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Barley X Rye Crosses
The Morphology and Cytology of the Hybrids and the Amphidiploids

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With 2 figures and 4 tables

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Production of interspecific and intergeneric hybrids, and the study of their chromosome behaviour can provide valuable information on the phylogenetic relationships of the species involved. For the plant breeder such hybrids can be used (1) for the transfer of desirable characters from one species to another; (2) for the formation of a new crop species, e.g. triticale; or (3) as a breeding technique, e.g. in barley, where chromosome elimination following hybridization between *Hordeum vulgare* and *H. bulbosum* has been used to produce haploids (Kasha and Kao 1970).

A hybrid between *Hordeum jubatum* L. ($2n = 28$) and *Secale cereale* L. ($2n = 14$) was reported by Brink et al. (1944). A cytological study on a hybrid of the same cross was made by Wagenaar (1959). Successful crosses between *H. vulgare* L. ($2n = 14$) and *S. cereale* L. have been reported on several occasions. Kruse (1967, 1976) produced true hybrids possessing chromosomes from each parent but, even after chromosome doubling, these were pollen sterile. Crosses between the same species made by Fedak (1977) yielded only haploid barley plantlets.

Our observations confirm the hybridity of plants from this intergeneric cross by studies of plant morphology and of the karyotype using Giemsa staining. Meiosis was also studied in both, the hybrids and amphidiploids.

Materials and Methods

Crosses were carried out in the glasshouse during April and May 1976, at temperatures of 15—25 °C and 16 h photoperiod. Florets of a range of *H. vulgare* cultivars and F_1 hybrids were emasculated using the technique of Pope (1944). One to five days later freshly collected pollen from *S. cereale* cv. 'Rheidol' (winter rye) was applied with a brush to receptive stigmas. On the day following pollination gibberellic acid (75 mg/l) with wetting agent was sprayed onto each floret. After 14—27 days, when seeds showed signs of deterioration, embryos were excised from the watery endosperm and cultured under sterile conditions. The B5 medium described by Gamborg et al. (1968) was used but modified by omitting the 2,4-D and by the addition of 7 g agar/litre.

One embryo, which callused, was placed on MURASHIGE and SKOOG's medium (1962) and sub-cultured twice. After 17 weeks a plant began to regenerate and it was transferred to MURASHIGE and SKOOG's medium with 0.2 mg/l kinetin. This plantlet and eleven others produced directly from embryos were transferred to pots of John Innes Nr. 3 compost. Six of the plantlets died, however, before it was found that survival was enhanced by covering each plantlet with a clear plastic beaker.

At the 5 leaf stage, plants were treated with colchicine using a modification of the technique of MORGAN (1976). Plants were removed from the soil, washed, and the roots cut back to 1—2 cm. A single incision was made with a scalpel at the stem base and the plants immersed to a depth of 5 cm in 0.1 % colchicine and 2 % DMSO for 5 h at room temperature before washing in tap water for 2—3 min and repotting.

For cytological studies actively growing root tips were removed and pre-treated in distilled water for 24 h at 1 °C before fixing in ethanol : acetic acid (3 : 1) for 2 h. For chromosome counting the root tips were hydrolysed in 1 N HCl at 60 °C for 10 min, stained in Feulgen and squashed in 1 % aceto-carmine. For Giemsa staining, root tips were treated as described by THOMAS (1977).

Inflorescences were taken just before emergence, fixed in Carnoy (6 : 3 : 1) solution for 24 h, then transferred to 70 % ethanol for storage. Anthers were stained in alcoholic hydrochloric acid-carmine (SNOW 1963) and squashed in 45 % acetic acid. For Giemsa staining, anthers were squashed in 1 % aceto-carmine to determine the stage, then preparations frozen and the coverslip removed. The technique used for root tips was then followed, the carmine being removed during the treatment.

Results and Discussion

At the time of excision most of the embryos were found to be globular with little differentiation and were very small (< 1 mm in length) although occasionally larger ones were obtained which resembled haploid barley embryos produced from *H. vulgare* × *H. bulbosum* crosses (KASHA and KAO 1970). Only embryos showing signs of differentiation were cultured and from these eleven plantlets developed; a 12th embryo produced a callus which regenerated to form a 12th plantlet (*Table 1*).

The morphology of all plants resembled that of rye, having hairy peduncles and stiff hairs on the lemma keel. Most spikelets consisted of 2 (3) florets though occasionally some spikelets had as many as 3 additional smaller florets. Auricles were intermediate in size between the two parent species. The vigour of the plants was variable, some tillering profusely but remaining in a vegetative state, probably an indication of the influence of the winter rye parent. Those that came to head were shorter than either parent (*Fig. 1*).

Tab. 1 Seed set and embryos cultured from crosses between *H. vulgare* and *S. cereale* cv. Rheidol

Cross No.	*H.vulgare* parent (♀)	Florets pollinated	Number of seeds set	Seed set %	Embryos cultured	Plantlets produced
1	(Mirena × Athos) F_1	211	199	94.3	42	4
2	(Mirena × Goldspear) F_1	194	186	95.9	24	1
3	(Athos × Goldspear) F_1	332	277	83.4	32	4
4	(Athos × CB 7432) F_1	121	90	74.4	17	1
5	Vada	60	46	76.7	3	0
6	Emir	26	11	42.3	5	2

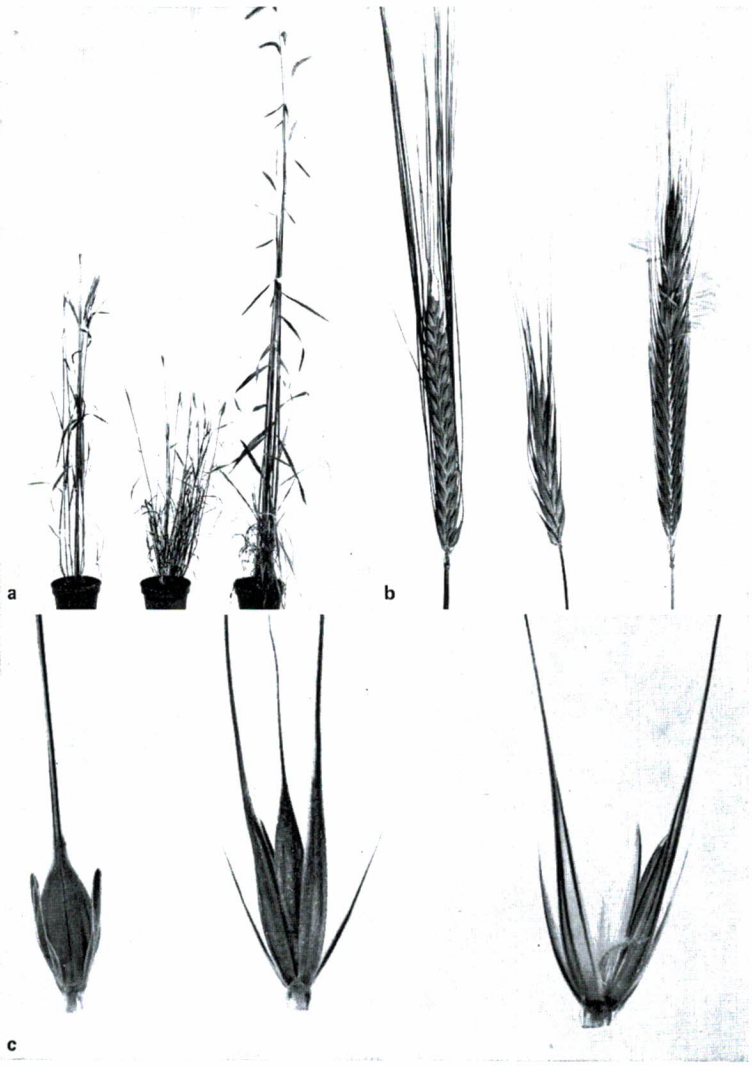

Fig. 1. Plants (a), ears (b), and spikelets (c) of a barley × rye hybrid (centre), and the respective parents barley (left) and rye (right)

Chromosome counts of the root tips indicated that five of the surviving plantlets had 14 chromosomes while the one which arose via a callus had 28 chromosomes. All plants, judging by chromosome morphology and size, contained chromosomes from both parents. Colchicine treatment produced 28 chromosome sectors in two of the 14 chromosome hybrids. Giemsa staining confirmed the presence of 7 barley and 7 rye chromosomes in the hybrids and 14 chromosomes of each species in the amphidiploids (doubled hybrids).

Root tip counts made periodically over a number of months showed some instability of chromosome numbers, possibly due to the presence of some anaphase cells with multipolar spindles (*Fig. 2 a*). Counts made after 12 months

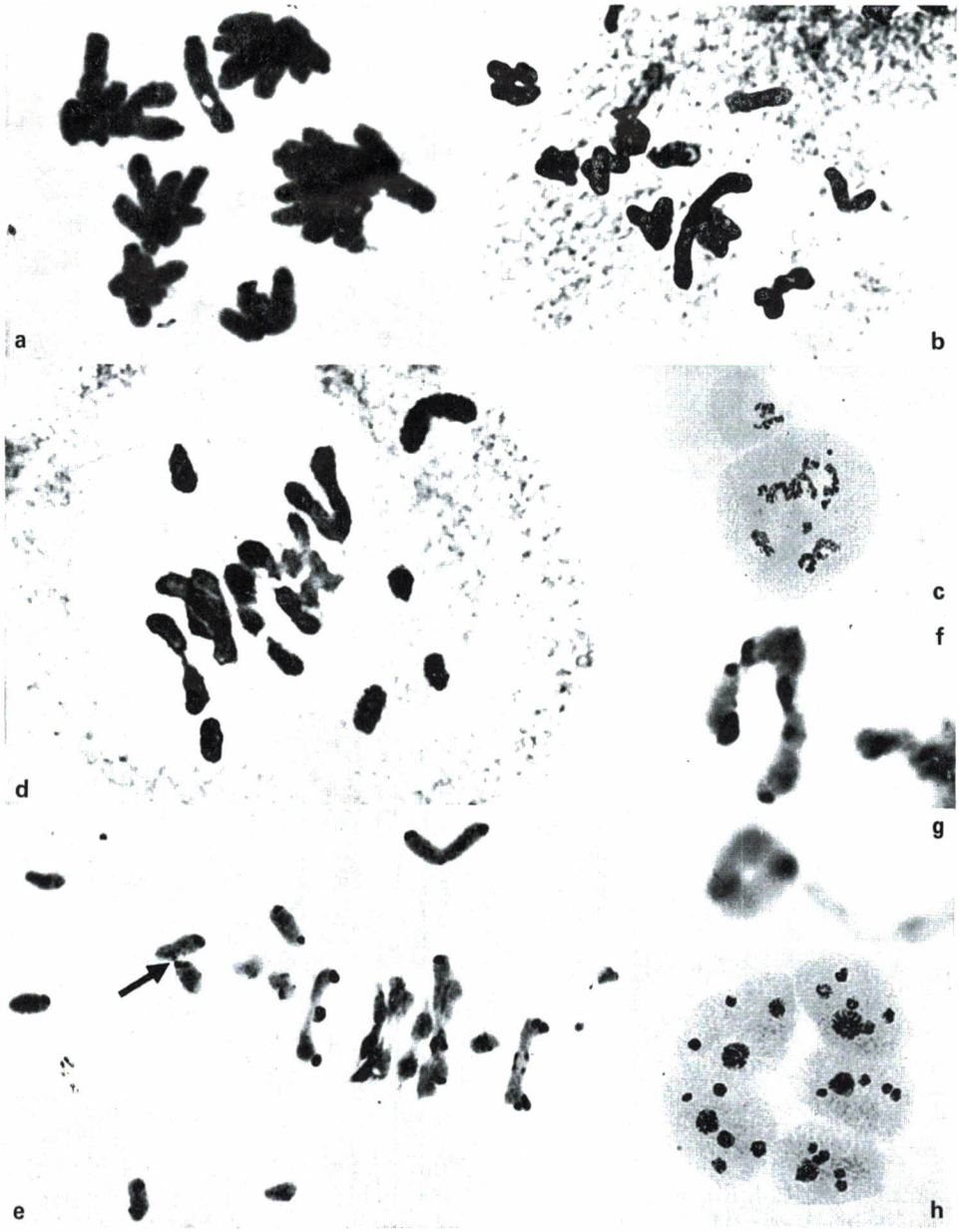

Fig. 2. a: Root tip anaphase of the hybrid with multipolar spindle. b: MI of the hybrid with rye-rye bivalent. c: 'Budded-off' meiotic cell in hybrid. d: MI of the amphidiploid with trivalent. e: MI of the amphidiploid stained with Giemsa (heterochromatic fusion in secondary association — arrowed). f: Trivalent involving 3 non-homologous rye chromosomes, stained with Giemsa. g: Secondary associations forming ring, stained with Giemsa. h: Five daughter cells with micronuclei in the amphidiploid. Magnifications a 1700×; b, d, e 1400×; c, h 550×; f, g 2600×

showed that most cells in the hybrids still had 14 chromosomes, and although more rye chromosomes appeared to have been lost than barley chromosomes, the ratio of barley to rye chromosomes per plant did not differ significantly from 1:1. There was no significant difference in chromosome stability between genotypes, but the hybrids appeared to be more stable than the amphidiploids. The number of cells counted in the latter was however small (*Table 2*).

Tab. 2 Chromosome counts of root tips taken after 12 months

Hybrids (5 plants)										
Chromosomes/cell (mean 12.9)	15	14	13	12	11	10	9	8		
No. of cells (total 100)	1	58	14	12	4	5	4	2		
Amphidiploids (2 plants)										
Chromosomes/cell (mean 24.8)	29	28	27	25	23	21	19	16	15	13
No. of cells (total 32)	1	11	9	2	2	2	1	1	1	2

Meiosis of the hybrids

A number of inflorescences of the 14 chromosome hybrids were fixed but most anthers were empty and no meiotic stages were found. Only one floret was observed with PMCs which ranged from first metaphase to tetrads but insufficient cells were found to score the pairing, or to enable an accurate assessment of the range of chromosome numbers. One bivalent was found and based on size, was judged to involve two rye chromosomes (*Fig. 2 b*). The chromosome numbers were variable but less than 14. At all stages, some cells were found with a part of the cell "budded off" (cf. WAGENAAR 1959). These "buds" always contained what appeared to be nuclear material (*Fig. 2 c*). Irregular second division resulted in triads as well as tetrads; but no stages later than these were found.

Meiosis of the amphidiploids

Two of the 28 chromosome plants produced ears, several of these were fixed from the plant derived from a callus (cross 1), and one from a colchicine treated plant (cross 6). The chromosome number was not consistent in the PMCs of either plant (*Table 3*) and pairing was reduced from the possible 14 bivalents (*Table 4*). The difference between anthers in the number of bivalents per cell was highly significant, indicating genetic instability in the material. Most of the chromosomes not involved in bivalent formation remained unpaired except for one ring quadrivalent and a few trivalents (*Fig. 2 d*).

Tab. 3 Chromosome number at MI of meiosis in the amphidiploids

	Mean	Range	No. cells scored
Plant 1	27.15	24—30	77
Plant 6	27.90	23—34	39

Giemsa banding of the meiotic chromosomes showed no pairing between chromosomes of barley and rye (*Fig. 2 e*) but pairing occurred between non-homologous chromosomes in the rye complement. One trivalent, stained by the Giemsa technique, was seen to be composed of three non-homologous rye chromosomes (*Fig. 2 f*). As this occurred in a 28 chromosome cell, these chromosomes had paired with each other in the presumed presence of their respective homologues, though it is not clear whether these three chromosomes paired by chiasmata or simply formed end-to-end associations. It appears that the presence of one genome not only suppressed pairing in the other genome but also interfered with the selectivity of the pairing mechanism.

Reduced chromosome pairing might be attributable to several factors. Firstly, it might be due to differences in the duration of meiosis in the two species, that of rye is 51 hours (BENNETT and KALTSIKES 1973) while that of barley is only 39 h (FINCH and BENNETT 1972). Recently, however, ROUPAKIAS and KALTSIKES (1977a and b) have reported that in triticale the duration of meiosis has no bearing on the degree of pairing. Secondly, REES (1955) found that homozygosity resulting from inbreeding in rye depressed pairing; a similar effect might be expected in the present material where homozygosity was created by doubling the haploid complement. This would not, however, explain the reduced pairing in the barley complement.

The presence of non-homologous pairing suggests that the reduction in pairing may have been the result of failure of chromosome movement and homologue recognition. HOTTA and STERN (1971) reported a protein with such a function in the meiotic cells of *Lilium* which may be lacking in the present material. Since differences in pairing were significantly greater between plants than they were between anthers, there is a possibility that other genotypes of barley and rye might be more compatible, and amphidiploids between such parents might produce fertile plants.

Tab. 4 Chromosome pairing per cell in the amphidiploids

	Configurations, mean (range)				Secondary associations*)							Toral number cells
	I	II	III	IV	s-s	s-s-s	e-s	e-e	e-e-e	e-e-e-e	ring	
Plant 1	11.68	7.75 (1—13)	0.05	—	0.17	0.01	0.23	0.87	0.17	—	0.13	77
Plant 6	18.65	4.38 (0—11)	0.03	0.03	0.10	—	0.59	0.85	0.13	0.05	0.03	39

*) s-s = side-to-side; e-s = end-to-side; e-e = end-to-end etc.

Secondary associations between univalents were common (*Table 4*). RILEY and CHAPMAN (1957) attributed these to fusion of heterochromatin, but not all heterochromatin is necessarily stained by the Giemsa technique, particularly in meiotic chromosomes. Some secondary associations clearly did involve fusion of heterochromatic areas, and darkly stained threads were seen to connect them (*Fig. 2 e*). Side-to-side, end-to-end, and end-to-side associations were observed, together with multiple associations of 3—4 chromosomes from both

species. The points of attachment of several ring associations resembled those of end-to-end associations. When stained with Giemsa it was clear that two rye chromosomes were held together by fusion of the heterochromatic bands at the telomeres (*Fig. 2 g*).

Restitution nuclei were formed both after MI and more commonly after MII resulting in monads and diads respectively. Any tetrads produced had large numbers of micronuclei. Occasionally second division led to groups of five or six daughter cells (*Fig. 2 h*). Later stages of development were not found, older anthers consisting only of empty pollen grains indicating a breakdown in cell development after meiosis. All plants were completely pollen sterile.

In conclusion, it is seen from these results that the difficulties of producing hybrids and amphidiploids between barley and rye have largely been overcome. However, any further development will be dependent on finding more compatible parents from both species and so enabling the production of fertile amphidiploids. The chromosome behaviour of the material studied in this investigation was highly irregular and led to a breakdown in pollen development after the tetrad stage.

Summary

Hybrids between *Hordeum vulgare* L. (2n = 14) and *Secale cereale* L. (2n = 14) were obtained by embryo culture. Plants with 28 chromosomes were obtained by spontaneous doubling and by colchicine treatment as well. The morphology of both the hybrids and the amphidiploids resembled rye, while Giemsa staining showed 7 barley and 7 rye chromosomes in the hybrids and 14 chromosomes from each species in the amphidiploids. Some instability of chromosome number was found over several months.

Meiosis in the amphidiploids showed a disruption of homologous pairing. However, Giemsa staining of meiotic cells showed that there was no pairing between barley and rye chromosomes and also demonstrated the nature of some of the secondary associations of univalents. Some possible causes of pairing failure are discussed.

The products of meiosis were highly irregular and both 14 and 28 chromosome plants were sterile.

Zusammenfassung

Gerste × Roggen-Kreuzungen
Morphologie und Zytologie der Hybriden und der Amphidiploiden

Durch Embryokultur wurden Hybriden zwischen *Hordeum vulgare* L. (2n = 14) und *Secale cereale* (2n = 14) hergestellt. Durch spontane Verdopplung und durch Colchicinbehandlung erhielt man Pflanzen mit 28 Chromosomen. Morphologisch ähnelten sowohl die Hybriden als auch die Amphidiploiden dem Roggen, dagegen zeigte die Giemsa-Färbung in den Hybriden sieben Gerste- und sieben Roggenchromosomen, in den Amphidiploiden

14 Chromosomen jeder Art. Im Laufe mehrerer Monate zeigte sich eine gewisse Instabilität der Chromosomen.

In der Meiose der Amphidiploiden kam es zu einem Auseinanderbrechen der homologen Paarung. Mit Hilfe der Giemsa-Färbung meiotischer Zellen konnte man zeigen, daß keine Paarung zwischen Gersten- und Roggenchromosomen auftrat, und daß sekundäre Assoziationen von Univalenten vorlagen. Einige mögliche Ursachen des Scheiterns von Paarungen werden diskutiert

Die Produkte der Meiose waren sehr irregulär und sowohl die 14- als auch die 28chromosomigen Pflanzen waren steril.

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References

BENNETT, M. D., and P. J. KALTSIKES, 1973: The duration of meiosis in a diploid rye, a tetraploid wheat, and the hexaploid triticale derived from them. Canad. J. Genet. Cytol. **15**, 671—679.

BRINK, R. A., D. C. COOPER, and L. E. AUSHERMAN, 1944: A hybrid between *Hordeum jubatum* and *Secale cereale*. J. Hered. **35**, 66—75.

FEDAK, G., 1977: Haploids from barley × rye crosses. Canad. J. Genet. Cytol. **19**, 15—19.

FINCH, R. A., and M. D. BENNETT, 1972: The duration of meiosis in diploid and autotetraploid barley. Canad. J. Genet. Cytol. **14**, 507—515.

GAMBORG, O. L., R. A. MILLER, and K. OJIMA, 1968: Nutrient requirements of suspension cultures of soyabean root cells. Exp. Cell Res. **50**, 151—158.

HOTTA, Y., and H. STERN, 1971: A DNA-binding protein in meiotic cells of *Lilium*. Dev. Biol. **26**, 87—89.

KASHA, K. J., and K. N. KAO, 1970: High frequency haploid production in barley (*Hordeum vulgare* L.). Nature (London) **225**, 874—876.

KRUSE, A., 1967: Intergeneric hybrids between *Hordeum vulgare* L. ssp. *distichum* (cv. Pallas, 2n = 14) and *Secale cereale* L. (cv. Petkus, 2n = 14). Roy. Vet. Agric. Univ. Copenhagen Yearbook, 82—92.

— —, 1976: Reciprocal hybrids between the genus *Hordeum, Secale* and *Triticum*. Hereditas **84**, 2, 224.

MORGAN, W. G., 1976: A technique for the production of polyploids in grasses. Euphytica **25**, 443—446.

MURASHIGE, T., and F. SKOOG, 1962: A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol. Plantarum **15**, 473—497.

POPE, M. N., 1944: Some notes on technique in barley breeding. J. Hered. **35**, 99—111.

REES, H., 1955: Genotypic control of chromosome behaviour in rye. I. Inbred lines. Heredity **9**, 93—116.

ROUPAKIAS, D. G., and P. J. KALTSIKES, 1977a: The effect of wheat cytoplasm on meiosis in hexaploid triticale. Canad. J. Genet. Cytol. **19**, 39—49.

— —, and — —, 1977b: Independence of duration of meiosis and chromosome pairing in hexaploid triticale. Canad. J. Genet. Cytol. **19**, 345—354.

RILEY, R., and V. CHAPMAN, 1957: Haploids and polyhaploids in *Aegilops* and *Triticum*. Heredity **11**, 195—207.

SNOW, R., 1963: Alcoholic hydrochloric acid-carmine as a stain for chromosome squash preparations. Stain Techn. **38**, 9—13.

THOMAS, H. M., 1977: Giemsa banding in *Lolium temulentum*. Canad. J. Genet. Cytol. **19**, 663—666.

WAGENAAR, E. B., 1959: Intergeneric hybrids between *Hordeum jubatum* L. and *Secale cereale* L. J. Hered. **50**, 192—202.

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