Theor Appl Genet (1985) 70: 199–206

© Springer-Verlag 1985

The influence of temperature on chromosome elimination during embryo development in crosses involving *Hordeum* spp., wheat (*Triticum aestivum* L.) and rye (*Secale cereale* L.)

R. A. Pickering* and P. W. Morgan

Welsh Plant Breeding Station, Plas Gogerddan, Near Aberystwyth, Dyfed, SY23 3EB, Wales, Great Britain

Received September 21, 1984; Accepted November 21, 1984
Communicated by G. Wenzel

Summary. Several interspecific and intergeneric crosses involving five *Hordeum* species, *Triticum aestivum* and *Secale cereale* were carried out to investigate the influence of two contrasting temperatures on chromosome elimination during embryo development. In four of the interspecific *Hordeum* crosses, chromosome elimination was significantly increased at the higher of the two temperatures, resulting in greater proportions of haploid plant progenies. However, there was no significant effect of temperature in the other interspecific cross between *H. lechleri* × *H. bulbosum* nor in the two intergeneric crosses between *H. vulgare* × *S. cereale* and *T. aestivum* × *H. bulbosum* whose progeny were exclusively hybrid and haploid, respectively.

Key words: Wide hybridizations – Triticeae – Temperature – Chromosome elimination – Embryo development

Introduction

Chromosome elimination of one parental genome has been shown to take place in several interspecific *Hordeum* crosses (Bennett et al. 1976; von Bothmer et al. 1983; Subrahmanyam 1982; Subrahmanyam and Kasha 1973) and intergeneric crosses in the Triticeae involving a *Hordeum* species as one of the parents (e.g. *Hordeum vulgare* L. × *Secale cereale* L., Fedak 1977; *Triticum aestivum* L. × *H. bulbosum* L., Barclay 1975). To exploit this phenomenon for practical purposes, hybridization between *H. vulgare* and *H. bulbosum* has been extensively used in breeding programmes.

It is already known that there is a marked temperature and genotypic effect on chromosome elimination in *H. vulgare* × *H. bulbosum* crosses (Pickering 1983, 1984; Simpson et al. 1980) and that by using particular environments and cultivars, the proportions of haploids, and hybrids which retain the *H. bulbosum* genome, can be varied (Pickering 1984).

In order to investigate more extensively the influence of temperature on chromosome elimination during embryo development, several other interspecific and intergeneric crosses in the Triticeae were attempted at two contrasting temperatures. Most of these crosses were chosen because both haploid and hybrid progeny have previously been obtained from them.

* Present address: Crop Research Division, Lincoln, DSIR, Private Bag, Christchurch, New Zealand

Materials and methods

Parental material

A list of genotypes used in the crossing programme, and their origin are shown in Table 1. The *H. bulbosum* Cb 2929/1, originally selected from a seed sample of GBC 281, has been vegetatively propagated for several years and bulbs were vernalized at 2 °C, 8 h daylength for 7–8 weeks to induce flowering. The remainder of the parental material was grown from seed in 1983 and vernalisation was found to be unnecessary.

Environmental conditions

All plants were raised in a heated glasshouse in 15 cm diameter plastic pots containing J. Innes No. 3 compost. The temperature was maintained at 23 ± 5 °C/13 ± 3 °C (day/night) but *H. lechleri* plants which were crossed with *H. bulbosum* were grown in a different season when glasshouse temperatures were 16 ± 2 °C/9 ± 2 °C (day/night). Natural daylight was supplemented with 400 W high pressure mercury fluorescent bulbs when necessary to provide a 16 h daylength. Just before spike emergence, pots containing the female parents were transferred to one of two controlled environments maintained at continuous temperatures for crossing (Table 2). A 16 h daylength was obtained using white fluorescent lamps giving an irradiance at spike height of 200–300 $\mu E/m^2/s$. Male parents were left in the glasshouse for pollen collection.

Pollination and embryo culture

All florets on spikes of the female parent were emasculated conventionally and pollinated, at the time of lemma and palea opening, with freshly collected pollen or dehiscing anthers. In case a temperature of 26 °C might have adversely affected pollen tube growth and fertilization, pollinations of barley with rye and *H. lechleri* with *H. bulbosum* were carried out at room temperature (20–22 °C) prior to returning the plants to 26 °C after 2 h. One and two days after pollination, a solution of gibberellic acid 75 mg/l (GA_3)+0.05% Tween 20 wetting agent was sprayed onto the florets and each spike covered with a brown paper bag (Pickering 1982). Once embryos had attained a size of 1–1.5 mm in length (17–19 days after pollination (d.a.p.) at 15 °C; 10–12 d.a.p. at 22 °C; 10 d.a.p. at 26 °C) the differentiated ones were excised and cultured, scutellum faced down, in vials containing 7 ml of a modified B5 medium (Gamborg et al. 1968) with 2% sucrose, 0.7% agar but without 2,4-D. The cultured embryos were incubated at 22 °C in darkness. After germination the tubes were removed to a 16 h daylength environment (150–200 $\mu E/m^2/s$ white fluorescent lamps) at room temperature (22±2 °C). At a height of 5–6 cm, the seedlings were transferred to J. Innes No. 3 compost in 10 cm diameter pots in the glasshouse. Undifferentiated embryos from crosses between *H. depressum* × *H. bulbosum*, *H. procerum* × *H. bulbosum*, *H. lechleri* × *H. bulbosum*, *T. aestivum* × *H. bulbosum* and *H. vulgare* × *S. cereale* were cultured on a B5 medium containing 2 mg/l 2,4-D in darkness at 22 °C. This was to induce callus formation prior to subculture onto B5 without 2,4-D in an attempt to regenerate plants as it is known that only a very low proportion of undifferentiated embryos regenerate plants directly (Pickering 1980).

Identification of progeny

Chromosome counts were carried out on root tips of all plants, generally about 8 weeks after embryo culture. As the two parental species involved in each of the crosses differed in their chromosome constitution (except *H. vulgare* × *S. cereale*), it was assumed that 21 chromosome progeny from crosses involving *H. procerum* and *H. lechleri* with *H. vulgare* and *H. bulbosum* were haploids of the former two species, and 28 chromosome progeny were hybrids. Plants with 14 and 21 chromosomes derived from *H. depressum* × *H. bulbosum* were classified as *H. depressum* haploids and hybrids, respectively. Vegetative characters (such as habit of growth and presence or absence of auricles) in addition to spike morphology also aided their identification. Haploid and hybrid plants derived from *H. vulgare* × *S. cereale* were to have been classified by their chromosome number (7-haploid, 14-hybrid) and morphology, but only hybrids were obtained (see "Results").

In all crosses, samples of hyperploid plants and those having an intermediate or variable chromosome number (aneusomics) were recounted after approximately 4 months to determine their stability.

For cytological analysis, root tips were removed from plants in pots. After pretreatment for 24 h in distilled water at

Table 1. List of genotypes used in the crossing programme

Species	Chromosome no.	Code no. WPBS	Code no. Original	Source
H. bulbosum L.	14	Cb 2929/1	Selection from GBC 281	Prof. K. J. Kasha Guelph University Canada
H. depressum (Scribn., & Sm.) Rydb.	28	Cb 3813	H 1980	Dr. R. von Bothmer Inst. of Crop Genetics Swedish Univ. of Agric. Sci. Svalöv, Sweden
H. lechleri (Steud.) Schenk	42	CB 3814	H 1765	Dr. R. von Bothmer Inst. of Crop Genetics Swedish Univ. of Agric. Sci. Svalöv, Sweden
H. procerum Nevski	42	CB 3815	H 1166	Dr. R. von Bothmer Inst. of Crop Genetics Swedish Univ. of Agric. Sci. Svalöv, Sweden
		Cb 3820	PI 266196	Dr. G. A. White USDA, Beltsville Agric. Res. Center Beltsville, USA
H. vulgare L.	14	cvs. 'Emir', 'Golden Promise', 'Sabarlis', 'Vada'		
T. aestivum L.	42	cv. 'Chinese Spring'		Mr. E. Simpson Plant Breeding Institute Cambridge, UK
S. cereale L.	14	cv. 'Rogo'		Dr. R. Cook Welsh Plant Breeding Stn Aberystwyth

Table 2. List of parents and environments used in the crossing programme

♀ Parent	♂ Parent	Temperature during embryo development	References[a] describing the presence (+) or absence (–) in the progeny of:		
			Haploids	Hybrids	Reference
T. aestivum	*H. bulbosum*	15±0.25 °C 22±1 °C	+	–	Barclay 1975; Zenkteler and Straub 1979
H. depressum	*H. bulbosum*	15±0.25 °C 22±1 °C	+	+	Subrahmanyam 1979
H. procerum Cb 3815 Cb 3820	*H. vulgare* cvs. 'Sabarlis' 'Vada'	15±0.25 °C 22±1 °C	+	+	Subrahmanyam 1977; Finch and Bennett 1980
			–	+	von Bothmer et al. 1983
H. procerum Cb 3820	*H. bulbosum*	15±0.25 °C 22±1 °C	+	–	Subrahmanyam 1977
H. lechleri	*H. vulgare* cvs. 'Emir' 'Sabarlis' 'Vada'	15±0.25 °C 22±1 °C	+	+	von Bothmer et al. 1983; Rajhathy and Symko 1974; Subrahmanyam 1982
			+	–	Subrahmanyam 1980
H. lechleri	*H. bulbosum*	15±0.25 °C 26±1 °C	+	–	Subrahmanyam 1980, 1982
H. vulgare cv. 'Golden Promise'	*S. cereale*	15±0.25 °C 26±1 °C	+	+	Fedak 1978; Kruse 1967
			+	–	Fedak 1977
			–	+	Cooper et al. 1978 Fedak 1979; Thomas and Pickering 1979

[a] Previous workers did not necessarily use the cultivars named in columns 1 and 2 as parents

1 °C and fixation in ethanol: acetic acid (3:1), they were stained by the Feulgen method and squashed in 1% acetocarmine.

Within each cross χ^2 contingency analyses were carried out to assess the influence of temperature on chromosome elimination by comparing the numbers of progeny which had haploid, hybrid or intermediate chromosome numbers. In all crosses except *H. procerum* × *H. bulbosum* the numbers of haploid and aneusomic plants (i.e. those showing chromosome elimination) were pooled to validate the analyses. When more than one genotype was used (*H. procerum* × *H. vulgare* and *H. lechleri* × *H. vulgare*), χ^2 analyses were partitioned to separate genotype and temperature effects. The small numbers of hyperploid plants with chromosome numbers greater than those expected from hybrid progeny, and those which were highly variable in this respect were excluded from the analyses.

Results

Seed setting and development

As there were large between spike variations in the levels of seed set, few conclusions about the genotypic or environmental effects on seed setting could be drawn. However, seed sets in *H. lechleri* × *H. bulbosum* were markedly reduced at 26 °C despite allowing fertilization to take place at room temperature (Table 3). It is possible that fertilization took place but that subsequent seed development at 26 °C was arrested. This explanation is supported by the fact that higher proportions of seeds had degenerated by the time of embryo culture at 26 °C (21.0%) compared with 15 °C (6.1%).

Endosperm and embryo development

Endosperms were predominantly watery apart from crosses between *H. procerum* × *H. vulgare*, and to some extent *H. lechleri* × *H. vulgare*, in which both watery and solid endosperms occurred (Table 3). Greater proportions of progeny derived from the solid endosperm seeds were hybrid compared with those from watery endosperms (e.g. *H. procerum* × *H. vulgare:* 59.6% and 21.6%, respectively). Chromosome retention may therefore result in a greater tissue stability and production of functional endosperm.

In the interspecific crosses, embryo differentiation rates were highest in crosses involving *H. procerum* and *H. lechleri* but relatively low in those between *H. depressum* × *H. bulbosum* (Table 3). In general, 15 °C seemed to favour embryo differentiation in most of the crosses except *H. vulgare* × *S. cereale*. Embryos from this cross frequently showed the presence of an epiblast, a feature absent from *H. vulgare* and *S. cereale* embryos although Norstog (1961) observed epiblasts on cultured barley embryos which were 0.5 mm in length at the time of excision.

Table 3. Seed setting, seed characteristics, embryo differentiation and plant regeneration rates from crosses between *Hordeum* spp., *T. aestivum* and *S. cereale* at two temperatures during embryo development

Cross	Temperature (°C)	No. of florets pollinated	Seed sets (%)	Solid endosperm seeds (%)	Shrivelled seeds (%)	Differentiated embryos (%)	Plant regeneration[a] (%)
T. aestivum × *H. bulbosum*	15	797	17.8	0.0	40.1	71.8	91.8
	22	598	9.9	0.0	32.2	62.5	64.0
H. depressum × *H. bulbosum*	15	261	83.9	0.0	27.9	79.1	52.0
	22	213	88.3	0.0	22.3	61.0	37.1
H. procerum × *H. vulgare*	15	827	37.7	72.2	4.5	99.7	87.9
	22	689	37.4	64.4	9.7	100.0	94.4
H. procerum × *H. bulbosum*	15	89	59.6	0.0	5.7	100.0	76.0
	22	143	62.9	0.0	11.1	78.8	87.3
H. lechleri × *H. vulgare*	15	349	43.0	17.6	4.0	100.0	97.9
	22	245	62.0	1.3	4.6	100.0	96.6
H. lechleri × *H. bulbosum*	15	199	73.9	0.0	6.1	91.3	67.5
	26	436	42.7	1.1	21.0	73.5	63.9
H. vulgare × *S. cereale*	15	384	96.9	0.0	86.8	20.4	63.6[b]
	26	395	92.7	0.0	71.0	21.7	

[a] % plant regeneration from differentiated embryos cultured on B5 medium without 2,4-D
[b] Combined data from 15 °C + 26 °C

Table 4. Plant regeneration from callus derived from undifferentiated embryos

Cross	No. of undifferentiated embryos cultured	No. of surviving plants regenerated	Chromosome constitution
T. aestivum × *H. bulbosum*	39	1	c. 42 chromosomes
H. depressum × *H. bulbosum*	90	3	14 chromosomes (haploid)
		2	21 chromosomes (hybrid)
		1	28 chromosomes (doubled haploid)
		1	variable, 15 + 26 chromosomes
H. procerum × *H. bulbosum*	17	0	
H. lechleri × *H. bulbosum*	51	1	c. 42 chromosomes (doubled haploid)
H. vulgare × *S. cereale*	122	14	14 chromosomes (hybrid)

Plant regeneration and genetic constitutions of the progeny

The data collated was mainly from plants regenerated from differentiated embryos and sufficient progeny were obtained from all cross combinations to determine the influence of temperature (and in some cases genotype) on chromosome elimination in the developing embryo. Plant regeneration was, however, disappointingly low from callus derived from undifferentiated embryos (Table 4).

Haploid and hybrid plant morphologies have been described previously (von Bothmer et al. 1983; Rajhathy and Symko 1974; Subrahmanyam 1977, 1979; Thomas and Pickering 1979) except for the new hybrids from *H. procerum* × *H. bulbosum* and *H. lechleri* × *H. bulbosum.* Hybrid spikes from these combinations were either intermediate between the parents (*H. procerum* × *H. bulbosum*) or more closely resembled the female (*H. lechleri* × *H. bulbosum*). In crosses between *H. procerum* × *H. vulgare* using two genotypes of each parent, hybrids from Cb 3815 as female were stunted in appearance (Fig. 1) with small weak spikes. Hybrids derived from Cb 3820 were however, vigorous and produced large well developed spikes. Anthers were indehiscent in all progenies except where stated.

In crosses between *H. procerum* × *H. vulgare,* chromosome elimination was influenced to some extent by both parental genotypes. Highest proportions of hybrids overall were obtained from Cb 3820 as female ($\chi^2 = 31.6$, 1 d.f., $P \leqq 0.001$) and 'Vada' as male ($\chi^2 = 4.7$, 1 d.f., $P \leqq 0.05$). In *H. lechleri* × *H. vulgare* there were also significant differences between pollinators in this

Fig. 1. Progeny from *H. procerum* × *H. vulgare* illustrating the influence of the female parental genotype on hybrid plant morphology. *Left to right:* Cb 3820 × *H. vulgare* cv. 'Vada', 28 chromosome hybrid; Cb 3815 × *H. vulgare* cv. 'Vada', 21 chromosome haploid; Cb 3815 × *H. vulgare* cv. 'Vada', 28 chromosome hybrid.

respect but only at 15 °C ($\chi^2 = 7.0$, 2 d.f., $P \leqq 0.05$). Chromosome elimination was greatest using 'Vada' as male and least with 'Emir'; 'Sabarlis' was intermediate.

There was no evidence of temperature influencing chromosome elimination in the two intergeneric hybridizations as all progeny were hybrid or haploid (Table 5). Haploids, aneusomics and hybrids were however obtained from all five interspecific *Hordeum* cross combinations but there were only very few hybrids regenerated from *H. lechleri* × *H. bulbosum* and temperature during embryo development had no significant effect on chromosome elimination. In the remaining four interspecific *Hordeum* crosses, significantly increased numbers of haploids and aneusomics were produced at 22 °C compared with 15 °C. Conversely, the latter temperature favoured chromosome retention in hybrids which retained both parental sets of chromosomes.

Aneusomic, hyperploid and polyploid progeny

Most aneusomic plants remained stable for 4 months (95% *H. procerum* × *H. vulgare*; 85% *H. procerum* × *H. bulbosum;* 68% *H. lechleri* × *H. vulgare*). The unstable ones usually shed further chromosomes although some increased in number.

Eight hyperploid progeny occurred at 15 °C (Table 5). Four out of five of them from *H. lechleri* crosses

Table 5. The numbers and proportions of haploid, aneusomic and hybrid plants obtained from various crosses at two temperatures during embryo development (analyses excluding hyperploid and highly variable plants). Pooled haploid + aneusomic data used in all crosses except *H. procerum* × *H. bulbosum*

Cross	Temperature during embryo development	Nos. (%) of plants with chromosome numbers of:					Others	Total no. of plants	χ^2	df	*P*
		14	14–21	21	21–28	28					
T. aestivum ×	15 °C			56 (98)			1 (2) c. 42 chromosomes ex callus	57			
H. bulbosum	22 °C			16 (100)				16			
H. depressum ×	15 °C	24 (35)	2 (3)	37 (54)	3 (4) hyperploid	2 (3) tetraploid	1 (1) highly variable	69	3.84	1	0.05
H. bulbosum[a]	22 °C	20 (55)	1 (3)	13 (36)	0	1 (3) doubled haploid	1 (3) mixoploid	36			
H. procerum ×	15 °C			29 (11)	78 (30)	143 (55)	11 (4) highly variable	261	17.5	1	<0.001
H. vulgare	22 °C			71 (32)	67 (31)	79 (36)	2 (1) highly variable 1 (0.5) pentaploid	220			
H. procerum ×	15 °C			22 (58)	5 (13)	10 (26)	1 (3) highly variable	38	8.5	2	0.05–0.01
H. bulbosum	22 °C			39 (71)	12 (22)	3 (5)	1 (2) highly variable	55			
H. lechleri ×	15 °C			1 (1)	15 (11)	122 (86)	2 (1.4) hyperploid; 1 (0.7) self	141	58.8	1	<0.001
H. vulgare	22 °C			30 (22)	48 (34)	60 (43)	2 (1) highly variable	140			
H. lechleri × *H. bulbosum*	15 °C			76 (88)	0	3 (4)	3 (4) hyperploid 2 (2) pentaploid 2 (2) 42 chromosomes (1 ex callus)	86	0.8	1	NS
	26 °C			60 (87)	3 (4)	6 (9)		69			
H. vulgare ×	15 °C	11 (100)						11			
S. cereale[a]	26 °C	24 (100)						24			

[a] Combined data from plants derived from callus and directly from differentiated embryos

which were rechecked after 4 months had remained stable, whereas the fifth from *H. lechleri* × *H. vulgare* had lost chromosomes and stabilised at 28.

Seven polyploid plants were obtained directly from differentiated embryos with chromosome numbers of 28 (*H. depressum* × *H. bulbosum*), 35 and 42 (*H. procerum* × *H. vulgare, H. lechleri* × *H. vulgare* and *H. lechleri* × *H. bulbosum* – Table 5). The 28 and 35 chromosome plants were hybrid-like in appearance. Two of the three pentaploids showed chromosome loss after 4 months, whereas the third from *H. lechleri* × *H. bulbosum* remained stable. The unstable ones were recounted as possessing 28 and 32–34 chromosomes.

One of the 42 chromosome plants from *H. lechleri* × *H. vulgare* was derived from a seed with solid endosperm and was probably a self or intraspecific out-pollination. However, the other 42 chromosome plant which resembled *H. lechleri,* was obtained from a seed with watery endosperm after pollinating *H. lechleri* with *H. bulbosum.*

The 28 and 42 chromosome fertile plants regenerated from calli of *H. depressum* × *H. bulbosum* and *H. lechleri* × *H. bulbosum* respectively and the infertile c. 42 plant from a callus of *T. aestivum* × *H. bulbosum* resembled the female parents.

Discussion

In order to determine the influence of temperature on chromosome elimination in the developing embryo, it was not thought practical to count individual embryonic chromosome numbers. Instead, it was decided to culture the embryos on a nutrient medium to obtain plant progenies and then to carry out root tip chromosome analyses. In crosses from which large proportions of differentiated embryos and high plant regeneration rates were achieved (*H. procerum* × *H. vulgare; H. procerum* × *H. bulbosum;* and *H. lechleri* × *H. vulgare*) the chromosome numbers of these plants would accurately reflect the overall numbers of haploid, aneusomic and hybrid embryos. However, in the case of *H. depressum* × *H. bulbosum* comparatively low regeneration rates and high proportions of undifferentiated embryos were obtained but their chromosome numbers could not be assessed reliably from the progeny as only seven plants were regenerated via callus. However, it has been reported that in interspecific *Hordeum* crosses, differentiated embryos regenerate both haploids and hybrids whereas undifferentiated ones are more likely to be haploid (Pickering 1984; Subrahmanyam 1983). Assuming this to be so, then as the proportions of undifferentiated embryos were larger at the higher temperature in *H. depressum, H. procerum* and *H. lechleri* all pollinated with *H. bulbosum,* the numbers of haploids are likely to be a greater underestimate at 22 °C and 26 °C than at 15 °C.

In four of the five interspecific crosses, chromosome elimination was enhanced during embryo development at the higher of the two temperatures. These data confirm the temperature effect on chromosome elimination observed previously in *H. vulgare* × *H. bulbosum* crosses (Pickering 1984). Although most aneusomics had remained stable after 4 months, many of the remainder exhibited further loss of chromosomes. However, some increased in number but this could be due to differences in chromosome number between tillers (see for e.g. Humphreys 1978). It seems therefore that a high temperature during embryo development is often able to 'trigger off' the process of chromosome elimination but that this process is sometimes arrested at an intermediate point. By way of contrast, in crosses where predominantly haploid or hybrid progeny are obtained (e.g. *T. aestivum* × *H. bulbosum, H. vulgare* × *S. cereale, H. lechleri* × *H. bulbosum*) the effect of temperature is not strong enough to overcome or modify the mechanism of chromosome elimination/retention.

Hierarchy of chromosome elimination in Hordeum

Subrahmanyam (1982) ranked *Hordeum* species according to their relative strength in eliminating chromosomes of other species as follows: *H. lechleri* > *H. procerum* > *H. depressum* > *H. vulgare* > *H. bulbosum.*

In the crosses of *H. procerum* and *H. lechleri* with *H. bulbosum* and *H. vulgare,* haploid formation was greater when *H. bulbosum* rather than *H. vulgare* was male parent indicating that the *H. bulbosum* genome is more readily eliminated than *H. vulgare* (Table 5). This confirms the preferential elimination of *H. bulbosum* chromosomes in crosses of this species with *H. vulgare* (Subrahmanyam and Kasha 1973). Using *H. bulbosum* as pollinator on three *Hordeum* species, greatest to least proportions of haploids were produced from *H. lechleri, H. procerum* and *H. depressum* respectively at both temperatures tested. However, comparing *H. procerum* × *H. vulgare* with *H. lechleri* × *H. vulgare* their relative positions were reversed in that greater haploid production occurred with *H. procerum* than *H. lechleri.* Crosses between *H. lechleri* and *H. procerum* would help to resolve their relative orders in the hierarchy, but from the data presented herein the order of placement would be: *H. lechleri* and *H. procerum* > *H. depressum* > *H. vulgare* > *H. bulbosum.*

Origin of hyperploid and polyploid plants

Hyperploids may have arisen by the increase of chromosomes from an unstable hybrid, or by a reduction of chromosomes from polyploid plants with chromosome numbers higher than those expected from hybrids.

The 28 and 35 chromosome polyploid hybrid-like plants derived directly from embryos probably arose

following the fertilization of the egg by an unreduced male gamete. Although only low proportions of these plants were obtained, it seems that the formation of unreduced male gametes may not be an uncommon event, but that in intraspecific pollinations they are at a selective disadvantage compared with haploid pollen.

The 42 chromosome plant derived directly from an embryo may have occurred after an unreduced *H. lechleri* egg was fertilized by a haploid *H. bulbosum* followed by elimination of the male genome. Alternatively fertilization of haploid gametes took place followed by elimination of the *H. bulbosum* chromosomes and subsequent spontaneous chromosome doubling within the embryo. The polyploid plants regenerated from calli could have been formed in a similar way. More probably they arose after chromosome elimination of the *H. bulbosum* genome in the embryo followed by spontaneous chromosome doubling in the callus (see for example Cattoir-Reynaerts and Jacobs 1978; Jensen 1981).

In conclusion, because of the genotypic and environmental effects on the proportions of haploid and hybrid progeny it is very difficult to make direct comparisons with other reports. Nevertheless, it has been amply demonstrated that temperature affects chromosome elimination in certain interspecific *Hordeum* crosses. This confirms a previous report concerning *H. vulgare* × *H. bulbosum* (Pickering 1984) and will aid haploid or hybrid development by manipulating both genotype and environment.

Acknowledgement. In addition to the suppliers of seed and plant material we should like to thank Mr. H. M. Thomas for useful discussions.

References

Barclay IR (1975) High frequencies of haploid production in wheat (*Triticum aestivum*) by chromosome elimination. Nature 256:410–411

Bennett MD, Finch RA, Barclay IR (1976) The time rate and mechanism of chromosome elimination in *Hordeum* hybrids. Chromosoma 54:175–200

von Bothmer R, Flink J, Jacobsen N, Kotimäki M, Landström T (1983) Interspecific hybridization with cultivated barley (*Hodeum vulgare* L.). Hereditas 99:219–244

Cattoir-Reynaerts A, Jacobs M (1978) 'In vitro' culture of barley. Barley Genet Newslett 8:23–35

Cooper KV, Dale JE, Dyer AF, Lyne RL, Walker JT (1978) Hybrid plants from the barley × rye cross. Plant Sci Lett 12:293–298

Fedak G (1977) Haploids from barley × rye crosses. Can J Genet Cytol 19:15–19

Fedak G (1978) Barley monoploids and hybrids from barley × rye crosses. In: Proc 8th Eucarpia Congr, Interspecific hybridization in plant breeding. Madrid, pp 269–273

Fedak G (1979) Cytogenetics of a barley × rye hybrid. Can J Genet Cytol 21:543–548

Finch RA, Bennett MD (1980) Mitotic and meiotic chromosome behaviour in new hybrids of *Hordeum* with *Triticum* and *Secale*. Heredity 44:201–209

Gamborg OL, Miller RA, Ojima K (1968) Nutrient requirements of suspension cultures of soybean root cells. Exp Cell Res 50:151–158

Humphreys MW (1978) Chromosome instability in *Hordeum vulgare* × *H. bulbosum* hybrids. Chromosoma 65:301–307

Jensen CJ (1981) Regenerating monoploid barley plants from cells, tissues and organs of various origins. In: Asher MJC, Ellis RP, Hayter AM, Whitehouse RNH (eds) Barley genetics, vol IV. Edinburgh University Press, Edinburgh, pp 677–685

Kasha KJ, Kao KN (1970) High frequency haploid production in barley (*Hordeum vulgare* L.). Nature 225:874–876

Kruse A (1967) Intergeneric hybrids between *Hordeum vulgare* L. ssp. *distichum* (v. 'Pallas', 2n = 14) and *Secale cereale* L. (v. 'Petkus', 2n = 14). Royal Vet Agric Univ Copenhagen Yearbook, pp 82–92

Norstog K (1961) The growth and differentiation of cultured barley embryos. Am J Bot 48:876–884

Pickering RA (1980) Use of the doubled haploid technique in barley breeding at the Welsh Plant Breeding Station. In: Rep Welsh Plant Breed Stn 1979, pp 208–226

Pickering RA (1982) The effect of pollination bag type on seed quality and size in *Hordeum* inter- and intraspecific hybridization. Euphytica 31:439–449

Pickering RA (1983) The influence of genotype on doubled haploid barley production. Euphytica 32:863–876

Pickering RA (1984) The influence of genotype and environment on chromosome elimination in crosses between *Hordeum vulgare* L. × *Hordeum bulbosum* L. Plant Sci Lett 34:153–164

Rajhathy T, Symko S (1974) High frequency of haploids from crosses of *Hordeum lechleri* (6x) × *H. vulgare* (2x) and *H. jubatum* (4x) × *H. bulbosum* (2x). Can J Genet Cytol 16: 468–472

Simpson E, Snape JW, Finch RA (1980) Variation between *Hordeum bulbosum* genotypes in their ability to produce haploids of barley, *Hordeum vulgare*. Z Pflanzenzücht 85: 205–211

Subrahmanyam NC (1977) Haploidy from *Hordeum* interspecific crosses. 1. Polyhaploids of *H. parodii* and *H. procerum*. Theor Appl Genet 49:209–217

Subrahmanyam NC (1979) Haploidy from *Hordeum* interspecific crosses. 2. Dihaploids of *H. brachyantherum* and *H. depressum*. Theor Appl Genet 55:139–144

Subrahmanyam NC (1980) Haploidy from *Hordeum* interspecific crosses. 3. Trihaploids of *H. arizonicum* and *H. lechleri*. Theor Appl Genet 56:257–263

Subrahmanyam NC (1982) Species dominance in chromosome elimination in barley hybrids. Curr Sci 51:28–31

Subrahmanyam NC (1983) Significance of embryo differentiation in barley interspecific hybridization and haploid formation. In: Sen SK, Giles KL (eds) Proc Int Symp Plant Cell Culture Crop Improv. Calcutta, pp 105–111

Subrahmanyam KC, Kasha KJ (1973) Selective chromosomal elimination during haploid formation in barley following interspecific hybridization. Chromosoma 42:111–125

Thomas HM, Pickering RA (1979) Barley × rye crosses the morphology and cytology of the hybrids and the amphidiploids. Z Pflanzenzücht 82:193–200

Zenkteler M, Straub J (1979) Cytoembryological studies on the process of fertilization and the development of haploid embryos of *Triticum aesticum* L. (2n = 42) after crossing with *Hordeum bulbosum* (2n = 14). Z Pflanzenzücht 82: 36–44