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Complex interspecific hybridization in barley (*Hordeum vulgare* L.) and the possible occurrence of apomixis

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Summary. Several complex hybrids were produced from the combination [(*Hordeum lechleri*, 6× × *H. procerum*, 6×) × *H. vulgare*, 2×]. Crosses with six diploid barley lines resulted in triple hybrids, most of which had a full complement of barley chromosomes (no. 1–7), but were mixoploid with respect to alien chromosomes (19–22). In one combination, chromosome no. 7 was duplicated. Meiosis in triple hybrids showed low, but variable pairing (1.3–5.5 chiasmata per cell). The syndesis probably did not include the barley chromosomes. Direct backcrosses to di- and tetraploid barley lines were unsuccessful. Chromosome doubling of the triple hybrid based on cv 'Pallas' resulted in a plant with $2n = 53-56$, which had an increased fertility. Backcrosses to one di- and one tetraploid barley line resulted in offspring. The cross made with the tetraploid line ('Haisa II'), produced a 28-chromosomic plant in which the male parental genome was absent. We suspect that this plant may have arisen through parthenogenetic development of a reduced female gamete. The other cross with a diploid line ('9208/9') resulted in plant with $2n = 51-53$. The most likely explanation for this second plant is that an unreduced gamete from the amphiploid was fertilized by a normal gamete from the backcross parent, and during early embryo development, some chromosomes were eliminated.

Key words: Barley – *H. vulgare-Hordeum* spp. – Interspecific hybridization – Apomixis – Aneuploidi – Meiosis.

Introduction

The transfer of genetic material from wild species of *Hordeum* into cultivated barley, *H. vulgare* L., is difficult and has met with very little success using conventional techniques, i.e. direct crosses followed by backcrosses (Bothmer and Hagberg 1983; Bothmer et al. 1983; Fedak 1985). Generally, primary hybrids are completely sterile, so that backcrosses seldom result in offspring. Only in crosses with the progenitor and closest relative of cultivated barley, *H. vulgare* ssp. *spontaneum* (C. Koch) Thell., is recombination in the fertile hybrids possible. In addition the amphiploid comprising tetraploid cultivated barley and tetraploid *H. bulbosum* L. is partly fertile (Szigat and Wustrack 1976; Szigat and Pohler 1982).

There are two major obstacles to alien gene transfer to barley: (1) the strict sterility barriers which make backcrossing particularly difficult (Fedak 1985; Bothmer et al. 1983, 1986), and (2) no or very limited chromosomal pairing between the genome of cultivated barley and the genomes of wild *Hordeum* species, (except *H. bulbosum*, Kasha and Sadisavaiah 1971; Lange 1971; Bothmer et al. 1983). These two obstacles must be tackled independently using unconventional methods. One possible way of circumventing the sterility barriers is to work with complex hybridization and amphiploids (Schooler and Anderson 1979).

The present paper describes the result of complex hybridization and the cytological variation and stability of the triple hybrids and plants derived from backcrosses.

Material and methods

After manual crossing, all embryos were rescued on solid media using the technique of Bothmer et al. (1983). The methods used for studying C-banding patterns are those of Linde-Laursen et al. (1980), for nucleolus forming capacity, Linde-Laursen (1984) and for meiotic analysis, Bothmer et al. (1986). The colchicine treatment of Jensen (1974) was used.

The primary hybrid *H. lechleri* × *H. procerum* (no. HH 364) was produced in the interspecific *Hordeum* crossing program by Bothmer and Jacobsen (1986).

The diploid barleys used in the crosses were the commercial European varieties 'Tellus', 'Welam', 'Pallas', 'Vada', 'Dissa', 'Maythorpe' and a landrace from Nepal (here called 'Nepal'). One diploid line, '*hap* E12-p14', containing the *hap* gene, was obtained from Prof. A. Hagberg, Department of Crop Genetics and Breeding, Svalöv, Sweden, and two tetraploid barley lines, 'Strengs Franken III', and 'D 8/55' from Dr. T. Konishi, Barley Germplasm Center, Kurashiki, Japan. Two lines were obtained from Dr. W. Friedt of the Institut für Resistenzgenetik. Grünbach, FRG: namely, the tetraploid 'Haisa II' and one line '5304/73' (in our experiments used under its German field number '9208/9') which contained both diploids and tetraploids. However, in our crosses, we mainly used the diploid ones. The pedigree of the crosses is shown in Fig. 1.

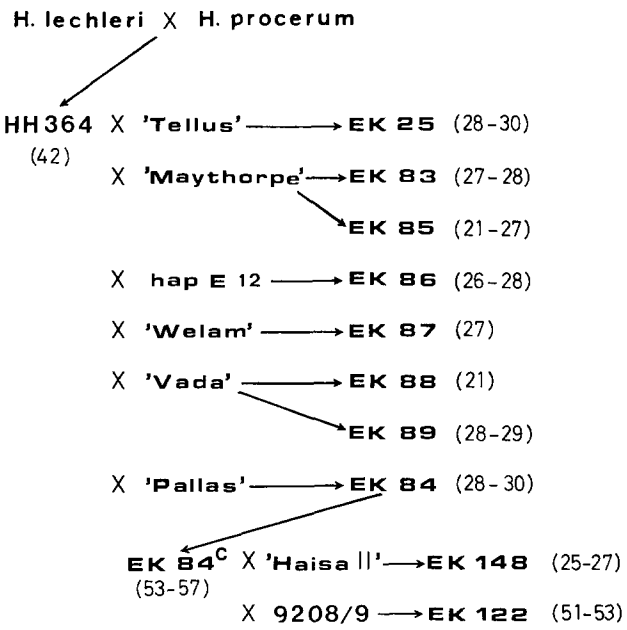

Fig. 1. Pedigree of the studied material; chromosome numbers in parentheses

Results

Crosses and backcrosses

The original F_1 hybrid, HH364 (*H. lechleri* × *H. procerum*, hereafter called "F_1"), was used as the female parent in crossing attempts with several barley lines. Successful triple hybrids were obtained with cv 'Tellus', 'Welam', 'Vada', 'Pallas' and 'Maythorpe', and with line '*hap* E12-p14' (Fig. 1, Table 1). The resulting triple hybrids were cytologically unstable (Table 4) and highly sterile.

The morphology of the triple hybrids was clearly dominated by the original "F_1" with its slender growth and spikes (Fig. 2), although a few traits from the cultivated barley parents were also present, i.e. the small auricles, somewhat larger spikelets and coarser awns (Fig. 2).

Direct backcrossing to *H. vulgare* in combinations with either di- or tetraploid barley (Table 2) failed to produce viable offspring. Only the two combinations with diploid barley, viz. ("F_1" × 'Tellus') × "Nepal" and with ("F_1 × 'Welam') × 'Welam', produced a few seeds which subsequently did not germinate.

Cytology of the triple hybrids

The karyotype of "F_1" (*H. lechleri* × *H. procerum*) showed a maximum of six satellited (SAT) chromosomes, and up to nine nucleoli in the interphases. This is in accordance with earlier results of Linde-Laursen and von Bothmer (1986a and unpublished) for these two species, but is two more than expected based on the results of Jessop and Subrahmanyam (1984). Pairing at MI was high, with an average of 22.9 chiasmata per cell (maximum 29) and up to 14 bivalents, rings and rods in equal frequency; bridges and fragments were rarely seen at A1 (Table 5). These values are higher than those reported by Reddy and Subrahmanyam (1985) and Bothmer et al. (1988).

Two different crossing attempts with cv 'Maythorpe' resulted in five plants (EK83, EK85). Two EK85 plants invariably had $2n = 21$. They were trihaploids of the original "F_1" and arose through selective elimination of the barley chromosomes. Three plants (from both EK83 and EK85) were true triple hybrids with $2n = 27-28$. The meiotic pairing in one of these plants was comparatively low with an average of 2.85 chiasmata per cell (maximum 11) and up to six bivalents and one trivalent (Fig. 5A). The C-banding patterns were studies in EK83 and one EK85 plant. The plants had $2n = 28$ and $2n = 27$, respectively. Both had a complete set of 7 barley chromosomes plus 21 or 20 alien chromosomes, respectively, i.e. one alien chromosome had been eliminated in the EK85 plant.

The cross with line '*hap* E12-p14' gave two adult triple hybrid plants, one with $2n = 27$ and one with $2n = 28$ (EK86). The first plant had 20–21 alien chromosomes and 6 barley chromosomes; chromosome number 7 had been eliminated (Table 4). The meiotic chromosome number in this plant was $2n = 26$, except in one cell that had $2n = 25$ (Table 5). The MI pairing was intermediate in comparison to that found in other triple hybrids, the chiasma frequency was 4.0 per cell (maximum 8) and each cell contained up to six bivalents, two trivalents and an occasional quadrivalent. One meiotic cell (not included in the analysis of pairing) was partly doubled and had 14 ring bivalents, one trivalent and an open quadrivalent.

The single successful cross with 'Welam' (EK87) yielded five triple hybrid seedlings, two of which grew into adult plants. One of the plants had $2n = 27$, comprising a complete set of seven chromosomes from *H. vulgare*

Fig. 2A–D. Spikes of **A** *H. lechleri* × *H. procerum* (F_1, HH 364), **B** (F_1 × '*hap* E12' (EK 86-1), $2n = 26–28$; **C** amphiploid of F_1 × 'Pallas' (EK 84[c]), $2n = 53–57$; **D** EK 84[c] × 'Haisa II' (EK 148), $2n = 25–27$. *Bar* = 1 cm

Table 1. Seed set and plant formation in crosses between the original "F_1" hybrid, HH 364 (*H. lechleri* × *H. procerum*) and different lines of cultivated barley

Combinations	Cross no.	No. of spikes pollinated	No. of flowers	Seed set		
				No.	%	Plants
"F_1" × 'Tellus'	EK 25	1	35	5	14	2
"F_1" × 'Nepal' landrace	EK 22	1	29	3	10	0
"F_1" × '*hap* E12'	EK 86	1	30[a]	3	10	2
"F_1" × 'Welam'	EK 87	1	30[a]	14	47	5
"F_1" × 'Vada'	EK 88, EK 89	2	60[a]	25	42	7
"F_1" × 'Pallas'	EK 84	1	30[a]	13	43	3
"F_1" × 'Maythorpe'	EK 83, EK 85	2	60[a]	21	35	5

[a] The exact number was not noted, but in every spike about 30 spikelets were emasculated

but lacking one chromosome of the wild species (Fig. 3C, Table 4). Meiosis of this plant showed $2n = 26–27$, a low pairing of 1.32 chiasmata per cell (maximum 4) and up to three mainly rod bivalents and one trivalent (Table 5).

Two crosses with 'Vada' yielded seven seedlings, three of which became adult plants (EK88, EK89). One plant was a trihaploid of the original "F_1" with $2n = 21$. The two other plants had $2n = 28–29$ and were triple hybrids. One of these, with $2n = 29$, had a comparatively high meiotic pairing of 5.48 chiasmata per cell (maximum 10) and up to six rod bivalents and two trivalents (Fig. 5B). A few cells also had fragments.

With 'Tellus', the "F_1" gave two mixoploid, triple hybrids (EK25). One plant was $2n = 28–29$ and always had a complete set of 7 barley chromosomes together with 21 or 22 alien chromosomes (Table 4). The other plant was $2n = 29–30$ with 8 chromosomes of cultivated barley, including chromosome 7 in duplicate, and 21–22 alien chromosomes. Meiosis in the first plant had either 29 chromosomes or, in a few cells, 28 + 1 fragment. The

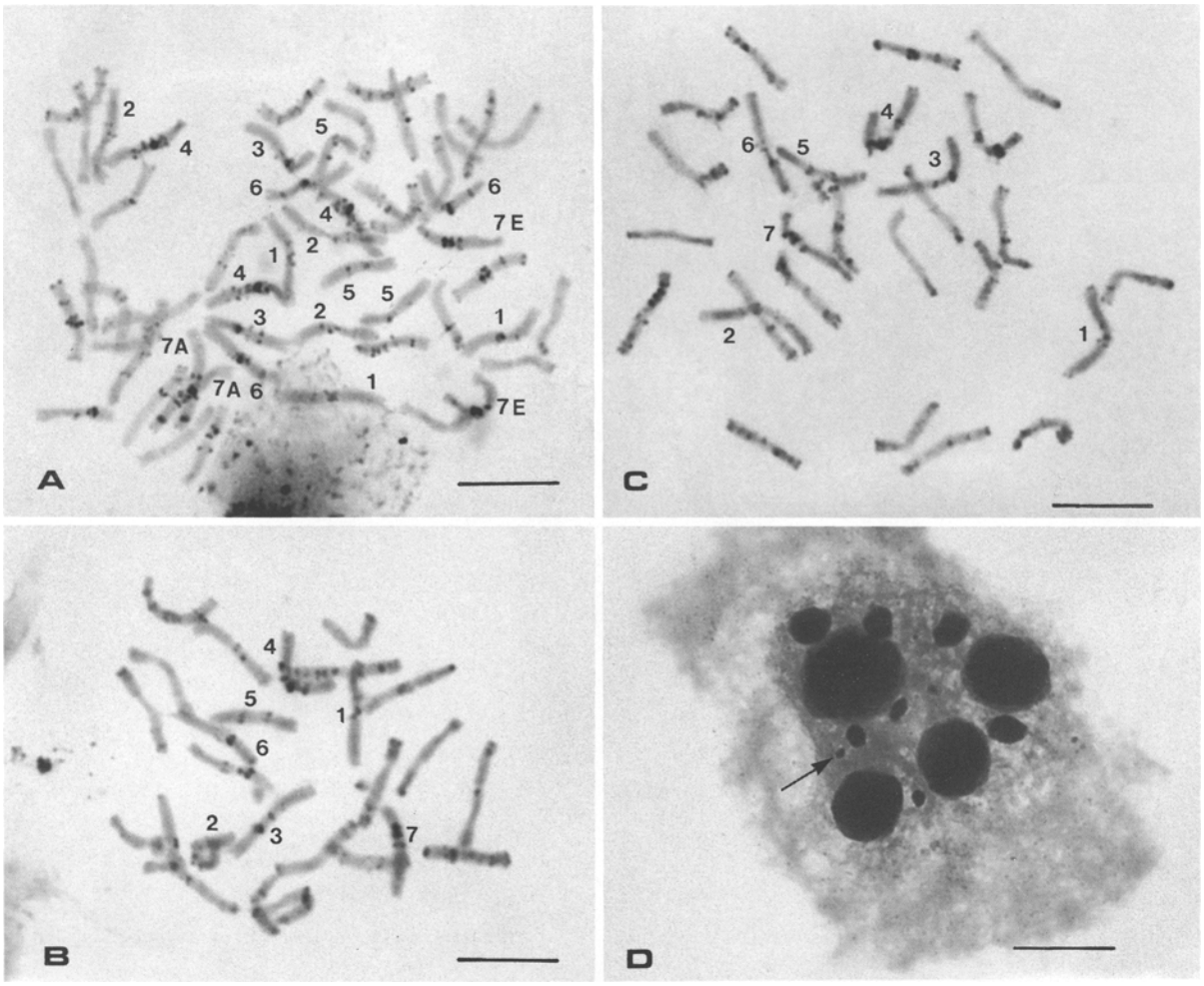

Fig. 3 A–D. Giemsa C-banded chromosomes at somatic metaphase of complex interspecific *Hordeum* hybrids. **A** EK 122 (EK 84^c × *H. vulgare* line 9208/9), $2n = 52$; **B** EK 148 (EK 84^c × *H. vulgare* 'Haisa II' 4 ×), $2n = 26$. In **A** banding pattern, 7A from line 9208/9 clearly deviates from banding pattern 7E from 'Pallas' (cf. Linde-Laursen et al. 1982); **C** EK 87-2 [(*H. lechleri* × *H. procerum*) × *H. vulgare* cv 'Maythorpe'], $2n = 27$. In **A–C**, *H. vulgare* chromosomes designated 1–7; **D** $AgNO_3$-stained cell at somatic interphase of the chromosome-doubled trispecific hybrid EK 84^c [(*H. lechleri* × *H. procerum*) × *H. vulgare* cv 'Pallas'] showing 4 large and 8 small nucleoli. *Arrow* points to very small nucleolus. *Bars* = 10 μm

Table 2. Seed set in direct backcrosses of triple hybrids comprising *H. vulgare* to diploid and tetraploid barley. No seeds germinated

Combinations		No. of barley lines	No. of spikes	No. of flowers	Seed set	
					No.	%
(F_1 × 'Tellus')	× *H. vulgare*, 2 ×	3	21	617	3	0.5
	× *H. vulgare*, 4 ×	2	28	434	0	
(F_1 × 'Maythorpe')	× *H. vulgare*, 2 ×	6	8	146	0	
	× *H. vulgare*, 4 ×	3	24	439	0	
(F_1 × 'Welam')	× *H. vulgare*, 2 ×	5	23	341	1	0.3
	× *H. vulgare*, 4 ×	4	69	930	0	
(F_1 × 'Vada')	× *H. vulgare*, 2 ×	5	12	182	0	
	× *H. vulgare*, 4 ×	3	10	132	0	
(F_1 × '*hap* E12')	× *H. vulgare*, 2 ×	1	2	27	0	
	× *H. vulgare*, 4 ×	3	4	62	0	
(F_1 × 'Pallas')	× *H. vulgare*, 2 ×	2	2	32	0	
	× *H. vulgare*, 4 ×	3	18	252	0	
Total			223	2,973	7	0.2

Table 3. Seed set, embryo formation, germination and plant production in backcrosses of the chromosome-doubled triple hybrid (*H. lechleri* × *H. procerum*) × 'Pallas' (EK 84°) to various lines of cultivated barley

Combinations	No. of attempts	No. of flowers	Seed set		Embryos	Plants	
			No.	%		Germinated	Adult
EK 84° × 'Haisa II', 4×	8	121	7	5.8	2	2	1
× 'D 8/55', 4×	4	64	2	3.1	1	1	0
× 'Str. Frank. III', 4×	1	36	1	2.8	0		
× '9208/9', 2×	13	218	8	3.7	6	4	2
× 'Welam', 2×	1	16	0				
× 'Dissa', 2×	1	13	3	23.1	1	0	
× 'Vada', 2×	2	31	1	3.2	0		

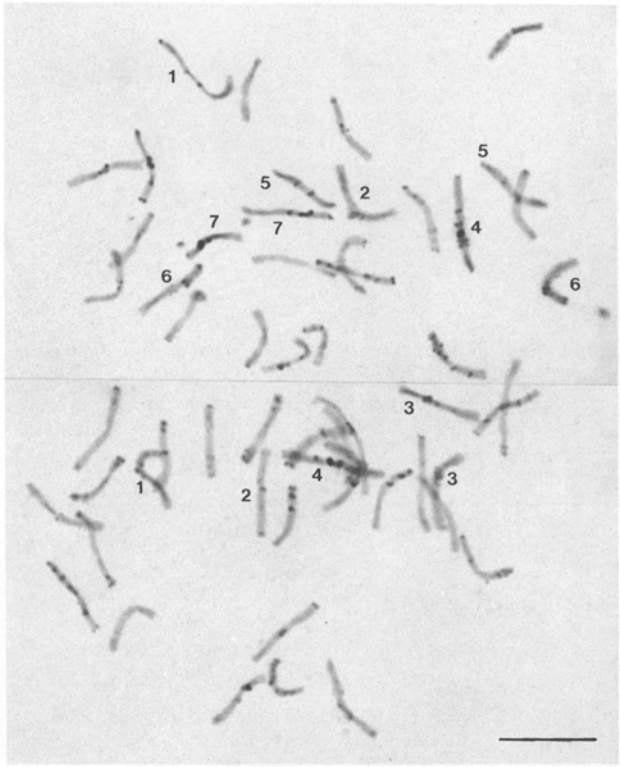

Fig. 4. Giemsa C-banded chromosomes at somatic metaphase of the chromosome-doubled, trispecific *Hordeum* hybrid EK 84° [(*H. lechleri* × *H. procerum*) × *H. vulgare* cv 'Pallas'], $2n=57$ (2 non-*H. vulgare* chromosomes outside field of vision). *H. vulgare* chromosomes designated 1–7. *Bar* = 10 μm

pairing was low with an average of 2.08 chiasmata per cell (maximum 6), and each cell had up to 5 bivalents and 1 trivalent (Fig. 5C).

The "F_1" by 'Pallas' gave five seedlings, two of which were subviable and died early (EK84). One of the three remaining plants was a trihaploid ($2n=21$) of "F_1", and two plants were mixoploid triple hybrids ($2n=28-30$). One of these latter plants (EK84-2) had $2n=28-30$ with a complete set of 7 barley chromosomes and 21 to 23 alien chromosomes (Table 4). All the meiotic cells examined from this plant were invariably $2n=28$, and the pairing was high, i.e. an average of 5.10 chiasmata per cell (maximum 8), and up to seven bivalents and one trivalent (Table 5, Fig. 5D). Several cells showed centromeric misdivisions.

Backcrosses on amphiploids

The triple hybrid EK84 [(*H. lechleri* × *H. procerum*) × 'Pallas'] was treated with colchicine. One gamete had its chromosomes doubled (EK84°) and was $2n=53-57$, with invariably 2 × 7 barley chromosomes and 39–43 alien chromosomes, and up to four large and eight small nucleoli (Figs. 3D, 4; Table 4). The analysed meiotic cells had $2n=54-56$ and, on an average, 31.4 chiasmata per cell with a maximum of 22 bivalents and some tri- and quadrivalents (Table 5, Fig. 5E).

Backcrossing with both diploid and tetraploid barley lines was attempted, and in all crosses but one, seed set took place (Table 3). The quality of the seeds varied from either small seeds, without endosperm but with a small and well-developed embryo, to large seeds with a well-developed endosperm but no embryo or a malformed one. Callus sometimes developed from the embryos. Seven seeds germinated and altogether three plants were raised. The other seedlings were unviable and died shortly after germination.

The cross with the tetraploid line of 'Haisa II' resulted in two seedlings, one of which was subviable and died early. The other plant (EK148) was mixoploid with $2n=25-27$. The C-banding pattern showed that one complete set of 7 barley chromosomes from 'Pallas' and 18–20 alien chromosomes were present (Table 4, Fig. 3B). The meiotic cell lines all had $2n=26$, a pairing average of 3.10 chiasmata per cell (maximum 11) and up to 6 bivalents and 2 trivalents (Table 5, Fig. 5F).

The second backcross to barley of $EK84^C$ was with the diploid line, '9208/9'. The single resulting plant (EK122) had $2n=51-53$ and three sets (3 × 7) of barley chromosomes. There were, however, only two homo-

Table 4. Chromosomal constitution of hybrids and backcrosses

Crossing number	2n	N[a]	No. of chromosomes		Specific *H. vulgare* chromosomes	Max. no. of satellited chromosomes[b]	Max. no. of Ag-stained NORs	Max. no. of nucleoli[c]
			alien	*H. vulgare*				
HH 364	42	6	42	0		6		9
EK 25-1	28–29	19	21–22	7	1–7	2 (6+7)	4	6 (21+4s)
EK 25-2	29–30	3	21–22	8	1–7+7	3 (6+7+7)	3	7 (31+4s)
EK 83-2	28	11	21	7	1–7	2 (6+7)		6 (21+4s)
EK 85-1	27	6	20	7	1–7	2 (6+7)	2	5 (21+3s)
EK 86-1	26–27	5	20–21	6	1–6	1 (6)	1	6 (11+5s)
EK 87-2	27	5	20	7	1–7	2 (6+7)	4	2
EK 84-2	28–30	7	21–23	7	1–7	2 (6+7)	4	6 (21+4s)
EK 84[c]	53–57	9	39–43	14	(1–7)×2	4 (6+7)×2[d]		12 (41+8s)
EK 122	51–53	4	30–32	21	–[e]	6 (6+7)×3		11
EK 148	25–27	17	18–20	7	1–7	2 (6+7)		7 (21+5s)

[a] Number of cells analysed
[b] Specific *H. vulgare* satellited chromosomes are in brackets
[c] l = large and s = small nucleoli
[d] Exclusive 5 satellited alien chromosomes
[e] The specific *H. vulgare* chromosomes of EK 122 are: [(1–7)×3]–3+7

Table 5. Meiotic MI pairing in parental material, triple hybrids and amphiploids. N is number of cells analysed, r = range, 2n is the chromosome number found in meiotic cells

Species/hybrid	2n	N	I	II			III	IV	Chiasmata/cell
				Total	Rods	Rings			
H. lechleri	42	20		19.25	1.25	18.00		0.80[a]	42.90
(H 504)		r		17–21	0–4	14–21		0–2	40–45
H. procerum	42	20	1.10	15.55	1.50	14.05	0.20	2.05[b]	39.50
(H 501)		r	0–4	13–19	0–5	11–18	0–2	0–4	33–44
F_1	42	28	15.04	11.89	6.21	5.68	2.11	0.29	22.93
(HH 364)		r	7–16	8–14	1–12	2–10	0–4	0–1	16–29
F_1 × 'Tellus'	28	25	25.00	1.88	1.76	0.12	0.04		2.08
(EK 25-1)		r	19–29	0–5	0–4	0–2	0–1		0–6
F_1 × 'Maythorpe'	28	27	22.85	2.52	2.26	0.26	0.04		2.85
(EK 83-1)		r	13–28	0–6	0–4	0–3	0–1		0–1
F_1 × '*hap* E12-p 14'	25–26	50	18.88	3.18	2.86	0.32	0.22	0.02	4.00
(EK 86-1)		r	14–24	1–6	1–5	0–2	0–2	0–1	1–8
F_1 × 'Welam'	26–27	22	24.23	1.18	1.14	0.05	0.05		1.32
(EK 87-1)		r	21–27	0–3	0–3	0–1	0–1		0–4
F_1 × 'Vada'	28–29	21	19.95	3.76	3.00	0.76	0.48		5.48
(EK 89-1)		r	14–24	1–6	1–6	0–4	0–2		3–10
F_1 × 'Pallas'	28	22	20.18	3.68	3.14	0.55	0.18		4.59
(EK 84)		r	14–24	2–7	1–7	0–2	0–1		2–8
EK 84[c]	56	14	14.07	18.43	9.00	9.43	1.50	0.14	31.43
		r	5–24	13–22	6–12	3–15	0–4	0–1	20–40
EK 84[c] × 'Haisa II'	25–27	50	20.94	1.80	1.44	0.38	0.50		3–10
(EK 148)		r	11–24	0–6	0–4	0–3	0–2		0–11

[a] In addition: VI 0.05 (r 0–1)
[b] In addition: V 0.10 (r 0–2), VI 0.10 (r 0–1)

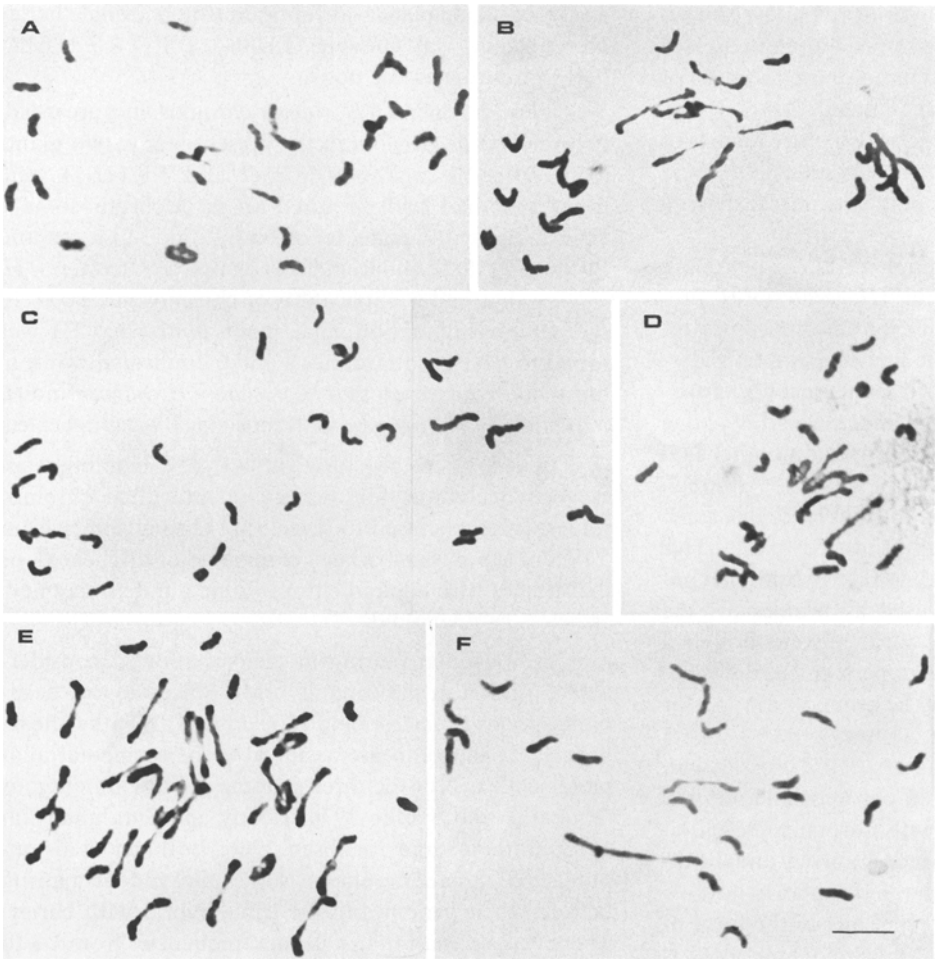

Fig. 5 A–F. Meiotic MI configurations in **A** EK 83-1 [(*H. lechleri* × *H. procerum*) × *H. vulgare* cv 'Maythorpe'], $2n = 28$, 22 I, 3 II (1 ring, 2 rods); **B** EK 89-1 [(*H. lechleri* × *H. procerum*) × *H. vulgare* cv 'Vada'], $2n = 29$, 21 I, 4 II (rods), 2 foldbacks and two secondary associations; **C** EK 25-1 [(*H. lechleri* × *H. procerum*) × *H. vulgare* cv 'Tellus'], $2n = 30$, 28 I, 1 II (rod), two foldbacks, 1 SS secondary association, **D** EK 84 [(*H. lechleri* × *H. procerum*) × *H. vulgare* cv 'Pallas'], $2n = 28$, 19 I, 3 II (1 ring, 2 rods) 1 III, 1 foldback; **E** EK 84^c [(*H. lechleri* × *H. procerum*) × *H. vulgare* cv 'Pallas'], chromosome doubled, $2n = 54$, 8 I, 23 II (19 rods 4 rings); **F** EK 148 [EK 84^c × *H. vulgare* cv 'Haisa II'] $2n = 25$, 20 I, 1 II (rod), 1 III. *Bar* = 10 μm

logues of chromosome 3 (chromosome 3B from '9208/9' and 3A from 'Pallas') and four homologues of chromosome 7 (two of variant 7A from '9208/9' and two of variant 7E from 'Pallas', Fig. 3A). Unfortunately this plant died before meiosis could be investigated.

In all the hybrids analysed, only the nucleolar constrictions of the *H. vulgare* SAT-chromosomes 6 and 7 were observed (Table 4). They may also have been involved in the formation of the large nucleoli seen in a corresponding number in silver nitrate stained interphases. The nucleolus-forming capacity of the alien SAT-chromosomes, however, was not completely suppressed. There were up to five (eight in EK84^c) additional small nucleoli, and more nucleolar organizers than corresponded with the number of *H. vulgare* SAT-chromosomes present in silver nitrate-stained metaphases.

Discussion

As shown by several authors, hybrids between cultivated barley and wild *Hordeum* species are, with exception of those obtained from crosses between diploids and those from crosses having barley as the female parent, comparatively easy to obtain (cf. Bothmer et al. 1983, 1986; Pickering and Morgan 1985; Fedak 1985; Bothmer and Jacobsen 1988). The primary hybrids, despite being vegetatively well-developed, appear to be completely sterile and to form no viable male or female gametes (Bothmer and Hagberg 1983). The present study clearly shows that obtaining triple hybrids comprising two closely related polyploid *Hordeum* species and cultivated barley is also rather easy. This agrees with the results of Schooler and Anderson (1979), and Starks et al. (1983).

Direct backcrosses of triple hybrids to barley, however, are also very difficult. Our attempts with both diploid and tetraploid lines of cultivated barley gave a seed set of only 0.2% (from almost 3000 pollinated flowers), and not one seed germinated. As there are no other reports of success with direct backcrosses to *H. vulgare*, this method doesn't appear to be realistic as a means of transferring alien genes to cultivated barley.

The production of an amphidploid (at the octoploid level) of the original triple hybrid [(*H. lechleri* × *H. procerum*) × *H. vulgare*] with around 56 chromosomes resulted in the formation of viable gametes at a considerably increased frequency. The seed set in most backcrosses to cultivated barley varied between 3% and 6%, one cross yielded no seeds (with 'Welam'), and another had an avarage seed set of 23% (with 'Dissa'). Although several of the seeds were formed without embryos, some contained viable embryos which produced plants. This encouraging result suggests that complex hybrids, in contrast to simple ones, may be used as bridges for the transfer of alien characters to barley. Even though a rather low number of crosses were performed, the varying results suggest that there may be genetic variation for crossability among different barley lines.

A characteristic feature of interspecific crosses with cultivated barley is a variation in chromosome number that is not necessarily linked with chromosome elimination (Bothmer et al. 1983; Linde-Laursen and Bothmer 1988). This is also evident in the complex hybrids of the present study. Mixoploidy, especially in the form of combinations of cells with hypo- and euploid numbers, is common, but combinations of cells with eu- and hyperploid numbers also occur. In many plants, a complete set of barley chromosomes (no 1–7) was present, but with 18–20 or 22–23 alien chromosomes instead of the expected 21. These data are similar to those reported by Finch and Bennett (1980) and Linde-Laursen and Bothmer (1986a, 1988).

Selective elimination of whole genomes also occurred in the present study. The two species *H. lechleri* and *H. procerum* sometimes eliminate the barley genome in interspecific hybrids (Subrahmanyam 1977, 1980; Bothmer et al. 1983; Subrahmanyam and Bothmer 1987; Linde-Laursen and Bothmer 1988). The frequencies at which the hybrids and haploids are formed are genotypically and environmentally determined (Pickering and Morgan 1985; Linde-Laursen and Bothmer 1986).

As reported by Linde-Laursen and Bothmer (1986b, 1988), the chromosomes of *H. vulgare* are rather stable in interspecific hybrids, but there may be losses or even gains of individual barley chromosomes in different plants within the same hybrid family. There seems to be a tendency that chromosomes 1, 5, 6 and 7 of *H. vulgare* are the first ones to be eliminated, but not in any specific order (Linde-Laursen and Bothmer 1988). In the present study, only one plant was found in which a single barley chromosome was missing: EK86-1 ("F_1" × '*hap* E12') had lost chromosome no. 7.

Gains of individual *H. vulgare* chromosomes are usually rarer than losses, but in the present study, two plants from different families (EK25-2 and EK122-1) were found that had both acquired an extra chromosome 7. This agrees with results reported by Linde-Laursen and Bothmer (1988), who found two plants in *H. lechleri* × *H. vulgare* that had a duplication of chromosome no. 7. In the same combination, one plant (with $2n=25$) was found to have chromosomes 4 and 6 duplicated, while in one plant from the crosses *H. parodii* × *H. vulgare* and *H. tetraploidum* × *H. vulgare*, chromosome 1 was duplicated.

The results of this study show that C-banding is an excellent technique for identifying individual chromosomes. It may be used to reveal that chromosomes from different genomes have been eliminated or duplicated, or that tissues with euploid chromosome number may nevertheless be aneuploid.

Chromosomal pairing in meiosis showed considerable variation in the triple hybrids. Since the two hexaploid species *H. lechleri* and *H. procerum* are rather closely related, and both are assumed to be segmental alloploids with two of the three genomes being homologous (Rajhathy and Symko 1974; Reddy and Surahmanyam 1985; Bothmer and Jacobsen 1986; Bothmer et al. unpublished), there should be some allosyndetic pairing between their genomes in the triple hybrid with barley. There was a variation in chiasma frequencies from 1.3 to 5.5 among the triple hybrids with different lines of barley. In comparison, trihaploids of *H. lechleri* had chiasma frequencies of 3.2–5.3, and trihaploids of *H. procerum*, 0.5 to 0.7 (Rajhathy and Symko 1974; Bothmer and Subrahmanyam 1988). So far, there is no evidence that barley chromosomes take part in any allosyndetic pairing configurations.

New methods for identifying individual chromosomes in meiosis need to be developed before this phenomena can be elucidated. The difficulty in using C-banding in detailed meiotic analysis in barley is a scarcity of the large blocks of constitutive heterochromatin that occurs, e.g. in rye (cf. Singh and Röbbelen 1975; Sybenga 1983) and in *H. brevisubulatum* (Linde-Laursen et al. 1980). Such heterochromatic blocks make possible a sophisticated interpretation of the meiotic configurations in hybrids.

The considerable variation in pairing found in triple hybrids comprising different accessions of barley is, at least partly, due to genetic variation in the ability to suppress meiotic pairing between the different lines of *H. vulgare* used in the present study. This confirms data from other authors (Bothmer et al. 1983; Gupta and Fedak 1985).

The interpretation of the two successful backcrosses of the amphiploid with $2n = 53-57$ to barley is not obvious. The backcrosses were performed with one diploid ('9208/9') and one tetraploid line ('Haisa II') of barley. Theoretically, the resulting backcrosses should have had 35 and 42 chromosomes, respectively, which in fact, none of them had. The one cross with '9208/9' (EK122) had $2n = 51-53$ with 21 *H. vulgare* chromosomes, 13 from 'Pallas', 8 from 9208/9, and 30–32 alien chromosomes. The most likely explanation is that an unreduced gamete of the amphiploid (EK84^c) was fertilized by a reduced gamete of the backcross parent. This was followed by the elimination of 10–12 alien chromosomes and one chromosome from 'Pallas' (no. 3), and the duplication of one chromosome (no. 7) from 9208/9 during embryo development.

In the cross with 'Haisa II' (EK148), the resulting plant had only $2n = 25-27$ with one complete set of seven barley chromosomes. Those were identified as the 'Pallas' genome (from the original triple hybrid, EK84). This plant also had 18–20 alien chromosomes. There are two possible explanations for this highly deviating plant: (1) fertilization through reduced gametes from both parents. During early embryogenesis selective elimination of the chromosomes of 'Haisa II' occurred, leaving the 'Pallas' genome. Even though there are many reports of chromosome elimination in interspecific and intergeneric crosses with *H. vulgare, H. bulbosum* and other species (see Fedak 1985, Subrahmanyam and Bothmer 1987 for references), there are no reports of the selective elimination of a specific barley genome leaving the other barley genome(s) intact. (2) The plant has arisen through spontaneous development of the reduced egg cell of the amphiploid without fertilization, possibly by the fertilization of the polar nucleus as a stimulus. We are inclined to believe that the second explanation is the most likely one, but further studies of the phenomenon are required.

So far there have been no reports of apomictic behaviour in pure *Hordeum* crosses. However, haploid parthenogenesis is known to occur in barley as a result of the action of the *hap*-gene (Hagberg and Hagberg 1980) and the fertilization of unreduced gametes through the *tri*-gene (Ahokas 1977). Mujeeb-Kazi (1981) reported deviating progeny in backcross families including barley and wheat, viz. (*H. vulgare* × *T. turgidum*) × *T. tugidum*, and (*H. vulgare* × *T. aestivum*) × *T. aestivum*. Plants of the first cross had $2n = 35$, and in the latter combination, $2n = 28$, instead of the expected $2n = 49$. These chromosome numbers were interpreted to be the result of an apomictic reproduction of the primary F_1. Mujeeb-Kazi (1981) also observed apomictic behaviour in a backcross of an apart hybrid (barley × wheat), i.e. with a similar genetic constitution as our complex *Hordeum* hybrid. It is probable that these more-or-less sterile and perhaps unbalanced "wide hybrids", form "U-type female gametes" (unreduced gametes, cf. Asker 1980) comparatively frequently which, after backcrossing to a stable and highly fertile male parent (in these cases, wheat and barley), initiate parthenogenesis or pseudogamic development through fertilization of the polar nucleus.

Other examples of apomixis in *Triticeae* are few. Kruse (1969) reported (a doubtful) pure wheat offspring in the cross *T. aestivum* × *Avena sativa*, and Tschermak-Seysenegg (1951) found parthenogenetic offspring in wheat. Autonomous endosperm development was found in wheat by Kandelaki (1976). In other grass groups, apomictic systems are more common, e.g. in *Poa* (Almgård 1966; Doll 1971), *Sorghum* (Hanna et al. 1970), *Panicum* (Pernes et al. 1975; Savidan 1978) and *Pennisetum* (Dujardin and Hanna 1986).

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