THESIS

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CYTOGENETICS OF HAPLOIDS AND HYBRIDS FROM THE CROSS, *HORDEUM PROCERUM* X *HORDEUM VULGARE*

By

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Abstract

Hordeum procerum Nevski (2n=6x=42) was crossed with three cultivars of barley, *H. vulgare* L. (2n=2x=14). Based on cytological and morphological evidence, two classes of plants resulted. Crosses made with *H. vulgare* cv. 'Larker' resulted in polyhaploids (2n=3x=21), while crosses made with other cultivars resulted in hybrids with variable chromosome numbers, 2n=23=29. The presence of genetic factors in the *H. vulgare* genome for resistance to chromosome elimination is proposed.

Microsporogenesis was studied in the polyhaploids and hybrids, as well as in both parental species. *H. procerum* is considered a segmental autoallohexaploid. In addition, a mechanism for the genetic control of meiotic pairing was inferred, which causes *H. procerum* to behave cytologically like a diploid. It is suggested that there is no appreciable homology between the genomes of *H. procerum* and *H. vulgare*.

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INTRODUCTION

The study of interspecific hybrids may yield information about taxonomic and evolutionary relationships between plants; and, when one of the species involved is an agronomically useful one, may lead to the transfer of genes which contribute to increased crop yields, improved quality, resistance to environmental stress, or resistance to pests. This generalization can be applied to most previous interspecific hybridization work in the genus Hordeum (Schooler and Anderson, 1979; Steidl, 1976; Starks, 1976; Morrison et al., 1959).

The most ambitious program of Hordeum interspecific hybridization to date was that initiated by D. G. Hamilton and carried out by J. W. Morrison and others at Ottawa, Canada. They attempted 111 interspecific cross combinations, about half of which involved cultivated barley, H. vulgare. They were able to produce fourteen viable hybrids by using embryo culture techniques (Morrison et al., 1959). All hybrids between H. vulgare and species outside section Cerealia were sterile, and studies of meiotic pairing indicated no homology between the parental genomes (Morrison and Rajhathy, 1959).

Nilan (1964) and Price (1968) reviewed the interspecific crosses attempted in Hordeum. They also pointed out that hybrids between barley and distantly-related species are sterile and exhibit no homology between genomes.

Schooler and Anderson (1979) have taken a somewhat different tack. Utilizing a fertile amphiploid from the cross (H. brachyantherum X H. bogdanii)(2n = 6x = 42) as the female parent and H. vulgare cv. 'Traill' (2n = 4x = 28) as the male parent, they were able to produce F_1 hybrids with a chromosome number which varied from 21 to 35. After backcrossing to diploid H. vulgare (2n = 2x = 14) and recovering diploid progeny, they reported some preliminary observations of gene transfer.

This same general approach, that is, crossing an amphiploid of a hybrid between two wild species to barley, followed by a backcrossing program, has been attempted by previous workers at this university (Huang, 1975; Steidl, 1976) without any appearance of startling success. Genome relationships have also been studied between Hordeum species, and between Hordeum and Agropyron species, by previous workers in this laboratory (Murry, 1975; Huang, 1975; Starks, 1976).

In the fall of 1978, I began a crossing program in an attempt to generate hybrids between H. vulgare and diploid wild species. This program was later expanded to include tetraploid and hexaploid species or cytotypes of species. Of the 74 crosses attempted, only those between cultivars or species from section Cerealia produced fully developed, viable seeds. Some other cross combinations did set seed, but although they included a reasonably well developed embryo, endosperm development was poor and the seeds did not germinate. Attempts to save some of these embryos via embryo culture, using

Norstog's Barley Medium II (Norstog, 1973) were wholly unsuccessful.

In the early winter of 1980, an encouraging development appeared which was to lead to further research. A hybrid seedling was obtained from the cross, H. procerum (2n = 6x = 42) X H. bulbosum (2n = 2x = 14) using a modified version of Norstog's medium.

This led to the consideration of the possibility of producing and studying hybrids between H. procerum (2n = 6x = 42) and H. vulgare (2n = 2x = 14). Subrahmanyam (1977) reported that this cross resulted in tetraploid hybrids, as well as triploid polyhaploids of H. procerum.

The questions which it was hoped the study would answer were:

1. Would the cross, H. procerum (6x) X H. vulgare (2x) yield hybrids and polyhaploids, as other workers have reported?
2. Would there be differences between barley cultivars in the degree and frequency with which their chromosomes were eliminated by the H. procerum genome?
3. What would cytogenetic studies of H. procerum hexaploids and polyhaploids reveal about the nature of its three genomes?
4. What would cytogenetic studies of the hybrids reveal about the degree of homology, if any, between the genomes of H. procerum and H. vulgare?

MATERIALS AND METHODS

PLANT MATERIALS

Two Hordeum species were used in this study. The first was a wild species collected in Argentina, Hordeum procerum (PI No. 266196), which was obtained as seed from the USDA Cereal Crops Research Branch, Beltsville, Maryland. Previous reports (Covas, 1950; Bowden, 1965) indicate a chromosome number of (2n = 6x = 42). The second species was barley, Hordeum vulgare L. (2n = 2x = 14) and three cultivars were used; 'Coho', 'Larker', 'Beacon'.

CROSSING TECHNIQUES

In all crosses performed as a part of this study, H. procerum was the female parent, and one of the three barley cultivars was the male parent. No reciprocal crosses were done because earlier experience with these plants indicated that the cross was more likely to be successful with H. vulgare as the male parent. This was also the case for similar interspecific Hordeum crosses (Steidl, personal communication).

For all crosses, the female parent was emasculated by first removing all spikelets above and below those judged to be at the right stage of development. Generally, this meant that only florets with pale-green to yellow anthers were used and the rest discarded. Lateral florets were removed from the remaining spikelets. The lemma and palea of each remaining floret was then cut off just above the

anthers with scissors, and the anthers removed with fine forceps. The spikes were then covered with a thin, cylindrical cap of aluminum foil. Aluminum foil was superior to other coverings, because it keeps the spikes cool, prevents dehydration, and prevents contamination by stray pollen.

For a source of fresh pollen, barley spikes were selected in which the anthers were just about to dehisce. The tops of the florets were cut off just above the anthers and the spike was placed in strong light, either sunlight or in close proximity to an incandescent bulb (about 50 cm.). This caused the anthers to be extruded from the florets and to dehisce. Pollen was collected by inverting spikes inside a small bag fashioned from dialysis tubing and tapping it to release pollen into the bag.

Previously emasculated spikes with receptive stigmas (stigmas spread out and florets open) were then inverted in one of these bags containing pollen, the opening of the bag was held closed, and the spike was returned to an upright position. This inversion-reversion process was then repeated until most of the pollen had come to rest on the florets; this resulted in the deposition of massive amounts of pollen on the stigmas, thus insuring that each stigma had an ample supply of viable pollen. This is also the quickest and simplest way to do these pollinations. After pollination, the aluminum foil cap was replaced.

EMBRYO CULTURE

Spikes were cut from the plants and taken to the laboratory for embryo culture 12 to 14 days after pollination. The percent seed set was also scored at this time.

All embryo culture work was done in a laminar flow hood using standard sterile techniques. The seeds were removed from their respective florets and sterilized in a 20% solution of commercial bleach (corresponding to a solution of roughly 1% sodium hypochlorite) for 20 minutes, then washed briefly in sterile, double-glass-distilled water. The embryos were dissected from the caryopses, and placed scutellum side down on a modified Norstog's medium, (Norstog, 1973; Taira and Larter, 1978; Appendix A) in slanted culture tubes. The embryo cultures were incubated in the dark at 27°C, then transferred to a lighted culture room at 25°C when they showed definite root and shoot (cotyledon) development. Some embryos produced only very short roots and shoots (about 2 - 5 mm) in the incubator, as if development was arrested at that point. These were transferred to a callus regeneration medium, designated B-5R (Orton, 1978; Appendix B), and placed in the lighted growth room to induce them to germinate further.

When the seedlings reached the three leaf stage, they were potted in plastic cups filled with vermiculite and placed in a growth chamber with a 16 hour day length and a 19° - 14°C day-night temperature regime. The plants were later transplanted to soil in

clay pots and placed in the greenhouse.

CYTOLOGICAL METHODS

Mitotic material was pretreated in two ways. Healthy root tips of growth chamber-grown plants were excised and pretreated in a solution of .01% colchicine at room temperature for 4 hours. Root tips from greenhouse-grown plants were excised and pretreated in distilled water at 2 - 4°C for 24 - 48 hours. In all cases, the root tips were fixed in 3:1 ethanol-acetic acid (Farmer's solution) at room temperature for 12 - 24 hours and then rinsed and stored in 70% ethanol at 4°C.

Root tips were prepared for squashing by hydrolyzing in 1N HCl for 10 minutes at 60°C, rinsing with distilled water, and staining in Feulgen's solution for 2 hours in the dark. Root tips were then rinsed with distilled water and further softened in 5% pectinase (SIGMA) for one hour at 30°C in a water bath. The pectinase was rinsed out with distilled water. The root tips were either squashed immediately or stored in 70% ethanol at 4°C until needed.

For observation of meiosis in pollen-mother-cells (PMC's), anthers were dissected from central florets and squashed in aceto-carmine stain.

Good cytological preparations were documented by photographing them with a Zeiss Photomicroscope II using Kodak Panatomic-X film in the built-in 35 mm camera.

Pollen stainability was determined by squeezing pollen out of mature anthers in a drop of I_2KI stain. Only darkly-stained grains were scored as stainable; a minimum of 300 grains per plant was scored.

RESULTS

A total of 19 spikes of H. procerum were pollinated by 3 different barley cultivars. The results of these hybridizations are presented in Table I. H. vulgare cv. 'Larker' was the male parent in 7 crosses, and the cultivars 'Coho' and 'Beacon' were used in six crosses each. A total of 242 florets was pollinated, 172 of which set seed; this is an average seed set of 71%. All of these seeds contained embryos, indicating there was no parthenocarpic fruit set. In all cases, also, the endosperm which developed was watery, and had begun to collapse by the time the embryos were excised for culture. One hundred and forty embryos were placed on embryo culture medium, and 21 were placed on callus initiation medium as part of another study. Eleven embryos were either too small to culture, or were damaged in the process of excision from the caryopsis. Seventy embryos, or 50% of the total, germinated almost immediately and were transferred to the lighted growth room for further development. Seventeen embryos seemed to stop development after only a day or two. Previous experience indicated that these would simply remain dormant, and eventually die. These were transferred to the B-5R medium, normally used for regeneration of plantlets from callus. All seventeen of these embryos germinated. In all, 87 embryos, or 62% of those cultured, germinated and were transplanted to vermiculite as seedlings, and placed in a growth chamber.

In the late summer of 1980 a growth chamber malfunction, resulting in extremely high temperatures, killed approximately one-half of these

TABLE 1

Crosses Performed With *Hordeum procerum* as Female Parent

Cross Number	Male Parent	Florets Pollinated	Seeds set #	Seeds set %	Seeds set range	Embryos Cultured	Seedlings Transplanted	%
1	Coho	14	11	79		10	7	
2	Coho	14	8	57		8	8	
4	Coho	14	13	93		12	11	
16	Coho	17	16	94		13	5	
18	Coho	11	7	64		5	5	
22	Coho	13	1	8		0	0	
Total		83	56	67	8- 94	48	36	75
5	Larker	8	6	75		6	2	
6	Larker	10	10	100		5	3	
7	Larker	4	4	100		4	3	
8	Larker	14	13	93		8	4	
9	Larker	13	6	46		6	3	
10	Larker	14	12	86		7	7	
11	Larker	17	13	76		13	9	
Total		80	64	80	46-100	49	31	63
13	Beacon	8	1	12		1	1	
14	Beacon	10	10	100		10	8	
15	Beacon	13	0	0		0	0	
17	Beacon	16	13	81		12	3	
20	Beacon	13	11	85		10	0	
23	Beacon	19	17	89		10	8	
Total		79	52	66	0-100	43	20	46
Grand Total		242	172	71	0-100	140	87	62

plants. All surviving plants were then transferred to the greenhouse and subsequently repotted in clay pots.

The mitotic chromosome numbers of 27 interspecific cross progeny, which resulted from five different crosses, are presented in Table 2. All ten plants which resulted from crosses 10 and 11, (H. procerum X H. vulgare cv. 'Larker') had 21 chromosomes in mitotic metaphase plates of root tip squashes (Figure 1, Table 2). This corresponds to the haploid chromosome number of the female parent. Morphologically, these plants closely resemble H. procerum; however, they are shorter in stature and their spikes and spikelets are smaller (Figure 2). All of these plants are sterile.

The eleven plants which resulted from crosses 1 and 4 (H. procerum X H. vulgare cv. 'Coho') had chromosome numbers which varied. Some plants had the same number of chromosomes in all cells studied, but in other plants, the number was variable. Overall, the number of somatic chromosomes in the plants from the Coho crosses ranged from 23 to 29 (Figure 1, Table 2), with a mean of 26.9. These plants have spike and spikelet morphology intermediate between the parents, and all are taller than either parent (Figure 2). A notable exception to this is plant number 4-2, which has 23 chromosomes (Figure 1). While its spike and spikelet morphology is intermediate, its stature and gross morphology more closely resembles that of the polyhaploids from the Larker crosses. All of these plants are sterile.

The six plants studied from cross 14, (H. procerum X H. vulgare cv. 'Beacon') also have variable chromsome numbers (Figure 1, Table 2),

TABLE 2

Chromosome Numbers of Progeny of Interspecific Crosses

(*H. procerum* (6x) X *H. vulgare* (2x))

Plant No.	Male Parent	Number of Cells						
		Chromosome Number						
		21	23	26	27	28	29	Total
1-1	Coho				15	11		26
2	"				25			25
3	"					5	3	8
4	"					29		29
5	"			8				8
6	"					25		25
7	"			21				21
4-2	Coho		33					33
8	"				29			29
10	"				48	19		67
11	"					34		34
Total	Coho							305
10-1	Larker	36						36
2	"	32						32
4	"	30						30
5	"	24						24
6	"	36						36
11-1	Larker	26						26
2	"	23						25*
3	"	15						15
4	"	26						26
5	"	29						29
Total	Larker							279
14-1	Beacon				19			19
2	"				19	24	1	44
3	"					5	14	19
4	"				25			25
6	"				30			30
7	"				53	17		70
Total	Beacon							207

*Includes 2 cells with 42 chromosomes each.

with a range of 27 - 29 and a mean chromosome number of 27.4. These plants also exhibit morphology which is intermediate between the parents, (Figure 2), are taller than both parents, and are sterile.

Seven plants, judged to be a representative sample of the variation in chromosome numbers and genetic background, were selected for meiotic studies. In addition, meiosis was also studied in H. procerum and H. vulgare cv. 'Larker'.

A summary of metaphase I chromosome associations in H. procerum and in H. vulgare is presented in Table 3.

Meiosis in H. vulgare cv. 'Larker' was essentially nominal (Figure 3). Metaphase I plates exhibited seven bivalents in 98% of the PMC's observed. Anaphase I was without laggards in the fifteen cells observed; segregations were the expected 7 - 7 in well-spread PMC's.

Metaphase, anaphase, and telophase II were also apparently normal and without bridges or laggards, (Table 4). Of the 320 tetrads observed, only one (0.3%) had micronuclei. No supernumerary dyads or tetrads were observed.

H. procerum appears to be a relatively normal hexaploid (Figure 3). In the 36 metaphase I cells studied, (Table 3), there was a mean of 19.6 bivalents and 2.4 univalents per cell. The maximum number of 21 bivalents occurred in 54% of the cells studied. Trivalents and quadrivalents were relatively rare, each occurring with a frequency of .06 per cell. All metaphase I cells had 42 chromosomes. Post-metaphase

I chromosome behavior, (Table 4) was relatively normal, with only a few bridges and laggards at anaphase I and telophase I, and a few micronuclei at the dyad stage. The second meiotic division, however, was characterized by bridges and laggards at anaphase and telophase, resulting in micronuclei in 24% of all tetrads studied. Pollen stainability was 76% (Table 5).

The plants which resulted from the crosses with 'Larker' as male parent were polyhaploids with $2n = 3x = 21$. The metaphase I chromosome behavior of the two plants studied, numbers 10-5 and 11-1, is presented in Table 6 and in Figure 4. Both plants had mean univalent frequencies of just over seventeen, and mean bivalent frequencies of just less than two. Multivalent frequencies were exceedingly low.

The combined post-metaphase I chromosome behavior is presented in Table 4 under the heading, 'Larker Crosses', and in Figure 4. Anaphase I is characterized by a high frequency of laggards, many of which divide precociously, as well as unequal segregations (other than 10 - 11) and tripolar division figures. Telophase I also exhibits bridges, laggards and tripolar divisions, resulting in dyads with micronuclei, as well as triads (dyad stage with three cells). Metaphase II seemed to occur as scheduled, despite the now-jumbled nature of the nuclei in a high proportion of dyads. Anaphase and telophase II were characterized by even higher percentages of bridges and laggards than anaphase and telophase I. There were no tripolar figures

observed at the second division; however, over 58% of the tetrads observed showed micronuclei, and over 10% of the "tetrads" had supernumerary cells (more than 4). Pollen stainability was 0.2% (Table 5).

Three plants were studied from the crosses with 'Coho' as the male parent. The metaphase I chromosome behavior of these plants, numbers 1-4, 4-2, and 4-8 is presented in Tables 7, 8, and 9, respectively, and in Figure 5. Note that the number of chromosomes in metaphase I PMC's varied within each plant. Plant number 1-4, which was determined to have a somatic chromosome number of 2n = 28, had a mean chromosome number in metaphase I cells of 25.9, with a range of 24 - 28. There was a mean number of 2.2 bivalents and 18.2 univalents per cell, with a trivalent frequency of .12. Plant number 4-2, with a somatic chromosome number of 2n = 23, had a mean metaphase I chromosome number of 23.5, with a range of 23 - 26. Mean bivalent and univalent frequencies were 1.8 and 19.9, respectively; no trivalents were observed. Plant number 4-8, with a somatic chromosome number of 2n = 27, had a mean chromosome number of 26.9 in metaphase I cells, with a range of 26 - 28. This plant had a mean of 19.9 univalents, 3.1 bivalents, .26 trivalents, and .02 quadrivalents per cell; this represents the highest frequency of multivalents of the five hybrid plants studied.

The post-metaphase I chromosome behavior of these plants is presented in Table 4 under the heading, 'Coho Crosses', and in Figure 5.

In many respects the meiotic behavior of these plants closely resembled that of the polyhaploids discussed previously. While tripolar first divisions seemed to decrease, the frequency of bridges and laggards increased at anaphase and telophase of both the first and second division and both dyads and tetrads had more micronuclei than the polyhaploids. The number of supernumerary cells at the tetrad stage was also greatly increased, and a few "tetrads" were observed with only three cells, as if the second division did not occur in one half of a dyad. Multinucleated cells were frequent. Pollen stainability was 0.19% (Table 5).

Two plants were studied from the crosses with 'Beacon' as the male parent, and these also had variable chromosome numbers in PMC's. Plant number 14-2 (Table 10), with a somatic chromosome number which varied from 27-29, (mean 27.6) had a mean metaphase I chromosome number of 26.9, and a mean of 22.7 univalents, 2.0 bivalents, and .06 trivalents per cell. Plant number 14-7 (Table 11) with a somatic chromosome number of 27-28, (mean 27.2) had a mean metaphase I chromosome number of 27.0. There was a mean of 23.4 univalents and 1.8 bivalents per cell. Photographs of meiotic phenomena from these plants are included in Figure 5.

The post-metaphase I chromosome behavior of these plants is presented in Table 4 under the heading "Beacon Crosses". Although the data in this case is rather sparse, the pattern established by the Coho crosses emerges. Tripolar first divisions, bridges and

laggards at anaphase and telophase of both divisions, tetrads with a high frequency of micronuclei, supernumerary cells, and multinucleated tetrads were all observed. Pollen stainability was 0% (Table 5).

TABLE 3

Summary of Metaphase I Chromosome Associations in *Hordeum vulgare* cv. 'Larker' and in *Hordeum procerum*

Plant	Chromosome Association				Number of cells	%
	I	II	III	IV		
H. vulgare cv. 'Larker'	2	6			1	2.2
		7			44	97.8
Total					45	
Mean per cell	0.04	6.9				
H. procerum		21			19	52.8
	2	20			5	13.9
	4	19			2	5.5
	6	18			3	8.3
	8	17			2	5.5
	12	15			1	2.8
	9	15	1		1	2.8
	8	15		1	1	2.8
	4	17		1	1	2.8
	1	19	1		1	2.8
Total					36	
Mean per cell	2.4	19.6	.06	.06		

TABLE 4

Summary of Post-Metaphase I Chromosome Behavior for All Plants Studied

Meiotic Stage and Phenomenon	Larker		H. procerum		Larker Crosses		Coho Crosses		Beacon Crosses	
	#	%	#	%	#	%	#	%	#	%
Anaphase I										
Normal	15	100	20	62.5	21	18.1			1	14.3
Unequal Seg.					11	9.5			3	42.9
Bridges			6	18.7			2	7.1	1	14.3
Laggards			6	18.7	64	55.2	1	3.5	2	28.6
Bridges and Laggards							24	85.7		
Tripolar					20	17.2	1	3.5		
Total	15		32		116		28		7	
Telophase I										
Normal			80	93.02	66	35.1	5	6.0	2	8.3
Bridges			2	2.33	29	15.4	12	14.5	15	62.5
Laggards			4	4.65	45	23.9	7	8.4		
Bridges and Laggards					3	1.6	47	56.6	7	29.2
Tripolar					45	23.9	10	12.0		
Linear							1	1.2		
Cytomictic							1	1.2		
Total			86		188		83		24	

TABLE 4 (cont.)

Meiotic Stage and Phenomenon	Larker #	Larker %	H. procerum #	H. procerum %	Larker Crosses #	Larker Crosses %	Coho Crosses #	Coho Crosses %	Beacon Crosses #	Beacon Crosses %
Dyads										
Normal			27	93.1	65	37.3	54	19.6		
With Micronuclei			2	6.9	52	29.9	108	39.3	47	48.4
Cytomictic							25	9.0		
Unequal Nuclei					12	6.9	5	1.8	5	5.2
Multinucleate							40	14.5	22	22.7
Triads							43	15.6	23	23.7
Total			29		174		275		97	
Metaphase II										
Normal	27	100	24	96	45	71.4	10	66.6	23	65.7
Micronuclei			1	4	11	17.5	5	33.3	12	34.3
In Triad					6	9.5				
Cytomictic					1	1.6				
Total	27		25		63		15		35	
Anaphase II										
Normal	29	100	4	80	1	6.7			10	43.5
Bridges										
Laggards			1	20	9	60.0				
Bridges and Laggards					5	33.3	5	100	13	56.5
Total	29		5		15		5		23	

TABLE 4 (cont.)

Meoitic Stage and Phenomenon	Larker #	Larker %	H. procerum #	H. procerum %	Larker Crosses #	Larker Crosses %	Coho Crosses #	Coho Crosses %	Beacon Crosses #	Beacon Crosses %
Telophase II										
Normal	70	100	32	76.0	16	19.0	13	34.2		
Bridges			1	1.5	31	36.9	8	21.0		
Laggards			34	50.7	21	25.0	8	21.0		
Bridges and Laggards					16	19.0	9	23.7	26	100.0
Total	70		67		84		38		26	
Tetrads										
Normal	319	99.7	432	76.0	197	41.7	61	25.9		
with Micronuclei	1	0.3	136	24.0	275	58.3	174	74.0		
Total	320		568		472		235			
Number of Cells										
3							20	4.0	1	2.4
4	320	100	568	99.8	456	89.6	322	64.9	28	66.7
5			1	.175	15	2.9	82	16.5	11	26.2
6					38	7.5	63	12.7	2	4.8
7							4	0.8		
8							5	1.0		
Total	320		569		509		496		42	

TABLE 5

Pollen Stainability

Plant	Stainable	Not Stainable	Total	% Stainable
H. procerum	1514	486	2000	75.7
'Coho' Crosses:				
1-4	0	300	300	0.0
4-2	3	297	300	1.0
4-8	0	1000	1000	0.0
Total	3	1597	1600	.2
'Larker' Crosses:				
10-5	0	300	300	0.0
11-1	1	299	300	0.33
Total	1	599	600	.1
'Beacon' Crosses:				
14-2	0	300	300	0.0
14-7	0	300	300	0.0
Total	0	600	600	0.0

TABLE 6

Summary of Metaphase I Chromosome Associations in Two Polyhaploids from the Cross H. procerum X H. vulgare cv. 'Larker'

Plant	Chromosome Association				Number of Cells	%
	I	II	III	IV		
10-5	21				14	15.7
	19	1			18	20.2
	17	2			36	40.4
	15	3			14	15.7
	13	4			4	4.5
	14	2	1		2	2.2
	16	2	1		1	1.1
Total					89	
Mean per cell	17.4	1.7	.03			
11-1	21				19	13.6
	19	1			35	25.2
	17	2			42	30.2
	15	3			21	15.1
	13	4			17	12.2
	11	5			2	1.44
	9	6			1	.72
	16	1	1		1	.72
	13	2		1	1	.72
Total					139	
Mean per cell	17.01	1.91	.007	.007		
Combined Total					228	
Combined Mean per cell	17.2	1.8	.017	.004		

TABLE 7

Summary of Metaphase I Chromosome Associations in 1-4 (*H. procerum* X *H. vulgare* cv. 'Coho')

Chromosome Association				Chromosome Number	Number of Cells	%
I	II	III	IV			
22	1			24	1	2.8
23	1			25	2	5.7
21	2			25	6	17.1
19	3			25	2	5.7
17	4			25	1	2.8
20	1	1		25	1	2.8
18	2	1		25	1	2.8
26				26	1	2.8
24	1			26	5	14.3
22	2			26	3	8.6
18	4			26	1	2.8
14	6			26	1	2.8
25	1			27	2	5.7
23	2			27	2	5.7
21	3			27	1	2.8
19	4			27	1	2.8
17	5			27	1	2.8
20	2	1		27	1	2.8
24	2			28	1	2.8
15	5	1		28	1	2.8
Total					35	
Mean per Cell	18.2	2.2	.11	25.9		

TABLE 8

Summary of Metaphase I Chromosome Associations in 4-2
(*H. procerum* X *H. vulgare* cv. 'Coho')

Chromosome Association				Chromosome Number	Number of Cells	%
I	II	III	IV			
23				23	4	11.8
21	1			23	8	23.5
19	2			23	8	23.5
17	3			23	5	14.7
15	4			23	1	2.9
20	2			24	1	2.9
23	1			25	1	2.9
21	2			25	2	5.8
17	4			25	1	2.9
15	5			25	1	2.9
22	2			26	1	2.9
26				26	1	2.9
Total					34	
Mean per Cell	19.9	1.8		23.5		

TABLE 9

Summary of Metaphase I Chromosome Associations in 4-8
(*H. procerum* X *H. vulgare* cv. 'Coho')

Chromosome Association				Chromosome Number	Number of Cells	%
I	II	III	IV			
24	1			26	2	4.6
22	2			26	1	2.3
20	3			26	2	4.6
18	4			26	1	2.3
17	3	1		26	1	2.3
27				27	2	4.6
25	1			27	1	2.3
23	2			27	4	9.3
22	1	1		27	2	4.6
21	3			27	5	11.6
20	2	1		27	4	9.3
19	4			27	3	6.9
17	5			27	4	9.3
17	3		1	27	1	2.3
17	2	2		27	1	2.3
16	4	1		27	1	2.3
15	6			27	4	9.3
10	7	1		27	1	2.3
22	3			28	2	4.6
20	4			28	1	2.3
Total					43	
Mean per Cell	19.8	3.1	.26	.02	26.9	

TABLE 10

Summary of Metaphase I Chromosome Associations in 14-2 (*H. procerum* X *H. vulgare* cv. 'Beacon')

Chromosome Association				Chromosome Number	Number of Cells	%
I	II	III	IV			
26				26	4	8.2
25	1			26	1	2.0
22	2			26	3	6.1
20	3			26	2	4.1
27				27	2	4.1
25	1			27	7	14.3
23	2			27	10	20.4
22	1	1		27	1	2.0
21	3			27	12	24.5
20	2	1		27	1	2.0
19	4			27	3	6.1
24	2			28	1	2.0
21	2	1		28	1	2.0
20	4			28	1	2.0
Total					49	
Mean per Cell	22.6	2.0	.06	26.9		

TABLE 11

Summary of Metaphase I Chromosome Associations in 14-7
(*H. procerum* X *H. vulgare* cv. 'Beacon')

Chromosome Association				Chromosome Number	Number of Cells	%
I	II	III	IV			
27				27	11	13.9
25	1			27	25	31.6
23	2			27	21	26.6
21	3			27	11	13.9
19	4			27	9	11.4
24	2			28	1	1.3
22	3			28	1	1.3
Total					79	
Mean per Cell 23.5	1.8			27.0		

Figure 1: Mitotic chromosomes of the polyhaploids and hybrids

a Plant #10-5 (H. procerum X 'Larker') with 2n=21. 830X.

b+c Plant #4-2 (H. procerum X 'Coho') with 2n=23. 830X.

d Plant #1-7 (H. procerum X 'Coho') with 2n=26. 830X.

e Plant #1-6 (H. procerum X 'Coho') with 2n=28. 830X.

f Plant #4-8 (H. procerum X 'Coho') with 2n=27. 830X.

g Plant #14-7 (H. procerum X 'Beacon') with 2n=27. 830X.

h Plant #14-7 (H. procerum X 'Beacon') with 2n=28. 830X.

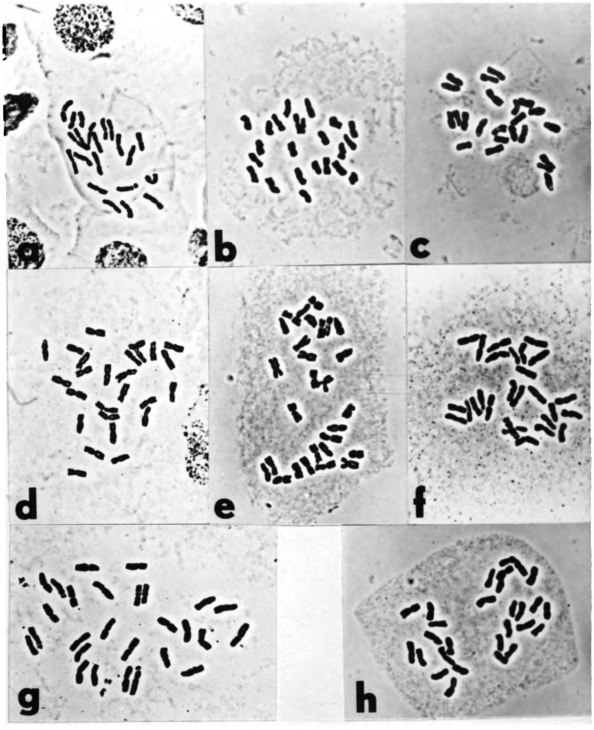
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Figure 2: Spike and Spikelet Morphology.

a *H. vulgare* cv. 'Beacon' spike.

b *H. vulgare* cv. 'Coho' spike

c *H. procerum* X 'Beacon' F_1 spike

d *H. procerum* X 'Coho' F_1 spike

e *H. procerum* spike

f *H. procerum* polyhaploid spike

a' 'Beacon' spikelet

b' 'Coho' spikelet

c' *H. procerum* X 'Beacon' F_1 spikelet

d' *H. procerum* X. 'Coho' F_1 spikelet

e' *H. procerum* spikelet

f' *H. procerum* polyhaploid spikelet

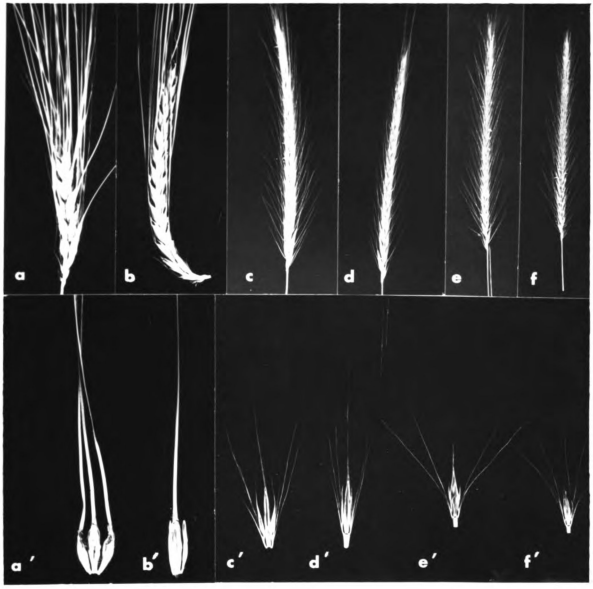
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Figure 3: Microsporogenesis in H. vulgare cv. 'Larker' and in H. procerum.

a 'Larker', metaphase I with 7II. 830X.

b 'Larker', normal tetra. 830X.

c H. procerum, prophase. 550X.

d H. procerum, diakinesis. 550X.

e H. procerum, metaphase I with 2II. 830X.

f H. procerum, telophase I with bridge. 830X.

g H. procerum, metaphase II. 550X.

h H. procerum, anaphase II. 550X.

i H. procerum, tetrad with micronuclei. 550X.

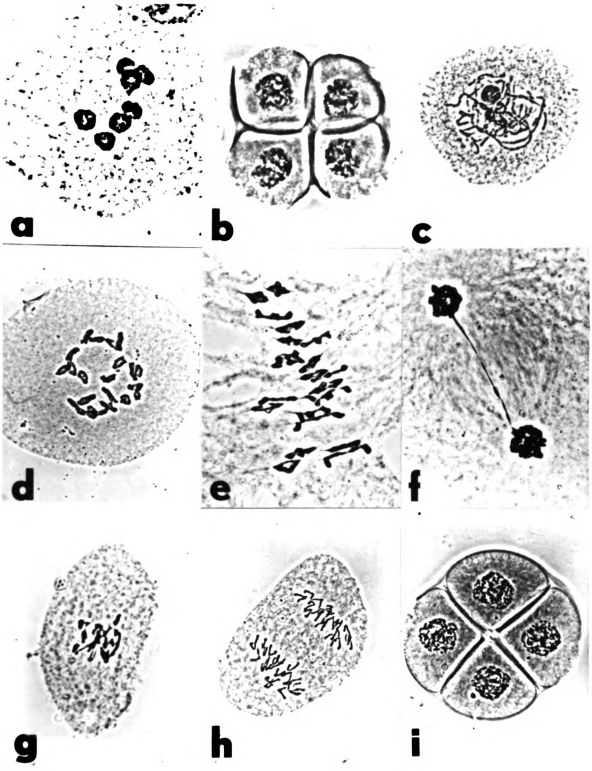
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Figure 4: Microsporogenesis in polyhaploids from the cross, *H. procerum* X *H. vulgare* cv. 'Larker'

a Plant #10-5; metaphase I with 1II + 19I. 830X.

b Plant #10-5; metaphase I with 1III, 1II, + 16I. 830X.

c Plant #10-5; metaphase I with 4II + 13I. 830X.

d Plant #11-1; metaphase I with 4II + 13I. 830X.

e Plant #11-1; metaphase I with 5II + 11I. 830X.

f Plant #11-1; metaphase I with 6II + 9I. 830X.

g Plant #11-1; anaphase I with laggards, some showing precocious division. 830X.

h Plant #10-5; anaphase I, tripolar showing 9-1-5 segregation. 660X.

i Plant #10-5; telophase I showing formation of 3 nuclei and bridge following a tripolar segregation.

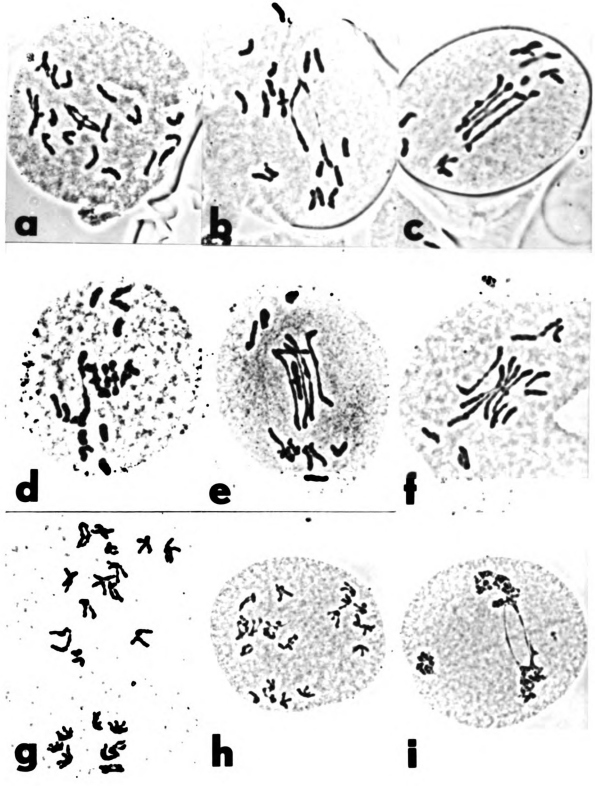
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Figure 4: continued.

j Plant #10-5; tripolar telophase I. 830X.

k Plant #11-1; three unequal-sized nuclei forming at telophase I. 830X.

l Plant #10-5; dyad with micronucleus and microcell. 660X.

m Plant #10-5; metaphase II in a triad, the result of a tripolar first division. 660X.

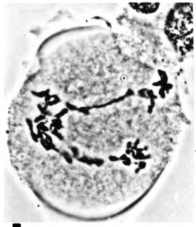
n Plant #10-5; telophase II with bridges. 660X.

o Plant #10-5; telophase II with laggards. 660X.

p Plant #11-1; tetrad with micronuclei. 830X.

q Plant #11-1; tetrad with microcell. 830X.

r Plant #11-1; tetrad stage with 6 cells. 830X.



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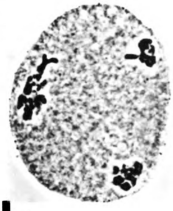

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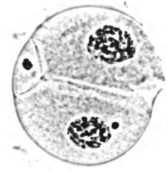

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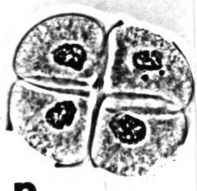

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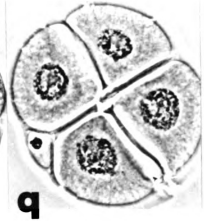

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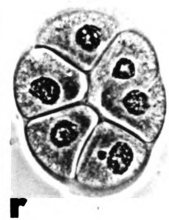

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Figure 5: Microsporogenesis in H. procerum X. H. vulgare hybrids

a Plant #4-8; metaphase I with 6II + 15I. 660X.

b Plant #14-2; metaphase I with 27I. 660X.

c Plant #4-2; anaphase I with laggards and precocious division. 660X.

d Plant #1-4; dyad with cytomixis, micronuclear, and microcell. 830X.

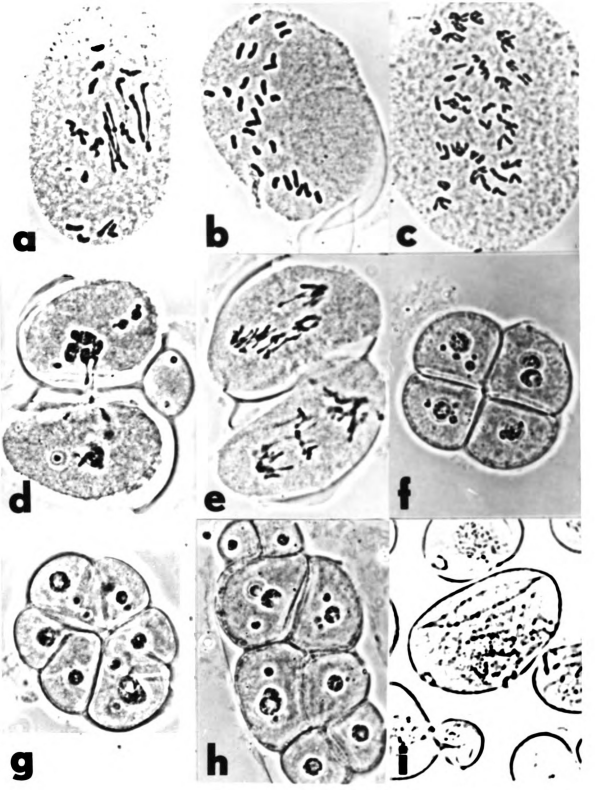
e Plant #4-2; telophase I with laggards. 830X.

f Plant #4-2; tetrad with multiple nuclei and micronuclei. 660X.

g Plant #4-8; tetrad stage with 6 cells. 830X.

h Plant #4-2; tetrad stage with 8 cells. 830X.

i Plant #14-2; Pollen abnormalities. Note supernumerary pollen pores in large irregular-shaped pollen grain. 660X.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DISCUSSION

There has been some confusion concerning the correct name for Hordeum procerum. According to Bowden (1965) the species was first described by Nevski (1941), but later independently described by Covas (1950) under the name Hordeum hexaploidum. Reports published prior to Bowden's article, eg. Rajhathy et al. (1964) refer to the material studied as H. hexaploidum. Later reports (Subrahmanyam, 1977) refer to the material as H. procerum. The sample used in this study, PI No. 266196, appears to fit the description of Covas (1950). Based on this and the priority of the name under which the plant was first described, PI No. 266196 is identified as Hordeum procerum Nevski.

One of the major stumbling blocks in an interspecific hybridization program is the low success rate of the crosses. Since these are usually hand pollinations, the production of even a few hybrids for further study is an exhaustive, labor intensive, frequently expensive endeavor. One of the original goals of this study was to attempt to define those conditions which would yield higher numbers of hybrid seedlings, with a minimum amount of effort. Comparing the results of this limited study with those of a previous one provides a basis for judging the success of these efforts.

Subrahmanyam (1977) was able to obtain seedlings from the cross H. procerum (2n=6x=42) X H. vulgare (2n=2x=14). He reported a seed set of 6.9%, and an embryo culture success rate of 51.9%; his overall yield of seedlings per floret pollinated was .03. In the present

study, an overall seedling per floret yield of .36 can be calculated (Table 1). This is a ten-fold improvement over the previous report. The major difference seems to be in the percent seed set obtained in this study, which averaged 71%; the embryo culture success rate was also slightly higher, at 62%. What, specifically, is the cause of these higher success rates?

The spike of H. procerum typically has in the neighborhood of 50 spikelets, which mature sequentially from top to bottom. On any given day, only 10 or 20 of the florets may contain newly mature, receptive stigmas which are prime candidates for fertilization. By selecting only these florets, time wasted in emasculation is saved and unnecessary eyestrain is avoided. This also results in a more favorable nutritional status for the developing caryopses. Another problem area is trying to get an ample amount of viable pollen onto receptive stigmas. Pollination is assured by simply shaking massive amounts of pollen on receptive stigmas.

The higher success rate of the embryo culture technique in the present study can be directly attributed to the 17 embryos which were forced to germinate on the B-5R medium. Similar cessation of germination on regular embryo culture media was reported by other workers (Steidl, 1976; Morrison, et al. 1959). The 17 recalcitrant embryos transferred to the regeneration medium, B-5R, in this study all went on to germinate completely and all plants which survived the growth chamber malfunction and were transferred to the greenhouse have reached

maturity. Whether this was due to the difference in sugar concentration (2% in B-5R vs. 5.1% in Norstog's) or to the presence of plant hormones in the regeneration medium (GA_3, IAA, Kinetin) is not known at this time. Schooler (1960) reported on the use of gibberellins in embryo cultures. It is known that gibberellins, auxins, and cytokinins have a role in seed germination (van Overbeek, 1968). It is probable that one or more of these substances may be lacking in those embryos which fail to germinate completely, and the B-5R medium may compensate for this deficiency.

It remains to be seen whether these procedures will produce similar results when applied to other interspecific crosses in Hordeum.

Cytological examination of the plants produced by the interspecific cross hexaploid H. procerum X diploid H. vulgare indicates that two basic types of plants resulted: polyhaploids with a stable chromosome number of 2n=3x=21, and hybrids with variable chromosome numbers which usually approach the tetraploid level (2n=23-29). This agrees to some extent with the results reported by Subrahmanyam (1977) for the same interspecific cross. He observed polyhaploids of procerum and tetraploid hybrids, but he did not report any aneuploidy or chromosome number variation in his hybrids.

The 10 polyhaploid plants closely resemble the hexaploids, with striking similarities in morphology. No characters are apparent which would indicate that H. vulgare has contributed in any way to their genetic make-up. The hybrid plants, on the other hand, have morphology

which is intermediate between the two parents, as shown most clearly by spikelet morphology. In the 'Beacon' hybrids (Table 1), for example, (Figure 2) the spikelets were sessile, a vulgare characteristic.

There are two possibilities by which such an interspecific cross may result in a polyhaploid; either no fertilization takes place and the egg develops parthenogenetically, or fertilization occurs but chromosomes from the male parent are eliminated during embryogenesis.

The production of haploids following interspecific hybridization has been extensively reported previously in Hordeum. The most notable cross involves H. vulgare X H. bulbosum, which results in vulgare-like plants (Symko, 1969; Kasha and Kao, 1970; Jensen, 1973). Using genetic marker stocks, Subrahmanyam and Kasha (1973a) showed that these plants did indeed include only vulgare chromosomes. Lange (1971) suggested that either vulgare or bulbosum chromosomes were eliminated during embryo development, but bulbosum chromosomes were eliminated more frequently. Subrahmanyam and Kasha (1973b), however, showed that double fertilization occurs, that there is no evidence of parthenogenesis, and that selective elimination of bulbosum chromosomes leads to haploid vulgare progeny. Bennett et al. (1976) showed that elimination of bulbosum chromosomes is nearly complete by the fifth day after fertilization.

The actual mechanism of chromosome elimination is still not known; however, several hypothesis have been put forth. Asynchrony of mitotic cell cycle times, (Gupta, 1969; Lange, 1971; Subrahmanyam and Kasha,

1973), and a "modification restriction system" (Davies, 1974) have been proposed. Bennett et al. (1976) showed that chromosomes of the species with the longer mean cell generation time either failed to congress at mitotic metaphase, or failed to move to one of the poles at anaphase, and were eliminated.

Haploids of other Hordeum species have been produced following interspecific hybridization (Rajhathy and Symko, 1974; Islam and Sparrow, 1974; Subrahmanyam, 1977, 1979, 1980), including polyhaploids of H. procerum (Subrahmanyam, 1977). Chromosome elimination was shown to occur in a complex interspecific Hordeum hybrid by Orton and Tai (1977), who proposed it may be due to the malfunctioning or disintegration of a proposed cytoplasmic organelle, the "spindle organizer (Tai, 1970)", or to a complex interaction between chromosomes and spindle-timing determinants. This theory seems consistent with the conclusions of Bennett et al. (1976).

It is apparent that fertilization of the H. procerum sample used in this study by H. vulgare is possible, since hybrid plants were obtained using the cultivars 'Beacon' and 'Coho' as the pollen parent. Chromosome elimination obviously occurs in these hybrids to some extent, because the F_1 plants have chromosome numbers which vary from 23-29. It is therefore believed that H. procerum was fertilized by H. vulgare cv. 'Larker'. Based on chromosome numbers and plant morphology, it is concluded that the 21 chromosome plants are polyhaploids (trihaploids) of maternal origin, and that the plants with higher chromosome numbers

are hybrids. In view of the overwhelming evidence that haploids are produced by chromosome elimination in Hordeum following interspecific hybridization, and in view of the fact that there is no evidence to the contrary, it is concluded that the 21 chromosome polyhaploids are the result of elimination of H. vulgare chromosomes during embryogenesis.

The appearnce of vulgare-like characters in all plants with more than 21 chromosomes indicates that the additional chromosomes are of paternal origin. The lack of aneuploidy in the polyhaploids indicates that aneuploidy in the hybrids is due to loss of individual vulgare chromosomes, and not as the result of aneuploid procerum egg cells.

The variation in chromosome numbers between hybrids, and between tissues in the same plant, is apparently a further manifestation of the elimination phenomenon. Similar variation has been reported for H. vulgare X H. bulbosum hybrids (Humphreys, 1978), as well as in a Nicotiana interspecific hybrid (Gupta and Gupta, 1973).

There were striking differences between barley cultivars used as the male parent in crosses. All ten plants with 'Larker' as male had a chromosome number of 2n=3x=21, and are polyhaploids. All 11 plants with 'Coho' as male were hybrids with variable chromosome numbers, with a mean in all cells studied of 26.9. If the exceptional plant with 23 chromosomes, Number 4-2 (Table 2), is excluded, the mean chromosome number for the Coho crosses is 27.4. All six plants with 'Beacon' as male were also hybrids, with variable chromosome numbers and a mean of 27.4. What the data point out is a difference in

resistance to chromosome elimination by the procerum genome, which resides in the vulgare genome. Such a difference would have to reside in nuclear genes, since H. vulgare is the male parent and there should be no effect of vulgare cytoplasm. Ho and Kasha (1975) have shown that genes for elimination of H. bulbosum chromosomes are located on chromosomes 2 and 3 of H. vulgare; these are offset by factors on the bulbosum chromosomes which, when in sufficient dosage, prevent the elimination of the bulbosum chromosomes. In this study, it appears that such factors, conditioning resistance to elimination of vulgare chromosomes by procerum, are either wholly lacking or are of insufficient dosage to be effective in 'Larker', as opposed to 'Coho' and 'Beacon'. No such differences between varieties were reported by Subrahmanyam (1977); it is therefore possible that there is a genetic difference between his procerum sample and PI No. 266196. Gene segregation in his pollen-parent population is also a possibility. He didn't use 'Larker' in his study, and he appeared to get polyhaploids and hybrids from the same pollinating variety.

The inheritance of this resistance to elimination could be studied, using trisomic stocks of 'Larker', if they were available, or simply by looking at hybrids between 'Beacon' and 'Larker'. The obvious morphological differences between plants (polyhaploids vs. hybrids) would make scoring the results relatively simple. If 'Larker' does, indeed, lack a genetic factor which resists chromosome elimination, as suggested by this study, it would be a good choice as a pollen

parent for the production of polyhaploids of wild Hordeum species.

Meiotic studies of H. vulgare cv. 'Larker' confirm that it is a regular diploid (Table 3), with 7II per cell and a negligible frequency of abnormalities at late stages of division. Meiosis of H. procerum (Table 3) reveals that it is a nearly regular hexaploid. Metaphase I plates studied reveal a bivalent frequency of 19.6, which approaches the expected value of 21 II. Frequencies for trivalent and quadrivalent associations were 0.06 each. These are so low that they may be insignificant in the interpretation of homology between genomes.

The meiotic studies done on the polyhaploids, however, provide additional information about the genome relationships in H. procerum. In these plants, there is a mean number of 1.9 bivalents per cell, with a maximum of 6 II observed in one cell. Further, 5 II were observed in two cells, and 4 II were observed in 21 cells or 9% of the total. Thus the maximum number of bivalents observed is six, which comes very close to the basic number for the genus of x=7. Based on this observation, the maximum association in the polyhaploids is 7II + 7I. Since it was established that the polyhaploids derived all three genomes of chromosomes from procerum in the previous section, it thus appears that there is a degree of homology between two of the three genomes present in H. procerum. Since the metaphase I chromosome pairing in these plants consisted mostly of rod bivalents, with only a few ring bivalents, it seems likely that the two homologous genomes are similar, but not identical. With this in mind, a preliminary

genome formula for H. procerum would be AAA'A'BB; thus, H. procerum should be considered a segmental autoallohexaploid.

This conclusion may seem incongruous with the observation that multivalents were at very low frequencies in the hexaploid H. procerum. This is explained by either of two possibilities. It was very difficult to obtain well-spread metaphase I cells when working with PMC's from the hexaploid. It is thus possible that multivalent formation occurs more frequently than observed in this study, but such cells weren't interpretable due to the resulting congested nature of the cell plate. The other possibility is that there is genetic control of chromosome pairing, which suppresses the pairing of homoeologous chromosomes in the hexaploid. Genetic influence on chromosome pairing has been well-documented in hexaploid wheat (Riley and Chapman, 1958; Feldman et al., 1966) and reported in other Hordeum species (Rajhathy et al., 1964). Starks and Tai (1974) proposed a system for control of pairing in Hordeum jubatum: "When a single dose of this gene (or a group of genes) is present, homoeologous pairing may prevail. When a double dose is present, only homologous chromosomes may pair." This same system appears to be operative in H. procerum. Previous studies of H. procerum (mistakenly referred to as H. hexaploidum Covas) have indicated that the two genomes of H. jubatum are included in H. procerum, based on karyomorphology (Rajhathy and Morrison, 1961; Rajhathy et al., 1964). It is thus logical to assume that the same genetic system proposed for H. jubatum by Starks and Tai (1974) is also present in

H. procerum. The consequences of this should be considered in future cytogenetic studies in Hordeum, since a hybrid between H. procerum and a diploid species should be expected to show a maximum of 7II if there is no homology, and maximum of 14II if there was complete homology between the genome of the diploid and the B genome of procerum.

This same situation, that is, a hybrid between hexaploid H. procerum and diploid H. vulgare, is in question in this study. If there is no homology between the genomes of the two parents, the hybrids should exhibit a maximum number of 7II, which coincides with the maximum number in the polyhaploids.

The mean frequency of pairing in the five hybrids studied was 2.1 II vs. a mean of 1.9 II in the polyhaploids, a difference of only .2. More importantly, the maximum of 7 II was observed in one plant, and the numbers of bivalents in all five plants clearly approach 7II, the maximum number in the polyhaploids. Thus, the conclusion that there is no homology between the genomes of H. procerum and H. vulgare.

APPENDICES

Appendix A

Formula for Embryo Culture Medium

	mg/liter
KH_2PO_4	910
KCl	750
$MgSO_4 \cdot 7H_2O$	740
$CaCl_2 \cdot 2H_2O$	740
$MnSO_4 \cdot H_2O$	3.0
H_3BO_3	0.5
$ZnSO_4 \cdot 7H_2O$	0.5
$CoCl_2 \cdot 6H_2O$	0.025
$CuSO_4 \cdot 5H_2O$	0.025
Na_2MoO_4	0.025
Fe-EDTA	25.0
Inositol (meso)	50.0
Thiamine-HCl	0.25
Pyridoxine-HCl	0.25
Ca-pantothenate	0.25
Casein hydrolysate (enzymatic)	2500.0
Malic acid	1000.0
	g/liter
Agar	10
Sucrose	51.3
pH	5.0

Appendix B

Formula for B-5R Medium

Major	mg/liter
KNO_3	2500
$CaCl_2 \cdot 2H_2O$	150
$MgSO_4 \cdot 7H_2O$	250
$(NH_4)_2SO_4$	134
$NaH_2PO_4 \cdot H_2O$	150
Minor	
KI	.75
H_3BO_3	3.0
$MnSO_4 \cdot H_2O$	10
$ZnSO_4 \cdot 7H_2O$	2.0
$Na_2MoO_4 \cdot 2H_2O$	0.25
$CuSO_4 \cdot 5H_2O$	0.025
$CoCl_2 \cdot 6H_2O$	0.025
$Na_2 \cdot EDTA$	37.3
$FeSO_4 \cdot 7H_2O$	27.8
Inositol	100
Nicotinic Acid	1.0
Pyridoxine HCl	1.0
Thiamine HCl	10.0
Kinetin	.3
GA_3	1.0
IAA	0.5
	g/l
Sucrose	10
Glucose	10
Agar	10

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