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Elimination and duplication of particular *Hordeum vulgare* chromosomes in aneuploid interspecific *Hordeum* hybrids

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Summary. Seeds formed in crosses *Hordeum lechleri* (6x) × *H. vulgare* (2x and 4x), *H. arizonicum* (6x) × *H. v.* (2x), *H. parodii* (6x) × *H. v.* (2x), and *H. tetraploidum* (4x) × *H. v.* (2x) produced plants at high or rather high frequencies through embryo rescue. Giemsa C-banding patterns were used to analyze chromosomal constitutions and chromosomal locations on the methaphase plate. Among 100 plants obtained from *H. vulgare* (2x) crosses, 32 plants were aneuploid with 2n = 29 (1), 28 (3), 27 (13), 26 (5), 25 (4), 24 (4), or 22 (2); 50 were euploid (12 analyzed), and 18 were polyhaploid (5 analyzed). Four plants had two sectors differing in chromosome number. Two of four hybrids with *H. vulgare* (4x) were euploid and two were aneuploid. Parental genomes were concentrically arranged with that of *H. vulgare* always found closest to the metaphase centre. Many plants showed a certain level of intraplant variation in chromosome numbers. Except for one *H. vulgare* (4x) hybrids, this variation was restricted to peripherally located non-*H. vulgare* genomes. This may reflect a less firm attachment of the chromosomes from these genomes to the spindle. Interplant variation in chromosome numbers was due to the permanent elimination or, far less common, duplication of the centrally located *H. vulgare* chromosomes in all 34 aneuploids, and in a few also to loss/gain of non-*H. vulgare* chromosomes. This selective elimination of chromosomes of the centrally located genome contrasts conditions found in diploid interspecific hybrids, which eliminate the peripherally located genome. The difference is attributed to changed "genomic ratios". Derivatives of various *H. vulgare* lines were differently distributed among euploid hybrids, aneuploids, and polyhaploids. Chromosomal constitutions of hypoploid hybrids revealed a preferential elimination of *H. vulgare* chromosomes 1, 5, 6, and 7, but did not support the idea that *H. vulgare* chromosomes should be lost in a specific order. *H. vulgare* SAT-chromosomes 6 and 7 showed nucleolar dominance. Aneuploidy is ascribed to the same chromosome elimination mechanism that produces haploids in cross-combinations with *H. vulgare* (2x). The findings have implications for the utilization of interspecific *Hordeum* hybrids.

Key words: *Hordeum* – Interspecific hybrids – Chromosomes – Elimination – Duplication

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Introduction

Hypo- and hyperploidy have been reported in hybrids resulting from interspecific crosses between various polyploid *Hordeum* sp. and cultivated barley *Hordeum vulgare* L. (Islam and Sparrow 1974; Barclay 1976; Islam et al. 1981; Bothmer et al. 1983, 1986; Linde-Laursen and Jensen 1984; Linde-Laursen and Bothmer 1984, 1986 a,b; Linde-Laursen et al. 1986 a; Pickering and Morgan 1985; Sethi et al. 1986). The deviations in ploidy have been ascribed to a particular hybrid being aneuploid and/or to intraplant variation in chromosome number as a result of elimination or duplication of the respective chromosome(s). Owing to selective elimination of the *H. vulgare* genome from certain of these interspecific hybrids (Rajhathy and Symko 1974; Barclay 1976; Subrahmanyam 1977, 1980; Bothmer et al. 1983), the aberrations are generally assumed to be restricted to the chromosomes of the *H. vulgare* genome. However, reliable identification of individual chromosomes at somatic metaphase using Giemsa C-banding has shown that this is not always true

(Linde-Laursen and Bothmer 1984, 1986 b; Linde-Laursen et al. 1986 a).

Interspecific hybrids having *H. vulgare* as one parent are characterized by having parental genomes concentrically arranged at somatic metaphase. The chromosomes of one parental genome are closer to the cell centre than those of the other. In diploid hybrids, the outer non-*H. vulgare* genome may be eliminated (e.g., Finch 1983; Linde-Laursen and Bothmer 1984). Polyploid interspecific hybrids, e.g., *H. lechleri* × *H. vulgare*, *H. arizonicum* × *H. vulgare*, and *H. parodii* × *H. vulgare*, on the other hand always eliminate the inner *H. vulgare* genome (Subrahmanyam 1977, 1980; Linde-Laursen and Bothmer 1986 a, b; Linde-Laursen et al. 1986 a). A further characteristic of both diploid and polyploid hybrids is that the satellite (SAT)-chromosomes of the inner genome have the more conspicuous or the only visible nucleolar constrictions and produce the major nucleoli – a phenomenon known as "nucleolar dominance" (Finch 1983; Finch and Bennett 1983; Fig. 1 in Linde-Laursen and Jensen 1984; Linde-Laursen and Bothmer 1984, 1986 a,b; Linde-Laursen et al. 1986 a, b).

Owing to their recent identification, the aneuploid interspecific hybrids have so far been studied only slightly with respect to such variables as frequency, chromosomal constitution, and stability (Barclay 1976). The aim of the present investigation was to study these items as well as to examine whether particular *H. vulgare* chromosomes were specifically eliminated or duplicated in polyploid interspecific *Hordeum* hybrids with aneuploid chromosome numbers. The answer to these problems would elucidate if special attention should be paid to the chromosomal constitution of hybrids exploited in backcrosses (Bothmer and Hagberg 1983) or callus cultures (Jørgensen et al. 1986) for transferring genetic material from wild species to *H. vulgare*.

Material and methods

Interspecific crosses were carried out using the embryo rescue technique (Bothmer et al. 1983). The crosses were performed either at the Agricultural Research Department, Risø National Laboratory, Roskilde, Denmark, or at the Department of Crop Genetics and Breeding, The Swedish University of Agricultural Sciences, Svalöv, Sweden. *H. vulgare* L. was always used as the paternal parent. The material of the wild species used came from our collections from native distribution areas (Argentina and the USA). It comprised ten populations of *H. lechleri* (Steud.) Schenck (6x), one population of *H. arizonicum* Covas & Stebbins (6x), one population of *H. parodii* Covas (6x), and two populations of *H. tetraploidum* Covas (4x). The names or designations of the lines of *H. vulgare* used are given in Table 1. Most lines came from stocks kept at Risø or Svalöv. Line 9208/9 was received from Dr. W. Friedt, Grünbach, GFR, and D8/55 from the Barley Germplasm Center, Okayama University, Japan.

The chromosomes of hybrids and haploids were assigned to the constituent genomes by their Giemsa C-banding patterns from normally not-less-than three somatic metaphases (Linde-Laursen et al. 1980; Linde-Laursen and Bothmer 1986 a). Normally, only *H. vulgare* chromosomes were identified individually. The number of SAT-chromosomes later identified in C-banded preparations was established in squashed, unstained cells. The number of chromosomes with nucleolus-forming capacity was checked by staining interphases with silver nitrate (Linde-Laursen 1984 a). In the same preparations, the number and relative size of the nucleolus organizer regions (NORs) were established – if metaphases with well-demarcated NORs were obtained. The relative positions of the genomes with respect to each other were estimated subjectively in C-banded metaphases.

Results

H. lechleri (6x) × H. vulgare (2x and 4x)

Ninety-nine spikes of *H. lechleri* (2n = 42) were pollinated, 70 with pollen from diploid *H. vulgare* and 29 with pollen from tetraploid *H. vulgare* (Table 1). Both with respect to the percentage of pollinated florets that produced seeds (with and without embryos) and the number of seeds that produced plants, the combinations with diploid *H. vulgare* were superior to those with tetraploid *H. vulgare*, 8% versus 4%, and 67% vs. 19%, respectively. The differences are obviously too large to be referred to as variations in "growing conditions". However, the latter probably account for at least part of the big differences in seed set and the minor differences in number of plants produced per seed among combinations with different diploid lines of *H. vulgare*. Frequently, callus formed from embryos on artificial medium.

H. lechleri (6x) × H. vulgare (2x)

Of the 95 plants produced, there were 87 in which the chromosomes could be counted. Forty-three had 2n = 28 suggesting that they were hybrids carrying the full complements (21 + 7) of the two parents; 17, one-fifth, had 2n = 21 suggesting they were haploids of *H. lechleri*; and 27, one-third, had aneuploid chromosome numbers. Of the latter plants, one, HH473-3, was a hyperploid with 2n = 29. The remainder, 26 plants, were hypoploids with 2n = 22–27 (Tables 1–3). Four plants were chimeric. In addition to a sector with 2n = 27, BB623-7 had a sector with 2n = 28. 2n = 23 was found in plant BB6207-14 alongside a sector with 2n = 24; BB6207-3 had sectors with 2n = 27 and 2n = 25; and BB649-1, sectors with 2n = 26 and 2n = 25 (Tables 2 and 3). Among the hypoploids, the most common group, comprising half the number of plants, had 2n = 27. The other chromosome numbers, except for 2n = 23, which was found in the chimeric hybrid only, were represented by between two and four plants. A few cells of some plants had one or at most two chromosomes less than the principal number. One plant only, BB6207-6, had a cell with an extra chromosome (Table 3). In all plants, chromosome numbers remained at a constant level over the short or longer period, up to several years, during which the single plant was analyzed.

Table 1. Results of crosses between *H. vulgare* (2x and 4x) (♂) and four polyploid wild *Hordeum* species (♀)

H. vulgare variety/line	No. of crosses	No. of florets	Seed set		Plants		Hybrids (no.)		Haploids (no.)	Dead (no.)
			No.	%	No.	%	Euploid[a]	Aneuploid		
H. lechleri × *H. vulgare* (2x)										
Gull	58	1470	67	4.6	41	61.2	23	12[b]		6
Vada	1	15	1	6.7	1	100.0				1
Hooded	1	20	0							
Foma	2	60	25	41.7	23	92.0		10[b]	13	
Tellus	1	39	6	15.4	4	66.7	2	2		
9208/9	4	163	18	11.0	14	77.8	14			
Manchuria	1	11	11	100.0	5	45.5		1	4	
Vogelsanger Gold	1	9	8	88.9	4	50.0	3	1		
HP40	1	14	5	35.7	3	60.0	1	1		1
Total	70	1801	141	7.8	95	67.4	43	27	17	8
H. lechleri × *H. vulgare* (4x)										
D8/55	10	256	13	5.1	0					
Haisa II	19	441	13	2.9	5	38.5	2	2		1
Total	29	697	26	3.7	5	19.2	2	2		1
H. arizonicum × *H. vulgare* (2x)										
Hooded	1	18	1	5.6	1	100.0			1	
spontaneum	1	12	11	91.7	2	18.2		2		
Total	2	30	12	40.0	3	25.0		2	1	
H. parodii × *H. vulgare* (2x)										
Vogelsanger Gold	1	4	3	75.0	1	33.3		1		
H. tetraploidum × *H. vulgare* (2x)										
Risø Mutant 1508	1	14	6	42.9	5	83.3	2	2		1
Foma	1	48	3	6.3	3	100.0	3			
Flare	2	16	3	18.8	2	66.7	2			
Total	4	78	12	15.4	10	83.3	7	2		1
Grand total	106	2610	194	7.4	114	58.8	52	34	18	10

[a] May include unidentified aneuploids with 2n = 28 or 2n = 21

[b] Two plants chimeric

Table 2. Distribution of *H. lechleri* × *H. vulgare* (2x) offspring by principal chromosome number and paternal parent

H. vulgare (♂) variety/line	No. of plants									Total
	2n = 29	28	27	26	25	24	23	22	21	
Gull		23	7[a]	1[b]	2	2				35
Foma			3[c]	1	2	2[d]		2	13	23
Tellus		2	2							4
9208/9		14								14
Manchuria				1					4	5
Vogelsanger Gold		3	1							4
HP40	1	1								2
Total	1	43	13	3	4	4		2	17	87

[a] One aneuploid chimeric for 2n = 28 and 2n = 27
[b] One aneuploid chimeric for 2n = 26 and 2n = 25
[c] One aneuploid chimeric for 2n = 27 and 2n = 25
[d] One aneuploid chimeric for 2n = 24 and 2n = 23

In three combinations with different lines of *H. vulgare* as the male parent, more than ten offspring were produced. The combinations differed significantly with respect to the frequency of euploid hybrids, aneuploids, and polyhaploids produced. The 14 hybrids with line 9208/9 as the male parent were all euploid; the 35 hybrids from 'Gull' showed half as many aneuploids as euploids; and the 23 from 'Foma', no euploid hybrids, but roughly equal numbers of aneuploids and polyhaploids (Table 1). Also, the distribution of aneuploids from 'Foma' was skewed more towards lower numbers than in those from 'Gull' (Table 2).

There were no combinations having different *H. lechleri* populations as the maternal parents, but with the same *H. vulgare* line as the paternal parent that were numerous enough to allow meaningful comparisons.

Giemsa C-banding and staining with silver nitrate were performed on all aneuploids, nine euploid hybrids,

Table 3. Chromosomal constitution of 32 aneuploid interspecific hybrids between *H. vulgare* (2x) (♂) and four polyploid wild *Hordeum* sp. (♀) analyzed by Giemsa C-banding

H. vulgare (♂) variety/line	Hybrid no.	No. of cells	2n[a]	No. of chromosomes									No. (max.) of SAT-chromosomes			No. (max.) of $AgNO_3$ stained	
				Alien (max.)	*H. v.*	*H. vulgare* chromosomes present							Alien	*H. v.*[b]	Total	NORs[c]	Nucleoli[c]
H. lechleri × *H. vulgare*																	
HP40	HH473-3[d]	9	29 (27–29)	21	8	1	2	3	4	5	6	7+7		3 (6+7+7)	3		8
Gull 9208/9 Vogelsanger Gold	6 euploids 1 euploid 2 euploids	62	28 (27–28)	21	7	1	2	3	4	5	6	7	0–3	2 (6+7)	2–5	2–7 (2l+5s)	5–7 (2l+5s)
Gull	BB623-7	8	28	22	6	1	2	3	4	5	6		2	1 (6)	3		6 (1l+5s)
		5	27 (26–27)	21	6	1	2	3	4	5	6			1 (6)	1		6 (1l+5s)
Gull	BB623-3	5	27	21	6	1	2	3	4	5	6		1	1 (6)	2		6 (1l+5s)
Gull	BB527-1	8	27	21	6	1	2	3	4	5	6			1 (6)	1		7 (1l+4s+2m)
Foma	BB6207-8	5	27	21	6	1	2	3	4	5	6		2	1 (6)	3	4 (1l+3s)	6 (1l+5s)
Vogelsanger Gold	HH1284-1[d]	9	27	21	6	1	2	3	4	5	6		4	1 (6)	5		6 (1l+5s)
Gull	BB623-2	12	27 (25–27)	21	6	1	2	3	4	5		7	4	1 (7)	5	3	6 (1l+5s)
Foma	BB6207-4	9	27 (26–27)	21	6	1	2	3	4	5		7	3	1 (7)	4		6 (1l+5s)
Tellus	51-2	10	27 (26–27)	21	6	1	2	3	4	5		7	1	1 (7)	2		6 (1l+5s)
Gull	BB538-1	6	27	21	6	1	2	3	4		6	7	2	2 (6+7)	4	6 (2l+4s)	7 (2l+5s)
Gull	BB538-2	10	27 (26–27)	21	6	1	2	3	4		6	7		2 (6+7)	2	7 (2l+5s)	7 (2l+5s)
Gull	BB623-10	9	27 (25–27)	21	6	1	2	3	4		6	7	2	2 (6+7)	4	3	5 (2l+3s)
Tellus	51-4	8	27 (26–27)	21	6	1	2	3	4		6	7		2 (6+7)	2		7 (2l+5s)
Foma	BB6207-3	4	27	21	6		2	3	4	5	6	7	1	2 (6+7)	3		
		4	25	21	4		2		4	5	6		1	1 (6)	2		6 (1l+5s)
Manchuria	HH2399-3[d]	13	26 (25–26)	20	6		2	3	4	5		7+7	4	2 (7+7)	6		7 (2l+5s)
Foma	BB6205-9	13	26	21	5	1	2		4	5		7	2	1 (7)	3	6	6 (1l+5s)
Gull	BB649-1	6	26	21	5		2	3		5	6	7	2	2 (6+7)	4	5 (2l+3s)	7 (2l+5s)
		10	25	21	4			3		5	6	7	1	2 (6+7)	3		
Gull	BB536-3	5	25 (24–25)	20	5	1	2	3	4		6			1 (6)	1		6 (1l+5s)
Gull	BB538-3	7	25 (24–25)	21	4	1	2	3				7	1	1 (7)	2	6 (1l+5s)	6 (1l+5s)
Foma	BB6205-10	7	25	21	4		2	3		5	6		1	1 (6)	2	6 (1l+5s)	6 (1l+5s)
Foma	BB6207-2	4	25	21	4				4+4		6+6			2 (6+6)	2		5 (2l+3s)
Gull	BB538-4	7	24	21	3	1	2	3					5		5	5	6 (5l+1m)
Foma	BB6207-6	13	24 (24–25)	21	3		2	3				7		1 (7)	1	5	5
Gull	BB540-2	5	24	21	3			3	4		6		2	1 (6)	3	3 (1l+2s)	6 (1l+5s)
Foma	BB6207-14	5	24	21	3			3			6	7		2 (6+7)	2		
		2	23	21	2			3				7	1	1 (7)	2	5 (1l+4s)	6 (1l+5s)
Foma	BB6205-1	3	22	21	1				4				2		2		5
Foma	BB6207-13	6	22	21	1				4				3		3		6 (5+1m)
Gull Foma Manchuria	2 haploids 2 haploids 1 haploid	19	21	21									4–5		4–5	4	5

Table 3. (continued)

H. arizonicum × *H. vulgare*															
spontaneum	HH2133-1[d]	4	26 (26–27)	21	6	1	2	3	5	6	7	2	2 (6+7)	4	3 (21+1s)	5 (21+3s)
spontaneum	HH2133-2[d]	8	26 (25–28)	22	6	1	2	3	5	6	7	2	2 (6+7)	4		
H. parodii × *H. vulgare*																
Vogelsanger Gold	HH1293-1	15	28 (27–33)	25	8	1+1	2	3	4	5	6	7	2	2 (6+7)	4	7 (21+5s)	7 (21+4s+1m)
H. tetraploidum × *H. vulgare*																	
[Foma	3 euploids	28	21 (20–22)	15	7	1	2	3	4	5	6	7		2 (6+7)	2		4 (21+2s)]
Mut. 1508	HH2441-1[d]	16	21	14	7[e]	1+1	2	3s	4	5		7	1	1 (7)	2		5 (11+3s+1m)
Mut. 1508	HH2441-4[d]	11	21	15	6	1	2	3	4	5		7	1	1 (7)	2		5 (11+3s+1m)

[a] Range in brackets
[b] Identified *H. vulgare* chromosomes in brackets
[c] l = large NORs/nucleoli; s = smaller NORs/nucleoli; m = micronucleoli
[d] Partly after Linde-Laursen and Bothmer (1986b)
[e] Inclusive one telocentric

and five polyhaploids (Table 3; Fig. 1). In all plants except the four chimeric ones, BB623-7, BB649-1, BB6207-3, and BB6207-14, banding patterns identified the chromosomes producing the intraplant variation as belonging to the *H. lechleri* genome exclusively. In the euploids, seven chromosomes were identified as *H. vulgare* chromosomes 1–7, and 20 or 21 chromosomes as belonging to *H. lechleri*; in the polyhaploids, all 21 chromosomes belonged to *H. lechleri*.

Among the 27 C-banded aneuploids, including BB623-7, only three had permanently gained or lost *H. lechleri* chromosomes. The sector of BB623-7 with 2n = 28 had a *H. lechleri* chromosome in duplicate, but had, like the sector with 2n = 27, lost *H. vulgare* chromosome 7. HH2399-3 and BB536-3 had both lost one *H. lechleri* chromosome. All other permanent aneuploidy was due to the gain or loss of *H. vulgare* chromosomes.

Duplications of *H. vulgare* chromosomes were uncommon: they were observed in three plants only, and in all but one of these it was coupled with the elimination of other *H. vulgare* chromosomes. HH473-3 with 2n = 29 had an extra chromosome 7; HH2399-3 with 2n = 26 likewise had an extra chromosome 7, but had lost chromosomes 1 and 6. BB6207-2 with 2n = 25 had chromosomes 4 and 6 in duplicate, but had lost the other five *H. vulgare* chromosomes (Table 3).

No hypoploid plants, or sectors, derived from the 6x × 2x crosses, except those with 2n = 22 and 2n = 27, i.e., with six and one chromosome eliminated, respectively, had identical chromosomal constitutions (Table 3). The two plants with 2n = 22 had *H. vulgare* chromosome 4 added to the *H. lechleri* complement. Of the 13 plants or sectors with 2n = 27, four had lost chromosome 5, three, chromosome 6, five, chromosome 7, and one only (BB6207-3), chromosome 1. The latter loss was restricted to a small sector of the hybrid; the remaining large sector had lost chromosomes 3 and 7 in addition. No 27-chromosome hybrids had lost chromosomes 2, 3, or 4 (Table 4).

This distinct differential elimination of chromosomes among aneuploids with 2n = 27 was not observed among aneuploids with 2n = 23 to 26. In these hybrids, elimination apparently affected chromosome 1 the most, and each of the six other *H. vulgare* chromosomes more or less at random. However, it might be significant that chromosome 5 was eliminated in all six aneuploids having 2n less than 25. Further, chromosome 1 was found in only one of them. The chromosomal constitution of the hypoploid hybrids gave no indication that the chromosomes were lost in a specific order. A single group of aneuploid sister plants comprising four individuals of BB538 had all lost chromosome 5; however, no other groups of sister plants showed a similar tendency (Table 3).

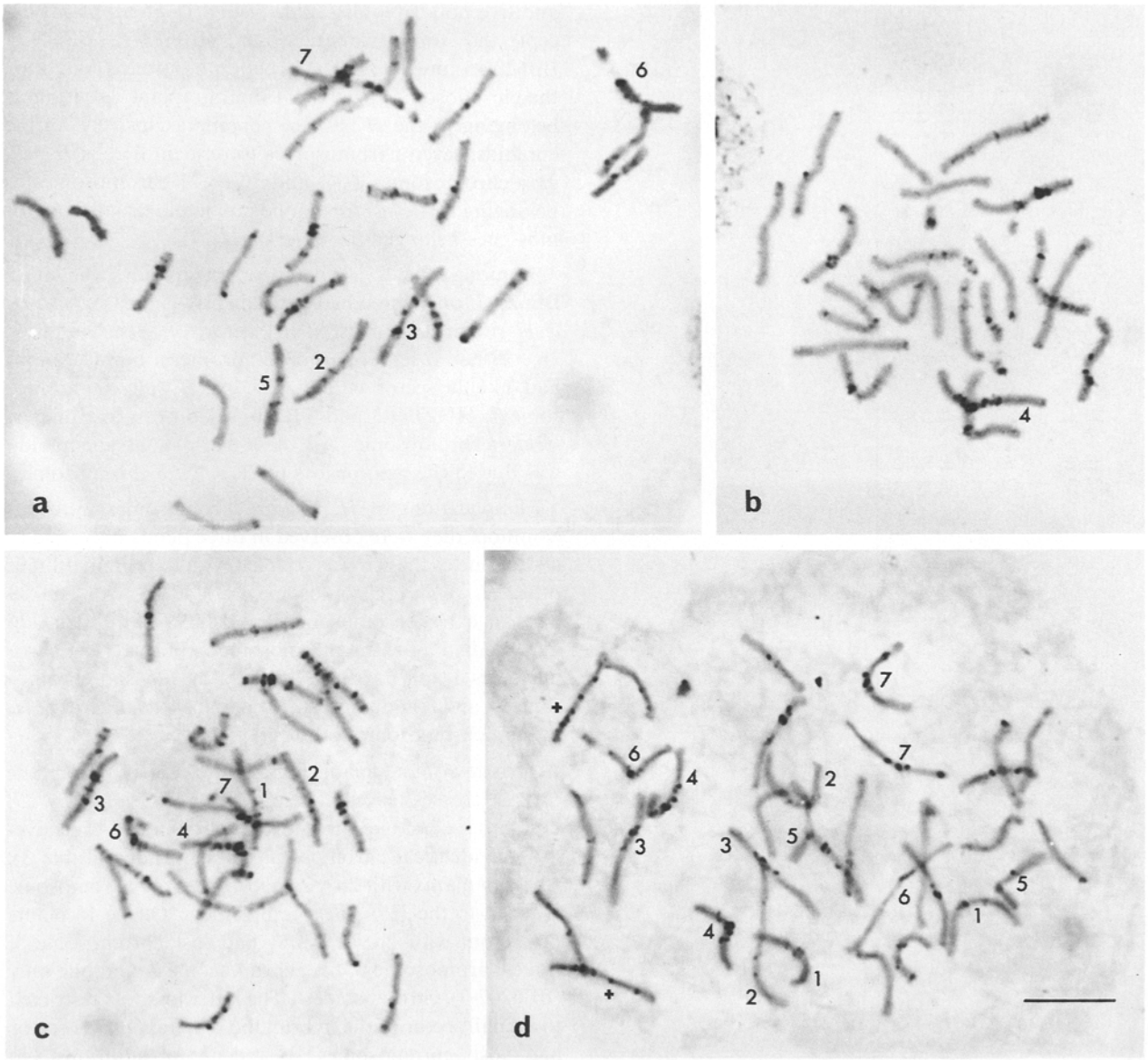

Fig. 1a–d. Giemsa C-banded chromosomes at somatic metaphase of interspecific *Hordeum hybrids.* **a** BB649-1 (*H. lechleri* × *H. vulgare* 'Gull') 2n = 26; b BB6207-13 (*H. lechleri* × *H. vulgare* 'Foma') 2n = 22 (one *H. lechleri* chromosome outside field of vision); **c** BB623-10 (*H. lechleri* × *H. vulgare* 'Gull') 2n = 27; **d** BB599-2 (*H. lechleri* × *H. vulgare* 'Haisa II' (4x)) 2n = 33. Two homologous *H. lechleri* chromosomes indicated by "+". *Figures* designate *H. vulgare* chromosomes. *Bar* = 10 μm

A comparison of the C-banded karyotypes of the *H. vulgare* lines 'Gull', 'Foma', and HP40 present in the hybrids with the karyotypes of these lines previously reported (Linde-Laursen et al. 1982; Linde-Laursen and Bothmer 1984) showed one discrepancy. The karyotype of the aneuploid BB649-1 derived from 'Gull' carried banding variant 6D instead of 6A.

H. vulgare chromosomes did not at any time show any detectable morphological modifications. This was apparently also the case with *H. lechleri* chromosomes. However, in the few cells in which centromere size was studied, *H. vulgare* chromosomes had on average, more conspicuous centromeres than *H. lechleri* chromosomes.

The nucleolar constrictions of the *H. vulgare* SAT-chromosomes were always more clearly expressed than those of the *H. lechleri* SAT-chromosomes, which were often suppressed, indicating nucleolar dominance of the *H. vulgare* SAT-chromosomes (cf. Linde-Laursen and Bothmer 1986 a) except in polyhaploids and aneuploids without *H. vulgare* SAT-chromosomes (Table 3). In these aneuploids and in polyhaploids, up to five *H. lechleri* chromosomes with nucleolar constrictions were present

Table 4. Elimination of particular *H. vulgare* chromosomes in hypoploid *H. lechleri* × *H. vulgare* (2x) hybrids

2n	No. of hybrids[a]	*H. vulgare* chromosome[b] eliminated							No. of chromosomes eliminated
		1	2	3	4	5	6	7	
27	13	1			(2)	4	3 (1)	5	13 (3)
26	4	2		1 (t)	1	1	2 (1)	1	8 (1+t)
25	4	3	1	1	3	1	1	2	12
24	4	3	2		3	4	2	2	16
23	2	2	2	1	1	2	1	1	10
22	2	2	2	2		2	2	2	12
Total	29	13	7	5 (t)	8 (2)	14	11 (2)	13	71 (4+t)

[a] BB6207-3, BB649-1, BB6207-14; each treated as two hybrids
[b] Chromosomes eliminated in three other aneuploid hybrids (cf. Table 3) in brackets

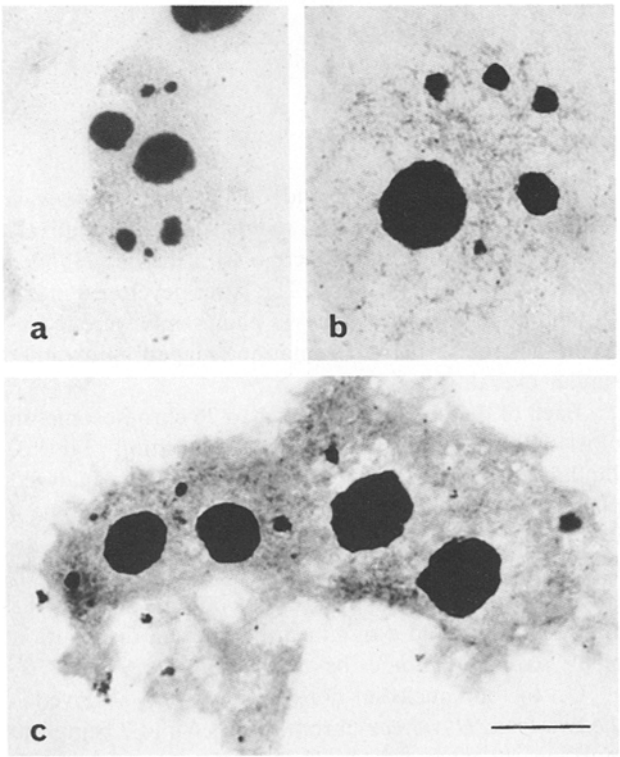

Fig. 2a–c. $AgNO_3$-stained cells at somatic interphase of interspecific *Hordeum* hybrids showing nucleoli. **a** BB538-1 (*H. lechleri* × *H. vulgare* 'Gull'). Two large plus five small nucleoli; **b** BB623-7 (*H. lechleri* × *H. vulgare* 'Gull'). One large plus five small nucleoli; **c** BB599-1 (*H. lechleri* × *H. vulgare* 'Haisa II' (4x)). Four large plus five small nucleoli. *Bar* = 10 μm

as expected (Linde-Laursen and Bothmer 1986 a). These observations were supported by the presence of silver nitrate-stained NORs in metaphases and of nucleoli in interphases (Table 3; Fig. 2). In hybrids containing *H. vulgare* SAT-chromosomes, large nucleoli were present in a corresponding number, and small nucleoli in a number maximally corresponding to the five *H. lechleri* SAT-chromosomes. In cells of three hybrids, BB527-1, BB538-4, and BB6207-13, one extra micronucleolus was observed, indicating the presence of one additional chromosome having a nucleolus-forming capacity. The derivation of this chromosome is unknown.

Observations of genome positions in the hybrids showed that the chromosomes belonging to the two parental genomes were generally differently dispersed, with the *H. vulgare* chromosomes closest to the cell centre surrounded by the *H. lechleri* chromosomes (Fig. 1; Linde-Laursen and Bothmer 1986 a). This applied also to aneuploids with only few *H. vulgare* chromosomes, but it could not be ascertained for the two plants with only one remaining *H. vulgare* chromosome 4 (BB6205-1 and BB6207-13) (Fig. 1 b).

H. lechleri (6x) × H. vulgare (4x)

Only the combination with the chromosome-doubled line of var 'Haisa II' gave viable offspring. The expected chromosome number in this combination is 2n = 35: 21 chromosomes from *H. lechleri* and 14 from *H. vulgare*. Some cells in two sister plants, BB599-1 and -2, had this number, whereas the maximum number observed in another pair of sister plants, BB600-2 and -3, were 2n = 34 and 2n = 32, respectively, an indication that some chromosomes had been eliminated (Table 5). The intraplant variation in chromosome numbers was larger than in hybrids with diploid *H. vulgare*. Thus, in BB599-1, the number varied between 2n = 28 and 2n = 35.

In the two hybrids, BB599-1 and -2, C-banding patterns identified 14 chromosomes as constituting two complete *H. vulgare* genomes (Fig. 1 d). In cells with 2n = 35, 21 chromosomes apparently consituted a complete *H. lechleri* genome. From cells containing fewer chromosomes, up to seven *H. lechleri* chromosomes, possibly consituting one full component genome of *H. lechleri*, had been eliminated. However, at least one hypo-

Table 5. Chromosomal constitution of four interspecific hybrids between *H. lechleri* and *H. vulgare* 'Haisa II' (4x)

Hybrid no.	No. of cells	2n[a]	No. (max.) of chr.		*H. vulgare* chromosomes present	No. (max.) of SAT-chromosomes			No. (max.) of $AgNO_3$-stained	
			H. lech.	*H. v.*		*H. lech.*	*H. v.*[b]	Total	NORs[c]	Nucleoli[c]
BB599-1	26	28–35	21	14	(1 2 3 4 5 6 7)×2		4 (6+6+7+7)	4	7 (4l+3s)	9 (4l+5s)
BB599-2	15	32–35	21	14	(1 2 3 4 5 6 7)×2		4 (6+6+7+7)	4		7 (4l+3s)
BB600-2	14	30–34	21	13	1 2 3 4 5 6 7 / 1 2 4 5 6 7	1	4 (6+6+7+7)	5		9 (4l+5s)
BB600-3	7[d]	28–30	17	13	1 2 3 4 5 6 7 / 1 2 4 5 6 7		4 (6+6+7+7)	4	9	9
	10[d]	28–32[e]	19	12+t	1 2 3 4 5 6 7 / 1 2 4 5 6 7s	1	4 (6+6+7+7s)	5		
	19[d]	27–31	19	12	1 2 3 4 5 6 7 / 1 2 4 5 6[f]	1	3 (6+6+7)	4		

[a] Range
[b] Identified *H. vulgare* chromosomes in brackets
[c] l = large NORs/nucleoli; s = smaller NORs/n
[d] Analyzed March – May 1986; May 12, 1986; and June 1986 – August 1987, respectively
[e] Inclusive one telocentric
[f] Three cells with a partly deleted long arm of chromosome 6

ploid cell with 2n = 33 had both gained and lost *H. lechleri* chromosomes (Fig. 1 d). Cells of the hybrid BB600-2 had 13 *H. vulgare* chromosomes. As in the sister plant BB600-3, one chromosome 3 had been eliminated. Cells with 2n = 34 probably had a complete *H. lechleri* genome, whereas those with fewer chromosomes lacked one or more *H. lechleri* chromosomes. BB600-3 was analyzed for three separate periods covering one year. During the first period, all cells had two full *H. vulgare* genomes minus one chromosome 3; in the second period, root-tips with cells having lost the long arm of chromosome 7 in addition to one chromosome 3 were observed; in the third period, the telocentric 7s was absent from all cells. In addition, all cells of one root-tip had a partly deleted long arm of chromosome 6. No cell had more than 19 *H. lechleri* chromosomes, which was an indication that two chromosomes were permanently lost.

In most cells, only nucleolar constrictions produced by *H. vulgare* SAT-chromosomes 6 and 7 were observed (Table 5). At most, only one *H. lechleri* SAT-chromosome had a visible nucleolar constriction. These observations were supported by finding a maximum of four large and five small nucleoli in silver nitrate-stained interphases (Table 5; Fig. 2 c).

As in the combinations with *H. vulgare* (2x), the *H. vulgare* (4x) chromosomes tended to be grouped together, nearer to the metaphase centre than the *H. lechleri* chromosomes.

H. arizonicum (6x) × H. vulgare (2x)

Two spikes only from one population of *H. arizonicum* (2n = 42) were crossed with two lines of *H. vulgare*, one belonging to ssp. *vulgare* and one to ssp. *spontaneum* (Table 1). The cross with ssp. *spontaneum* gave relatively many embryos, whereas the cross with the line Hooded resulted in one embryo only. The latter developed into a haploid plant with 2n = 21. Two plants only were recovered from the former combination, suggesting a rather similar overall success rate.

Each of the two latter plants had 26 chromosomes in most cells; however, there was some variation (Table 3; Linde-Laursen et al. 1986 a). All cells of both analyzed plants had six *H. vulgare* chromosomes; chromosome 4 was missing. Thus, all intraplant variation pertained to the *H. arizonicum* genome. It went from the loss of two chromosomes to the duplication of one. In none of the analyzed cells could a significantly different distribution of the parental genomes be ascertained.

Up to four nucleolar constrictions were observed – the two from *H. vulgare* chromosomes 6 and 7 being the most clearly expressed. As *H. arizonicum* has five SAT-chromosomes per genome (Linde-Laursen et al. 1986 a), the nucleolus-forming capacity of three appeared suppressed. However, observations of up to two large and three small nucleoli in silver nitrate-stained interphases indicated a less extensive suppression.

H. parodii (6x) × H. vulgare (2x)

The single hybrid produced from one crossing attempt proved to have a variable number of chromosomes, with the majority of the cells analyzed having 2n = 28, the expected euploid chromosome number. However, irrespective of chromosome number, all cells proved to be aneuploid with eight *H. vulgare* chromosomes – one full

genome plus an extra chromosome 1 – and from 19 to 25 *H. parodii* chromosomes (Table 3). Thus, they showed a wider intraplant variation in the number of non-*H. vulgare* chromosomes than the other tetraploid hybrids studied.

Up to four satellited chromosomes were observed. The two having the longest and most often seen nucleolar constrictions were *H. vulgare* chromosomes 6 and 7. The presence of these two SAT-chromosomes with clearly expressed nucleolar constrictions corresponded with observations of two metaphase chromosomes having large silver nitrate-stained NORs and, maximally, two large nucleoli in interphases. In addition to the large NORs and nucleoli often produced, up to five smaller NORs and five smaller nucleoli were observed; these correspond to the number of *H. parodii* SAT-chromosomes (Linde-Laursen unpublished).

The genomes of the two component species were concentrically arranged, with that of *H. vulgare* closest to the metaphase centre sourrounded by that of *H. parodii*.

H. tetraploidum (4x) × H. vulgare (2x)

Four spikes from two populations of *H. tetraploidum* were pollinated with three different lines of *H. vulgare*. The result was nine surviving hybrids, all with 2n = 21 as is expected for euploids; however, they showed some intraplant variation (Tables 1 and 3). The complements of five plants were analyzed: three proved to be true euploid hybrids, whereas the other two, although having the euploid chromosome number, were aneuploids. HH2441-4 had lost *H. vulgare* chromosome 6, but gained a *H. tetraploidum* chromosome. The constitution of HH2441-1 was more complex. It probably had a complete *H. tetraploidum* genome. Of the *H. vulgare* complement, it had lost chromosome 6 and the long arm of chromosome 3, but acquired an extra chromosome 1.

In the euploid hybrids only two SAT-chromosomes were observed. They were *H. vulgare* chromosomes 6 and 7. In the aneuploids also up to two SAT-chromosomes were observed: the one normally observed and having the longest nucleolar constriction was *H. vulgare* chromosome 7; the other belonged to *H. tetraploidum*. In agreement with this, only one large nucleolus was observed in interphases of HH2441-1 and -4. In addition, up to three smaller nucleoli and one micronucleolus were seen. Three corresponds to the number of *H. tetraploidum* SAT-chromosomes (Linde-Laursen and Bothmer 1986 a). The presence of a micronucleolus indicates the presence in one of the parental genomes of an additional SAT-chromosome with a too low nucleolus-forming capacity to result in the formation of a nucleolar constriction.

The genomes of the two component species were concentrically arranged, with that of *H. vulgare* being closest to the metaphase centre.

Discussion

The occurrence of stable aneuploid chromosome numbers at high frequencies in hybrids between wild tetra- and hexaploid *Hordeum* species and diploid cultivated barley, *H. vulgare*. was first reported by Barclay (1976). Both the present and some previous studies confirm and extend his list of *H. secalinum* (4x), *H. lechleri* (6x), and *H. murinum* (6x) by adding *H. tetraploidum* (4x), *H. arizonicum* (6x), *H. parodii* (6x), and *H. procerum* (6x) (Bothmer et al. 1983, 1986; Linde-Laursen and Bothmer 1986 a,b; Linde-Laursen et al. 1986 a; Pickering and Morgan 1985). In addition, we found aneuploid hybrids in the combination *H. lechleri* × *H. vulgare* (4x), in which Barclay (1976) reported euploid hybrids only. Aneuploidy in these polyploid hybrids thus seems to be a general trait as exemplified most extensively by the *H. lechleri* (6x) × *H. vulgare* (2x and 4x) combinations.

As stable aneuploid hybrids resulted form crossing combinations practically all of which are known to produce haploids of the non-*H. vulgare* parent through to complete elimination of the *H. vulgare* genome (Rajhathy and Symko 1974; Barclay 1976; Subrahmanyam 1977, 1980; Bothmer et al. 1983; Pickering and Morgan 1985), it is tempting to assume that hypoploid hybrids owe their formation to the same elimination mechanism. However, this elimination mechanism stops at an early zygotic division in the hybrid embryos (cf. Islam et al. 1981; Pickering and Morgan 1985). Further, the stage is apparently influenced by genetic background. As the elimination mechanism interferes with spindle formation, which is subsequently expressed by the different sizes of the centric constrictions of the chromosomes of the parental genomes (Finch and Bennett 1983; Linde-Laursen and Bothmer 1984), the production of chromosome duplications and intraplant chromosomal variation may have the same cause.

One reason why stable aneuploid interspecific hybrids occurring at a high frequency were observed only recently may be that the genomes of different *H. vulgare* lines are differently disposed to become either not at all, partly, or wholly eliminated. Thus, combinations with the diploid line 9208/9 produced no aneuploids or polyhaploids; those with 'Gull', rather many aneuploids, but no polyhaploids; and those with 'Foma', many aneuploids with, on average, a higher level of chromosome loss than those with 'Gull', and many polyhaploids. In previous investigations, var 'Edda' and probably also 'Emir' reacted like 9208/9, and var 'Ymer' and 'Vada', like 'Foma' (Barclay 1976; Pickering and Morgan 1985). Further, the genotype of the alien species and environmental conditions may also have influenced chromosome elimination (e.g., Jensen 1977; Pickering and Morgan 1985). Another reason for our finding stable aneuploid hybrids may be recent improvements in the embryo

rescue technique which allows a higher number of seedlings to be recovered, possibly by way of callus formation, as was often observed in the present study. Tissues and plants produced through callus growth in tissue culture of interspecific hybrids often show aneuploidy or chromosomal rearrangements (e.g., Orton 1980; Lapitan et al. 1984; Jørgensen unpublished); however, the apparent absence of chromosomal rearrangements and the near absence of deletions make it less likely that the induction of aneuploidy and callus growth are related.

Highly variable chromosome numbers have been reported in some interspecific *Hordeum* hybrids by Pickering and Morgan (1985). Just why such plants were absent from our material may be due to the fact that we analyzed far fewer plants, or that chromosome numbers had stabilized because our plants were older than theirs when analyzed.

The interplant variation in chromosome numbers mostly affected the *H. vulgare* genome, which is in good agreement with the tendency of this genome to become eliminated in polyploid interspecific hybrids studied so far (Rajhathy and Symko 1974; Barclay 1976; Subrahmanyam 1977, 1980; Bothmer et al. 1983; Pickering and Morgan 1985). Previously, it was supposed that only *H. vulgare* chromosomes were affected (Barclay 1976; Sethi et al. 1986), however, these authors did not identify any chromosomes, or only a few, and our study clearly shows that non-*H. vulgare* chromosomes may also be lost permanently from aneuploid hybrids, although to a much lesser extent than *H. vulgare* chromosomes. It is not possible to attribute the permanent loss or gain of non-*H. vulgare* chromosomes to the chromosome elimination mechanism active in species hybrids, as aneuploid plants having lost or gained a chromosome have been observed in some polyploid *Hordeum* species (Linde-Laursen et al. 1980, 1986 b).

In contrast to the permanent losses or gains that mostly affected *H. vulgare* chromosomes, intraplant variation in combinations with *H. vulgare* (2x) affected only chromosomes of the wild-type species. Although some of the intraplant variations observed can be ascribed to chromosome losses caused by the squashing procedure, reports of these variations in interspecific *Hordeum* hybrids are plentiful enough to substantiate its existence (Kasha 1974; Bothmer et al. 1983). Difference between two constituent genomes may be due to: (1) that chromosomes of the *H. vulgare* genome with its central position round the nucleolus are somehow protected by the peripherally located genome of the wild-type species; and/or (2) the chromosomes of the latter may be less firmly attached to the spindle than the *H. vulgare* chromosomes (Finch 1983; Finch and Bennett 1983; Linde-Laursen and Bothmer 1984).

Nevertheless, in spite of their central position, the chromosomes of the *H. vulgare* genome are the ones which normally become eliminated. This situation is opposite to that found in the diploid hybrids studied (Finch and Bennett 1983; Linde-Laursen and Bothmer 1984). In the latter case, the chromosomes of the inner genome are normally retained. These contrasting results are rather similar to those obtained in crosses of diploid and tetraploid *H. bulbosum* with diploid *H. vulgare* and, as in these, can probably be related to the changed ratio between the basic genomes (genomic balance) – 1:1 in the diploid and 2:1 or 3:1 in the polyploid hybrids (Lange 1971; Kasha 1974). In comparison to the intraplant variation present in hybrids with diploid *H. vulgare*, this variation in hybrids with tetraploid *H. vulgare* was on the whole much wider: some cells of one hybrid even had lost seven *H. lechleri* chromosomes, a suggestion that a component genome was lost.

From the chromosomal constitution of the hypoploid interspecific hybrids, we can surmise that one of *H. vulgare* chromosomes 1, 5, 6, or 7 – the two latter being the SAT-chromosomes – are generally eliminated first, followed by a more-or-less random elimination of the remaining chromosomes of the cell. However, the loss of chromosome 1 was always accompanied by the loss of other chromosomes except in one small sector of one plant. Thus, it appears that chromosome 1 per se confers a higher degree of chromosomal instability when lost first than the loss of any one of chromosomes 5, 6, or 7. The reason why no plant had lost chromosome 2 as the only one is probably coincidental.

The differential distribution of *H. vulgare* chromosomes into two groups, the first comprising the more frequently eliminated chromosomes 1, 5, 6, and 7, and the second the less frequently eliminated chromosomes 2, 3, and 4 supports our previous suggestion of a preferential loss of particular *H. vulgare* chromosomes in interspecific *Hordeum* hybrids (Linde-Laursen and Bothmer 1986 b). A preferential elimination of SAT-chromosomes has been reported previously in callus-derived *Solanum tuberosum* + *S. phureja* somatic hybrids (Pijnacker et al. 1987). Our study does not support the proposal of Finch (1983) who, based on his studies on chromosome elimination in endosperms of *H. marinum* × *H. vulgare* hybrids, suggested that *H. vulgare* chromosomes tend to be eliminated in a specific order. Because Finch (1983) used a *H. vulgare* line with three translocations, the two studies cannot be compared directly. However, it is striking that both studies have three comparable chromosomes among the first four eliminated, viz., the SAT-chromosomes and the partly homologous shortest chromosomes.

In the hypoploid sister plants, chromosome 5 was eliminated in the four *H. lechleri* × *H. vulgare* (2x) BB538 hybrids, chromosome 4 in the two *H. arizonicum* × *H. vulgare* (2x) HH2133 hybrids, chromosome 6 in the two *H. tetraploidum* × *H. vulgare* (2x) HH2441 hybrids, and chromosome 3 in the two *H. lechleri* × *H. vulgare*(4x) BB600 hybrids (Tables 3 and 5). The

concurrent loss of the same *H. vulgare* chromosome in a group of sister plants has no ready explanation.

The much lower number of chromosome duplications than eliminations was expected, as duplications of chromosomes have been infrequently observed in polyploid interspecific *Hordeum* hybrids (Bothmer et al. 1983; Linde-Laursen and Bothmer 1984, 1986 b; Pickering and Morgan 1985). The distribution of the six duplications on the four *H. vulgare* chromosomes 1, 4, 6, and 7 provided no safe opportunity for inferring a preferential involvement of any of them. However, it may be significant that chromosome 7 was the only *H. vulgare* chromosome duplicated in three of ten complex interspecific *Hordeum* hybrids (Bothmer et al. in preparation). Further, the exclusive duplication of a SAT-chromosome, although not that of a *H. vulgare* SAT-chromosome, has been previously observed in aneuploid *H. vulgare* × *Secale cereale* hybrids (Ramsay and Dyer 1983; Ramsay 1984; Wojciechowska 1985). These observations suggest that SAT-chromosomes may generally be more involved in numerical chromosome aberrations than other chromosomes.

The material analyzed was limited in size by the number of interspecific hybrids available and by the laborious C-banding technique applied. However, it was large enough to establish that some *H. vulgare* chromosomes were preferentially eliminated, and also that they were lost in a certain order. Moreover, the few plants of cross-combinations other than *H. lechleri* × *H. vulgare* that were analyzed gave no indication of elimination/duplication patterns different from those of the latter combination.

Differential amphiplasty in the form of a complete suppression of the nucleolus-forming capacity of one genome was not observed. However, a partial nucleolar dominance of the *H. vulgare* SAT-chromosomes was observed in all plants having at least one *H. vulgare* SAT-chromosome. This observation lends support to the view that the phenomenon is probably widespread (Linde-Laursen et al. 1986 a).

Both embryos and plants formed readily in most crossing combinations attempted, indicating that crossing barriers between these polyploid wild-type *Hordeum* species and diploid and tetraploid *H. vulgare* are not strong enough to prevent the formation of offspring when embryo rescue is performed (for references, see Bothmer et al. 1983; Pickering and Morgan 1985).

The present study showed that the chromosomal constitution of polyploid interspecific *Hordeum* hybrids used for attempted transfers of genetic material to cultivated barley (Bothmer and Hagberg 1983; Jørgensen et al. 1986) may influence both the results obtained and their interpretation by mimicing effects of the treatments used. Thus, material to be exploited in such approaches ought always at least to be chromosome-counted.

The investigation was based on Giemsa C-banding of chromosomes at somatic metaphase supplemented with silver nitrate staining of the interphase nucleoli. In contrast to previous studies on the chromosomal constitution of polyploid interspecific *Hordeum* hybrids (e.g., Barclay 1976; Subrahmanyam 1977, 1980; Bothmer et al. 1983; Pickering and Morgan 1985; Sethi et al. 1986), these techniques used together rendered it possible to discriminate reliably between the genomes of the constituent species in spite of similar chromosome sizes and also to identify each particular *H. vulgare* chromosome. Our study showed, for example that chromosomes of both genomes may be eliminated and duplicated, and that tissues with a euploid chromosome number may nevertheless be aneuploid (see also Linde-Laursen 1984 b), thus confirming the usefulness of the Giemsa C-banding method in studies in which the identification of single chromosomes is indispensable.

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