The Time Rate and Mechanism of Chromosome Elimination in *Hordeum* Hybrids

M. D. Bennett, R. A. Finch and I. R. Barclay

Plant Breeding Institute, Maris Lane, Trumpington, Cambridge CB2 2LQ, England

**Abstract.** Seed development at 20 ± 1°C in continuous light was studied during the first 5 days after pollination in diploid *Hordeum vulgare*, diploid *H. bulbosum* and the cross, *H. vulgare* × *H. bulbosum*, where *H. bulbosum* chromosomes were eliminated. Developing seeds were fixed and stained at known intervals after pollination and the embryo sac contents dissected out for cytological examination. — In all cases, the pattern of development was similar to that previously described for the Triticeae. After intraspecific pollination, the rate of endosperm and embryo development was significantly faster in *H. vulgare* than in *H. bulbosum*. In hybrid tissues, the rate was intermediate, but often much nearer to that of *H. vulgare* at first. Elimination of *H. bulbosum* chromosomes occurred only during endosperm and embryo mitoses. Usually, 0–3 chromosomes were lost at any one division but up to 7 were lost at some. Elimination, which occurred as early as zygotic anaphase, was nearly or quite complete in all dividing cells in both embryo and endosperm after 5 days. The mean number of chromosomes lost per nucleus per nuclear cycle was low at first but rose rapidly and stayed high for about a day in each tissue before falling quickly. The rate of elimination in each tissue was maximal when that tissue first synthesized significant amounts of new cytoplasm (day 2 after pollination in the endosperm and day 3 in the embryo). At mitosis, chromosomes being eliminated differed from others only in failing to congress at metaphase or to reach a pole at anaphase or both. — It is noted that in several widely different examples where either haploids are produced when only hybrids are expected, or where chromosomes of one species are preferentially eliminated from hybrid cells, nucleolar activity was suppressed in chromosomes of the genome which was selectively or preferentially eliminated. Consequently, it is suggested that chromosome elimination in *Hordeum* hybrids may be caused by a disturbed control of protein metabolism in hybrid seeds and perhaps *H. bulbosum* chromosomes are selectively eliminated because they are less efficient than *H. vulgare* chromosomes at forming normal attachments to spindle protein.

**Introduction**

Davies (1958) crossed tetraploid *Hordeum bulbosum* (female) with tetraploid *H. vulgare* (male) and obtained diploid plants with the morphology, meiosis and breeding behaviour of diploid *H. vulgare*. Later studies included reciprocal interspecific crosses both within and between the tetraploid and diploid levels (Symko, 1969; Lange, 1971; Kao and Kasha, 1971) and showed that in 2x × 2x and 4x × 4x crosses, the progeny included a large proportion of haploid and dihaploid *H. vulgare* plants, respectively.

At first Davies (1958) invoked male parthenogenesis to explain his results, but, the later work has provided results strongly implying that normal double fertilization invariably precedes the production of plants with reduced chromosome numbers, and that the reduction in chromosome number is the result of preferential elimination of *H. bulbosum* chromosomes (Kasha and Kao, 1970; Subrahmanyam and Kasha, 1973). Subrahmanyam and Kasha (1973) showed...
that substantial amounts of chromosome elimination occurred during days three to eleven after pollination, however, no quantitative studies of chromosome elimination at earlier stages have been made. It seemed worthwhile therefore to undertake another investigation of early seed development in *H. vulgare* × *H. bulbosum* crosses including the earlier stages not previously described in detail and to attempt to define quantitatively the time and rate of chromosome elimination.

**Materials and Methods**

1. **Materials.** In intraspecific and interspecific pollinations of *Hordeum vulgare* L. (*2n = 2x = 14*), the two-rowed cultivars, Sultan and Vada were used. Clone HB2004 of *H. bulbosum* (*2n = 2x = 14*), kindly supplied by Dr. W. Lange (Wageningen), was used to determine the rate of seed development in *H. bulbosum*. As this species is self-incompatible, this clone was pollinated with mixtures of pollen from the diploid clones HB2005, HB2032, HB2062, HB2078 and HB2082, also from Dr. Lange.

   Hybrid embryos and endosperms were produced on *H. vulgare* spikes after pollination with any one of three *H. bulbosum* clones (S14, S18, S19) raised from seed of line CPI 18968 kindly supplied by Dr. D. H. B. Sparrow (Adelaide). For the sake of simplicity, such embryos and endosperms on Sultan and Vada spikes are always termed Sultan and Vada hybrids, respectively, even when all seven *bulbosum* chromosomes may have been eliminated from many nuclei leaving them pure *H. vulgare* (see p. 185).

2. **Plant Culture and Seed Production.** Plants were grown in a glasshouse until development in leading tillers was about 1 week prior to anther dehiscence. They were then transferred to a growth room at 20 ~: 1 ~ C and given continuous light.

   For controlled pollinations, florets in spikes of seed parents were emasculated 2–3 days before their anthers were due to dehisce. The entire top of each spike and several retarded florets at its base were cut off and discarded, leaving about 20 well-developed florets for use. Emasculated *H. vulgare* spikes were enclosed in plastic pollination bags to prevent desiccation and stray pollination. Those of *H. bulbosum* were enclosed in “cellophane” pollination bags. About 3–5 days after each emasculation, the bag was removed while the stigmas were hand pollinated with fresh pollen and then the bag was immediately replaced and the time noted. Each pollinated spike was fixed in 1:3 acetic alcohol at a known interval up to 5 days after pollination. Hence developmental times given in this paper are times after pollination.

3. **Cytological Preparations.** Fixed ovaries from known florets were hydrolysed for 10 minutes at 60 ~C in 1N HCl and stained for two hours in leuco-basic fuchsin. The ovule was excised from each ovary under a dissecting microscope (magnification 10–200 ×) and the embryo sac contents were gently extruded or dissected out into a drop of aceto-carmine on a glass slide. If the embryo was unsquashed and unbroken, its maximum diameter (D) and the maximum diameter at right angles to this (d) were measured with a Vickers moving scale micrometer eyepiece. The preparation was then squashed under a coverslip for further cytological examination.

4. **Counting Cells and Nuclei.** The number of cells and nuclei in each complete embryo and endosperm was counted in squashed preparations. As the endosperm was coenocytic until about 72 hours, only nuclear number was counted during this period, but thereafter cell number was counted. In embryos and endosperms, micronuclei (see p. 189) were omitted from counts of nuclei. In the hybrids, many cells had been digested in endosperms fixed at 96 and 120 hours while others continued division (see p. 183). Thus counts of endosperm cell number at these times are not an accurate measure of the total number of endosperm nuclei formed, but are the resultant of the addition of some cells by mitosis and the loss of others by digestion.

5. **Counting Chromosome Numbers.** Chromosome number at metaphase was counted in all suitable embryo and endosperm nuclei on each slide and in some micronuclei. Mitotic chromosomes which had failed to congress with the others in the same nucleus were included in the chromosome count for that nucleus. Since embryo and endosperm nuclei had 14 or 21 chromosomes before elimination, respectively, mean chromosome loss per nucleus was estimated by