosome stability or elimination in different *Hordeum* interspecific hybrids suggest that the mechanism of elimination is similar to that for *vulgare-bulbosum* hybrids. Various hypotheses, such as asynchrony of mitotic cell cycle times due to differences between parental species (Gupta 1969; Lange 1971b; Subrahmanyam and Kasha 1973a), spindle abnormalities (Handmaker 1973) and a 'modification-restriction system' (Davies 1974) have been proposed. Bennett et al. (1976) found that the chromosomes of the parent with a longer mean cell generation time are eliminated. They also pointed out the failure of chromosomes to initiate or complete either congression at metaphase or migration to the poles at anaphase as the cause of elimination. Treatment of *bulbosum* and *vulgare* root-tip cells with bacterial restriction endonucleases results in chromosome degradation (Subrahmanyam et al. 1976) which is similar to the occasional degradation of whole chromosomes in *vulgare-bulbosum* hybrid cells (Subrahmanyam and Kasha 1973a). Whether a selective endogenous nuclease activity in interspecific hybrids leads to the failure of specific chromosomes to congress or migrate during cell division, or the chromosomes that fail to congress or migrate are degraded, remains to be resolved.

The polyhaploids and hybrids thus obtained are being utilized for meiotic studies.

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