STERILITY OF HYBRIDS BETWEEN
BARLEY AND RYE

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Abstract

Features of barley x rye hybrids which may influence their viability or fertility have been investigated. Fertility depends on the development of reproductive structures and balanced meiosis. Disrupted floral development in one group of hybrids prevented male and female meiosis from occurring. In the other hybrids meiosis was similar to that which would be expected for a wide hybrid. Univalents were formed and regular disjunction of chromosomes did not occur. Pollen grains and embryo sacs aborted before maturity. To attempt to restore balanced meiosis through amphidiploidy, colchicine was used to treat hybrids. These treatments were unsuccessful. The reasons for this were investigated.

Cytological features associated with hybridity which may be important during vegetative development were also studied. Karyotype change occurred, both in vivo and during embryo and callus culture. There was an interesting trend of the gain of rye NOR-bearing chromosome segments. The chromosome instability which gave rise to these variants continued in various meristems of the hybrids. Both chromosome loss and gain occurred. The degree of instability differed between tissues and genotypes. The suppression of the rye NORs was investigated and confirmed using several techniques. The effect of this NOR suppression on the number and size of the nucleoli in the hybrids was investigated.

Several theories exist to explain the causes of chromosome
instability in hybrids. In the final chapter the results from this thesis and other evidence collated from the literature were used to appraise some of these theories. Evidence was presented for a strong link between chromosome instability and NOR suppression. Experimental approaches to elucidate the nature of this link were proposed.
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Declaration

This thesis has been composed by myself and the work reported herein is my own.

Gavin Ramsay
May 1984.
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1. INTRODUCTION

1.1 Background

A diversity of interspecific hybrids has been deliberately made by man. Among plant sexual hybrids, intergeneric hybrids are generally much more difficult to produce and maintain than interspecific ones. In higher plants, most, possibly all, intergeneric hybrids are intratribal. Examples are triticale (x Triticosecale Wittmack) (Gustafson 1976), hordecale (Hordeum x Secale) (Cooper 1978) and raphanobrassica (Raphanus x Brassica) (McNaughton and Ross 1978). One exceptionally wide plant hybrid was that described by Ammal et al. (1972) between Saccharum and Zea. This hybrid was produced in 1938 and induced to flower with GA₃ in 1970.

Wide hybrids are not restricted to the plant kingdom. Animal breeders can boast intergeneric hybrids of which sheep x goat and cattle x bison are representatives (Hare and Singh 1979). Animal intergeneric hybrids other than between domesticated animals are rare.

Somatic cell fusion techniques have enabled some much wider hybrid cell cultures to be produced but these generally do not regenerate into organs. Plant x animal heterokaryons can be relatively easily produced but nuclear fusion and division have not been observed. Plant interfamilial somatic hybrid cells, such as

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1. 'Wide hybrid' is a convenient term for a hybrid between different genera or contrasting species within a genus.
that between Nicotiana (Solanaceae) and Glycine (Leguminosae), reported by Kao (1977), have been observed to undergo mitosis on a common spindle and retain partial hybridity for six months. However, such wide somatic hybrids do not undergo organogenesis. In general, somatic cell fusion techniques only rarely give hybrid plants unattainable by sexual means (Harms 1983).

Sexual crosses may fail due to crossing barriers in the style and so are potentially less able to give wide hybrids than somatic cell fusion. Techniques for producing sexual wide hybrids have, however, advanced considerably in the last decade or two and, as a result, wider interspecific crosses have recently been achieved. These techniques have been developed to overcome the two major classes of barrier to the production of interspecific hybrids: pre- and post-fertilisation barriers. Pre-fertilisation barriers are frequently an inability of pollen tubes to grow successfully in an alien style. Post-fertilisation barriers frequently involve embryo abortion and, in wide hybrids, this is usually due to early endosperm degeneration. Special techniques are available to overcome both of these barriers. Larter and Chaubey (1965) demonstrated that growth substance applications can help the growth of pollen tubes in an alien style. Embryo abortion due to abnormal endosperm can also be delayed using growth substances (Kruse 1976). Rescuing the embryos and culturing them on a suitable medium was first employed by Laibach (1929) on Linum hybrids. This technique has relatively recently been more widely applied with the refinement of media for
the growth of young embryos (e.g. Davies 1960).

With the breaking of previously existing barriers between species by these techniques, novel combinations of genomes have become possible. These hybrids are, as expected, generally sterile in the dihaploid form with partial fertility often restored in the amphiploids. In addition to meiotic irregularities these wide hybrids often exhibit a range of other problems and phenomena unique to wide hybrids. These phenomena are concerned with cell division and genome function and arise because of interactions between two diverse genomes. It is towards these problems, their causes and their effects on the viability and sterility of barley x rye hybrids, that this thesis will be directed.

1.2 Wide hybrids and agriculture

Man has been using hybrid crops since the early days of agriculture. Many important crops, including wheat and maize, have hybrid origins (Simmonds 1976). These crops have evolved from natural hybrids and were selected by early farmers.

Apart from these ancient hybrids, a large number of agriculturally useful hybrids have been deliberately produced during the last century. The great majority of these hybrids have been backcrossed to one parent to introduce desirable characters to a crop species. Of these hybrids, most have been used to provide sources of resistance to disease (e.g. *Avena*, Thomas and Aung 1977; *Beta*, Savitsky 1975; *Lycopersicon*, Maxon-Smith 1978 and Rick 1979; *Triticum*, Sears 1978). A wide range of other characters of
agronomic potential, including protein quality, cold tolerance, drought resistance, male sterility and different plant architecture have been transferred from related species or have been identified as being candidates for transfer.

A small number of hybrids have proved to be directly useful as new crops. Of these, triticale is the most important. A fertile triticale was first made by Rimpau in 1891 (Muntzing 1979) but only in the last ten years has it become successful as a crop. Problems encountered by triticale breeders included partial sterility, variability due to aneuploidy, and grain shrivelling. Partial sterility and aneuploidy were found to be due to meiotic abnormalities, particularly the occurrence of univalents (Merker 1973). Octoploid triticale (AABBDdRR) was more prone to give univalents than hexaploid triticale (AABBRr), possibly due to the influence of pairing suppressor genes in hexaploid wheat (AABBDD) (Merker 1973). These problems have, however, shown some response to selection and more stable types are now available. Grain shrivelling is the other main problem for triticale breeders. Bennett (1981) has shown that the number of aberrant divisions in the endosperm correlates well with (and implies a causal relationship with) the degree of grain shrivelling. It was suggested that the late replicating heterochromatin in the rye genome was responsible for these abnormalities. More recent work (Bennett and Gustafson 1982; Gustafson and Bennett 1982) has conclusively shown that the removal of certain telomeric blocks of heterochromatin from the rye
chromosomes reduces the percentage of aberrant endosperm nuclei and, in turn, increases kernel weight. These recent improvements in triticale have been responsible for its increased importance in several countries in recent years.

1.3 History of Hordeum × Secale crosses

A fertile hybrid between barley and rye has great potential for plant breeding. *Hordeum* and *Secale* are phylogenetically less close than *Triticum* and *Secale* (Bendich and McCarthy 1970; Appels and Dvorak 1982) so a fertile and directly useful hordecale is less likely than triticale. The potential of a fertile *Hordeum × Secale* hybrid is much more likely to lie in enabling the introgression of characters from one species to another. There are several characteristics found in rye which may be simply inherited and would be extremely valuable if transferred to barley. Rye is much more tolerant of extremes of climate than barley and is reputed to be particularly resistant to cold and drought. There are a number of diseases which afflict barley and significantly depress yields. Some, such as powdery mildew, also affect rye. Rye does seem to be resistant to several viruses and to *Rhynchosporium*, however, and, although the mechanisms of these resistances are not known, the possibility exists of transferring them to barley. The quality of the seed proteins of barley is another concern of the plant breeder. Barley seed proteins, unlike those of rye, are deficient in lysine (Miflin et al. 1981). Introgression of seed protein genes from rye to barley
would be a good way of improving the nutritional quality of barley.

There is less interest in using introgression to improve rye. This is, no doubt, partly due to the fact that rye is a much less important crop than barley. The weaknesses of rye, including allogamy, low yield and poor harvest index, are less likely to be improved by the introgression of characters from barley because they are complex, multi-gene characters.

The transfer of useful characters could be done by crossing and backcrossing or by recently developed molecular genetic engineering techniques. The former method has the advantage that the genetic and physiological mechanisms of the various characters do not have to be well understood for them to be successfully transferred.

The potential value of a barley x rye hybrid has encouraged several workers to attempt to produce them. Early attempts with *H. vulgare* and *S. cereale* failed (Quinke 1940), and this failure was attributed to early endosperm breakdown (Thompson and Johnston 1945; Odenbach 1965). Successful crosses between *H. vulgare* and *S. cereale* were not made until a combination of post-pollination treatment with GA$_3$ and embryo culture enabled hybrid seedlings to be raised by Kruse (1967; 1976), Cooper et al. (1978), Thomas and Pickering (1979), and Fedak (1979 a,b).

Hybrids have also been produced between other species of the two genera. *H. jubatum* and *S. cereale* can make hybrids without recourse to growth substances or embryo culture (Brink and
Ausherman 1944; Wagenaar 1959; Wojciechowska 1978). Six different hybrids between the two genera were reported by Finch and Bennett (1980) and two others were reported by Fedak and Armstrong (1981) and Fedak and Nakamura (1982). Hybrids between Hordeum and Triticum were also reported by Islam, Sheperd and Sparrow (1975) and Finch and Bennett (1980). Similar hybrids have been fertile enough to allow trigeneric (Triticum x Hordeum x Secale) hybrids to be produced (Kimber and Sallee 1979; Fedak and Armstrong 1980).

All H. vulgare x S. cereale hybrids produced so far have been completely sterile, even though Thomas and Pickering (1979) were able to produce the amphidiploid. Backcrosses have been possible with other hybrids. Wojciechowska (1978) has backcrossed the H. jubatum x S. cereale hybrid to the S. cereale parent. Backcrosses to wheat have also been made with barley x wheat crosses and addition lines have been produced (Islam and Sparrow 1981 a). Transmission of the barley chromosomes is difficult (Islam and Sparrow 1981 b).

The history of barley x rye hybrids at the University of Edinburgh, Department of Botany, began in 1975. Dr. K. V. Napier (then Miss K. V. Cooper) investigated the production of hybrids between H. vulgare and S. cereale for three years until 1978. Hybrids were produced, via embryo-derived callus (Cooper 1978; Cooper et al. 1978) and studies of embryo and endosperm development after crossing 2x and 4x parents were made (Napier et al. 1981).

Two projects ran concurrently from 1978. Dr. Brian Forster
studied the production of hybrids (Forster 1982). He investigated chemical applications to aid fertilisation and early embryo development, embryo and callus culture and the effect of parental cell cycle times on hybrid embryo development (Forster and Dale 1983 a, b).

The other project, being reported here, was to investigate the sterility of the hybrids. It had been planned to compare hybrids of different parental genotypes and ploidy. However, it proved impossible at that time to produce additional hybrids and only the hybrids produced by Dr. Napier and one hybrid clone, kindly donated to Dr. Napier by Dr. A. Kruse, Risö, Denmark, were available for study.

1.4 Cytological phenomena associated with wide hybrids

Wide hybrids, whether sexual or somatic, exhibit a range of cytological phenomena not generally found in intraspecific hybrids or hybrids between closely related species. The presence of these phenomena has been noted, in some cases 50 years ago, but explanations for them or studies of their effect on the organism are mostly lacking. These cytological phenomena comprise chromosome size changes (amphiplasty), nucleolar organiser suppression (differential amphiplasty), pistillody, genome elimination and chromosome number instability.

There is no reason to assume a common cause of these phenomena. It is only to be expected that diverse genomes, adapted
to different conditions and carrying out different functions, will not work efficiently in combination. Moreover, even in some hybrids where the two genomes cooperate sufficiently well to allow near normal development of the plants, certain characteristic cytological abnormalities are regularly found. It is interesting to speculate that the extent of one or more of these abnormalities may be responsible for determining which hybrids are attainable and which are not. For this reason, study of such phenomena as chromosome instability or nucleolar organiser suppression may be worthwhile, especially if this leads to an understanding of how to manipulate parents or crossing techniques to improve viability. In addition, such studies may reveal hitherto unknown detail about normal cytological processes.

It is not easy to determine how widespread these phenomena are in interspecific hybrids. Pistillody (the replacement or partial replacement of male floral structures by female ones), for example, occurs infrequently in hybrids and is often more prevalent in backcrosses than in F₁ hybrids. This has provoked the suggestion that pistillody is due to an interaction between foreign nuclei and cytoplasm. Other phenomena, such as nucleolar organiser suppression and chromosome instability, are often difficult to detect. Nucleolar organiser suppression is often partial and, as such, is often difficult to identify. Chromosome number instability is easily dismissed as being artefactual unless it is unusually prevalent or it is being specifically searched for.
It is possible, though not easily proven, that in many cases the appearance of one or more of these phenomena in a hybrid marks the boundary between species. Certainly some pairs of closely related species, such as *Vicia angustifolia* and *V. sativa* (Watanabe and Yamada 1958), considered by some to be conspecific, and *Capsicum chinense* and *C. baccatum* (Gonzalez de Leon, pers. comm.), exhibit nucleolar organiser suppression in the hybrid, so these phenomena are not restricted to wider hybrids.

Little consideration has been given to the likely effects of some of these phenomena. Pistillody, of course, causes male sterility. There are several cases where this has caused the sterility of hybrids or backcrosses. Many plants with pistillody remain fertile on the female side. In this case, the possibility exists to transfer the factors responsible for male sterility to breeding lines so that F1 seed can be more easily produced.

Amphiplasty, meaning the change in relative length of one chromosome set to another in a hybrid (Navashin 1934), is not a regularly reported phenomenon. It is likely that amphiplasty simply reflects a slight asynchrony of chromosome contraction.

Genome elimination has obvious effects: hybrids will be infrequently produced, if produced at all, and haploids will predominate. This phenomenon (followed by chromosome doubling) is being put to good use to produce 'instant homozygosity' in barley breeding programmes (Snape and Simpson 1981).

The significance of chromosome number instability and
nucleolar organiser suppression is less clear. Instability of chromosome number must have effects on the vigour of tissues. Those aneuploid cells formed are likely to be less efficient at carrying out their functions than euploid ones. The tolerance of this will vary with tissue type and plant species. In addition, if germ line cells are affected, the fertility of the plant will be decreased.

The effects of NOR suppression are less easy to predict. Whether or not this is important to a plant depends on whether the r RNA genes of the 'NOR-dominant' genome can increase synthesis sufficiently enough to compensate for the suppression of those of the other genome. It also depends on whether any other functions of the nucleolus are specific to its own genome.

Several authors have attempted to explain the reasons for chromosome instability and genome elimination. Most of these explanations remain untested. Possible explanations for chromosome elimination in hybrids involving Hordeum vulgare include cell-cycle asynchrony (Humphreys 1978), endonuclease damage by one genome to another (Davies 1974), 'genetic factors' (Barclay et al. 1972), competition for limited components or metabolites (Bennett et al. 1976), interaction between spindle organisers (Orton and Tai 1977) and genome separation on metaphase plates (Finch and Bennett 1981). The causes of chromosome elimination or instability may, of course, vary with circumstances and not all explanations are mutually exclusive so several of these possibilities may operate.
Nucleolar organiser suppression, one of the earliest documented of these hybrid phenomena, remains unexplained. Some recent work (Flavell et al. 1983) has uncovered part of the molecular mechanism behind nucleolar organiser suppression in hybrids, so further advances can be expected.

Some intriguing links exist between these phenomena. For instance, in hybrids with nucleolar organiser suppression in addition to either chromosome instability or genome elimination, the same genome is dominant for both. These links will be explored more fully in the last chapter of this thesis.

1.5 Objectives of this project

The broad objectives of this project were to investigate sterility and other causes of inviability in hybrids between barley and rye. Barriers to viability are found at all stages of the life cycle of the hybrid: at pollination and fertilisation, early embryo and endosperm development, seed maturation or embryo culture, germination of seed or embryo or growth of callus, regeneration of callus, plantlet establishment, continuing vegetative growth, reproductive development, meiosis and subsequent development and fertilisation. Failure can occur at any of these stages. As has been mentioned before, a parallel Ph.D. project (Forster 1982) was concerned with the fate of the hybrids until plantlet establishment. This thesis will discuss problems occurring during the subsequent development of the plants.
Two major subject areas will be dealt with: vegetative growth, including the phenomena associated with hybrids at this stage, and meiosis together with pre- and post-meiotic development.

Good vegetative growth and relative genotypic stability are essential for a successful plant. Several phenomena have already been mentioned which may have an influence on vegetative growth in a hybrid plant. Mitotic instability is likely to be a major problem because of the physiological disturbance to the resulting aneuploid cells. Other phenomena, listed before, have unknown effects or causes. It is essential to investigate these to explain why they occur, how extensive they are, what effects they may have and how they may be interrelated.

Meiosis, and its prerequisite reproductive development, are crucial to any sexually propagated plant. Both sometimes fail in hybrids. This thesis will explore how and why these processes fail and will suggest ways to overcome these problems.

To begin, colchicine doubling techniques, intended to induce amphiploidy and hence some fertility, will be explored (Chapter 3). Several techniques will be compared and the hybrids' responses to them will be examined. Meiosis and development leading up to, and proceeding from, meiosis will be described with a view to the possibility of restoring fertility (Chapter 4).

Cytological abnormalities associated with hybridity and having
the potential to affect vegetative growth will then be discussed. The
changes in karyotype of the hybrids, both during tissue culture and
subsequently, will be described and the trends of these changes
discussed (Chapter 5). The chromosome instability found in these
hybrids will be investigated and different hybrids and cell types
compared (Chapter 6). Various theories to account for chromosome
instability will be introduced. Differential amphiplasty and its
effect on nucleolus activity will also be described (Chapter 7). In
the final chapter, the phenomenon of chromosome instability will be
explored more fully and the existing explanations of it will be
critically discussed. The close correlation of differential amphiplasty
with directional chromosome instability in hybrids will be pointed out
and the possible interactions between the two will be discussed.
2. MATERIALS AND METHODS

2.1 Materials

Grain of various cultivars and lines of barley (Hordeum vulgare L.) and rye (Secale cereale L.) were held at the Department of Botany, University of Edinburgh. Stocks were maintained at the Bush Estate, Roslin, Edinburgh and the greenhouses of the Department of Botany, University of Edinburgh. The grain was stored in cloth bags in a refrigerator at 4°C until required.

Various barley x rye hybrids were available in the Department. One embryo from the cross diploid barley (cultivar Sundance) female x diploid rye (cv. Petkus Somro) male gave rise to callus (Cooper 1978). The callus and plants regenerated from it were maintained in the department. Additional hybrid plants were kindly donated by Dr. Anton Kruse, the Royal Veterinary and Agricultural University, Tastrup, Denmark to Dr. Napier (née Cooper) during the period of her Ph.D. at Edinburgh. The rye pollen parent was probably cv. Petkus; the barley parent was not known.

2.2 Plant culture

Plants were grown in John Innes No. 1 compost, barley and rye in 5 inch pots and hybrids in 4 inch to 5 inch pots according to plant size. Plants were grown in the Department of Botany greenhouses and transferred to growth rooms or growth cabinets as required. Greenhouse temperatures fluctuated but were maintained at around 19°C in the winter months. Natural daylight was
supplemented with mercury vapour lamps to give a minimum daylength of 16 hours. Growth room and growth cabinet temperatures were at 20 ± 1°C and incandescent tungsten lamps and fluorescent tubes gave 300 μEm⁻² sec⁻¹ at flag leaf height for 16 hours per day.

Hybrid plants were perennial and tillered freely. Plants were vigorous for most of the time but some lines died out after losing vigour. Representatives of several lines were grown outside from September 1980 but did not survive the winter. Plants were clonally propagated by splitting large plants into four or five smaller ones, each with some roots, potting and transferring to the mist propagator for about one week. The success rate was about 50%. Plants of less than five green tillers were not usually viable.

Plants of winter varieties of barley and rye were vernalised as follows. Grain was surface-sterilised by immersing in absolute ethanol for 10-15 seconds, rinsing in distilled water, soaking in 5% (w/v) sodium hypochlorite for 15 minutes and rinsing in distilled water. They were then placed in petri dishes with moistened filter paper and left to germinate in the refrigerator at 4°C for 9 weeks. The vernalised young plants were then potted and allowed to recover in the mist propagator for a few days.

Adiocarb crystals were watered into the soil to help prevent red spider mite attack. Permasect and Persulon were used to control aphids and mildew respectively.

When root-tip material of barley and rye was required, grain was surface-sterilised as above, imbibed on filter paper for 24 hours...


in a refrigerator and germinated in the dark at 20°C for a further 1-2 days.

2.3 Callus culture

Barley x rye hybrid callus was maintained on Norstog's B II medium without growth substances (Cooper 1978; Forster 1982). Callus was periodically subdivided and placed on new media. It was grown in 5cm. plastic petri dishes in continuous light. Cytogenetical studies were performed on callus 1-2 weeks after subculturing as these calli contained nodes of dividing cells.

2.4 Colchicine treatment

Chromosome doubling, using colchicine, was attempted. The technique used initially was one described by Jensen (1974) for chromosome doubling of barley haploids. Young plants were immersed in 0.05% (w/v) colchicine and 2% (v/v) dimethylsulphoxide (DMSO) for 5 or 7 hours, rinsing in running water for 30 minutes, planted into perlite and kept in a humid chamber for one week.

This basic technique, several modifications of it, and other methods of treating with colchicine were tried and found to be unsuccessful. The techniques used will be fully described later (Chapter 3) along with experimental investigations of the effect of varying concentration of colchicine and mode of infiltration and comparing the response to colchicine of barley, rye and hybrid material.
2.5  **Standard cytogenetical techniques**

a)  **Root-tip pectinase plus orcein schedule**

    **Material:** This technique was developed for studying chromosome morphology and making chromosome counts from root-tips. It is based on the method of Dyer (1963) but incorporates an enzyme wall-softening step as acid hydrolysis leaves the cell walls of this material brittle and does not allow adequate cell separation.

    **Pretreatment:** Excised root-tips were placed on filter paper moistened with 0.05% colchicine (w/v in water) in a petri dish in the dark for 4 hours.

    **Fixation:** Root-tips were transferred to 3:1 ethanol:acetic acid fixative for at least one hour. Material not used within 24 hours was transferred to 70% ethanol and stored at 4°C.

    **Enzyme:** After rinsing, root-tips were placed in pectinase (12 units ml\(^{-1}\) or approximately 1% w/v in water) at room temperature for 1½ hours, then rinsed in water.

    **Acid:** The water was replaced with 6M HCl at room temperature for 5 minutes before rinsing twice.

    **Stain:** The meristem was excised and placed in small drop of lacto-propionic orcein (35% of a 2% stock made up as in Dyer 1963). Cells were tapped out, more stain was added and the preparation was covered with a coverslip. After at least 5 minutes the slide was squashed between two layers of filter paper.

b)  **Pollen mother cell orcein schedule**

    **Material:** Male meiosis and pollen development were studied
using the following method. Meiotic spikes were selected. Hybrids enter male meiosis just before the swelling spike emerges sideways through the 'boot' (the final sheath). Rye enters meiosis earlier, during late flag leaf emergence. Barley undergoes meiosis even earlier, normally during the first stage of flag leaf emergence. Rye anthers can be stained without prior fixation and hydrolysis but barley and hybrid pollen mother cells are too delicate for this.

**Fixation:** Whole florets were fixed in 3:1 ethanol:acetic acid for at least one hour.

**Hydrolysis:** Anthers were dissected out, rinsed and hydrolysed in 1M HCl at 60°C for 10 minutes.

**Staining:** Anthers were rinsed then placed singly in a drop of lacto-propionic orcein on a slide. They were then held at one end with fine forceps and an eye scalpel was used to remove the tip from the opposite end of the anther. The back of the scalpel was then used to gently stroke the anther to make the locules slide out into the stain. A coverslip was carefully positioned and the slide was left to stain before squashing gently.

c) **Feulgen schedule**

**Material:** Feulgen's method was used to stain ovaries for sectioning, root-tip material when chromosome measurements were required and callus where Feulgen enabled the small meristematic areas, with higher nuclear density, to be identified.

**Fixation:** For material for sectioning see 2.7. Otherwise 3:1 ethanol:acetic acid fixative was used for one hour.
Hydrolysis: Trials showed that 12 minutes in 1M HCl at 60°C, using prewarmed tubes, gave the best staining.

Staining: After rinsing in distilled water, Schiff's reagent (Darlington and LaCour 1942) was used to stain the material for 2 hours. Meristems were excised in 45% acetic acid, tapped out and squashed. Alternatively the stained material was rinsed and processed for sectioning (see 2.7).

d) Acetocarmine schedule

Material: Counts of nucleoli in the interphase nucleus were made from root-tip material using this technique. These experiments were concluded before silver staining techniques were published for plant chromosomes. These new techniques stain both nucleoli and chromosomal nucleolar organisers simultaneously (see 2.6 b).

Pretreatment, fixation, enzyme treatment and acid hydrolysis were as in section 2.5 a.

Staining was performed in a drop of acetocarmine stain (Haskell and Wills 1968) with a small drop of saturated ferric acetate added with the stain. The slide was warmed over a spirit lamp and squashed.

e) Making preparations permanent

All preparations (except sections) could be made permanent in two ways. If untreated coverslips had been used stained preparations were frozen on top of a block of solid CO₂, the coverslip was removed with a scalpel and the slide with the still frozen cells
attached was quickly immersed in absolute ethanol. After 10 minutes in ethanol the slide was removed and mounted in Euparal.

Alternatively coverslips were smeared with a thin film of glycerine albumen which was dried over a spirit lamp. The stained preparation was then squashed, warmed over a spirit lamp and placed in a ridged dish containing 45% acetic acid. When the coverslip had floated off it was removed and placed in 50% ethanol for 5 minutes. It was then transferred, for 5 minutes each, to 75% ethanol, 95% ethanol, absolute ethanol and fresh absolute ethanol. The coverslip was then removed and mounted on a slide with Euparal. This second method, although more time-consuming, was preferred because it gave better preparations.

2.6 Chromosome banding techniques

a) Giemsa C-banding

In early 1980 visits were made to the laboratories of Dr. M. D. Bennett, P.B.I., Cambridge and Dr. C. G. Vosa, Botany School, Oxford to learn several cytogenetical techniques. Giemsa C-banding was demonstrated and practised in both laboratories but only Vosa's technique was found to work well in Edinburgh. The following technique was developed from that of Vosa (1974, 1976). Each stage is fully explained because many research workers experience difficulty in obtaining good Giemsa C-banding.

Material: Healthy (white with yellowish meristems) root-tips were selected. Germinating grain roots or adventitious roots arising from the bases of tillers of mature plants were used in
preference to roots from the soil because the larger meristems which such roots contain are more likely to give satisfactory preparations.

Pretreatment: The root-tips were placed on filter paper dampened with 0.05% (w/v) colchicine in a petri dish. They were left in the dark at room temperature from 3 to 3 $\frac{1}{2}$ hours, less time than for normal preparations. Staining is best on under-contracted chromosomes.

Fixation: The root-tips were transferred to 3:1 ethanol:acetic acid fixative for 18 hours at room temperature then rinsed twice in distilled water. The length of fixation is not critical but should not be more than one day.

Hydrolysis: 1M HCl at 60$^\circ$C was added to the material in a cold 5 cm. glass vial. The acid was stirred and left for 40 seconds before being pipetted off and replaced with distilled water.

Coverslip preparation: The meristem was excised with an eye scalpel in a small drop of 45% acetic acid, tapped out with a brass rod and covered with a glycerin albumen coated coverslip. The coverslip was tapped to spread the cells further before the preparation was squashed moderately hard in a folded piece of filter paper. The slide was flamed on the underside before placing in a ridged dish containing absolute ethanol. When the coverslip floated off, usually about 20 minutes later, it was transferred to fresh absolute ethanol for 30 minutes before drying, cells upward, in an oven at 60$^\circ$C for 5 minutes then at room temperature for 2
hours. Slides can equally well be treated immediately or stored for an indefinite period although Fiskesjo (1974) reports that storage improves banding in Allium. It is probable that adequate dehydration is the only requirement at this stage.

Treatment: Saturated Ba(OH)$_2$ solution and 2 x SSC (double strength saline sodium citrate) solution were prepared. At least 10g of Ba(OH)$_2$ were added to 100 ml distilled water in a 100 ml conical flask kept for the purpose. The flask was corked and shaken for a few minutes before standing in a water bath at the required temperature. The 2x SSC solution (0.3M NaCl and 0.03M Na citrate) was prepared by adding 3.506g NaCl and 1.765g Na citrate to distilled water and making up to 200 ml. The solution was warmed and kept in an oven at 60°C. Coverslips were treated in new petri dishes by pouring in Ba(OH)$_2$ solution and placing in a water bath at 19°C for rye, 45°C for barley for 5 minutes. Most of the Ba(OH)$_2$ was then replaced by running distilled water into the petri dish before the coverslips were lifted out, one at a time, and rinsed in running distilled water for 3-4 seconds only. Coverslips were then placed, face upwards, in a new petri dish and covered with 2x SSC at 60°C for 30 minutes. The optimum temperature for the Ba(OH)$_2$ treatment differs from that of Vosa (1974, 1976) probably because of differences in the degree of squashing.

Staining: 1% Giemsa stain was prepared and filtered shortly before use. Gurr's Improved G66 Giemsa stock was used and was kept in a refrigerator and replaced every 9 months, following the
advice of I. Linde-Laursen. Dilution to 1% was achieved by mixing 1 ml of stock with 99 ml pH 6.8 buffer from a buffer tablet and filtering through Whatman No. 1 filter paper. After 2x SSC treatment, coverslips were rinsed in distilled water and transferred to a petri dish containing stain. Staining was monitored by examining wet coverslips from time to time until good band differentiation was seen. This could take from 10 minutes to several hours but if the preparations were under stained after about two hours then 2% or 5% stain was used. Coverslips were then rinsed in distilled water, air dried overnight and mounted in Euparal. The use of a yellow gelatin filter, Kodak Wratten No. 12, was found to increase the contrast of pale preparations.

Experience showed that to overcome difficulty in obtaining good banding with this technique, it was important to observe the following points: hydrolysis should be kept short; preparations should have reasonable numbers of metaphases (use phase-contrast to check); prepared coverslips should be dried adequately; new petri dishes should be used for each stage; the Ba(OH)_2 solution temperature should be adjusted so that staining takes about 30 min. to one hour.

b) Silver staining

In recent years silver staining methods for chromosomes, giving bands at active nucleolar organisers, have been developed (Goodpasture and Bloom 1975). Two published methods for plant chromosomes have been tried here (Von Kalm and Smyth 1980;
Hizume, Sato and Tanaka (1980). The method of Von Kalm and Smyth gave good results but the later one of Hizume et al. was eventually preferred because of its simplicity, lower silver usage and undistorted chromosomes. Modified versions of both techniques are given below.

**Von Kalm and Smyth method**

**Pretreatment and fixation:** As the Giemsa C-banding schedule but fixation limited to 1 hour.

**Hydrolysis:** After rinsing twice in distilled water, 0.1 M HCl at 60°C was added and the vial placed in the oven at 60°C for 10 minutes before rinsing twice in distilled water.

**Slide preparation:** Excised root-tips were tapped out and squashed in 45% acetic acid before freezing on a block of solid CO₂, flicking off the coverslip and plunging the still-frozen slide into absolute ethanol for 1-5 minutes.

**Pre-staining:** After air-drying the slide for 1 hour, four drops of 50% AgNO₃ (w/v, filtered through a 0.22 μm Millipore filter attached to a syringe) were placed on each slide and a coverslip placed on top. The slide was incubated in a petri dish, containing moistened filter paper to prevent drying (see fig. 2.1), in an oven at 65°C for 15-20 minutes before rinsing in distilled water and air-drying for 2-4 hours.

![Fig. 2.1: Humid chamber for Ag-staining](image-url)
Staining: Ammoniacal silver (AS) and neutralised formalin were prepared shortly before staining (Bloom and Goodpasture, 1976). AS was prepared by dissolving 0.8 g AgNO₃ in 1 ml distilled water and 1 ml concentrated NH₄OH. The AS solution was filtered by passing through a 0.22 μm Millipore filter attached to a syringe before use. Neutralised formalin was prepared by neutralising (to pH 7.0) 3% aqueous formalin with sodium acetate crystals, then adjusting to pH 4.5 with formic acid. To stain the slides, two drops of AS solution were placed on the slide followed immediately by two drops of neutralised formalin then quickly covered with a coverslip. Chilling the solutions in an ice-bath beforehand slowed the reaction so that it can be monitored under the microscope before terminating it by rinsing in distilled water. The reaction normally took about one minute although considerable variation was found. Preparations were remounted in water and photographed. All attempts at making permanent, using a variety of washing regimes and mountants, resulted in greatly distorted chromosomes. The best way of preserving the slides was found to be rinsing overnight in running water then drying and viewing, under oil, without a cover slip. Some distortion and silver grain deposition inevitably occurred.

Hizume et al. method

The modifications to this method used here include the use of pectinase alone, without cellulase, and the omission of the hydration series. This simplified method worked very well.

Pretreatment and Fixation: As for Giemsa C-banding but
fixation limited to 1 hour.

**Enzyme:** After rinsing twice in distilled water, treat in pectinase (36 units ml\(^{-1}\), equivalent to about 4% w/v), adjusted to pH 4.5 with 0.1M HCl, at 35-37°C for 1\(\frac{1}{2}\) hours. Rinse twice in distilled water.

**Slide preparation:** Excise the meristem on a clean slide. Blot dry and tap out with a brass rod in a very small drop of fixative. Add another drop of fixative, mix and ignite over a spirit lamp. Allow to burn out. Slides may be stored or stained immediately.

**Staining:** Add two drops of AgNO\(_3\) (0.5g in 1ml distilled water initially but later 0.65g ml\(^{-1}\) was required to give good staining within 1-3 hours. Hydration of the AgNO\(_3\) crystals may account for this. Filtration was not usually required) and quickly cover with a coverslip. Exposure to air at this stage speeds staining and destroys banding. Place in a petri dish, containing moist absorbent paper to prevent drying (see fig. 2.1), in an oven at 60°C and monitor from time to time until chromosomes become yellow or light brown and NORs black. This usually takes between one and three hours. The reaction can be held at any point by placing the petri dishes in the refrigerator. Preparations were photographed immediately and preserved, if required, by washing overnight in running water and drying. Subsequent viewing was under oil without a coverslip.

2.7 **Ovary sections**

Sections through young florets were prepared for light
microscopy so that the development of the ovaries, female meiosis and embryo sac structure could be studied. Sections were made on a Beck rotating microtome after embedding in paraffin wax. The schedule used was one developed by Dr. R. F. Lyndon for class work at Edinburgh.

Fixation: Navashin's fluid (Sharma and Sharma 1965) was mixed immediately before required and material was fixed for 24 hours in vials at room temperature. Whole florets of the younger stages were fixed but ovaries were dissected out from pre-fertilisation florets. Single anthers were removed before fixation and stained in lacto-propionic orcein (see 2.5 b) to check the stage of the floret. Male and female meiosis are almost synchronous in several species of the Triticinae (Bennett et al. 1973).

Staining: Fixed material was rinsed in 3 changes of distilled water, leaving each for 15-30 minutes. Staining with Feulgen (see 2.5 c) at this stage was performed in preference to the usual method of staining after sectioning. Crystal violet was used to stain some preparations after sectioning (see later) but this practice was abandoned after difficulty in preventing some sections floating off the slide during staining.

Infiltration: Material, whether stained by the Feulgen method or unstained, was then taken through an alcohol series (10 minutes each of 30%, 50%, 70%, 85%, 95%, absolute and fresh absolute ethanol). After leaving for 1 hour, the ethanol was replaced by xylene:ethanol (1:3), xylene:ethanol (1:2), xylene:ethanol (1:1), then
pure xylene for 1 hour each. The xylene was then poured off except for just enough to cover the material and the tube was filled with wax shavings (Paraplast, 54°C m.p.). The unstoppered tube was left in an oven at 60°C overnight and the wax replaced with fresh pure molten wax and again left overnight in the oven at 60°C.

Embedding: Two metal 'Ls' and a brass plate were smeared with glycerine and arranged to receive molten wax. Molten wax was poured in and the plant material was transferred with warm forceps and arranged in the molten wax. The brass plate was cooled on ice then plunged into ice-water mixture when partially solidified. Submerging it too early caused bubbles of water to enter the wax. After about 15 minutes the wax block was removed from the water and carefully trimmed with a scalpel. The finished block had the material just showing at one end and the sides trimmed parallel and fairly close to the floret or ovary.

Sectioning: The block was mounted on the holder of the microtome using molten wax. The holder was adjusted so that the face and sides of the block were parallel to the knife edge. All sections were cut at 15μm thickness and transferred, after cutting the ribbons into appropriate lengths, to a water bath at 45°C. Slides, previously cleaned in chromic acid and ethanol and smeared with glycerine albumen, were positioned under the floating sections and the sections arranged on the slides. Excess water was blotted off and the slides left to dry overnight at room temperature. The wax was then melted in an oven at 60°C to help stick the sections to
the slides before immersing in xylene to remove the wax. After 30 minutes in two changes of xylene, sections previously stained by the Feulgen method were mounted in Canada Balsalm. Unstained sections were passed through ethanol:xylene (1:1), ethanol, 95%, 90% (15 minutes each), 80%, 70%, 50%, and 30% ethanol then distilled water (5 minutes each).

Staining: The hydrated, unstained slides were then placed in 1% chromic acid for 1½ hours and rinsed in two changes of distilled water for 5 minutes each. Slides were stained in 0.5% aqueous crystal violet for 20 minutes and rinsed in distilled water. Single slides were removed from the water and treated as follows. Mordanting solution of 1% I₂ and 1% KI in 80% ethanol was dropped on to the slide and left for 30 seconds. The slide was very quickly rinsed in a few drops of ethanol. The ethanol was shaken off and clove oil added and left for 30 seconds. The slide was then immersed in three changes of xylene and mounted in Canada Balsalm.

2.8 Photomicrography

All photomicrographs were taken on a Zeiss photomicroscope. Ilford Pan F 35mm film was used throughout and given standard processing in Ilford Microphen developer and Ilford IF 23 fixer at 20°C. Printing, using a Krokus 44 enlarger with a Minolta Rokkor 50mm f4.5 enlarging lens, was done on Ilfobrom single weight glossy paper using Ilfobrom developer and Ilford IF 23 fixer. Photographs of a micrometer slide with all combinations of photomicroscope lenses were used to calibrate the enlargements.
3. CHROMOSOME DOUBLING

3.1 Introduction

Exploitation of a hybrid in a breeding programme requires that the hybrid possesses at least some fertility. Fertility depends on regular meiosis, which does not normally occur in dihaploids. The complete sterility found in many dihaploids is often relieved by chromosome doubling. In the amphiploid, regular bivalents can be formed by homologous chromosome pairing which allows regular segregation of chromosomes. Amphidiploids can be produced by two routes: using autotetraploid parents or doubling the chromosome complement of diploid (dihaploid) hybrids.

There are several advantages in using tetraploid parents. Heterozygosity in the parents would be carried into the hybrids and may be useful. The need to double the chromosome complement of valuable hybrids is eliminated. There may be unexpected benefits from using tetraploid parents, such as a fortuitous improvement in crossability (e.g. Sanchez-Monge and Martín 1982). Two drawbacks of this approach are, however, the increased time required to generate tetraploids (unless they are already available) and the reduced fertility of autotetraploids.

The alternative approach, doubling the chromosome complement of hybrids, also has problems. The hybrids are often weak. Large amounts of material are required to offset high mortality and low success rate. Many authors have admitted failure in doubling the chromosome complements of interspecific cereal hybrids, i.e.
Fedak and Nakamuro (1981), Finch and Bennett (1980), Islam et al.
(1975), Mujeeb et al. (1978), Stebbins and Fung (1953) and Wagenaar
(1959), although there are examples of successful doubling of similar
hybrids, e.g. Sachs (1952) and Thomas and Pickering (1979).

Because the production of more hybrid plants was proving to
be difficult (Forster 1982), most chromosome doubling effort was
directed at the existing diploid hybrid plants rather than trying to
use tetraploid parents.

Successful methods for doubling the chromosome complement
of Poaceae haploids and hybrids mostly involve colchicine. Seeds
or seedlings can be soaked in colchicine solution (Speckmann 1975).
Colchicine solutions can be injected into tillers (Chase 1969).
Tillers can be 'capped' with colchicine-filled vials. Whole plants
may be 'crowned' with colchicine-soaked cotton wool (Siddiqui 1971),
immersed while in culture vials (Thiebaut and Kasha 1978),
immersed after uprooting (Ho et al. 1978; Subrahmanyam and
Kasha 1975; Thiebaut et al. 1979; Thomas and Pickering 1979),
or inverted into solution (Siddiqui 1967, 1971). A more unusual
technique is cutting stems and placing in colchicine solution during
premeiotic mitosis (Toledo et al. 1979). The various techniques
applied to barley haploids were reviewed by Jensen (1974).

For hybrids, treatment of seeds or seedlings is seldom
desirable. In addition, plants are much more likely to be success-
fully treated than seeds or seedlings (Siddiqui 1971). The
techniques for plants are mostly either immersion, or inversion
which gives some protection for the roots. Many minor modifications are employed. Dimethylsulphoxide (DMSO) is frequently used as an adjuvant. It is reputed to aid the penetration of colchicine and this may be due to its ability to make plasmalemmas leaky (Delmer 1979). Kaul and Zutshi (1971) and Subrahmanyam and Kasha (1975) have demonstrated that DMSO increases the efficiency of colchicine. A second compound, Tween 80 (polyoxy-ethylenesorbiton monooleate) is often added as a wetting agent. Nethery (1967) has demonstrated that it does not cause significant mitodepression. Thiebaut and Kasha (1978) have claimed that the addition of gibberellin decreases mortality and Thiebaut et al. (1979) recommend increasing the temperature during incubation in colchicine. Slitting tillers to aid penetration is a modification adopted by Thomas and Pickering (1979).

Given the wide range of available methods of application of colchicine, the best choice for barley x rye hybrids was one which had been used on plants at a suitable growth stage, which had given good results with wide hybrids and which had been successfully applied to congeners. Variations on the immersion and inversion techniques satisfied these criteria.

3.2 Colchicine treatment of barley x rye hybrids

Altogether 57 plants were treated with colchicine in an attempt to induce amphidiploidy (see table 3.1). Newly divided and repotted plants with several vigorous young tillers were selected. The majority were treated with 0.05% (w/v) colchicine and 2% (v/v) DMSO
for 5 hours, rinsed in tap water for about 30 minutes, potted into perlite, watered with half-strength Hoagland's solution and maintained in high humidity. Very few hybrid plants survived this treatment, and those that did were confirmed as being still diploid by chromosome counts from adventitious roots or meiotic analysis. All barley and rye plants subjected to inversion treatment (e) survived. Having shown that reduced concentrations of colchicine were still effective in inducing C-mitosis (see section 3.3), these were then tried. Hybrid mortality was greatly reduced but no plants were found to have doubled sectors. Vacuum infiltration (see section 3.3) of these reduced concentrations (Hearson vacuum chamber, evacuated and repressurised gradually over 15-20 minutes, then incubated for 5 hours) simply restored high mortality.

Another technique investigated was standing detached tillers in colchicine solution. Tillers judged to be 4 to 5 days before meiosis were treated for 24 hours in 0.05% colchicine to double the chromosomes in pre-meiotic mitosis. All tillers stopped growing and all pollen mother cells found were degenerating.

3.3 The cause of failure of colchicine doubling in barley x rye hybrids

Colchicine dosage

The repeated failure of the colchicine technique for chromosome doubling prompted some experimental analysis of some of the factors which might be involved. It seemed that the hybrids were being affected by the toxicity of the colchicine so an experiment
<table>
<thead>
<tr>
<th>Method</th>
<th>Material</th>
<th>Colch. %</th>
<th>DMSO</th>
<th>Duration</th>
<th>No. plants surviving</th>
</tr>
</thead>
<tbody>
<tr>
<td>a) Tiller injection (Chase 1969)</td>
<td>20xR</td>
<td>0.05</td>
<td></td>
<td>3 days</td>
<td>1/10 tillers</td>
</tr>
<tr>
<td>b) Immersion (Jensen 1974)</td>
<td>20xR</td>
<td>0.05</td>
<td>✓</td>
<td>5+7 hours</td>
<td>1/5</td>
</tr>
<tr>
<td>c) Immersion with slits (Thomas and Pickering 1979)</td>
<td>20xR</td>
<td>0.05</td>
<td>✓</td>
<td>5 hours</td>
<td>0/7</td>
</tr>
<tr>
<td>d) Immersion with GA₃ and Tween 80 (Thiebaut &amp; Kasha 1978)</td>
<td>20xR</td>
<td>0.05</td>
<td>✓</td>
<td>5 hours</td>
<td>0/5</td>
</tr>
<tr>
<td>e) Inversion (Siddiqui 1967)</td>
<td>barley</td>
<td>0.05</td>
<td>✓</td>
<td>5 hours</td>
<td>0/7</td>
</tr>
<tr>
<td>rye</td>
<td>0.05</td>
<td>✓</td>
<td></td>
<td>5 hours</td>
<td>3/3</td>
</tr>
<tr>
<td>f) Inversion</td>
<td>20xR</td>
<td>0.01</td>
<td>✓</td>
<td>5 hours</td>
<td>9/15</td>
</tr>
<tr>
<td>20xR</td>
<td>0.005</td>
<td>✓</td>
<td></td>
<td>5 hours</td>
<td>3/5</td>
</tr>
<tr>
<td>g) Inversion with vacuum infiltration</td>
<td>20xR</td>
<td>0.01</td>
<td>✓</td>
<td>5 hours</td>
<td>0/6</td>
</tr>
<tr>
<td>20xR</td>
<td>0</td>
<td>✓</td>
<td></td>
<td>5 hours</td>
<td>0/2</td>
</tr>
<tr>
<td>20xR</td>
<td>0.01</td>
<td>✓</td>
<td></td>
<td>5 hours</td>
<td>0/4</td>
</tr>
<tr>
<td>20xR</td>
<td>0</td>
<td>✓</td>
<td></td>
<td>5 hours</td>
<td>1/2</td>
</tr>
</tbody>
</table>

Total no. of colchicine-treated hybrid plants: 57

total survivors: 14
amphiploids: 0
was designed to determine the lowest efficacious colchicine
centration. Apical meristems from 3-leaf tillers were dissected
out and randomly allocated to petri dishes with 2 sheets of Whatman
no. 1 filter paper and 3 mls colchicine solution. They were
incubated for 4 hours, fixed and stained in lacto-propionic orcein
(see section 2.5 a). Cells in abnormal metaphase were scored as
'affected' and cells in normal metaphase, anaphase or telophase as
'unaffected'. Random samples were scored from each of 6 apices
for each colchicine concentration.

The dose response curve (fig. 3.1) shows a clear abrupt
decline in affected mitoses below 0.005% colchicine. The continuing
increase with increasing concentration probably reflects improved
penetration of the higher concentrations to cells in the centre of the
meristem. This confirmed that reduced concentrations of
colchicine (0.005% and 0.01%) could be used in chromosome doubling
attempts.

Penetration and vacuum infiltration

An aqueous dye, toluidine blue, was used to check whether
there was infiltration of solutions to the apex when intact tillers
were submerged for 4 hours. It was shown that the dye reached the
apex in small amounts in controls. Prior vacuum infiltration
greatly increased the amount of dye reaching the meristem.

Comparison of colchicine sensitivity in parents and hybrids

Following the failure to achieve doubling, even after vacuum
infiltration of weaker colchicine solutions (see section 3.2), the
Fig. 3.1 - Dose-response curve for colchicine on excised hybrid apices

<table>
<thead>
<tr>
<th>Treatment</th>
<th>No. of mitotic cells</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Affected</td>
<td>Total</td>
<td>%</td>
<td>S. E.</td>
</tr>
<tr>
<td>Distilled H2O</td>
<td>5</td>
<td>109</td>
<td>4.6</td>
<td>-</td>
</tr>
<tr>
<td>0.001% colchicine</td>
<td>16</td>
<td>179</td>
<td>8.9</td>
<td>-</td>
</tr>
<tr>
<td>0.005% &quot;</td>
<td>95</td>
<td>177</td>
<td>53.7</td>
<td>3.8</td>
</tr>
<tr>
<td>0.01% &quot;</td>
<td>160</td>
<td>224</td>
<td>71.4</td>
<td>3.2</td>
</tr>
<tr>
<td>0.05% &quot;</td>
<td>127</td>
<td>165</td>
<td>77.0</td>
<td>-</td>
</tr>
<tr>
<td>0.1% &quot;</td>
<td>258</td>
<td>265</td>
<td>97.4</td>
<td>-</td>
</tr>
</tbody>
</table>

* From table W, Sokal and Rohlf (1969)
responses of hybrids and parents to colchicine were compared. Root tips from mature plants of hybrid '20 x R', barley (cv. Sundance) and rye (cv. Petkus Somro) were randomly allocated to 5 colchicine solutions and one distilled water control (5 replicates each) and incubated for 4 hours in the dark at 22°C. The roots were then fixed and a standard length (= width of root) of the tip was stained in lacto propionic orcein (see section 2.5 a). About 100 cells in metaphase, anaphase or telophase from each slide were scored as "affected" or "unaffected". In addition over 1,000 cells from each slide (i.e. between 5382 and 5985 in total for each treatment) were scored as interphase, prophase, metaphase, anaphase or telophase.

The results are presented in figs. 3.2, 3.3 and 3.4. In fig. 3.2 the distribution of the different stages of mitosis is shown. In general the barley root tips had a lower mitotic index (0.52% to 1.48%) than rye or hybrid ones (0.73% to 3.33%). In all cases the numbers of telophases declined with increasing colchicine concentration and there was a concomitant increase in metaphases. This trend is more easily analysed by referring to the metaphase:telophase ratio (fig. 3.3). The results are plotted on log scales. A clear trend of increasing metaphase:telophase ratio is shown, as expected. The results for the hybrid fall within the range of the parents. At lower concentrations of colchicine the metaphase:telophase ratio for barley seems to be smaller than that of rye. The hybrid was similar to rye at these concentrations.
Fig. 3.2 Frequency of mitotic stages after various colchicine treatments

% BARLEY cv. SUNDANCE

0.05

0.01

0.005

0.001

0.0005

H₂O

% RYE cv. PETKUS SOMRO

0.05

0.01

0.005

0.001

0.0005

H₂O

% HYBRID 20xR

0.05

0.01

0.005

0.001

0.0005

H₂O

M.I. - Mitotic Index
Fig. 3.3  

a) Metaphase:telophase ratio after various colchicine treatments

<table>
<thead>
<tr>
<th>Material</th>
<th>Colchicine concentration (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Barley Sundance (O)</td>
<td>0.05  0.01  0.005  0.001  0.0005  0</td>
</tr>
<tr>
<td></td>
<td>5.00  0.50  0.04  0.18  0.10</td>
</tr>
<tr>
<td>Rye Petkus Somro (□)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>12  2.20  1.55  1.33  0.23  0.37</td>
</tr>
<tr>
<td>Hybrid 20xR (Δ)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>96  5.33  0.77  0.17  0.75  0.42</td>
</tr>
</tbody>
</table>

b) Log/Log plot of colchicine concentration against metaphase:telophase ratio

Note: lines fitted by eye.
Fig. 3.4 Dose-response curve for colchicine on excised roots of barley, rye and hybrid.

- **barley Sundance**
- **rye Petkus Somro**
- **hybrid 20 x R**

95% confidence limits

Log$_{10}$ colch %

Affected mitoses %
significance of this is not known but may reflect either differences in the relative lengths of the stages of mitosis or partial synchrony in the population of cells sampled.

The second method of demonstrating the effect of colchicine on dividing cells was to score sensitive stages (metaphase, anaphase and telophase) as affected or unaffected. The dose-response curves are given in fig. 3.4. Barley and rye respond similarly at all concentrations. The hybrid appears to be less sensitive than the parents to intermediate levels of colchicine, but as this is significant at only one concentration (0.005%) this result may be spurious. Any decrease in sensitivity of the hybrid as compared to the parents would be somewhat surprising as the hybrids have a higher mortality than barley or rye when exposed to colchicine. It does not seem likely that hybrid spindles are less affected by colchicine than barley or rye spindles.

3.4 Discussion

The techniques selected for use here were those using injection of colchicine solution and immersion and inversion into colchicine solutions. Injection was technically difficult with the small tillers produced by the hybrids so work concentrated on immersion and inversion of plants into colchicine solutions. Mortality of the hybrids was high in all treatments but more plants survived inversion techniques, where some protection is given to the roots, than immersion techniques.
Roots are particularly susceptible to colchicine (McLeod 1971). The greater survival after inversion of plants into lower concentrations of colchicine indicated that the colchicine was responsible for the mortality of the hybrids. Vacuum infiltration increased it again, presumably partly by increasing the concentration, or rate of entry, of colchicine to the apex.

It is interesting that the plants of barley and rye survived the inversion technique more readily than the hybrids. This is either because the hybrids are generally weak or there is a specific physiological reason for this increased mortality. Hybrid spindles are frequently suggested to be deficient in some way (e.g. Bennett, Finch and Barclay 1976). It is possible that hybrid spindles may be more sensitive to colchicine than those of the parents. An experiment to investigate this did not identify any increased susceptibility of the hybrids to colchicine. The metaphase:telophase ratio varied with colchicine concentration in a manner very similar to that of rye but slightly different to that of barley. Interestingly, the percentage of mitotic cells which were visibly affected by colchicine at various concentrations in hybrids was different to that of barley and rye but not in the manner expected. Cell division in the hybrids appeared to be less sensitive to colchicine than in the parents. It seems, therefore, that the increased susceptibility of the hybrids to colchicine is not due to hybrid spindles being unduly affected by weak concentrations of colchicine. It is more likely that the low vitality of the
hybrids is accentuated by stresses such as uprooting, washing, and treatment with toxic chemicals such as colchicine and that this combination of stresses causes the death of the plant.

The repeated failure to double the chromosome complement of the hybrids under study contrasts with the results of Thomas and Pickering (1979) who were also working with barley x rye hybrids. They successfully treated two plantlets by immersion in colchicine and, in addition, found one spontaneous callus-derived tetraploid. Their hybrids were euploid whereas ours were aneuploid which may partially explain the differences in ease of chromosome doubling.

To summarise, the failure to successfully double the chromosome complement of the hybrids under study here has been tentatively ascribed to several causes: the hybrids are inherently weak; colchicine is toxic to the plants; reduced concentrations of colchicine are ineffective; and the plants are aneuploid.

Future attempts to produce tetraploid hybrids could use several approaches. Several alternatives to colchicine could be considered, especially N₂O (Östergren 1954; Zeilinga and Schouten 1968). However, Speckmann (1975) and Subrahmanyan and Kasha (1975) report that N₂O is much less effective than colchicine. Other spindle inhibitors such as griseofulvin and vinblastine (Malawista et al. 1968), many benzene derivatives (Östergren 1944) and even cold or heat (Muntzing et al. 1936) have not been routinely applied for chromosome doubling. It
seems, therefore, that colchicine treatment remains the best way to produce amphidiploids. If parental genotypes can be selected which will give more vigorous hybrids, colchicine doubling of these hybrids may prove easier. In addition, any future crossing programme should include tetraploid parents. The depressed fertility of autotetraploids may be ameliorated by using tetraploids of F1 intraspecific hybrids of contrasting genotypes for each species.
4. **REPRODUCTIVE DEVELOPMENT AND MEIOSIS**

4.1 **Introduction**

Interspecific hybrids, once produced, must be at least partially fertile to be of any use in plant breeding. The major use of such an intergeneric hybrid as *Hordeum x Secale* would be the introgression of characters to one parent. The fertility essential for introgression depends on a sequence of events, including transition to flowering, differentiation of floral organs, development of meiocytes, balanced meiosis and the subsequent development of pollen and embryo sacs.


Many interspecific and intergeneric hybrids fail at meiosis. The pairing of disparate genomes is often almost completely lacking (e.g. hybrids involving *Hordeum*: Cauderon et al. 1978; Fedak 1977a, 1977b, 1979a; Finch and Bennett 1980; Kimber and Sallee 1976; Morrison and Rajhathy 1959; Rajhathy and Morrison 1959; Thomas and Pickering 1979; Wojciechowska 1979; and...
other intergeneric hybrids: *Raphanus x Brassica*, McNaughton 1973; *Secale x Agropyron*, Stebbins and Fung 1953; several other intergeneric hybrids in the *Triticeae*, Ahokas 1970). Failure of pairing of homoeologues and the subsequent lack of chiasma formation results in univalents at metaphase I and, therefore, irregular disjunction and sterility. Asynaptic hybrids may be rendered fertile by chromosome doubling, as discussed in Chapter 3. Such amphiploids frequently show autosyndesis and bivalent formation which restore fertility but also reduce the possibility of introgression. Chromosome doubling does not necessarily substantially increase bivalent formation (e.g. *Hordeum x Secale*, Thomas and Pickering 1979) or, if it does, the meiotic products may still abort (e.g. *Raphanus x Brassica*, McNaughton 1973).

The hybrids under study were sterile. To assess the possibility of overcoming the sterility, it was necessary to investigate its causes. This involved investigating the reproductive development and meiosis of the hybrids. A study of meiosis was likely to be particularly valuable as the prospects for chromosome doubling could be assessed and chromosome homologies perhaps could also be determined.

4.2 Pre-meiotic development

All hybrids tillered and flowered freely throughout the year. Some tillers in all hybrids were blind but this also commonly

---

1. defined in the glossary
occurred in the parental species.

**Hybrid cytotypes A, B, C and D** (See Chapter 5)

These hybrids, from the cross barley cv. Sundance x rye cv. Petkus Somro, displayed pronounced pistillody, i.e. several supernumerary pistils or pistil-like structures were adpressed to the ovary. The numbers of these structures were variable: commonly 3 to 5 per floret but occasionally 6. It was probable that these structures had arisen from the three anther primordia and that lodicule and other primordia were sometimes also involved. The florets were completely female (fig. 4.1 e) or, very rarely, had signs of male-ness on the lower portion of the outer segments (fig. 4.1 d).

![Fig. 4.1 Intermediates between normal stamens and secondary pistils.](image)
The internal anatomy of the primary and secondary pistils was investigated by sectioning. Both primary (fig. 4.2) and secondary (fig. 4.3) pistils were grossly abnormal. The primary pistils were larger than those of both parents and had irregular outlines. It was often difficult to distinguish the nucellus from other tissues and the integuments grew erratically instead of enveloping the nucellus. Embryo sac mother cells and embryo sacs were never found. Secondary pistils (fig. 4.3) were generally more disturbed than the primary pistils. Integuments were less frequently detected and large air spaces were often found inside the pistil.

Meiosis did not occur in these hybrids. The lack of stamens precluded male meiosis and although female organs were present, meiocytes were never seen in them.

Hybrid 20 x R

Pistillody occurred in this hybrid but was never as severe as in the other hybrids. In many florets, pistillody was undetectable. Where pistillody was found it was similar to stamen types b and c in fig. 4.1. The majority of stamens had only two locules (fig. 4.4) in contrast to rye and barley, both of which have four.

The structure of the ovary was investigated by sectioning. Development up to meiosis resembled that of rye. Normal nucellus and integuments were present. The ovule was anatropous, bitegmic and with a single embryo sac mother cell towards the
fig. 4.2  Longitudinal section through primary pistil of hybrid cytotype A at anthesis. Stained by Feulgen's method. (Ov - ovule containing degenerating nucellus).

fig. 4.3  Longitudinal section through secondary pistil of hybrid cytotype B at anthesis. Feulgen.

fig. 4.4  Transverse section through anther of hybrid 20xR, showing two locules containing immature pollen grains. Feulgen.

Scale bars 100 μm.
micropylar end of the nucellus and about three cells below the surface of the nucellus (figs. 4.5, 4.6). Male and female meiosis were approximately synchronous in this hybrid, as they are in barley and rye. These hybrids underwent meiosis late in the development of the floret, after completion of the anatropous movement of the ovule and the growth of the integuments. Rye undergoes meiosis at a similar developmental stage but barley meiosis occurs much earlier. A barley spike in pre-meiotic interphase is shown in fig. 4.7. The integuments have yet to grow round the nucellus and the ovule has not yet become fully anatropous.

4.3 Meiosis

As previously stated, the barley cv. Sundance x rye cv. Petkus Somro cytotypes did not reach meiosis. The hybrid 20xR, produced by Kruse in Denmark, underwent both male and female meiosis.

Male meiosis

The analysis of hybrid pollen mother cells in stages between leptotene and late pachytene was difficult due to the condensed nature of the nucleus. Chromosome pairing could not be detected during pachytene and a large single nucleolus was often present (fig. 4.8). The nucleolus decreased in size through diplotene (figs. 4.9, 4.10) and disappeared during diakinesis. As the chromosomes became resolvable at diplotene, they were seen to occur mainly as univalents with one bivalent present in a minority of
fig. 4.5 Hybrid 20xR ovary at female meiosis showing position of ovule (Ov). Feulgen. Scale bar 100 μm.

fig. 4.6 Hybrid 20xR ovule with integuments (I) nucellus (N) and embryo sac mother cell (ESMC) at meiosis. Feulgen. Scale bar 10 μm.

fig. 4.7 Premeiotic barley floret with partially developed integuments (I) and embryo sac mother cell (ESMC). Feulgen. Scale bar 100 μm.
fig. 4.8 Hybrid 20xR PMC at pachytene. Lacto-propionic orcein.

fig. 4.9 Hybrid 20xR PMC at diakinesis with nucleoli (N) Lacto-propionic orcein.

fig. 4.10 Hybrid 20xR PMC diakinesis with nucleoli (N). Lacto-propionic orcein.

Scale bars 10 μm.
pollen mother cells. Some chromosomes were joined together by "pseudochiasmata" or secondary associations. During diakinesis, spiralling of the chromatin of bivalents and univalents was seen (figs. 4.11, 4.12). The bivalents and univalents were scattered throughout the pollen mother cell at this stage. Normal migration of all the chromosomes to the metaphase plate at metaphase I did not occur. The bivalent normally became centrally positioned but the univalents remained dispersed (figs. 4.13, 4.14, 4.15). All chromosomes continued to condense and the smaller univalents eventually had a diameter greater than half of their length.

Meiosis in the parental species was typical of normal diploids. Bivalents with chiasmata in both arms were found at diakinesis (fig. 4.16) and by metaphase seven bivalents, usually all ring bivalents, became aligned in the centre of the pollen mother cell (fig. 4.17).

Hybrid 20xR was aneuploid, being disomic for 1R (section 5.2). It would be expected that the two 1R chromosomes would pair and form a bivalent at meiosis. An analysis of pairing in pollen mother cells at metaphase I was made (table 4.1). Aneusomaty was present and was greater than in root tips of the same plants (see section 6.2). The number of metaphase bound arms (MBA), which approximates the number of chiasmata, was 0.22 per cell and hence 0.22 per homologous chromosome pair. The number of MBAs in barley was 13.89 per cell and 1.99 per homologous chromosome pair.
figs. 4.11 - 4.14 Hybrid 20xR PMCs at meiotic metaphase I showing spiralled univalents and bivalents (4.11 and 4.12) and spindle attachments on central univalents (4.13 and 4.14). Maximum of one bivalent. Lacto-propionic orcein. Scale bars 10 \( \mu \text{m} \).
fig. 4.15 Hybrid 20xR PMC at meiosis with one ring bivalent (arrow). Lacto-propionic orcein.

fig. 4.16 Rye cv. Petkus Somro PMC at diplotene/diakinesis with bivalents. Lacto-propionic orcein.

fig. 4.17 Barley cv. Sundance PMC at meiotic metaphase I with bivalents. Lacto-propionic orcein.

fig. 4.18 Hybrid 20xR PMCs at tetrad stage with irregular numbers of major and micronuclei. Lacto-propionic orcein.

Scale bars 10 μm.
Table 4.1 - 20xR meiotic configurations at metaphase I

<table>
<thead>
<tr>
<th>Chromosome no.</th>
<th>Configuration</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>All I</td>
<td>one II 'rod'</td>
</tr>
<tr>
<td>13</td>
<td>4</td>
<td>-</td>
</tr>
<tr>
<td>14</td>
<td>26</td>
<td>1</td>
</tr>
<tr>
<td>15</td>
<td>185</td>
<td>57</td>
</tr>
<tr>
<td>16</td>
<td>8</td>
<td>1</td>
</tr>
<tr>
<td>Total</td>
<td>223</td>
<td>59</td>
</tr>
</tbody>
</table>

Following metaphase I the bivalent, if present, divided normally. Univalents either divided precociously at anaphase I or coalesced into groups and re-entered interphase. Some of these nuclei underwent further division to give cells with variable numbers of large and small nuclei. Representatives of these 'telophase II' cells can be seen in fig. 4.18. These observations are summarised in diagrammatic form in fig. 4.19.

Chromosome disjunction at anaphase I in the parental species is normal (fig. 4.20) and regular tetrads are formed at telophase II (fig. 4.21).

All rye chromosomes are larger than all barley chromosomes but there is no marked discontinuity between them. Nevertheless, univalents can often be assigned to one genome. In the majority of metaphase pollen mother cells the barley chromosomes were in a relatively small group near the centre of the cell and the rye chromosomes were more peripherally disposed (figs. 4.13, 4.14).
Fig. 4.19   Diagram of male meiosis in hybrid 20xR

early prophase

pachytene/diplotene

One biv.  20%

-20%

No bivs.  80%

metaphase I

meta/anaphase I

anaphase II
fig. 4.20  Barley cv. Sundance PMC at early anaphase I. Lacto-propionic orcein. Scale bar 10 µm.

fig. 4.21  Barley cv. Sundance tetrads. Lacto-propionic orcein. Scale bar 100 µm.

fig. 4.22  Hybrid 20xR embryo sac (ES) with micronuclei. Feulgen. Scale bar 10 µm.
In addition the most central barley univalents had clear spindle attachments and were apparently under some tension.

Female meiosis

A small number of female meiocytes of hybrid 20xR were found by sectioning. Two metaphase I embryo sac mother cells were seen. They were very similar to male meiocytes: all chromosomes were present as short, broad and oval univalents (fig. 4.6). Embryo sac mother cells in post-meiotic interphase had several variously-sized nuclei, which again followed the pattern of development of the male meiocytes (fig. 4.22).

4.4 Post-meiotic development

Pollen

The male meiotic products had a variable number of major nuclei and micro nuclei. Walls were laid down between nuclei to give between two and six young pollen grains per pollen mother cell. These young pollen grains contained between about one and nine nuclear fragments (fig. 4.23). Some thickening of the pollen grain wall occurred but the pollen grains aborted and degeneration of the anther followed (fig. 4.24).

Pistil

After meiosis, embryo sac mother cells contained micronuclei and failed to develop further. Degeneration of the nucellus began within a few days of the end of meiosis and was complete by flowering (fig. 4.2). The rest of the ovary continued development
fig. 4.23  Hybrid 20xR young pollen grains with many micronuclei. Lacto-propionic orcein. Scale bar 10 μm.

fig. 4.24  Hybrid 20xR abortive pollen grains from mature anther. Lacto-propionic orcein. Scale bar 10 μm.
and was outwardly normal at the gaping of the floret. The stigma was intermediate between that of barley and rye, being feathery and partially exerted.

Backcrosses of about 500 hybrid florets to barley or rye, in the hope that some ovaries were fertile, failed to produce any appreciable swelling of the ovary.

Illustrations of mature embryo sacs of barley and rye are presented for comparison (figs. 4.25, 4.26, 4.27, 4.28).

4.5 Discussion

Two major causes of sterility in these hybrids have been identified, pistillody and meiotic failure.

Pistillody

Pistillody (carpellloid stamens, teratological stamens, multiovary or feminised anthers) has been widely reported from other hybrids. Tsitsin and Lubimova (1959) reported that it was most severe in first generation hybrids whereas the majority of reports suggest that severity increases in later generations, especially when hybrids are backcrossed (Gerstel et al. 1978; Islam et al. 1975; Kihara and Tsunewaki 1961 and Pearson 1972). Several workers have concluded that pistillody is commonly caused by interactions between genomes or specific genes and alien cytoplasm.

Other causes of pistillody have been reported. A single recessive gene mutant in barley is known to cause pistillody (Moh
fig. 4.25  Barley cv. Sundance mature embryo sac with antipodals (A) and polar nuclei (PN). Feulgen.

fig. 4.26  Barley cv. Sundance mature embryo sac with egg apparatus (EA) and polar nuclei (PN). Feulgen.

fig. 4.27  Rye cv. Petkus Somro mature embryo sac with antipodals (A). Feulgen.

fig. 4.28  Rye cv. Petkus Somro mature embryo sac with egg apparatus (EA). Feulgen.

Scale bar 100 μm.
and Nilan 1953). A similar mutant in tomato can be restored to normality by the addition of gibberellin (Phatak et al. 1966). It has also been noted that short days can induce pistillody in wheat (Fisher 1972) and can accentuate it in hybrids expressing pistillody (Tsitsin and Lubimova 1959). Heslop-Harrison (1972) has noted that the application of IAA to *Hyoscyamus niger* and *Silene pendula* promotes 'feminisation' of their flowers.

Pistillody does not inevitably cause sterility. Backcrosses from a *Triticum* × *Aegilops* hybrid gave seed set from primary pistils in florets with pistillody (Kihara and Tsunewaki 1961). Tsitsin and Lubimova (1959), working with hybrids between the same genera, obtained some double and triple kernels showing that even the secondary pistils can be fertile.

Pistillody has not been reported for other * Hordeum* × *Secale* hybrids. It has already been stated that backcrosses are more prone to pistillody than *F*₁ interspecific hybrids. Only Wojciechowska (1979) has managed to produce a *Hordeum* × *Secale* backcross and she did not report any pistillody. The paucity of backcrosses with *Hordeum* × *Secale* hybrids may be responsible for the lack of reported pistillody in this cross. All cytogenetical studies of *Hordeum* × *Secale* hybrids have shown that they were euploid except for those under study here (see Chapter 5) and also one hybrid reported by Pickering and Thomas (1979) which did not flower. The aneuploidy of the hybrids studied here may have been partially responsible for the pistillody.
Sectioning primary and secondary pistils of cytotype A demonstrated that their internal anatomy was severely disturbed and that they were likely to be totally sterile. Three features of these hybrids indicated some physiological abnormality which may be mediated by growth substances: the callus from which the hybrid plants regenerated did not require exogenous growth substances for sustained growth (Cooper et al. 1978); pistillody was found; and the growth of integuments and other constituents of the ovary was irregular.

Meiosis

Both male and female meiosis were studied in hybrid 20xR. Meiosis occurred approximately in phase after completion of the growth of the integuments in these hybrids. Meiosis in the hybrid is at a much later developmental stage than in barley and at the same stage as in rye (Bennett et al. 1973).

Meiosis in wide hybrids typically has little chromosome pairing between genomes. This is particularly true for intergeneric hybrids. The meiotic chromosome behaviour of wide hybrids is comparable to that of haploids. Studies on haploids of maize (Chase 1969), barley (Tomentorp 1939; Sadasivaiah and Kasha 1971, 1973) and rye (Nordenskiöld 1939; Levan 1942) generally show very few multivalents. The behaviour of the chromosomes during meiosis is very similar to that described for hybrid 20xR. Some non-homologous chromosome pairing is often seen in haploids and some synaptonemal complex is formed (Gillies 1974). Weak
chromosome attachments, not thought to represent chiasmata, persist as secondary associations at diakinesis and metaphase (Sadasivaiah and Kasha 1973). Chromosome disposition at metaphase I and the subsequent formation of micronuclei in hybrid 20xR (see fig. 4.18) closely parallels that seen in haploids.

The similarity of meiosis of the hybrid to that of haploids gives some hope for the success of colchicine doubling in restoring fertility. An indication of the likely behaviour of the amphidiploid may be obtained from the study of the disomic chromosome in the aneuploid hybrid. Chromosome 1R, present in two copies, was able to pair with itself but an estimate of the frequency of chiasmata (i.e. metaphase bound arms) indicated that chiasmata were much rarer than expected (about 0.22 per homologous chromosome pair). If this frequency of chiasmata was maintained in the amphidiploid then it could be argued that $(0.22)^{14}$ of pollen mother cells would have no univalents and hence about one in $6.2 \times 10^{10}$ meiocytes would give balanced products. It is somewhat dubious to extrapolate these results from an aneuploid dihaploid to an amphidiploid. Thomas and Pickering (1979) have, however, studied meiosis in an amphidiploid H. vulgare x S. cereale hybrid and found that pairing is reduced and also disturbed, even occurring between non-homologous chromosomes of the same genome.

The lack of pairing between barley and rye chromosomes can be ascribed to three causes. Overall pairing may be suppressed
(Thomas and Pickering 1979). There may also be insufficient homoeology between chromosome segments to allow pairing. Giemsa C-banding studies support this suggestion (see Chapter 5). A further cause of the lack of allosyndesis may be the spatial separation of the Hordeum and Secale genomes first noted by Finch and Bennett (1981 a, b). The spatial separation of genomes has been confirmed for hybrid 20xR. It also seems that the more central genome, Hordeum, has earlier and/or stronger centromere-spindle attachments. This may be partially responsible for the preferential instability of Secale chromosomes in these hybrids (see Chapter 6).

Male and female meiosis may differ in hybrids. Restitution nuclei seem to be formed more frequently from female meiocytes than male meiocytes and using hybrids as female in backcrosses is sometimes effective (e.g. Wojciechowska 1979). Another reason for the greater success of the female side could be that male organs abort if a high proportion of spores are inviable whereas all female organs normally continue to develop. If a low percentage of meiocytes does produce viable spores, then backcrossing with the hybrid as female may be the only way to obtain F2s. However, no evidence for this was obtained for this in these hybrids. Female meiosis was as disturbed as male meiosis. All post-meiosis ovules sectioned were degenerating and all attempts at backcrossing failed.
Restoration of fertility

Two main causes of sterility have been discussed: pistillody and failure of chromosome pairing at meiosis. One group of hybrids was sterile because of complete pistillody accompanied by disturbed morphogenesis of the pistils. Aneuploidy may be partly responsible for this pistillody because other workers have produced euploid hybrids and have not reported pistillody. Genic or cytoplasmic factors in the parental cultivars may also be partly responsible for the pistillody. The prospects for overcoming such pistillody are not high because the physiological reasons for it are not understood. It has been noted, however, that growth substances may be involved in the expression of sex. Application of gibberellin restored normality in a pistilloid tomato mutant, so this may be one way to restore normal morphogenesis in barley x rye hybrids.

Normal pre-meiotic reproductive development does not lead to fertility however. A further barrier to fertility occurs at meiosis, as seen in the hybrid which does not suffer from pistillody. Meiosis in this hybrid resembled that of a haploid, as is expected in such a hybrid. Chromosome doubling of hybrids with no alloosyndesis often leads to some fertility. The effect of chromosome doubling in this hybrid remains untested because of the difficulty in producing the amphidiploid (see Chapter 3). There are two reasons for predicting that such chromosome doubling would not lead to fertility. The hybrid was disomic for 1R (see Chapter 5) but a minority of pollen mother cells at meiotic metaphase I had this chromosome
pair as a bivalent. In addition, Thomas and Pickering (1979) obtained the amphidiploid which was found to be sterile because of meiotic faults. However, it is worth producing more amphidiploids. Some parental genotypes may give fertile amphidiploids. Hybrids only have to be partially fertile, as male or as female, to allow gene flow to take place.
5. KARYOTYPE ANALYSIS IN HYBRIDS AND PARENTS

5.1 Introduction

As indicated earlier (see 2.1), hybrid plants came from two sources. A stock of plants growing at Edinburgh was originally produced by A. Kruse at Risö, Denmark. The plants, labelled 20xR, were all morphologically very similar and were assumed to be descended from one original plant by repeated vegetative propagation. The other hybrid plants were produced in the Department of Botany, University of Edinburgh, during 1977-78 (Cooper et al. 1978, Cooper 1978, Napier et al. 1981). Hybrid embryos, excised and cultured at an early stage of growth, proliferated to produce callus, even without added growth substances, instead of germinating directly. One embryo from the cross barley cv. Sundance x rye cv. Petkus Somro produced callus which, when subcultured on Norstog's B II medium (Norstog 1973), regenerated albino then green plantlets. About fifty plants were established following regeneration and, contrary to expectation, preliminary cytogenetical studies revealed 6 barley and 8 rye chromosomes present in at least some of the plants (Cooper et al. 1978).

Detailed cytogenetical study of these plants was desirable for several reasons. Callus is known to be chromosomally unstable (Bayliss 1973, Kao et al. 1970, Singh et al. 1975) and there was the possibility of further aneuploid lines existing in the stock of
lines regenerated from callus. Alternatively, aneuploid tillers could have arisen within unstable hybrid plants, subsequently segregated due to continued division of the crown for propagation, and now be present as karyologically distinct plants. Full description of any such karyotype changes may reveal whether or not there are any trends of chromosome change.

The hybrids were investigated with a view to:

i) assessing how many different karyotypes were present;

ii) identifying, using Giemsa C-banding, all chromosomes and producing C-banded karyotypes of the aneuploid complements;

iii) determining whether chromosome change occurred during the callus phase, during normal vegetative growth, and/or was still continuing; and iv) noting the relationship between karyotype and plant morphology. With these aims in mind, the hybrid plants were initially surveyed using standard cytogenetical techniques and classified according to their karyotypes.

5.2 Categories of hybrid karyotypes

Of the three techniques used to study non-banded root-tip chromosomes (see section 2.5), a modified version of Dyer's (1963) lacto-propionic orcein squash technique was most useful. Acid hydrolysis does not adequately soften barley and rye root-tips so a pectinase digestion step was added to allow cells to be separated and squashed without rupturing them. The analysis of orcein stained chromosomes gave some surprising results. Of
the lines from one barley cv. Sundance × rye cv. Petkus Somro embryo, 21 were surviving. The same karyotype was found in 20 lines (cytotype A, fig. 5.1) and the one remaining line contained plants with three distinct karyotypes (cytotypes B, C and D, figs. 5.2, 5.3 and 5.4). The chromosome numbers of these cytotypes were 2n = 14 (cytotypes A and C), 2n = 14 plus an acrocentric chromosome (cytotype B) and 2n = 15 (cytotype D). The plants of the clone produced by Kruse (20xR) had a constant karyotype of 2n = 15.

Cytotypes A and C, though both having 2n = 14, were readily distinguishable due to the presence of a marker chromosome in cytotype A. This chromosome (see fig. 5.1) had a higher arm ratio (1:1.9) because of a smaller short arm than any present in barley or rye (see figs. 5.5 and 5.6). This chromosome was not present in any other cytotype but was still to be seen in the callus 18 months after it had stopped regenerating plantlets. In cytotype A, no normal barley chromosome 7 was seen but small knobs of chromatin were occasionally noted distal to a secondary constriction on the short arm of this chromosome (see fig. 5.7). These may represent rRNA genes which are not always active and occasionally appear as extra chromatin. Later observations confirmed that this chromosome was barley chromosome 7. It follows from these observations that the missing barley chromosome must be one other than a nucleolar organiser chromosome.

Cytotype C had 2n = 14 but both barley nucleolar organisers
fig. 5.1 Cytotype A with 6B and a chromosome with a short short arm (arrow). Feulgen.

fig. 5.2 Cytotype B with an acrocentric chromosome (arrow). Lacto-propionic orcein.

fig. 5.3 Cytotype C, derived from cytotype B, with no acrocentric. Chromosomes 6B and 7B visible. Lacto-propionic orcein.

Scale bars 10 μm.
fig. 5.4  Cytotype D  2n = 15.  Feulgen.

fig. 5.5  Barley cv. Sundance.  2n = 14.  Feulgen.

fig. 5.6  Rye cv. Petkus Somro.  2n = 14.  Feulgen.

Scale bars 10 μm.
fig. 5.7 Cytotype A showing distal small satellite (arrow). Feulgen.

fig. 5.8 Hybrid 20xR (2n = 15). Feulgen.

fig. 5.9 Hybrid callus. (2n = 16). Feulgen.

Scale bars 10 μm.
were visible and the marker chromosome in cytotype A was absent (see fig. 5.3).

Cytotype B had a conspicuous acrocentric chromosome (see fig. 5.2) consisting of one arm the length of a rye short arm or a barley long arm and a small segment on the other arm. The complement appeared to be that of cytotype C (both barley nucleolar organiser chromosomes being present) with the addition of the acrocentric chromosome.

Cytotype D (see fig. 5.4) also had both barley nucleolar organiser chromosomes present but had 2n = 15.

Kruse's plants also had 2n = 15 with both barley nucleolar organiser chromosome visible (see fig. 5.8).

The callus which gave rise to cytotypes A to D was no longer regenerating plantlets. Pieces of callus, about 14 days after sub-culturing, were stained by Feulgen's method so that the small meristic areas could be identified and excised. The karyotype varied between cells (see chapter 6) but the commonest chromosome number was 16 (fig. 5.9). A dicentric chromosome was seen in several cells and a small fragment was also seen. One interesting finding was that barley chromosome 7 was in the altered form which was present in cytotype A. At one time the callus must have contained an unaltered barley chromosome 7 to have regenerated the cytotypes of the B/C/D group.

The results at this stage showed that aneuploidy was common (it was, in fact, pandemic - see next section) in these hybrids and
that at least two chromosomes were present in an altered state. However, the identification of individual chromosomes in orcein stained preparations was difficult because all the unaltered chromosomes were metacentric or submetacentric and of similar size. Relative lengths (i.e. % of diploid genome length) of individual rye chromosomes ranged from 8.0 to 6.7. Barley chromosomes had an even smaller range of relative lengths with the largest being 7.8 and the smallest being 6.7. This means that only the largest and smallest chromosomes, along with any marker chromosomes, can be recognised with any certainty. Fortunately chromosome banding techniques are available and can be used to distinguish barley chromosomes from rye chromosomes and, with care, each chromosome can be identified.

5.3 Chromosome identification using Giemsa C-banding

Introduction

The development within the last decade and a half of techniques to band chromosomes has revolutionised cytogenetics. The first of the new banding methods used quinacrine mustard, a fluorochrome, to give Q-bands (Caspersson et al. 1968). Pardue and Gall (1970) were first to use Giemsa to demonstrate C-banding. Other banding techniques have been developed since but C-banding remains the most useful one for identification of plant chromosomes. Plant Giemsa C-banding involves treating cells with alkali, usually barium hydroxide, then incubating in saline sodium citrate before staining in Giemsa. Constitutive heterochromatin (hence C-banding) is
normally stained preferentially.

The mechanism of C-banding is related to the fact that heterochromatin contains repeated sequences. It was originally thought (Hsu 1973) that the alkali treatment denatured DNA and the single-stranded molecules re-annealed in the saline sodium citrate (SSC). Repetitive DNA will re-anneal more quickly and if the stain binds preferentially to double-stranded DNA these regions will form bands. However Comings et al. (1973) demonstrated that the DNA does not re-anneal in SSC but that DNA is removed from the chromosomes during the alkali and SSC stages. It seems likely that DNA is preferentially removed from non C-banded regions because of different DNA-protein interactions in the heterochromatic and euchromatic areas. DNA staining by Giemsa is dependent on the density of DNA fibres. Sumner and Evans (1973) demonstrated that the Giemsa staining reaction depends on a new molecule being formed in situ as a bridge between two strands of DNA. Pale areas on chromosomes are likely to correspond to areas with DNA strands too dispersed to allow 'bridging'. Although the mechanism of Giemsa C-banding seems to be largely explained, some mysteries about the technique remain. Preparations must be rigorously dehydrated before treating with alkali and the initial hydrolysis must be kept very mild. In addition, techniques and technicians are not always successfully transferred from one laboratory to another even though materials are standard and all water distilled and de-ionised.
Giemsa C-banding, whether in plants or animals, tends to be largely telomeric or largely pericentromeric in any one genome. However, even within a genome if banding is adequate all chromosomes can usually be identified. This means that the technique can often permit distinguishing between chromosomes indistinguishable on morphological grounds. It is particularly useful in plants which have genomes of similarly-sized metacentric chromosomes like most cereals. The chromosomes of the two parental genomes of these barley x rye hybrids are of very restricted size ranges and arm ratios so Giemsa C-banding is essential to identify the chromosomes involved in aneuploidy and to discover the origin of the altered chromosomes.

Results

Giemsa C-banded preparations from *Hordeum vulgare* cv. Sundance grain root-tips were prepared using the method in section 2.6. Barley C-banding was found to be less reproducible than that of rye but sufficient good cells could be found to prepare the karyotype given in fig. 5.10. Five good diploid cells (e.g. figs. 5.12 and 5.13) were used to prepare this karyotype which was then checked against other cells. The karyotype of Sundance as prepared by Linde-Laursen (1978a) is given in appendix 1 for comparison. All the major bands agree well but some differences of the minor bands may be due to slight differences in technique. The chromosomes are numbered 1B to 7B, as in Linde-Laursen (1975), and the numbers do not necessarily correspond to the linkage groups or wheat homoeology (Appendix 2).
Fig. 5.10  C-banded karyotype of barley cv. Sundance

Chromosome number (as Linde-Laursen 1975)

<table>
<thead>
<tr>
<th></th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
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<tbody>
<tr>
<td>Relative mean</td>
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<td>7.0</td>
<td>7.2</td>
<td>7.1</td>
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</tr>
<tr>
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<td>0.22</td>
<td>0.11</td>
<td>0.20</td>
<td>0.23</td>
<td>0.18</td>
<td>0.19</td>
</tr>
<tr>
<td>Arm Ratio mean</td>
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<td>1.14</td>
<td>1.10</td>
<td>1.12</td>
<td>1.11</td>
<td>0.96</td>
<td>1.23</td>
</tr>
<tr>
<td>SE</td>
<td>0.03</td>
<td>0.03</td>
<td>0.04</td>
<td>0.04</td>
<td>0.04</td>
<td>0.04</td>
<td>0.08</td>
</tr>
</tbody>
</table>

Fig. 5.11  C-banded karyotype of rye cv. Petkus Somro

Chromosome number (as Zeller et al. 1977)

<table>
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<tr>
<th></th>
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<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
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<tbody>
<tr>
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<td>7.3</td>
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<td>6.7</td>
<td>6.9</td>
<td>6.9</td>
<td>6.9</td>
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<td>length (%) SE</td>
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<td>0.18</td>
<td>0.25</td>
<td>0.24</td>
<td>0.17</td>
<td>0.18</td>
<td>0.15</td>
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<tr>
<td>Arm Ratio mean</td>
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<td>1.03</td>
<td>1.07</td>
<td>1.17</td>
<td>1.14</td>
<td>1.13</td>
<td>1.09</td>
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<tr>
<td>SE</td>
<td>0.05</td>
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<td>0.05</td>
<td>0.05</td>
<td>0.06</td>
<td>0.09</td>
<td>0.07</td>
</tr>
</tbody>
</table>
fig. 5.12 Barley cv. Sundance C-bands.

fig. 5.13 Barley cv. Sundance C-bands.

fig. 5.14 Rye cv. Petkus Somro C-bands.

Scale bars 10 μm.
The Giemsa C-banded karyotype of rye (fig. 5.11) was prepared from five good diploid cells from grain root-tips of Secale cereale cv. Petkus Somro (e.g. fig. 5.14) and, as with barley, then checked against additional cells. No differences between different plants of Petkus Somro were noticed although band polymorphism in an outbreeding cultivar must be possible. The karyotype agrees well with several published C-banded karyotypes and the correspondence between this and other karyotypes is shown in appendix 2. The chromosomes are numbered 1R to 7R to indicate their homoeology with wheat.

Root-tips from each hybrid cytotype (excluding cytotype C which had by then died out) were used to obtain Giemsa C-banded preparations. Karyotypes were produced by comparing several cells from each plant with the parental karyotypes. The results for the plants derived from the 'Sundance' x 'Petkus Somro' cross are presented in fig. 5.15. The presumed karyotype for cytotype C is also given because it is known to be derived from cytotype B by the loss of the acrocentric chromosome (see later). A C-banded karyotype is also presented for hybrid 20xR. This karyotype was not prepared by comparing the hybrid with barley and rye because its parental cultivars may have had different C-banding patterns to Sundance and Petkus Somro. Examples of cells and interpretative drawings from all the cytotypes are given (figs. 5.16, 5.17, 5.18 and 5.19).

The results for the Sundance x Petkus Somro hybrids show
Fig. 5.15  Barley cv. Sundance x rye cv. Petkus Somro hybrid karyotypes

CYTOTYPE A  $2n = 14$

CYTOTYPE B  $2n = 14 - ac$

CYTOTYPE C  $2n = 14$

CYTOTYPE D  $2n = 14$

- Deviation from n·n
fig. 5.16 Giemsa C-banded karyotype of cytotype A.
Scale bar 10 μm.
fig. 5.17  Giemsa C-banded karyotype of cytotype B.
Scale bar 10 μm.
fig. 5.18  Giemsa C-banded karyotype of cytotype D.  Scale bar 10 μm.
fig. 5.19 Giemsa C-banded karyotype of 20xR. Scale bar 10 μm.
that all lines had only six barley chromosomes. The missing barley chromosome in each case was one of the intermediate sized chromosomes, 2B. The marker chromosome in cytotype A was one of the nucleolar organiser chromosomes, 7B, and the deletion covers the entire satellite on the short arm. The acrocentric chromosome in cytotype B was shown to be the entire short arm of 1R with a small portion of the long arm. The only extra chromosomes were 1R (the rye nucleolar organiser chromosome) or a derivative of 1R.

Hybrid 20xR had all seven barley chromosomes present and unaltered. Two changes had affected rye chromosomes: a) the addition of one 1R, as in cytotypes A and C, and b) the apparent loss of a terminal block of heterochromatin from chromosome 2R. With a telomeric band the chromosome would closely resemble 2R. The arm ratio of this chromosome is about 1.2. In fig. 5.20 the effect on arm ratio of deletions of telomeres of 2R are given for Petkus Somro. The expected arm ratios indicate that the deletion of the long arm telomere was the most likely of the two but they differ only slightly and Petkus Somro was quite possibly not the rye parent for this hybrid. The suggestion that the long arm telomere was deleted was backed up when the banding pattern was compared with published results (e.g. Lelley et al. 1978). 2R had an interstitial band on the long arm which this chromosome appeared to have on the arm without a telomeric block of heterochromatin.
Fig. 5.20  Telomere deletion in 2R in hybrid 20xR

Observed 2R arm ratio : 1.2

<table>
<thead>
<tr>
<th></th>
<th>Predicted arm length</th>
<th>Predicted arm ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(from fig. 5.11)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Short arm</td>
<td>Long arm</td>
</tr>
<tr>
<td>Normal 2R</td>
<td>18.0</td>
<td>18.6</td>
</tr>
<tr>
<td>Short arm telomere deleted</td>
<td>16.5</td>
<td>18.6</td>
</tr>
<tr>
<td>Long arm telomere deleted</td>
<td>18.0</td>
<td>15.0</td>
</tr>
</tbody>
</table>

5.4 Discussion of the karyotypes and karyotype change

Barley

Giemsa C-banding in plants tends to be either largely pericentromeric or largely telomeric. Barley and rye each show one of these types: barley C-bands are mostly near the centromere and rye C-bands are mostly telomeric. Published work on barley C-bands has come from three groups (Linde-Laursen 1975, 1978a, b, 1979, Noda 1975, Noda and Kasha 1978 a, b, Vosa 1976). The karyotypes produced by each group are similar to each other and, with the exception of Vosa's, follow the same nomenclature (see appendix 2). The chromosome numbers were originally thought to represent the known linkage groups but Noda and Kasha (1978 b) have now proposed a revision of this (see appendix 2). A putative scheme of homoeologous classification of barley with wheat (and
hence also rye) has been prepared (Islam and Shepherd 1981 a) but the validity of this is doubtful partly because of insufficient evidence and partly because it is likely that several translocations will separate both barley and rye from wheat.

Banding patterns have been compared for different species of *Hordeum* (Linde-Laursen et al. 1980, Vosa 1976) as well as various cultivars of *H. vulgare* (Linde-Laursen 1978 a). Although there are some major differences between the species, particularly in the presence of telomeric bands in some species, the banding patterns of cultivars of *H. vulgare* are very similar. Differences between cultivars involve only minor band variations and many European spring cultivars are the same.

**Rye**

the size of the terminal heterochromatic blocks (Bennett et al. 1977, Singh and Röbellen 1975). The differences between cultivars of *Secale cereale* are also pronounced though all chromosomes remain readily identifiable (Giraldez et al. 1979). Even inbred lines of rye contain polymorphisms for some bands (Lelley et al. 1978).

Many workers have not attempted to match their rye karyotypes to the wheat homoeologous numbering system and there are disagreements amongst those who have (compare Zeller et al. 1977 with Bennett et al. 1977 and deVries and Sybenga 1976). Lelley et al. (1978) and others agree with Zeller's nomenclature and this has been adopted here.

The karyotypes presented here are similar to the published ones and there was no problem assigning chromosomes to the published numbering systems (see appendix 2). The differences between the karyotype presented here and a published karyotype (Linde-Laursen 1978 a) for Sundance were slight, as were the differences between this karyotype for Petkus Somro and published karyotypes (Bennett et al. 1977, Sarma and Natarajan 1973 and deVries and Sybenga 1976) for Petkus rye (which gave rise to Petkus Somro).

**Hybrids**

The results for the hybrids show that all plants, even those with $2n = 14$, were aneuploid. This surprising finding contrasts with the few published chromosome studies of barley x rye hybrids. Plants from the cross *Hordeum vulgare* (2x) x *Secale cereale* (2x)
have been reported from three laboratories other than ours (Fedak 1979, Kruse 1967, Thomas and Pickering 1979). In every case the chromosome number was reported to be $2n = 14$, and in one of these (Thomas and Pickering 1979) was confirmed as 7 barley + 7 rye by Giemsa C-banding.

Pickering and Thomas (1979) also reported a hybrid with about seven chromosomes, most of them barley, from a tetraploid barley x diploid rye cross. Crosses between other species of the same genera, Hordeum and Secale, (Finch and Bennett 1980, Wagenaar 1959, Wojciechowska 1978, 1979) have yielded hybrids with the expected chromosome number. It would appear that the loss or gain of chromosomes is not necessary for a viable hybrid to be produced, although the hybrids seem to be able to tolerate a higher degree of aneuploidy than the parents. The aneuploid hybrids grown from an embryo of the Sundance x Petkus Somro cross were much more vigorous than Kruse's hybrid. This could be due to different parental genotypes being used or could be the effect of losing 2B. Both types of hybrid had an extra 1R so the difference in vigour could not be due to different dosages of 1R.

It is possible to work out the timing of most of the chromosome changes which occurred in the Sundance x Petkus Somro hybrids given the following facts: cytotypes B, C and D were derived from one original regenerated plant; cytotype B was much more numerous than cytotypes C and D; the callus contained an altered 7B which must have been unaltered earlier in the growth of the callus.
summary of these events is given in fig. 5.21. The chromosome changes found in all plants, i.e. loss of 2B and gain of 1R, could have occurred at any time before the regeneration of the first plant but probably occurred after the formation of the hybrid zygote because aneuploid gametes from normal diploid parents are very rare. It should be noted that some chromosome changes must have occurred in the callus and others (only aneuploidy, not chromosome breakage) must have occurred in growing plants. A certain amount of karyotype change has not prevented regeneration in the callus, and indeed may have encouraged it, but the karyotype last seen in the callus (2n = 16, including a dicentric) is probably too altered to allow further regeneration. Regeneration in this callus has now ceased despite experiments with growth substances (Forster 1982). The chromosome variation that occurs during the growth of callus can be seen as a barrier to the use of callus as an intermediary stage between embryo and plant. An alternative view would be that callus can be used to produce a range of aneuploid cytotypes, some of which may be more vigorous than the original embryo and hence be more likely to survive. Without knowing in advance which parts of a genome will be undesirable in a certain hybrid, one way to achieve the most compatible combination of chromosomes is to allow the (presumably random) chromosome changes in the callus to create various combinations then regenerate the best of them!

It is surprising that some of the hybrids (cytotypes A to D)
Fig. 5.21  The timing of karyotype changes in hybrid cytotypes A to D

Chromosome events
1. Loss of 2B
2. Gain of 1R
3. Loss of part of 7B
4. Gain of part of 1R
5. Loss of part of 1R
6. Gain of further 1R
7. Gain of additional chromosomes including dicentric
have lost one barley chromosome or more without suffering any loss of vigour. Barley is tolerant of hyperaneuploidy (Fedak and Tsuchiya 1975, Sandfaer 1979, Tsuchiya 1960, 1969) but diploid hypoaneuploids (e.g. monosomics) are not known. It is possible that the addition of a 1R will compensate for the loss of 2B if there is any homoeology between the two. The information available on homoeology (see appendix 2) does not, however, indicate that this is the case. The hybrids have many morphological features closer to rye than barley (see section 5.5). The barley genome is probably expressed less than the rye hence the loss of a barley chromosome may be less critical.

The apparently greater vigour of the hybrids without 2B, as compared to Kruse's hybrid, may indicate that 2B carries genes deleterious to a hybrid. It is interesting that Kasha, Kao and Reinbergs (1970) reported that 4x interspecific Hordeum crosses usually produce 2x _H. vulgare_ plants but may occasionally produce 4x - 1 hybrids. The implication was that a specific chromosome may carry factors which control chromosome elimination. Two groups (Barclay, Shepherd and Sparrow 1972, Ho and Kasha 1975) have now shown that genes affecting chromosome elimination in _H. vulgare_ x _H. bulbosum_ crosses lie on both arms of 2B and also on the short arm of 3B. If the loss of 2B in our hybrids increased vigour because of the loss of 'eliminating' genes then it would be expected that the karyotype would remain stable. Chromosome loss and gain has been shown to occur in these plants. Chromosome
instability also continues in these plants giving hyperaneuploid and hypoaneuploid cells within meristems (see Chapter 6). This makes it rather difficult to accept that the loss of 2B simply reduces chromosome elimination in these hybrids.

One small change has been shown to affect one of the rye chromosomes in hybrid 20xR. 2R has lost the block of heterochromatin from the telomere of the long arm. This could have been a change in the hybrid or could have been present in the original rye parent. Giemsa C-banding studies of many rye cultivars (Giraldez et al. 1979, Lelley et al. 1978, Singh and Robellen 1975 and Vosa 1974) have failed to show that 2R exists in this form in normal rye so it is likely that the deletion of the telomere occurred in the hybrid. Deletion of this telomere has been reported from triticale. Seal and Bennett (1981) reported that in spring triticales one of the 2R telomeres is the most frequently deleted telomere. Deletion of rye telomeres has occurred in several triticale genotypes. The absence of one or more rye telomeres has been shown to reduce mitotic aberrants in the endosperm and reduce grain-shrivelling (Bennett and Gustafson 1982, Gustafson and Bennett 1982). The loss of a rye telomere in hybrid 20xR may have been a spontaneous but beneficial event or may have been caused by chromosome breakage at anaphase following incomplete separation of the telomeres.

The last, and most striking, of the chromosome changes which have occurred in these hybrids is the addition of extra copies
of 1R, either in its entirety or as an acrocentric chromosome with most of the long arm deleted. This change occurred in two different hybrids produced in two different places by different workers. In some of the plants three copies of the chromosome, or part of it, were present.

There are some indications in the literature that this chromosome, 1R, has a greater tendency towards aneuploidy than the other rye chromosomes. In a study of trisomics of rye produced from autotriploids, Kamanoi and Jenkins (1962) found that the rye nucleolar organiser-bearing chromosome was involved in trisomics about twice as frequently as any other chromosome. In addition 1R appears to give rise to derivatives by breaking on the long arm near the centromere. Singh and Robellen (1975) report that the four B-chromosomes in rye cultivar Transbaikal resemble the short arm of 1R plus a small segment of the long arm. Zeller et al. (1977) found two unusual aneuploids during a search for trisomics in cultivar Heines Hellkarn. One of these was a telocentric 1R short arm very similar to the one in cytotype B; the other resembled a 1R short arm isochromosome.

Even with this increased tendency towards aneuploidy of 1R the boosting of this chromosome, or its acrocentric derivative, to three copies in an otherwise dihaploid background may indicate that 1R confers some advantage on hybrids carrying more copies than expected. Genome dosage is important in Hordeum vulgare x H. bulbosum crosses with only a 1:2 ratio of H. vulgare to
H. bulbosum giving hybrids (Kasha and Sadasivaiah 1971). There is no reason to suppose that the whole genome has to be present - perhaps one chromosome extra would be sufficient. It also appears that if this is the case then the short arm of 1R carries the useful genes. One intriguing possibility is that boosting the number of rye nucleolar organisers compensates for the fact that the rye nucleolar organisers in these hybrids are suppressed. This topic will be more fully explored in Chapters 7 and 8.

5.5 Plant morphology

Introduction

A study of the morphology of the hybrids can be useful for several reasons. The hybrids are known to be aneuploids. Any features found in hybrids with a specific change affecting one chromosome may be attributable to genes on that chromosome. In this way, a contribution may be made to the available knowledge on linkage groups and gene mapping.

Hybrids between species of equal chromosome number ought to be intermediate in morphology. It is worth comparing the hybrids with barley and rye to investigate this. Many Gramineae (Poaceae) hybrids do tend to resemble one parent more than another, so some kind of 'genome dominance' may occur.

Hybrids may also have features not inherited from either parent but produced as a result of the interactions between genomes or between one genome and the cytoplasm of the other species. The barley x rye hybrids will carry cytoplasm from both parental
species; barley cytoplasm through the oocyte and some rye cytoplasm will be inherited from the pollen (Fröst, Vaivars and Carlbom 1970). If the reciprocal cross had been possible, the hybrid would have inherited only rye cytoplasm. Male sterility, for example, (anthers poorly developed or transformed to other structures) has been attributed to nucleus/cytoplasm interactions in hybrids (Islam, Shepherd and Sparrow 1975; Pearson 1972).

Results

The different hybrids have been assessed for various characters, both quantitative and qualitative, and compared with each other and the parents. The results are summarised in table 5.1 and 5.2. Photographs of spikes of barley, rye and hybrid cytotype A are provided (figs. 5.22, 5.23 and 5.24). One line (cytotype C) had died out before its morphology could be studied but it was similar to the other Sundance x Petkus Somro cytotypes except in its shorter stature and freer tillering habit.

Cytotype B had been measured for tiller, ear and awn length at the beginning of the project and hence could be compared with plants of the cytotype 2 1/2 years later. The subjective impression that the plants were reduced in stature with time was confirmed. The reason for the reduction in vigour is not known.

The only characters which separated the three Sundance x Petkus Somro cytotypes were height (B>D>A), ear length (B>D, A), tillering rate (A>B, D) and number of nodes (B, D>A). These characters vary with time and growth conditions and could not be
fig. 5.22  Spike of barley
  cv. Sundance.

fig. 5.23  Spike of rye
  cv. Petkus Somro.

fig. 5.24  Spikes
  of hybrid cytotype A.
relied upon to distinguish the cytotypes. The three cytotypes were identical in terms of all the more taxonomically reliable characters.

Hybrid 20xR differed from the above in several respects. It was smaller in all its dimensions and in the following characters was different to the Sundance x Petkus Somro cytotypes: fewer and smaller lemma hairs, pubescence on leaf blades and below collar, paler in colour, rye-like auricles, profuse tillering, ligule present, larger awn spicules and reflexing mature spike.

Table 5.2 shows the comparisons between the hybrids and the parents. In some respects the hybrids were intermediate but many characters were more rye-like. The sizes of the plants were less than barley or rye indicating a reversal of hybrid vigour. The genomes are probably too distant to be able to work together well to produce healthy, vigorous hybrids.

Some features of the hybrids were not found in either parent: they had more surface wax than rye or barley, pistillody (see glossary) was found and some other occasional morphological aberrations were seen. The most frequent of these aberrations are presented in fig. 5.25. These aberrations were found much more frequently in the Sundance x Petkus Somro cytotypes (about 10% of tillers had at least one aberration) but also occasionally appeared in hybrid 20xR.
Table 5.1  Morphology of Hybrid Cytotypes

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<tr>
<th></th>
<th>Cytotype A</th>
<th>Cytotype B</th>
<th>Cytotype B</th>
<th>Cytotype D</th>
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<td>2B</td>
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<td>Chromosomes Gained</td>
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<td>1\frac{1}{2} @ 1R</td>
<td>2 @ 1R</td>
<td>1R</td>
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<td>1) Tiller height (cm)</td>
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<td>52</td>
<td>58</td>
<td>47</td>
<td>31</td>
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<td>2) Ear Length (mm)</td>
<td>49</td>
<td>49</td>
<td>55</td>
<td>38</td>
<td>32</td>
</tr>
<tr>
<td>3) Spikelets per Spike</td>
<td>10</td>
<td>11</td>
<td>-</td>
<td>11</td>
<td>11</td>
</tr>
<tr>
<td>4) Awn Length (mm)</td>
<td>31</td>
<td>45</td>
<td>49</td>
<td>35</td>
<td>18</td>
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<tr>
<td>5) Flag Leaf Length (mm)</td>
<td>83</td>
<td>88</td>
<td>-</td>
<td>83</td>
<td>77</td>
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<tr>
<td>6) Largest Glume (mm)</td>
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<td>19</td>
<td>-</td>
<td>28*</td>
<td>10</td>
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<td>7) Lemma Hairs</td>
<td>30</td>
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<td>-</td>
<td>30</td>
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<td>- No.</td>
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<td>- Size (mm)</td>
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<td>8) Pubescence below collar</td>
<td>No</td>
<td>No</td>
<td>-</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>+ lf. blade</td>
<td>++</td>
<td>++</td>
<td>-</td>
<td>++</td>
<td>+</td>
</tr>
<tr>
<td>9) Waxiness</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>10) Hairs on Rachis</td>
<td>Yes</td>
<td>Yes</td>
<td>-</td>
<td>Yes</td>
<td>Yes(larger)</td>
</tr>
<tr>
<td>11) Awn Spicules</td>
<td>Inter-</td>
<td>Inter-</td>
<td>-</td>
<td>Inter-</td>
<td>Rye-like</td>
</tr>
<tr>
<td>12) Auricle</td>
<td>med.</td>
<td>med.</td>
<td>-</td>
<td>med.</td>
<td></td>
</tr>
<tr>
<td>13) Ligule</td>
<td>very</td>
<td>very</td>
<td>-</td>
<td>very</td>
<td>1 mm</td>
</tr>
<tr>
<td>small</td>
<td>small</td>
<td>small</td>
<td>-</td>
<td>small</td>
<td></td>
</tr>
<tr>
<td>14) No. of nodes</td>
<td>3-4(-5)</td>
<td>4(-6)</td>
<td>-</td>
<td>4</td>
<td>(2-)3(-4)</td>
</tr>
<tr>
<td>15) Reflexion of mature ear</td>
<td>No</td>
<td>No</td>
<td>-</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>16) Leaf colour</td>
<td>Dark</td>
<td>Dark</td>
<td>-</td>
<td>Dark</td>
<td>Light</td>
</tr>
<tr>
<td>17) Tillering</td>
<td>Few</td>
<td>Few</td>
<td>-</td>
<td>Few</td>
<td>Many</td>
</tr>
<tr>
<td>(but B)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>18) Spikelet Structure</td>
<td>Rye-like</td>
<td>Rye-like</td>
<td>-</td>
<td>Rye-like</td>
<td>Rye-like</td>
</tr>
<tr>
<td>19) Lemma shape</td>
<td>Rye-like</td>
<td>Rye-like</td>
<td>-</td>
<td>Rye-like</td>
<td>Rye-like</td>
</tr>
<tr>
<td>20) Pistillody</td>
<td>Yes</td>
<td>Yes</td>
<td>-</td>
<td>Yes</td>
<td>Occasional</td>
</tr>
</tbody>
</table>

* Possibly an abnormal glume. All measurements means of ten samples.
Discussion

The results have shown that there are a number of characters which vary between the different aneuploid hybrids. The differences between cytotypes A, B and D however were minimal and related to

<table>
<thead>
<tr>
<th>Category</th>
<th>Character</th>
</tr>
</thead>
<tbody>
<tr>
<td>Barley-like</td>
<td>Reflexion of mature spikes (hybrid 20xR only)</td>
</tr>
<tr>
<td>Rye-like</td>
<td>Awn length, awn spicules, glume size, lemma shape, hairs on rachis, spikelet structure, auricle (20xR only)</td>
</tr>
<tr>
<td>Smaller</td>
<td>Tiller height, ear length, spikelet number, flag leaf length</td>
</tr>
<tr>
<td>Unlike both parents</td>
<td>Waxiness, pistillody</td>
</tr>
</tbody>
</table>

differences in vigour, leading to different tiller and spike sizes.

The only useful indications of genetic differences between aneuploid lines can be gained by comparing Kruse's hybrid with the Sundance x Petkus Somro hybrids. Unfortunately the parental cultivars of hybrid 20xR were not known with certainty so the differences due to different genetic background and the differences due to aneuploidy (loss of 2B) cannot be separated. Knowing the morphological differences between a range of rye cultivars it was possible to tentatively assign some of these characters to the different rye parent and hence others to the presence of chromosome 2B. The
results must, however, be used with care. I would tentatively suggest that the following characteristics of hybrid 20xR, not found in the other cytotypes, were because of a different rye parent: differences in lemma ciliation, pubescence on leaf blades and below collar, larger ligule and larger awn spicules. Characters more likely to be due to the presence of 2B included: reflexing of the mature spike and, possibly, more rye-like auricles.

All hybrids had extra copies or an extra copy of 1R so comparisons between hybrids will not indicate the effect of increased dosage of 1R. The hybrids can, however, be compared with normal rye and rye trisomics for 1R. Kamanoi and Jenkins (1962) have described the 1R trisomic which they call 'bush'. There were a few differences between trisomics at the seedling stage, which cannot be used with this clonally propagated material, but the mature plants had fewer, thinner and more erect tillers than normal diploids. Characters such as these were not easily identified in hybrids where two genomes are interacting.

An attempt was made (table 5.2) to assign characters of the hybrids to barley or rye. This table confirmed the rye-like nature of the hybrids. This can be explained in two ways. The hybrids were aneuploid with less barley DNA and more rye DNA than the predicted gametic sum. Alternatively, rye genes may be 'dominant' to barley genes. Other workers have reported that their euploid barley x rye hybrids tended to resemble rye more than barley (Fedak 1979, Kruse 1976, Thomas and Pickering 1979).
cereal hybrids also resembled one parent more than the other
(Ahokas 1970, Lange 1971 a). There are no explanations for this
except that one genome may be dominant over another in a similar
way to allelic dominance within species. If genes of one species
do tend to suppress those of another then it would be expected that
the r RNA genes at the nucleolar organiser would follow the same
fate. Most interestingly, in the following hybrids: Hordeum
vulgare x Hordeum bulbosum (Lange 1971 b), Triticum ovatum (L.)
Raspail (Aegilops ovata L.) x Elymus arenarius L. (Ahokas 1970),
and Hordeum vulgare x Secale cereale (Fedak 1979; Thomas and
Pickering 1979; this thesis - Chapter 7), the genome apparently
more expressed in the hybrid had suppressed nucleolar organiser.


There is, as yet, no adequate explanation for this:

A range of morphological aberrations was found in the
hybrids (see fig. 5.25). They can be classified into two distinct
groups. One group, containing the majority of these aberrants,
has a variety of structures split or duplicated. The other group
contains structures converted, or partially converted to different
organs.

The first of these two groups of aberrants have several
features in common. In most circumstances one organ has become
two. These are often of a similar size, comparable with a normal
one, and are always at the same stage of development. One
explanation could cover all of these aberrations. A split in the
meristem, for whatever reason, could result in any of the observed
**Morphological aberrations in barley x rye hybrids**

Fig. 5.25

a) Normal tiller  
b) Double leaf sheath and blade.  
c) Split tiller  
d) Double spike  
e) Split spike  
f) Supernumerary spikelets

Other aberrations are: malformed nodes, collar part sheath, twisted spike, supernumerary glumes or lemmas, twisted awns and pistillody.

Aberrations, depending on the stage of development of the meristem when the split occurred. The conjectured mechanism for this is given in fig. 5.26. The event giving rise to a group of inviable cells in the centre of the meristem could be a change in karyotype, possibly the loss of chromosomes, in a cell in the centre of a meristem. Chromosome instability in meristems of these hybrids.
does occur (Chapter 6). Moreover, Kruse's hybrid (20xR) has a lower frequency of aberrations and also shows a reduced incidence of chromosome instability (Chapter 6), which supports the suggestion of a connection between chromosome instability and morphological aberrations.

**Fig. 5.26** Possible cause of some morphological aberrations

Double or split structures in hybrids may be caused by a cytological lesion dividing a meristem in two.

| Initial event, possibly chromosome loss or change | Formation of a lesion | Cessation of division of the lesion | Splitting of the meristem |

Such a split in a primary meristem could cause a split tiller, double spike or split spike, whereas a similar event in a ridge could cause double leaf sheath and blade, supernumerary spikelets or supernumerary glumes or lemmas.

Other types of morphological aberrations, for example, where one structure has become converted to another, occurs exclusively with reproductive structures. This has been fully described in Chapter 4.
6. MITOTIC CHROMOSOME INSTABILITY

6.1 Introduction

Reports of the occurrence of chromosome instability occur almost as far back as the beginnings of cytogenetics. Instability of chromosome numbers falls into three partially overlapping categories: instability between generations caused by meiotic anomalies; instability within plants affecting large groups of chromosomes; and instability within plants affecting one or more chromosomes at a time. Only the last two categories will be considered here. Chromosome instability manifested by euploid jumps in chromosome number has been called polysomaty. The alternative type of somatic chromosome instability, fluctuations in chromosome complement around a euploid number, has been given the term aneusomaty (Duncan 1945). In practice there is no clear distinction between the two as polysomaty is often accompanied by aneusomaty.

Both types of chromosome instability, as well as other chromosome changes such as translocations, are so common in cultured plant cells that they are considered normal (for reviews see Partanen 1963, and Sunderland 1973). Chromosome instability has been noted in callus cultures of both barley (Novak 1980, Saalbach and Koblitz 1977, and Scheunert et al. 1978) and rye (Asami et al. 1976). Chromosome instability in vitro is thought to occur because the normal restraints on cell division are lifted, due to the influence of the growth substances used to induce proliferation. However, genetic changes during callus culture may
also be important because chromosome instability has been noted in
some plants regenerated from callus, e.g. *Saccharum* (Heinz and
Instability did not affect all regenerated plants in the above examples.

Other cases of chromosome instability are usually associated
with high polyploidy or, more usually, hybridity. Some early reports
of aneusomaty, however, referred to apparently normal diploid
plants (Duncan 1945, Sharma and Bhattcharya 1956). Many more
reports referred to both polysomaty and aneusomaty in high polyploids
(e.g. Carlbom 1962, Fukumoto 1962, Gottschalk 1971, Hegwood and
Hough 1958, Jones and Bamford 1942, Khoshoo and Raina 1971, Lewis
1962, Snoad 1955, Tan and Dunn 1977, Thompson 1962; and Vaarama
1949).

Hybrids are rich sources of various types of chromosome
instability. Many hybrids are polyploid and some have been through
a phase of tissue culture so the true causes of chromosome instab-
ility are often obscured. However it is clear that the largest
category of reports of chromosome instability refer to hybrids whose
parental species are otherwise chromosomally stable. The
majority of these cases of chromosome instability are found in wide
species hybrids or intergeneric hybrids. Chromosome instability
has been seen in somatic tissues of hybrids (Dowrick 1953, Finch
1969, Menzel and Brown 1952, Moav and Cameron 1960, Murrey
and Craig 1964, Nielsen and Nath 1961, Ono and Sakai 1952,

Chromosome instability in hybrids often causes the elimination of one of the contributing genomes. Interspecific hybrids in the genus Hordeum often lose chromosomes of one genome - approximately 20 reports of this phenomenon will be discussed in Chapter 8. Other hybrids showing genome elimination have been reported by Barclay 1975, Brown 1947; Fedak 1977 b, Islam and Shepherd 1981 b, Ladizinsky and Fainstein 1978, Shigenobu and Sakamoto, 1977, and Szilagyi 1975. Genome elimination often occurs during embryogenesis but may also occur later in development.

There are several old reports of androgenic progeny from interspecific crossing. It was once thought that these were due to pollen nuclei developing into embryos but it now seems more likely that elimination of the maternal genome, following fertilisation, occurred in most or all of these cases.

Somatic cell hybrids, produced by fusing cells of different species, also show chromosome instability. These include both plant hybrids (Kao 1977) and animal hybrids (Croce et al. 1977, Graves 1972, Pontecorvo 1971, Miller et al. 1976, and Weiss and Green 1967).
Chromosome instability can be produced in several ways. Non-disjunction can arise if both sides of the centromere attach to spindle fibres from one pole, or if spindle fibres become attached to one side of the centromere only. This would produce two aneuploid daughter cells, one with an extra chromosome and one with one chromosome missing. Alternatively, chromosomes can fail to move to the metaphase plate and give rise to lagging chromosomes which may then be eliminated as micronuclei or included in one of the two daughter nuclei. Another cause of chromosome instability is split spindles. In cells with a split spindle one to several chromosomes may be eliminated in the form of a micronucleus. Examples of each of these mitotic faults can be found in the literature. Non-disjunction was noted by Dowrick (1953). Lagging chromosomes at anaphase have been seen in many hybrids (Barclay 1975, Bennett et al. 1976, Cooper 1978, Dowrick 1953 and Thomas and Pickering 1983 a). Split spindles are also reported from hybrids (Ladizinsky and Fainstein 1978, Nielsen and Nath 1961 and Thomas and Pickering 1983 a).

Such instability of chromosome numbers in hybrids has several consequences. Certain genome combinations may be impossible unless chromosome instability is understood and overcome. The vigour of plants with a tendency to lose or gain chromosomes may be reduced. Sexually propagated plants will suffer reduced fertility and increased variability if chromosome instability persists in the germ line.
Thus it is important to assess the extent of chromosome instability in any new hybrid. In addition, few studies have attempted to explain why the chromosomes in a hybrid are unstable and those that have are based almost entirely on speculation. An explanation of both how and why such instability occurs is essential before success in improving crops through wide crosses can be achieved.

A study of the extent of aneusomy in the barley x rye hybrids was undertaken for the above reasons. Chromosome change in these hybrids was known to have occurred both before and after regeneration from the callus (see Chapter 5). The next step was to determine if the instability which generated chromosome change persisted in the hybrids and, if it did, to discover which tissues were involved, whether the karyotype had any effect, and how such instability in chromosome number arose.

6.2 Observations on the hybrids

The pectinase/orcein technique (see section 2.5) was used for hybrid plants. The boundaries of each cell could be seen using this method and chromosome counts were made from unbroken cells only. Callus was stained by the Feulgen method hence the chromosome counts for callus may be less reliable due to undetected breakage of cells during the preparation of slides.

The results are presented in fig. 6.1. The callus had the highest degree of aneusomy with nearly half of all cells having chromosome numbers other than the most frequent which was
Fig. 6.1 Somatic and germ-line aneusomaty in barley x rye hybrids

**CALLUS**

<table>
<thead>
<tr>
<th>CHROMOSOME NUMBER</th>
<th>% ANEUSOMATY</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td></td>
<td></td>
</tr>
<tr>
<td>11</td>
<td></td>
<td></td>
</tr>
<tr>
<td>12</td>
<td></td>
<td></td>
</tr>
<tr>
<td>13</td>
<td></td>
<td></td>
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<tr>
<td>14</td>
<td></td>
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</tr>
<tr>
<td>15</td>
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<td></td>
</tr>
<tr>
<td>16</td>
<td></td>
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<tr>
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<tr>
<td>18</td>
<td></td>
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</tr>
<tr>
<td>28</td>
<td></td>
<td></td>
</tr>
<tr>
<td>30</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

n = 63

% ANEUSOMATY 46%

**HYBRID CYTOTYPE A**

<table>
<thead>
<tr>
<th>CHROMOSOME NUMBER</th>
<th>% ANEUSOMATY</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>12</td>
<td></td>
<td></td>
</tr>
<tr>
<td>13</td>
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<td></td>
</tr>
<tr>
<td>14</td>
<td></td>
<td></td>
</tr>
<tr>
<td>15</td>
<td></td>
<td></td>
</tr>
<tr>
<td>16</td>
<td></td>
<td></td>
</tr>
<tr>
<td>17</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

n = 366

% ANEUSOMATY 18%

**HYBRID CYTOTYPE B**

<table>
<thead>
<tr>
<th>CHROMOSOME NUMBER</th>
<th>% ANEUSOMATY</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>14</td>
<td></td>
<td></td>
</tr>
<tr>
<td>15</td>
<td></td>
<td></td>
</tr>
<tr>
<td>16</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

n = 77 (64)

% ANEUSOMATY 32.7% (19:1)

--- (ignoring acrocentric loss)

**HYBRID 20xR**

<table>
<thead>
<tr>
<th>CHROMOSOME NUMBER</th>
<th>% ANEUSOMATY</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>14</td>
<td></td>
<td></td>
</tr>
<tr>
<td>15</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

n = 46

% ANEUSOMATY 9%

**HYBRID 20xR**

<table>
<thead>
<tr>
<th>CHROMOSOME NUMBER</th>
<th>% ANEUSOMATY</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>13</td>
<td></td>
<td></td>
</tr>
<tr>
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<td></td>
<td></td>
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<tr>
<td>15</td>
<td></td>
<td></td>
</tr>
<tr>
<td>16</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

n = 283

% ANEUSOMATY 14%
$2n = 16$. Most of this observed aneusomaty is likely to be genuine and not due to cell breakage because there were many cells with more than the most frequent number (i.e. $2n > 16$). Only two petri dishes of callus were sampled, and the karyotype differed from those of the callus-derived plants. The degree of aneusomaty seen in the callus is typical of that of cultured plant cells and is likely to be representative of the barley x rye hybrid callus as a whole.

Hybrid plants from the cross Sundance x Petkus Somro belonged to four cytotypes but for only two of these, cytotypes A and B, was sufficient material available for this study. These plants did not produce anthers (see section 6.2) so only root-tips and not pollen mother cells were used. Aneusomaty in cytotype A ($2n = 14$) root-tips affected 18% ($± 4\%$ at 95% confidence limits) of all dividing cells (fig. 6.1, an example given in fig. 6.2). This was likely to be an under-estimate of the total number of cells affected because the event causing aneusomaty must have occurred at the previous mitosis and it was likely that a greater proportion of affected cells will fail to re-enter mitosis. In cytotype A there was a more or less even distribution of aneuploid cells above and below the modal number. Micronuclei were very rare in these plants.

Cytotype B ($2n = 15$) had a karyotype which differs from that of cytotype A by the presence of a rye-derived acrocentric chromosome and the lack of a deletion on chromosome 7B (see section 5.3). This cytotype had a much higher rate of aneusomaty at 32% (fig. 6.2, an example given in fig. 6.3). A large part of this aneusomaty can
fig. 6.2  Hypermodal cell (2n = 15; one extra) of cytotype A. Feulgen.

fig. 6.3.  Hypermodal cell (2n = 15½; one extra) of cytotype B. Feulgen.

fig. 6.4  Lagging acrocentric chromosome at metaphase in root tip meristem of cytotype B. Probable source of occasional micronuclei seen in this cytotype. Acetocarmine.

Scale bars 10 µm.
be explained by the loss of the acrocentric chromosome. If the cells with 14 chromosomes and no acrocentric chromosome were discounted, the rate of aneusomaty fell to a level very close to that of cytotype A, 19%. The anomalous behaviour of the acrocentric chromosome was confirmed by looking at normal metaphases. On several occasions the acrocentric chromosome was seen to be lying away from the metaphase plate (see fig. 6.4). This non-congregation of the acrocentric chromosome often resulted in the formation of a micronucleus and approximately 1% of all interphase cells in the root-tip meristem had a micronucleus. Some root-tips had a modal chromosome number of 2n = 14 and appeared to have lost the acrocentric chromosome entirely.

Barley x rye hybrid 20xR (2n = 15) also showed chromosome instability. In root-tips only 9% of all mitotic cells had chromosome counts other than 2n = 15, the modal number. This was distinctly lower than in the root-tips of cytotypes A and B but the small sample size (46) means that the difference may not be significant. Pollen mother cells, scored at diakinesis or metaphase I, had 14% of cells aneuploid. This rate of aneusomaty was intermediate between root-tips of the Sundance x Petkus Somro and hybrid 20xR. It appeared that aneusomaty in hybrid 20xR was greater in pollen mother cells than in root-tip meristem cells. Analysis of interphase cells failed to reveal any micronuclei in somatic or pre-meiotic cells.

The counts of chromosome numbers in pollen mother cells
were obtained from cells with all chromosomes together in one group. Very occasionally pollen mother cells were seen with a distinct group of extra chromosomes towards the side of the cell (see fig. 6.5). These were probably inherited from a neighbouring cell during a previous division by cytomixis (as described by Sarvella 1958) and were excluded from the counts.

Confirmation that aneusomaty exists in hybrid 20xR has come from electron microscope studies. Dr. M. D. Bennett (pers. comm.) has studied the relative positions of chromosomes in root-tip metaphases in these plants using serial sections and has found aneuploid cells.

The pattern of aneusomaty can be seen from the histograms in fig. 6.1. All hybrids gained as well as lost chromosomes. This implies that non-disjunction of metaphase chromosomes was taking place rather than (or as well as) the elimination of lagging chromosomes. This is reinforced by the lack of micronuclei in most of the hybrids. Amongst dividing cells in cytotype A, hypomodal and hypermodal chromosome numbers were found in approximately equal proportions, and cytotype B was the same if the figures were adjusted by omitting the loss of the acrocentric chromosome.

---

1. The terms hypomodal and hypermodal have been used to indicate variations from the chromosome number found in most cells of a cytotype. As the cytotypes are themselves aneuploid, the terms hypoaneuploid and hyperaneuploid are misleading.
fig. 6.5  Hybrid 20xR hypermodal PMC (2n = 20; 5 extra). Lacto-propionic orcein.

fig. 6.6  Hybrid 20xR hypermodal root tip meristem cell (2n = 16; one extra). Probably 7 barley + 9 rye. Giemsa.

Scale bars 10 μm.
Hybrid 20xR and the callus which gave rise to cytotypes A to D displayed a different pattern of chromosome instability with more cells hypomodal than hypermodal.

The identification of chromosomes involved in aneusomaty could not be done on the basis of chromosome size (see section 5.2). The C-banding technique was found to be best with chromosomes extruded from cells, so the aneuploidy of many cells may have been an artefact. Very few complete, hypermodal cells remained and only two of these from cytotype A and two from 20xR (e.g. fig. 6.6) were banded. All cases of extra chromosomes involved the addition of one rye chromosome. There was some suspicion that barley chromosomes were also involved in aneusomaty from counts of nucleoli in interphase cells (see Chapter 7).

One observation of relevance to the chromosome instability in these hybrids was the presence of variegation. A small number of tillers (around 1%) contained white sectors of varying sizes. These usually took the form of streaks running the length of a leaf blade and sheath and sometimes extended to more leaves or part of the spike. This pattern means that the white areas originated in the apex or in a sub-apical node and appeared along the length of organs developed from these meristems. The variegation could have resulted from segregation of mutant chloroplasts or could have been caused by chromosome instability.

6.3 Discussion
Aneusomaty

The degree of aneusomaty in mitotic cells of these hybrids was surprisingly high at 10 to 20%. The figure for interphase cells may have been even higher because of the possibility that fewer variant cells will re-enter mitosis and will be detected by chromosome counts. Aneusomaty has already been seen in barley x rye hybrids. Fedak (1979 a) obtained a $2n = 14$ hybrid and found chromosome numbers between 14 and 18 in meiocytes. Finch and Bennett (1980) found a few hypo- and hyperaneuploid cells in hybrids between wild species of Hordeum and Secale. Thomas and Pickering (1979) reported aneusomaty in root-tips of hybrids between Hordeum vulgare and Secale cereale cultivars.

The amount of aneusomaty found in our hybrids must have implications for the vigour of the plants. Tissues containing a high proportion of aneuploid cells are unlikely to be as efficient as those containing entirely euploid cells. The barley x rye hybrids had much poorer root systems than the parental species. The roots were often smaller and were much less numerous, rarely forming a matted mass in the pot. Poor root systems have been recorded for other interspecific hybrids (Brock 1954; and Vosa 1966). Such a depression of growth of the roots could have been due to the effects of aneusomaty.

It is possible that groups of cells or even whole tissues of a variant karyotype could arise if such cells continued dividing. To distinguish between repeated chromosome loss or gain giving rise
to many small groups of variant cells and rare events but with continued division giving large areas of variant karyotype would require sectioning. However, if this was the case then these karyotypes would probably have been found repeatedly within one root-tip. Different chromosome numbers appeared randomly and were not clustered in one preparation so a mosaic distribution was more likely. Cells with aberrant karyotypes are likely to require plasmodesma contacts with neighbouring cells to replace some missing gene products so could only exist in small groups. In addition such cells may have been less able to undergo division.

The situation in green tissue may be different. Stripes of white tissue appeared in leaves from time to time. They probably indicated the presence of sectorial or mericlinal chimaeras in some meristems. The cause could have been either cytoplasmic or chromosomal. Unstable chromosomes have been recorded as causing variegation in leaves from many plants, including hybrids (Moav and Cameron 1960; Monti and Saccardo 1969; McClintock 1932, 1938; and Saccardo 1971). Petals from hybrid Nicotianas can show colour variegation (Sand 1957). The presence of chromosomal chimaeras in the barley x rye hybrids could explain the observed variegation. Other morphological aberrants seen much more frequently were the split or extra structures as described in section 5.5. These could have arisen from aborted incipient chimaeras as depicted in fig. 5.26.

The aneusomaty found in these barley x rye hybrids was not
accompanied by the presence of micronuclei nor were any lagging chromosomes found except in cytotype B. Here there was a clear link between the acrocentric chromosome not congressing at metaphase, the increased frequency of hypomodal cells and the presence of micronuclei in about 1% of interphase cells. So, with the exception of the acrocentric chromosome, instability of chromosome number was caused, at least largely, by something other than the elimination of lagging chromosomes at telophase. One possibility is that one or more chromosomes did not congress normally and tended to lie nearer to one pole than the other, so that both daughter chromosomes became included in one group of chromosomes at telophase. Analysis of large numbers of metaphase cells from several hybrids without colchicine treatment failed to demonstrate any abnormal chromosome behaviour, except that of the acrocentric chromosome. An alternative way of generating aneusomaty without visibly abnormal metaphases would be to attach the centromeres from both chromatids of one chromosome to spindle fibres from one pole. At anaphase both chromatids would migrate to the same pole. This would generate hyper- and hypomodal cells without micronuclei or unusual metaphase plates. It is tentatively suggested that this is the mechanism of aneusomaty in these hybrids.

The relative numbers of hyper- and hypomodal cells varied between hybrids. The Sundance x Petkus Somro cytotypes had equal amounts of both. Hybrid 20xR, on the other hand, had more hypomodal than hypermodal cells. Hybrid 20xR had 15 chromosomes
so any increase in chromosome number will mean that the complement was further from the euploid number whereas the loss of one chromosome could possibly be equivalent to the gametic sum. This karyotype may have been "fitter" and divided more readily causing a skewed distribution of aneusomaty with a tendency towards hypomodality.

The results for the pattern of aneusomaty for the barley x rye hybrids presented here differ from those of Thomas and Pickering (1979). They found a marked tendency towards hypomodal complements in their barley x rye hybrids, with only 58 out of 100 cells diploid and euploid. Both genotype and karyotype differed from the hybrids in this study. Their staining method was also different and it is conceivable that broken cells have been included in their counts.

The degree of aneusomaty in pollen mother cells and in root-tip meristems was compared in hybrid 20xR. Pollen mother cells were more variable than root-tip meristem cells (fig. 6.1). Increased instability in meiocytes as compared to somatic cells has been recorded in interspecific hybrids in Hordeum (Orton 1980 and Noda and Kasha 1981 a, b) and Nicotiana (Gupta and Gupta 1973). Pre-meiotic mitosis in anthers is synchronous, at least in cereals (Bennett et al. 1971), and it is possible that mitotic faults become more serious in synchronous tissues. Endosperm has synchronous divisions and often has seriously disturbed mitosis in hybrids.

The occurrence of extra groups of chromosomes in pollen
mother cells of hybrid 20xR has already been mentioned. These were found very rarely and do not appear to be a major cause of aneusomaty. The occurrence of chromatin from the nucleus of one pollen mother cell in the cytoplasm of an adjacent pollen mother cell was described and termed cytomixis by Sarvella (1958) in *Gossypium* hybrids. The mechanisms involved are probably split spindles and aberrant wall formation.

The sort of chromosome instability seen in these hybrids, small variations above and below the modal chromosome number, could easily have given rise to plants with different karyotypes. A chromosome change which did not adversely affect the ability of the affected cell to grow, divide and differentiate could easily have spread throughout a meristem and hence have given rise to a 'sport'. Some bud sports in chrysanthemums are thought to have arisen in this way (Dowrick 1953). There is evidence that this did take place in the barley x rye hybrids. Both cytotype C and cytotype D were derived from cytotype B. Chromosome loss (cytotype C) and chromosome gain (cytotype D) were involved (see Chapter 5).

**The relationship between aneusomaty and elimination of genomes in hybrids**

Before considering the causes of chromosome instability it is desirable to determine whether the two main categories of hybrid chromosome instability, aneusomaty and genome elimination, are symptoms of the same cause or are entirely different. Aneusomaty, present in some tissues of a hybrid, can be transformed into genome
elimination in other tissue types. There are several ways in which this transformation could take place, depending on the cause of aneusomaty (fig. 6.7).

**Fig. 6.7** Hypothetical relationship between aneusomaty and genome elimination

<table>
<thead>
<tr>
<th>ANEUSOMATY</th>
<th>GENOME ELIMINATION</th>
</tr>
</thead>
<tbody>
<tr>
<td>non-disjunction</td>
<td>increase in non-disjunction and/or selection for lower euploid complements.</td>
</tr>
<tr>
<td>lagging chromosomes</td>
<td>lagging chromosomes excluded from daughter nuclei.</td>
</tr>
<tr>
<td>included in daughter nuclei</td>
<td></td>
</tr>
<tr>
<td>small extra spindles</td>
<td>large extra spindles</td>
</tr>
</tbody>
</table>

The switch from aneusomaty to genome elimination would be favoured by selection for haploid complements (through an increased rate of cell division) and by increasing the amount of chromosome instability. In addition, a simple change, such as increasing the cell size and hence increasing the likelihood of lagging chromosomes being eliminated, may be important.

**Theories on the causes of chromosome instability**

Chromosome instability is found in a variety of types of cell including cell cultures, regenerated plants, polyploids and hybrids. Chromosome instability appears to be nearly ubiquitous in cells in culture. This instability is sometimes passed on to plants regenerated from callus cultures, although only some plants are affected. This has been explained variously as an indication that regenerated plants are chimaerical for different karyotypes or that
'chromosome stability' genes have undergone mutation during a callus phase (Ogihara 1981; Ogura 1978). This instability appears to occur in only a few regenerated plants and is unlikely to be the origin of instability in the barley x rye hybrids because chromosome instability is known from barley x rye hybrids which grew, in culture, directly from embryos (Thomas and Pickering 1979).

The chromosome instability found in hybrids has been the subject of much speculation. Most published suggestions of causes of instability could apply equally well to aneusomaty and genome elimination, although some refer specifically to elimination. Not all of these suggestions are mutually exclusive and several combinations are possible.

Among the more speculative suggestions is that of Davies (1974). He postulates that 'endonuclease attack' by one genome on another may take place. Higher plants could have restriction enzymes similar to those of certain bacteria. A non-heritable modification of the DNA (e.g. methylation of bases) of one genome may confer immunity (or susceptibility) to enzyme attack. A similar hypothesis has been put forward to explain the loss of chloroplast genes in Chlamydomonas following sexual fusion of different mating types.

Some suggestions for the existence of chromosome instability in hybrids concern faulty spindles. Orton and Tai (1977) describe two alternatives. One is that there may be a 'spindle-organiser' organelle in plants, equivalent to the centriole of
animal cells, which determines spindle construction and can be adversely affected by hybridity. One genome's 'spindle-organiser' may be dominant or 'spindle-organisers' may be maternally inherited. The alternative is that each genome is autonomous for the timing of spindle formation and/or that spindle components are species-specific. One spindle would be made first and the chromosomes of the second genome failed to attach to the spindle or became attached to a second, weaker spindle. The drawbacks about these suggestions are that present knowledge would indicate that spindle components are the same in all organisms, spindle structure in different species is probably very similar and visibly split spindles are rare except in high polyploids. Any specificity is more likely to be found in centromeres or spindle attachment proteins about which little is known.

Bennett et al. (1976) have suggested that chromosome elimination may be mediated by a shortage of mitotic components such as spindle attachment proteins, centromeric proteins and spindle components. If one genome had a greater affinity for these components than the other, or required more of them, preferential elimination could take place.

Finch and Bennett (1981 a, b) have demonstrated a spatial separation of the chromosomes of the two genomes in Hordeum x Secale hybrids, including barley x rye hybrid 20xR. This could provide the basis for selective instability of chromosomes from the two genomes. For instance, if the spindle was weaker towards the
periphery then the chromosomes found there (Secale chromosomes) would be more unstable.

However, if defective spindles are the cause of chromosome instability it might be expected that metaphases would be susceptible to lower concentrations of colchicine than those that are normally required to disrupt the spindle. An experiment to test the sensitivity of the barley x rye hybrid spindles to colchicine did not detect any lowering of the minimum effective concentration (see section 3.3). This means that it may be centromeres, rather than spindles, which are important in chromosome instability.

The occurrence of genes controlling chromosome elimination in Hordeum vulgare x Hordeum bulbosum hybrids has been investigated by Barclay et al. (1972) and Ho and Kasha (1975). Trisomics and telotrisomics were used to demonstrate that there are genes on both arms of chromosome 2B and the short arm of chromosome 3B which control chromosome elimination. It is not clear how these genes operate.

The most widely accepted explanation of the origin of chromosome instability in hybrids is that first proposed by Gupta (1969). Hybrids between Nicotiana plumbaginifolia and N. tabacum show chromosome instability. The duration of the cell cycle is similar in these two species but the lengths of G1 and G2 are different. Gupta proposed that the late replication of the Nicotiana plumbaginifolia DNA was the cause of chromosome instability in these plants. This idea has become widely accepted though
cytological evidence for it is restricted to a few cases. The validity of this theory will be explored in Chapter 8.

A new idea, that there may be a link between chromosome instability and nucleolar organiser suppression, will also be discussed in Chapter 8. First, the extent of differential amphiplasty in the barley x rye hybrids and its possible effects will be explored.
7. NUCLEOLUS ORGANISER ACTIVITY IN PARENTS AND HYBRIDS

7.1 Introduction

The role of the nucleolus as the site of production and assembly of ribosomes in the eukaryotic cell has been reviewed by Busch and Smetana (1970), Gimenez-Martin et al. (1977) and Jordan and Cullis (1982). The nucleolus consists of chromosomal, fibrillar and granular components representing rRNA genes with associated DNA, and zones of rRNA processing, and of ribosome assembly respectively. In addition to its major role as a producer of ribosomes for export to the cytoplasm, the nucleolus may also be involved in the expression of structural genes (Deák, Sidebottom and Harris 1972; Das 1962), possibly via processing or export of mRNA. The activity of the nucleolar genes is reflected by the size of the nucleolus, which is controlled in part by the number of rRNA genes (Flavell and Martini 1982) and also depends on the metabolic activity or mitotic cycling rate of the cell (Gimenez-Martin et al. 1977). Flavell et al. (1983) have shown that methylation of certain bases in the spacer regions between the rRNA genes may control the activity of the genes.

The region of the chromosome responsible for producing the nucleolus, and associated with the nucleolus from mid-telophase to late prophase (De La Torre and Giménez-Martín 1982), is called the nucleolus organiser region (NOR), SAT (sine acido thymo-nucleinico - Heitz 1931) or the secondary constriction. The region of chromatin distal to the NOR is commonly called the satellite. In conventionally stained chromosome preparations the NOR is an unstained
portion of chromosome whose length is approximately proportional to its activity (Givens and Phillips 1976; Gimenez-Martin et al. 1977; Warburton and Henderson 1979).

Hybridity affects nucleoli. Navashin (1934) coined the term differential amphiplasty to describe the suppression of the NORs of one genome in a hybrid. The phenomenon has been widely noted in interspecific hybrids but little is known about its causes or consequences. Many reports involve cereals (Heneen 1963; Ahokas 1970; Kasha and Sadasivaiah 1971; Lange 1971a, b; Lange and Jocomsen 1976; Wojciechowska 1978; Finch and Bennett 1980; Fedak and Nakamuro 1981; Martini et al. 1982). Other examples are found in interspecific hybrids from across the animal and plant kingdoms: Crepis (Navashin 1934; Wallace and Langridge 1971; Doerschug et al. 1976); Lilium (von Kalm and Smyth 1980); Nicotiana (Gerstel et al. 1978); Ribes (Keep 1960, 1962 and 1971); Salix (Wilkinson 1944); Solanum (Yeh and Peloquin 1965); Vicia (Watanabe and Yamada 1958); Drosophila (Bicudo and Richardson 1977; Durica and Krider 1978); Xenopus (Honjo and Reeder 1973) and mammalian somatic cell hybrids (Miller et al. 1976; Croce et al. 1977).

Most reports of differential amphiplasty merely note its presence. It is not known if differential amphiplasty affects the size of the nucleolus or the output of ribosomes. If differential amphiplasty causes a deficit of ribosomes this could, in turn, compromise the plant's metabolism. If one genome's rRNA genes
are suppressed then roughly half of the nucleolus organising capability is lost. This may not be important, at least in the majority of tissues, as many genes are unused (Givens and Phillips 1976).

Regulation of nucleolus activity (i.e. maintenance of normal size) has been reported for a *Xenopus* mutant with 50% of the normal rRNA gene number (Barr and Esper 1963). In addition, Martini et al. (1982) have reported that nucleolus size in wheat/*Aegilops umbellulata* substitution lines showing differential amphiplasty was normal. The work of Shermoen and Kiefer (1975) showed that there is a limit to the regulation possible. In *Drosophila* rRNA gene deficient mutants ('bobbed' mutants) occur but the deficiency never exceeds 50%. It would, therefore, be useful to determine the sizes of nucleoli in parents and hybrids to see if differential amphiplasty causes total nucleolar size to be reduced, or if the hybrid cells are able to regulate nucleolar activity and form a normal amount of nucleolar material.

There is a conflict in the literature on the effect of nucleolar fusion. Sacristan-Garate et al. (1974) compared fused and unfused nucleoli in *Allium* and concluded that the total nucleolar volume was regulated (i.e. increased) on fusion to give a constant surface area. However, Jordan et al. (1982) studied nucleolar fusion in wheat and have come to the opposite conclusion: that the volumes of nucleoli are additive on fusion. Data produced to study the effect of differential amphiplasty on nucleolar size in hybrids could also be used to resolve this contradiction.
Even if differential amphiplasty is not a cause of hybrid dysfunction, it may provide a useful means of studying interaction between genomes. It has been proposed (Finch and Bennett 1983) that methylation of DNA, already linked with differential amphiplasty, may also reduce the efficiency of centromeres and allow chromosome elimination. There is certainly some link between differential amphiplasty and chromosome elimination as hybrids may have both.

Preliminary investigation revealed that the barley x rye hybrids under study had fewer nucleoli than the maximum number possible from the number of NORs present in the karyotypes. Three techniques can be used to determine which genome possesses active nucleolar organisers: a) **Conventional** (Feulgen, orcein or aceto-carmine) staining of mitotic chromosomes. Constrictions are observed at active nucleolar organisers. There are advantages in using this method. Preparations used for karyotype work can be used. Organisers can be assigned to particular chromosomes relatively easily as long as they can be unambiguously identified (as they can be in this case). Disadvantages include confusion with centromeres or with other constrictions. Rye, for example, frequently has 'extra' constrictions (Heneen 1963). In addition, nucleolar organisers are susceptible to distortion on squashing, so the activity of an organiser (length of constriction) may be difficult to ascertain.

b) **Meiosis.** In pollen mother cells of barley the nucleolus persists, attached to the NOR, until late diakinesis (Schultz-
Schaeffer 1980). Drawbacks of this technique include difficulties in interpretation (particularly identification of chromosomes), limitation to one specialised cell type and a dependence on the availability of meiocytes (only one hybrid clone underwent meiosis).

(c) **NOR-specific banding.** A banding technique specifically for NORs has been developed (Goodpasture and Bloom 1975) and recently modified for use with plant chromosomes (von Kalm and Smyth 1980; Hizume, Sato and Tanaka 1980; Lacadena et al. 1984). Silver ions bind to a protein associated with actively transcribing nucleolar regions (Hubbell, Rothblum and Hsu 1979) which remains attached to the NOR through mitosis. The bands produced by this technique are found only at active sites and are at least partially quantitative (von Kalm and Smyth 1980, Hizume et al. 1980).

Drawbacks of this technique are that the method is, in common with other banding techniques, somewhat time consuming and sometimes no differential staining is obtained.

The range of cytotypes of barley x rye hybrids available provided ideal material for a study of nucleolar behaviour in hybrids and their parents. Features of nucleolar behaviour of interest included whether differential amphiplasty was detectable, what effect this had on nucleolar fusion and nucleolar size and whether there was any correlation between extent of differential amphiplasty and chromosome instability.

7.2 **Observations on nucleoli**
Numbers of nucleoli

To test for differential amphiplasty, nucleoli were counted in root-tip meristem cells. Root-tip meristems were standardised by excising the terminal portion of the root equal to the width of the root. Acetocarmine (section 2.5 d) was used because it stains nucleoli and nuclei, unlike other nuclear stains. Random samples of 200 cells were taken from each of five slides for each genotype. The results are presented in table 7.1.

Rye has one NOR in the haploid set, so the maximum number of nucleoli in the diploid is two. Barley has two NORs in each haploid set, giving a maximum number of nucleoli of four.

Both hybrid genotypes under study here had one extra rye NOR-bearing chromosome and so carried four NORs. The full complement of four nucleoli was never seen. The majority of nuclei had only one nucleolus and a substantial number had two nucleoli. Other categories were rare with less than 1% of cells having more than two major nucleoli. In addition many cells had one or two micronucleoli, a phenomenon very rare in the parental species. The rarity of cells with more than two major nucleoli indicates that most cells had only two active NORs, though four NORs were present (table 7.2). Other evidence (see later) indicates that the active NORs belong to the barley genome. The micronucleoli seen probably result from the partial suppression of the rye NORs. The presence, in a few cells, of three active nucleoli could be due to the occasional full expression of a rye...
Table 7.1  

a) Counts of nucleoli

<table>
<thead>
<tr>
<th>Genotype</th>
<th>No. of nucleoli or micronucleoli (m) per cell</th>
<th>1</th>
<th>1+m</th>
<th>1+2m</th>
<th>2</th>
<th>2+m</th>
<th>3</th>
<th>3+m</th>
<th>4</th>
<th>TOTAL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hybrid '20xR'</td>
<td></td>
<td>909</td>
<td>26</td>
<td>2</td>
<td>166</td>
<td>7</td>
<td>7</td>
<td>1</td>
<td>-</td>
<td>1118</td>
</tr>
<tr>
<td>%</td>
<td></td>
<td>81.3</td>
<td>2.3</td>
<td>0.2</td>
<td>14.8</td>
<td>0.6</td>
<td>0.6</td>
<td>0.1</td>
<td>-</td>
<td>99.9</td>
</tr>
<tr>
<td>Hybrid 'Cyto A'</td>
<td></td>
<td>1175</td>
<td>14</td>
<td>3</td>
<td>118</td>
<td>2</td>
<td>3</td>
<td>-</td>
<td>-</td>
<td>1315</td>
</tr>
<tr>
<td>%</td>
<td></td>
<td>89.4</td>
<td>1.1</td>
<td>0.2</td>
<td>9.0</td>
<td>0.2</td>
<td>0.2</td>
<td>-</td>
<td>-</td>
<td>100.1</td>
</tr>
<tr>
<td>2x rye</td>
<td></td>
<td>877</td>
<td>2</td>
<td>-</td>
<td>370</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>1249.1</td>
</tr>
<tr>
<td>'Petkus Somro'</td>
<td></td>
<td>70.2</td>
<td>0.2</td>
<td>-</td>
<td>29.6</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>100.0</td>
</tr>
<tr>
<td>2x barley</td>
<td></td>
<td>510</td>
<td>-</td>
<td>-</td>
<td>414</td>
<td>-</td>
<td>112</td>
<td>-</td>
<td>18</td>
<td>1054</td>
</tr>
<tr>
<td>'Sundance'</td>
<td></td>
<td>48.4</td>
<td>-</td>
<td>-</td>
<td>39.3</td>
<td>10.6</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>1.7 100.0</td>
</tr>
</tbody>
</table>

b) Comparison of proportion of cells with unfused nucleoli in genotypes with 2 nucleoli

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Total</th>
<th>Obs.</th>
<th>Exp.</th>
<th>Dev.</th>
<th>D^2</th>
</tr>
</thead>
<tbody>
<tr>
<td>20xR</td>
<td>1075</td>
<td>166(15.4%)</td>
<td>194.5</td>
<td>-28.5</td>
<td>812.2</td>
</tr>
<tr>
<td>Cytotype A</td>
<td>1293</td>
<td>118(9.1%)</td>
<td>233.9</td>
<td>-115.9</td>
<td>13432.8</td>
</tr>
<tr>
<td>Petkus Somro</td>
<td>1247</td>
<td>370(29.7%)</td>
<td>225.6</td>
<td>+144.4</td>
<td>20851.4</td>
</tr>
</tbody>
</table>

X^2 (with correction for continuity) = 154.0

d.f. = 1   \( P < 0.001 \)

...very highly significant deviation from uniformity.
NOR or the fusion of two partially active rye NORs. Neither of these seem likely as NORs on rye chromosomes were never very clear and the micronucleoli seen were not large enough to give rise to a full nucleolus through fusion. A more likely source of these extra nucleoli is the aneusomaty known to occur in these hybrids (see Chapter 6) which results in 0-10% of root-tip cells (depending on genotype) having one or more extra chromosomes. An extra barley NOR chromosome would presumably give rise to an extra nucleolus.

Table 7.2 Summary of nucleolus counts

<table>
<thead>
<tr>
<th>Genotype</th>
<th>No. of NORs in karyotype</th>
<th>Max. no. of nucleoli</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hybrid '20xR'</td>
<td>4 (6B, 7B, 1R, 1R)</td>
<td>2</td>
</tr>
<tr>
<td>Hybrid 'Cytotype A'</td>
<td>4 (6B, 7B, 1R, 1R)</td>
<td>2 1.</td>
</tr>
<tr>
<td>2x Rye 'Petkus Somro'</td>
<td>2 (1R, 1R)</td>
<td>2</td>
</tr>
<tr>
<td>2x Barley 'Sundance'</td>
<td>4 (6B, 6B, 7B, 7B)</td>
<td>4</td>
</tr>
</tbody>
</table>

1. Less than 1% had 3 or more.

In addition to the information on the suppression of NORs, these results show the degree of fusion of nucleoli. Comparison can be made between genotypes with two active NORs. Table 7.1b shows that there are highly significant differences in the degrees of fusion of nucleoli between the three genotypes considered, with the two hybrids being different from each other as well as from rye.
Nucleolus size

To determine how the sizes of nucleoli were affected in barley x rye hybrids, measurements were made on silver stained spreads of root-tip meristems of parents and hybrids (see section 2.6 b - Hizume method). The individual volumes and surface areas of every nucleolus for 60 cells of each genotype were calculated and the means and standard errors are presented in table 7.3. The results have also been displayed graphically for easy interpretation (fig. 7.1).

The results have several interesting features. Barley and rye, though differing substantially in DNA content, have remarkably similar nucleolar dimensions. It was expected that the dimensions of the nucleoli of the hybrids would be either smaller than (no regulation) or similar to (regulation) those of the parents. The results show that both hybrids have significantly larger nucleoli than either parent (tables 7.3, 7.4, fig. 7.1, Ramsay and Dyer 1983).

The data have been used to calculate volumes and surface areas of nucleoli for fused and unfused nucleoli. Comparison of these parameters would be interesting because there are conflicting reports of changes in volume and surface area following fusion of nucleoli, as noted in the introduction.

The results presented here strongly support the suggestion that surface areas, not volumes, are additive. In all four genotypes the total surface areas were very similar for cells with one, two or,
<table>
<thead>
<tr>
<th>Genotype</th>
<th>Dimension</th>
<th>No. of nucleoli per cell</th>
<th>1</th>
<th>2</th>
<th>3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hybrid '20xR'</td>
<td>Total volume (μm^3)</td>
<td>158.6 ± 11.9</td>
<td>110.6 ± 9.7</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Total surface area (μm^2)</td>
<td>136.7 ± 7.0</td>
<td>131.7 ± 8.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hybrid 'Cytotype A'</td>
<td>Total volume (μm^3)</td>
<td>214.7 ± 23.3</td>
<td>141.7 ± 20.4</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Total surface area (μm^2)</td>
<td>163.2 ± 11.0</td>
<td>151.5 ± 12.9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rye 'Petkus Somro'</td>
<td>Total volume (μm^3)</td>
<td>81.4 ± 4.9</td>
<td>47.3 ± 3.7</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Total surface area (μm^2)</td>
<td>88.7 ± 3.5</td>
<td>75.6 ± 4.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Barley 'Sundance'</td>
<td>Total volume (μm^3)</td>
<td>80.8 ± 8.0</td>
<td>47.1 ± 5.2</td>
<td>42.1 ± 4.0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Total surface area (μm^2)</td>
<td>85.7 ± 5.4</td>
<td>78.7 ± 6.6</td>
<td>78.2 ± 5.1</td>
<td></td>
</tr>
</tbody>
</table>

mean of 60 cells (± standard error)
Table 7.4  Statistics on nucleolus dimensions

Analysis of variance: Comparison of surface areas

<table>
<thead>
<tr>
<th>Genotypes</th>
<th>Hybrid '20xR'</th>
<th>Hybrid 'Cyto. A'</th>
<th>Rye</th>
<th>Barley</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean surface areas</td>
<td>1 nucleolus</td>
<td>163.2</td>
<td>88.7</td>
<td>85.7</td>
</tr>
<tr>
<td></td>
<td>2 nucleoli</td>
<td>151.5</td>
<td>75.6</td>
<td>78.7</td>
</tr>
<tr>
<td></td>
<td><strong>\bar{x}</strong></td>
<td>157.35</td>
<td>82.15</td>
<td>82.20</td>
</tr>
<tr>
<td></td>
<td>a</td>
<td>b</td>
<td>c</td>
<td>c</td>
</tr>
<tr>
<td>Sources of Variation</td>
<td>Sums of Squares</td>
<td>Degrees of Freedom</td>
<td>Mean Squares</td>
<td>F</td>
</tr>
<tr>
<td>Treatments</td>
<td>8625.845</td>
<td>3</td>
<td>2875.28</td>
<td>60.14</td>
</tr>
<tr>
<td>Error</td>
<td>191.25</td>
<td>4</td>
<td>47.81</td>
<td>**</td>
</tr>
</tbody>
</table>

A different letter with a genotype mean indicates a significant difference at \( p = 0.05 \) using the Multiple Range Test (Parker 1973).
in the case of barley, three nucleoli but there was a marked change (approx. 60% increase) in volume on fusion. (fig. 7.1; Ramsay and Dyer 1983).

7.3 Observations on nucleolar organizers

Conventional staining of mitotic chromosomes

The position of the NORs in barley and rye were determined from Feulgen, orcein and acetocarmine preparations and verified by comparison with published karyotypes (see Chapter 5, esp. figs. 5.5 and 5.6). Barley was confirmed to have two NORs: a longer one on 6B and a slightly shorter one on 7B. Rye has only one NOR per genome, on chromosome 1R. All NORs were readily visible in the parents and all are distinguishable by a combination of satellite size, arm ratio and NOR size.

All hybrid genotypes were studied and all were found to have expressed barley NORs but weak or undetectable rye NORs in root-tip cells. These can be seen in figs. 5.1, 5.3 and 5.7. In cytotype A the 7B NOR was not normally visible because of a terminal deletion covering all of the satellite. Activity in a few cells of this NOR was, however, confirmed by the very occasional presence of some chromatin distal to the organizer (fig. 5.7). It is probable that this chromatin represents inactive rRNA genes. It is well known that heterochromatin adjacent to NORs contains inactive rRNA genes (Givens and Phillips 1976). It is likely, therefore, that surplus parts of NORs can become heterochromatin.
Meiosis

Further evidence of the expression of barley NORs and the suppression of rye NORs came from a study of male meiosis. The only hybrid to reach male meiosis (pistillody prevented male meiosis in the other hybrids) was 20xR. Attachment of nucleoli to chromosomes was seen throughout meiotic prophase and the chromosomes could sometimes be assigned to genomes, particularly with preparations of diakinesis. Examples are given in figs. 4.9 and 4.10, where the attachments to barley chromosomes can be seen. These observations directly linked specific chromosome sites with nucleoli but interpretation was frequently difficult.

Silver NOR banding

The use of AgNO₃-based staining procedures gives a very specific and partially quantitative measure of NOR activity. The results, obtained mostly with the modified Hizume method (see section 2.6 b), clearly back up other evidence that barley NORs are active and rye NORs suppressed. Banding patterns for hybrids 20xR and cytotype A were compared with rye and barley. Active rye NORs can be seen in fig. 7.2 and compared with hybrids 20xR (fig. 7.3) and cytotype A (fig. 7.4). The results were complicated by some staining of rye heterochromatin but this was easily distinguished by its sub-terminal position. The position of the rye NOR was much more median and a faint band was sometimes seen there. Barley NORs were as clearly visible in hybrids as they were in barley. The activity of the now terminal NOR on 7B in cytotype A
fig. 7.2  Rye cv. Petkus Somro with Ag stained NORs on 1R (arrows).  Hizume method.

fig. 7.3  Hybrid 20xR with Ag stained NORs on 6B and 7B but weakly stained NOR on 1R.  Some heterochromatin (h) also stained.  Von Kalm and Smyth method.

fig. 7.4  Hybrid cytotype A with Ag stained NORs on 6B and terminal on 7B.  Adjacent nucleus has strongly stained nucleoli.  Hizume method.

Scale bar 10 μm.
was also confirmed (see fig. 7.4).

In addition, hybrid leaf base meristem cells and root-tip meristems subjected to 24 hours at 4°C were stained with AgNO₃ to verify that NOR suppression continued at low temperatures. All were found to have the same degree of suppression of rye NORs.

A summary of these results is presented in table 7.5.

Table 7.5 Observations on hybrid NORs

<table>
<thead>
<tr>
<th>Hybrid Genotype</th>
<th>Tissue</th>
<th>Method</th>
<th>Rye NORs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cytotype A</td>
<td>Root-tips</td>
<td>C, Ag</td>
<td>S</td>
</tr>
<tr>
<td></td>
<td>Leaf bases</td>
<td>C</td>
<td>S</td>
</tr>
<tr>
<td>Cytotype B</td>
<td>Root-tips</td>
<td>C</td>
<td>S</td>
</tr>
<tr>
<td>Cytotype C</td>
<td>Root-tips</td>
<td>C</td>
<td>S</td>
</tr>
<tr>
<td>Cytotype D</td>
<td>Root-tips</td>
<td>C</td>
<td>S</td>
</tr>
<tr>
<td>20xR</td>
<td>Root-tips</td>
<td>C, Ag</td>
<td>S</td>
</tr>
<tr>
<td></td>
<td>Root-tips at 4°C</td>
<td>Ag</td>
<td>S</td>
</tr>
<tr>
<td></td>
<td>Pollen mother cells</td>
<td>C</td>
<td>S</td>
</tr>
<tr>
<td></td>
<td>Leaf bases</td>
<td>Ag</td>
<td>S</td>
</tr>
</tbody>
</table>

Key: C - conventional staining (Feulgen, lacto-propionic orcein, acetocarmine)
Ag - Ag NOR banding
S - Suppressed

7.4 Discussion

Differential amphiplasty has been recorded from many hybrids, both interspecific and intergeneric (listed in section 7.1). In Ribes (Keep 1971), wider crosses have stronger differential amphiplasty
than crosses between closely related species. A hierarchy of NOR dominance is apparent in some genera, e.g. *Crepis* (Wallace and Langridge 1971), with some species regularly dominant.

The phenomenon is widespread in hybrids and it is possible that all interspecific hybrids have some degree of differential amphiplasty. Some examples of interspecific or even intergeneric hybrids apparently lack differential amphiplasty (e.g. *Hordeum vulgare* x *Triticum turgidum*, Mujeeb et al. 1978) but it is rarely made clear what proportion of cells was seen with full expression of NORs. Variation within each meristem may account for some of these anomalous results.

It is possible that differential amphiplasty can act as a barrier to gene flow between species. Hybrids between species like barley and rye may be difficult to produce because differential amphiplasty causes abortion at certain stages of the life cycle. Studies with deletion mutants in *Drosophila* (Shermoen and Kiefer 1975) have shown that deletions of greater than 50% of the complement of rRNA genes are lethal. The effect of differential amphiplasty may be similar.

However, it does not seem likely that differential amphiplasty has been developed as an isolating mechanism. The true cause of differential amphiplasty remains obscure. It may be due to the processes of gene dominance, which normally act within a genome, causing dominance between genomes in a hybrid. An alternative to this is that the molecular mechanisms which control nucleolus
formation at telophase (as yet unknown) diverge in some way in
different species. Once they have diverged, it may be possible for
interference to occur between the two control systems.

Whatever the ultimate cause of differential amphiplasty, little
is known about its effects, if there are any. For this reason, the
effect of differential amphiplasty on the sizes of nucleoli in barley x
rye hybrids was investigated. It would be expected that total
nucleolar dimensions would be reduced, or, if compensation occurred,
the total size of nucleoli in the hybrids would be the same as that
of the parents. It was found (fig. 7.1) that the hybrids had more
nucleolar material than either parent. This surprising result is
not easily explained but three possibilities are worth considering:

a) regulation of nucleolus activity has been upset by
hybridity and rRNA gene activity has increased more than is
required to compensate for differential amphiplasty,

b) genetic control over rRNA genes is affected by aneuploidy,
as in wheat (Mohan and Flavell 1974; Liang, Wang and Phillips
1977),

c) a partial blockage of ribosome export leads to nucleolus
hypertrophy.

It would be expected that if the last possibility was true then
the ratio of fibrillar to granular portions of the nucleolus would be
upset. This was difficult to measure, due to the convoluted nature
of the boundary, but no difference was immediately apparent.

This increase in size of nucleoli in the hybrids, as compared
to the parents, was not predicted. The effect of it can only be
guessed at. If the large size of hybrid nucleoli reflects high
ribosome output, then it is possible that this nucleolar hypertrophy
is a cause of heterosis. It is worth noting that the barley x rye
hybrids produced by Dr. Napier (cytotypes A to D) were initially
more vigorous than both parents (Cooper 1978).

The data also show, for all four genotypes, that total
nucleolar surface area is conserved after fusion. This result
agrees with Sacristan-Garate et al. (1974) but not with Jordan et al.
(1982). The slight increase in surface area on fusion (fig. 7.1)
may be accounted for by the gradual increase in nucleolar size
through interphase. Cells with fused nucleoli were more likely to
be in late interphase than cells with unfused nucleoli. There must
be some reason for the dramatic change in nucleolar volume on
fusion, apparently maintaining a constant surface area. It may be
that the final stages of ribosome assembly, taking place around the
periphery of the nucleolus, limit the rate of ribosome production.
This would mean that ribosome production would depend on surface
area, not volume. This is clearly an issue requiring further study.

Differences in nucleolus fusion rates between genotypes have
already been commented on. The three genotypes with two active
NORs have different degrees of nucleolus fusion and this can be
compared with nucleolus size. It has already been shown that
surface areas are additive on fusion. The mean surface areas can
be subjected to analysis of variance using the one and two nucleolus
classes as replicates (table 7.4). Significance testing, using the Multiple Range Test, shows that not only were the hybrid nucleoli larger than the parents' nucleoli, but there was also a significant difference (at p =0.05) between the hybrids. Plotting fusion rate against surface area for the three genotypes with two active NORs (fig. 7.5) gives a very good correlation, albeit with only three points. It appears from this that the size of the nucleolus has an important bearing on nucleolus fusion. A previous study (Lange and Jochemsen 1976) failed to detect any influence on nucleolus fusion of nuclear size using 2x and 3x *Hordeum vulgare* × *H. bulbosum* hybrids. Their study took no account of nucleolar size.

The suggestion (Deak et al. 1972) that nucleoli process mRNA leads to the possibility that, in hybrids with differential amphiplasty, the genome with the suppressed nucleoli may also have overall genetic suppression. This does not appear to be the case: the hybrids under study here have rye NORs suppressed but are morphologically slightly closer to rye than barley (see section 5.5). Two other *Triticinae* hybrids have morphologies which tend to resemble the parent donating the suppressed NORs (Ahokas 1970; Lange 1971b).

Differential amphiplasty does not have to be complete. In the hybrids under study here small Ag bands were sometimes seen on rye satellite chromosomes. Micronucleoli also indicated that suppression was not always complete. Other interspecific hybrids similarly show incomplete suppression: this has been noted in
Fig. 7.5  Correlation between nucleolar fusion and nucleolar size

NUCLEOLUS FUSION RATE
% (TABLE 7.1a)

NUCLEOLUS SURFACE AREA PER CELL (NUCLEI WITH FUSED NUCLEOLI) μm³ (TABLE 7.2)
Capsicum (Pickersgill and Gonzalez de Leon, pers. comm.), Lilium (von Kalm and Smyth) and triticale (Scoles and Kaltsikes 1974).

The degree of suppression seen here did not vary with genotype. Perhaps the increase in dosage of rye NORs (to two or three) partially balances out their suppression. It is clear that the trend of aneuploidy in these hybrids was towards an increase in dosage of rye NORs.

The causes of differential amphiplasty, a phenomenon first noted in 1928 (Navashin 1934), are still unknown. It seems likely that further advances may come from molecular biology. Flavell et al. (1983) have shown that suppressed rRNA genes in a hybrid with differential amphiplasty have extensive methylation of the cytosine residues. Elucidation of the control mechanisms of this methylation may be possible. In addition, it is now possible to compare differential amphiplasty with other phenomena, e.g. chromosome elimination or instability, to see if DNA methylation is responsible for other anomalies of hybrid chromosome behaviour as has been proposed by Finch and Bennett (1983).

The possibility that the position of the chromosome on the metaphase plate may control whether or not chromosome elimination takes place has been discussed by Finch and Bennett (1983). In hybrids, one genome tends to be located peripherally on the metaphase plate (Finch and Bennett 1981 a, b), and the peripheral genome is subjected to both chromosome elimination and differential amphiplasty. Anastassova-Kristeva et al. (1977) and Nicoloff
et al. (1979) have shown that barley NORs involved in translocations are suppressed in a manner similar to that in hybrids. Given that the chromosomes in a nucleus are in a defined order, predicted by arm sizes (Bennett 1981; 1983), it may be that this is further evidence for a positional effect on NOR expression.

Present knowledge of differential amphiplasty is, therefore, somewhat patchy. It is known that it occurs in most, if not all, interspecific hybrids. It is also known that the ability to make sufficient nucleolar material is not compromised. The biochemical basis of the suppression is known but the control of it is not. It is also suspected that there is a common explanation for differential amphiplasty, chromosome elimination and genome separation in interspecific hybrids but, as yet, the links between them are obscure. Further consideration of chromosome instability, its correlates and possible causes will be found in Chapter 8.
8. DISCUSSION OF THE CAUSES OF MITOTIC INSTABILITY IN HYBRIDS AND GENERAL CONCLUSIONS

8.1 Introduction

Mitotic chromosome instability (aneusomaty) and genome elimination are phenomena which are characteristic of many hybrids. This topic has been introduced in Chapter 6. To summarise the points raised in that chapter, in plant hybrids many reports exist of aneusomaty (18 were listed) and genome elimination (7 reports, excluding those involving Hordeum, of which there are many). In addition, there are many old reports of 'patrogenesis' or 'androgenic progeny' following interspecific pollination which could be ascribed to genome elimination. Instability of chromosome number is not restricted to plant hybrids, being found also in cell cultures, some plants regenerated from callus, high polyploids, animal somatic hybrid cells and a few apparently normal diploid plants. It is likely that genome elimination is an extreme form of mitotic chromosome instability which operates at certain points in the life cycle. Examples exist of both taking place in the same hybrid.

The characteristics of aneusomaty in barley x rye hybrids have been described. Aneusomaty was present in all hybrids and in all tissues examined. It was greater in pollen mother cells than in roots and greater in the Sundance x Petkus Somro hybrid than in Kruse's hybrid. In one hybrid, which had an acrocentric rye chromosome, 32% of root-tip meristem cells had chromosome complements other than the modal number. The chromosome
number varied in both directions, although loss was commoner than
gain, and rye chromosomes were more commonly involved but
probably both genomes were affected.

Aneusomaty must be an important phenomenon in plants where
it occurs. Variation in chromosome number must reduce vigour
and viability. Genome elimination can prevent some hybrids being
produced but, in some circumstances, is useful to a breeder for
producing haploids (e.g. Snape and Simpson 1981). Consequently,
the mechanisms behind these phenomena are well worth investigating.
Some of the many hypothetical explanations from the literature for
mitotic chromosome instability and genome elimination have been
discussed in Chapter 6. One major theory from the literature
remains. Differences in cell cycle times or the late replication of
DNA in one genome have been proposed to be major causes of
chromosome elimination in hybrids (e.g. Gupta 1969). The purpose
of this chapter is to explore this suggestion, to assemble data from
the literature on chromosome instability and genome elimination
and to suggest alternative causes of them.

8.2 Genome elimination in interspecific hybrids of the genus Hordeum

In hybrids between species of Hordeum both chromosome
instability and genome elimination are found (Lange and Jochemsen
1976). However, chromosome instability has been investigated
infrequently, probably because chromosome number instability,
unlike genome elimination, is easily overlooked because mechanical
breakage of cells in normal squashes commonly occurs.

Genome elimination is frequent in crosses between *Hordeum* species. Jacobsen and von Bothmer (1981) reported plants from 292 interspecific combinations of parental species of *Hordeum*. Of these, more than 10% underwent genome elimination to give haploids. Unfortunately, the direction of genome elimination was not recorded. Subrahmanyam (1982) gave information on the direction of genome elimination in hybrids of *H. vulgare* and *H. bulbosum* with 11 other species of *Hordeum*. A hierarchy of genome elimination was proposed. His data have been combined with data collated from 19 other papers to produce the diagram showing the hierarchy of genome elimination in fig. 8.1.

Collation and presentation of the data in this manner brings out several interesting points about genome elimination. The 'eliminating potential' (defined as the ability of one genome to remain stable in a hybrid combination where the other genome becomes unstable and is eliminated) is remarkably consistent for any given species. For instance, *H. procerum* and *H. lechleri* genomes remain stable in hybrid combinations with *H. vulgare* where *H. vulgare* chromosomes are lost; *H. vulgare* (2x) is dominant to *H. bulbosum* (2x); and *H. procerum* and *H. lechleri* are dominant to *H. bulbosum* (2x).

The stability of the chromosomes of a species strongly depends on its ploidy. Higher ploidy levels tend to be found higher up in fig. 8.1. Where different ploidies of individual species exist (e. g.
H. vulgar 2x and 4x and H. brachyantherum 4x and 6x), the higher ploidy level has a higher 'eliminating potential'. In addition, the H. jubatum x H. compressum amphiploid has a greater 'eliminating potential' than H. jubatum or H. compressum.

Interestingly, there is no correlation between genome elimination and taxonomy of the genus. The taxonomic groupings of von Bothmer et al. (1981) have been used. Closely related species, such as H. brachyantherum and H. depressum or H. lechleri and H. procerum behave similarly. However some species from the same group, e.g. H. vulgar and H. bulbosum, undergo genome elimination in the hybrid. On the other hand, pairs of distantly related species, such as H. bulbosum and H. chilense, may form hybrids.

To summarise the available data on genome elimination in interspecific Hordeum crosses: taxonomy is not important; 'eliminating potential' is consistent for each species with some being consistently recessive (e.g. H. bulbosum) and some being consistently dominant (e.g. H. lechleri); and the ploidy level strongly modulates the pattern, with both autoploids (e.g. 4x H. vulgar) and alloploids (e.g. H. jubatum x H. compressum amphiploid) being stronger eliminators than their counterparts of lower ploidy.

A number of theories have been proposed to explain why genome elimination occurs. Most of these referred to interspecific Hordeum crosses. Many of these theories (i.e. endonuclease
Fig. 8.1 Hierarchy of genome elimination in Hordeum

This diagram shows the direction of elimination of chromosomes when various genotypes of Hordeum are used to produce hybrid combinations. These hybrid combinations are not shown. The relative stabilities of the genomes are displayed by placing the more stable genotype in a hybrid combination above the less stable (i.e. eliminated) one. Genotypes joined by a solid line (——) form haploids of the higher genotype when combined. Genotypes joined by a dashed line (---) form relatively stable hybrids. Several combinations of genotypes within boxes also form hybrids. In addition, H. bulbosum (2x) chromosomes are eliminated from hybrid combinations with H. vulgare (4x), H. procerum (6x) and H. lechleri (6x) but these have been omitted for clarity.

Sources:
Bennett, Finch and Barclay (1976) Rajhathy, Morrison and Symko (1963)
Lange (1971 a,b) Subrahmanyan (1977), (1978), (1979)
Kasha and Sadasivaiah (1971) Subrahmanyan and Kasha (1973)
Morrison and Rajhathy (1959) Symko (1969)
Orton and Tai (1977) von Bothmer et al. (1983)
Rajhathy and Morrison (1959), (1961)
attack, interaction between hypothetical spindle organisers, competition for spindle attachment proteins and genetic factors on particular chromosomes) were discussed in Chapter 6 and will not be elaborated further here.

One widely accepted explanation for genome elimination, which suggests it is caused by differences in cell cycle times between the two genomes, fits well with the data presented here on interspecific Hordeum crosses for two reasons. Each species has a consistent position in the hierarchy, which supports a genetic or nucleotypic explanation of genome elimination. In addition, the known effects of an increase in ploidy include a decrease in mean cell doubling time (MCDT) (Bennett and Smith 1972). Presumably a higher ploidy plant would have a shorter MCDT and hence be less susceptible to genome elimination in a hybrid (see next section for reasoning).

There is, however, no direct evidence to support this hypothesis. Data on MCDTs of the various species of Hordeum are not available. The production of these data would be a worthwhile project.

8.3 Are cell cycle times important for chromosome stability?

Predicted effects of cell cycle asynchrony

There are three types of possible DNA cycle asynchrony which would be expected to create instability for one genome in a hybrid cell at mitosis. These three possibilities are: differences in the length of the DNA synthesis phase ('S' phase); differences in the duration of the constituent parts of the S phase; and differences in the position of the S phase during interphase. The first of these,
differences in S phase duration, may be due to different DNA amounts per genome. The second, differences in the constituent parts of the S phase, may be due to different amounts of different types of DNA. Heterochromatin is known to be late-replicating in some plants, including rye (Ayonoadu and Rees 1973), and so is a likely cause of problems of asynchrony in hybrids. The third possibility, that differences in the position of the S phase may affect the mitoses of a hybrid, will occur when the relative lengths of G1 and G2 vary. It has been suggested that this is responsible for chromosome elimination in *Nicotiana* hybrids (Gupta 1969).

DNA synthesis is the most important of the periodic events which occur during interphase, so it seems likely that any effects of cell cycle asynchrony will be mediated by asynchronous DNA synthesis. However, there are other cell cycle events which have important effects. Some of these, e.g. histone synthesis and synthesis of some enzymes, are periodic and so may be affected by asynchrony.

The cytological effects of asynchrony could fall into two categories. If DNA replication, or subsequent chromosome packing, was incomplete at mitosis it would be expected that chromatid separation would be prevented and anaphase bridges would be formed. The effect would be similar to the adhesion loci reported by Shaw (1958) in *Trillium*. The other possible cytological effect of cell cycle asynchrony would be the incomplete assembly of centromeres of one genome resulting in a failure of chromosomes to congress
normally at metaphase.

If asynchronous DNA synthesis is the cause of chromosome instability in hybrids, then changing the rate of cell division may have an effect on chromosome instability. If all parts of the cell cycle retain the same proportions when the MCDT changes, then it is unlikely that chromosome instability would be affected. However, some parts of the cell cycle may be dispensable or may be more able to be accelerated. The minimum MCDT in plants appears to be limited by DNA amount (Bennett 1972) and so the duration of the S phase may be relatively inflexible. Bennett et al. (1972) demonstrated that mitotic cycle duration differed in different meristems of *Vicia faba* and that the difference was due solely to differences in the length of G1. MCDT in *Zea* was shown to be highly dependent on temperature (Verma 1980). The proportion of the cell cycle spent in the S phase remained constant but, as the temperature and MCDT was increased, the proportion in G1 fell and the proportion in G2 rose. It is clear from this that changes in MCDT do not affect all parts of the cell cycle equally. If DNA synthesis asynchrony is an important cause of chromosome instability, then it would be expected that a reduction in the proportion of the cell cycle occupied by G2 would bring the DNA synthesis overlap closer to mitosis and cause greater mitotic faults. It is possible that this reduction in the proportion of the cell cycle occupied by G2 may occur in some fast-dividing tissues.
Hybrid endosperm

Endosperm is one of the fastest dividing tissues in higher plants, so if cell cycle time asynchrony is important in hybrids, endosperm is the most likely tissue to have problems at mitosis. Breakdown of hybrid endosperm following mitotic abnormalities has long been recognised as a major cause of hybrid embryo abortion (Brink and Cooper 1947). The abnormalities seen include bridges, dumbell nuclei and highly polyploid nuclei, all of which could be due to problems of asynchrony between genomes. These mitotic abnormalities are frequent in the endosperm of crosses between barley and rye (Napier et al. 1981; Forster and Dale 1983 b) and probably cause the subsequent abortion of the hybrid embryo.

Experimental evidence exists for an effect of mean cell doubling time on endosperm development. Bennett et al. (1976) found more aberrant endosperm nuclei after crossing Hordeum bulbosum with H. vulgare cv. Vada than when cv. Sultan was used. The mean cell doubling time of Sultan (13.7 hrs) was more similar to H. bulbosum (20 hrs) than Vada (11.5 hrs). Forster and Dale (1983 a) determined the mean cell doubling times of a range of cultivars of barley and rye. Based on these MCDTs, crosses were made which were predicted to be compatible, intermediate and incompatible. The endosperm of the predicted compatible crosses developed further and enabled embryos to grow for longer than in the predicted incompatible crosses (Forster and Dale 1983 b).

Another indication that disparity of cell cycle time is an
important cause of hybrid endosperm breakdown comes from work on rye heterochromatin in triticale. As has been previously stated, rye heterochromatin has been shown to be late-replicating. Rye has a longer cell cycle than wheat (Bennett et al. 1971) and so mitotic problems involving late-replicating rye heterochromatin may be expected. In triticale, grain shrivelling is correlated with aberrant endosperm nuclei and bridges involving rye heterochromatin can be seen in many aberrant anaphases (Bennett 1977). Triticale lines with certain rye heterochromatic telomeres deleted have fewer aberrant endosperm nuclei and less grain shrivelling (Bennett 1981b). The effects of deletion of rye telomeric heterochromatin are additive (Bennett and Gustafson 1982; Bennett and Seal 1982; Gustafson and Bennett 1982).

Varghese and Lelley (1983), however, disagree that heterochromatin is important in grain shrivelling in triticales. They selected plump and shrivelled lines from four genotypes and found no clear correlation between grain shrivelling and heterochromatin loss. However, this does not disprove Bennett and Gustafson's work but indicates that other factors may also be important.

Other evidence for the importance of cell-cycle time disparity

The evidence for an important role of cell cycle time disparity in endosperm mitosis anomalies has been discussed. Endosperm is, of course, a special tissue, being coenocytic during its dividing phase and possessing two maternal and one paternal genomes. It is also the fastest dividing tissue in many plants, with a MCDT of
3-4 hours during the first day of growth in barley (Forster and Dale 1983a). Barley embryos of a comparable age have a MCDT of about 10 hours. Barley seedling root-tip meristems have a MCDT of 10-12 hours (Bennett and Finch 1972; Schwammenhoferova and Ondrej 1978). It is, therefore, pertinent to review the evidence for an effect of cell cycle time disparity on tissues other than endosperm.

In general, there is no convincing evidence to link chromosome instability or other mitotic anomalies with cell cycle disparity. There are several tentative indications of a link, however.

In hybrids with some chromosome instability, the MCDT of the more stable parental genome is shorter in the few cases where both are known. This is the case in the following crosses:

Hordeum bulbosum x H. vulgare (Bennett et al. 1976); H. vulgare x Secale cereale (Forster and Dale 1983a) and Triticum aestivum x S. cereale (Bennett et al. 1971).

In Nicotiana plumbaginifolia x N. tabacum hybrids, which show chromosome instability, Gupta (1969) found that the cell cycle times were similar but the G1 and G2 durations were different so that N. plumbaginifolia had a later S phase than N. tabacum.

Temperature is known to greatly affect MCDTs. Verma (1980) demonstrated that increasing the temperature from 20°C to 30°C decreases the MCDT in Zea from 16.5 hours to 7 hours. It is not known how temperature might affect the different genomes in a hybrid but one possibility is that an increase in rate of division
may lead to an increased instability. Humphreys (1978) has indeed shown that the chromosome elimination in *Hordeum vulgare* × *H. bulbosum* crosses is accelerated by increasing temperature. This can be construed as evidence that MCDTs are important in chromosome elimination but other explanations are possible.

Another indication that MCDTs are important comes from the work on genome elimination in *Hordeum* hybrids discussed in the previous section. It has already been stated that polyploids tend to have shorter MCDTs than equivalent diploids (Bennett and Smith 1972). In interspecific *Hordeum* crosses, higher ploidies tend to have stronger eliminating potentials. This may be because of their reduced MCDTs, but increased dosage of certain genes is an equally likely possibility.

**Contradictory evidence**

There are reasons for thinking that cell cycle asynchrony is not the only, or even the major, cause of chromosome instability and genome elimination in tissues other than endosperm. There are several points which lead to this conclusion.

Stress has been placed on the importance of the length or position of the S phase as a cause of chromosome instability in hybrids. Graves (1972) investigated mouse x Chinese hamster somatic hybrid cells and found that both S phases were initiated simultaneously but that the mouse S phase lasted longer. G2 was long in relation to the overlap in S phases and chromosomes of both genomes were lost. So, in these somatic hybrids showing
chromosome instability, the length of the S phases were unrelated to chromosome instability.

In crosses between *H. vulgare* and *H. bulbosum* the chromosomes of *H. bulbosum* are eliminated during early embryogenesis. If the chromosomes are eliminated because of cell cycle asynchrony, then it would be expected that maximum elimination would be found when the rate of division was at a maximum. Bennett et al. (1976) considered that cell cycle asynchrony was not directly involved because: i) elimination began at first zygotic metaphase, before the first hybrid S-phase, and ii) rate of elimination was initially slow, then increased, unlike the rate of division. Interestingly, the rate of chromosome elimination in the endosperm increases after five days, which is also against the trend of rate of division.

If cell cycle asynchrony is the cause of chromosome elimination then it would be predicted that one genome will be exclusively affected. There is some evidence to the contrary. Humphreys (1978) and Lange and Jochemsen (1976) have noted that there is aneusomaty affecting *H. vulgare* chromosomes as well as elimination affecting *H. bulbosum* chromosomes in the hybrid between the two. *H. vulgare* x *Secale cereale* hybrids suffer from both genome elimination affecting *Secale* chromosomes (Fedak 1979 a) and aneusomaty affecting primarily *Secale* but also *Hordeum* (see Chapter 6) chromosomes.

To summarise, the suggestion that cell cycle asynchrony is a major cause of genome elimination and aneusomaty in interspecific
hybrids is well supported by evidence in the special case of hybrid endosperm. The evidence for the same in other tissues is equivocal.

A range of alternative explanations for genome elimination and aneusomaty has been discussed in Chapter 6. The most attractive of these is that proposed by Finch and Bennett (1983). They note that eliminated genomes tend to be peripheral on the metaphase plate. The chromosomes of the eliminated genomes also tend to have suppressed centromeres and nucleolar organisers. The correlation between NOR suppression and chromosome elimination has not been discussed in detail in the literature and it is to this that I now propose to turn.

8.4 The link between differential amphiplasty and chromosome instability

Differential amphiplasty, as discussed in Chapter 7, is a phenomenon found in many hybrids. The term is applied to hybrids which show the suppression of the activity of the nucleolar organiser regions (NORs) of one genome. It is found in many types of interspecific and intergeneric hybrids, both plant and animal. The effects of differential amphiplasty have been, until now, assumed to be restricted to nucleolar function.

As demonstrated in Chapter 7, differential amphiplasty occurs in barley x rye hybrids. The rye NOR is suppressed, as indicated by nucleolus counts, Feulgen staining, and silver banding, but the suppression is not complete. The nucleoli of the hybrid were,
surprisingly, larger than those of either parent. It is most interesting that the trends of aneuploidy seen in the barley x rye hybrids (Chapter 5) included four separate events where a rye NOR was gained by the hybrids. It is possible that gain of a rye NOR will compensate for the partial suppression of rye NORs.

It was noted that some pairs of closely related species display differential amphiplasty in their hybrid. It is possible that most (or all) interspecific hybrids have at least partial differential amphiplasty. This may be important in some barriers between species, particularly if there are stages in the life cycle which require a full complement of rRNA genes to be active. It is worth noting that deletions of more than 50% of rRNA genes in Drosophila are lethal (Shermoen and Kiefer 1975).

A link between chromosome instability and differential amphiplasty was suspected. In barley x rye hybrids, the Secale genome is more unstable and carries a suppressed NOR. A literature search was undertaken to investigate the coincidence of chromosome instability and differential amphiplasty. There are three reasons why few cases of correspondence of chromosome instability and differential amphiplasty should be expected: i) chromosome number instability is difficult to link with one genome unless the genomes are chromosomally distinct; ii) when genome elimination takes place, differential amphiplasty cannot be investigated unless some hybrids are produced; iii) differential amphiplasty is easily overlooked, especially if it is only partial.
and the investigator is not searching for it. Nevertheless, there are 10 reports of hybrids in the Triticeae where some form of chromosome instability has been reported and nucleolar organiser activity investigated (fig. 8.2). In five of these cases the genome which suffered genome elimination or chromosome instability also had suppressed nucleolar organisers, in three cases the chromosome instability was not ascribed to a particular genome and in two cases chromosome elimination was found but all NORs were reported to be active. As has been previously stated, partial differential amphiplasty can be overlooked, so this appears to support the assertion that chromosome instability and differential amphiplasty are closely linked.

This same link can also be found in other hybrids. In Crepis, for example, Hollingshead (1928) reported haploids of *C. capillaris* following crosses between *C. capillaris* and *C. tectorum*. Wallace and Langridge (1971), however, were able to obtain the hybrid and found that the *C. tectorum* NOR was suppressed.

Similar corroboration comes from work on mammalian somatic cell hybrid lines. Human x mouse somatic hybrid cells normally lose human chromosomes (Weiss and Green 1967) and also have the human NORs suppressed (Miller et al. 1976). Using different cell lines, Croce et al. (1977) also made a human x mouse somatic hybrid but, exceptionally, in their hybrids it was the mouse chromosomes which segregated. It is, therefore, all the more significant that even when all the mouse NOR chromosomes were
### A. Interspecific Hybrids

<table>
<thead>
<tr>
<th>Cross</th>
<th>Ref.</th>
<th>Nature of Instability</th>
<th>NORs</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>H. vulgare</em> x <em>H. bulbosum</em></td>
<td>3, 5, 13, 15, 16, 31, 32</td>
<td><em>bulbosum</em> eliminated, also some <em>vulgare</em> instability.</td>
<td><em>bulbosum</em> suppressed.</td>
</tr>
<tr>
<td><em>H. vulgare</em> x <em>H. brachyantherum</em></td>
<td>24</td>
<td>PMC aneusomaty</td>
<td>-</td>
</tr>
<tr>
<td><em>H. vulgare</em> x <em>H. depressum</em></td>
<td>18</td>
<td>&quot;</td>
<td>-</td>
</tr>
<tr>
<td><em>H. vulgare</em> x <em>H. jubatum</em></td>
<td>24</td>
<td>&quot;</td>
<td>-</td>
</tr>
<tr>
<td><em>H. vulgare</em> x (<em>H. jubatum</em> x <em>H. compressum</em>)</td>
<td>21</td>
<td>aneusomaty affecting <em>vulgare</em></td>
<td>-</td>
</tr>
<tr>
<td><em>H. vulgare</em> x <em>H. lechleri</em></td>
<td>25</td>
<td><em>vulgare</em> eliminated</td>
<td>all active</td>
</tr>
<tr>
<td><em>H. vulgare</em> x <em>H. leporinum</em></td>
<td>12</td>
<td>vegetative segregation</td>
<td>-</td>
</tr>
<tr>
<td><em>H. vulgare</em> x <em>H. marinum</em></td>
<td>35</td>
<td><em>vulgare</em> eliminated</td>
<td>-</td>
</tr>
<tr>
<td><em>H. vulgare</em> x <em>H. procerum</em></td>
<td>28</td>
<td><em>vulgare</em> eliminated</td>
<td>-</td>
</tr>
<tr>
<td><em>H. bulbosum</em> x <em>H. arizonicum</em></td>
<td>30</td>
<td><em>bulbosum</em> eliminated</td>
<td>-</td>
</tr>
<tr>
<td><em>H. bulbosum</em> x <em>H. brachyantherum</em></td>
<td>29</td>
<td>&quot;</td>
<td>-</td>
</tr>
<tr>
<td><em>H. bulbosum</em> x <em>H. brevisubulatum</em></td>
<td>30</td>
<td>&quot;</td>
<td>-</td>
</tr>
<tr>
<td><em>H. bulbosum</em> x <em>H. depressum</em></td>
<td>29</td>
<td>&quot;</td>
<td>-</td>
</tr>
<tr>
<td><em>H. bulbosum</em> x <em>H. jubatum</em></td>
<td>25</td>
<td>&quot;</td>
<td>all active</td>
</tr>
<tr>
<td><em>H. bulbosum</em> x <em>H. parodii</em></td>
<td>28</td>
<td>&quot;</td>
<td>-</td>
</tr>
<tr>
<td><em>H. bulbosum</em> x <em>H. procerum</em></td>
<td>28</td>
<td>&quot;</td>
<td>-</td>
</tr>
<tr>
<td><em>H. bulbosum</em> x <em>H. secalinum</em></td>
<td>4</td>
<td><em>secalinum</em> eliminated</td>
<td><em>secalinum</em> suppressed</td>
</tr>
</tbody>
</table>

*Hordeum* spp. (several hybrids)  

<table>
<thead>
<tr>
<th>Cross</th>
<th>Ref.</th>
<th>Nature of Instability</th>
<th>NORs</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>T. dicoccum</em> x <em>T. timopheevi</em></td>
<td>26</td>
<td>PMC aneusomaty</td>
<td>-</td>
</tr>
<tr>
<td><em>T. dicoccoides</em> x <em>T. timopheevi</em></td>
<td>26</td>
<td>PMC aneusomaty</td>
<td>-</td>
</tr>
<tr>
<td><em>T. dicoccoides</em> x <em>T. boeticum</em></td>
<td>26</td>
<td>PMC aneusomaty</td>
<td>-</td>
</tr>
<tr>
<td><em>T. timopheevi</em> x <em>T. durum</em></td>
<td>26</td>
<td>PMC aneusomaty</td>
<td>-</td>
</tr>
<tr>
<td><em>T. timopheevi</em> x <em>T. turgidum</em></td>
<td>26</td>
<td>PMC aneusomaty</td>
<td>-</td>
</tr>
</tbody>
</table>

### B. Intergeneric Hybrids

<table>
<thead>
<tr>
<th>Cross</th>
<th>Ref.</th>
<th>Nature of Instability</th>
<th>NORs</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>H. vulgare</em> x <em>S. vavilovii</em></td>
<td>6</td>
<td><em>Secale</em> eliminated</td>
<td>-</td>
</tr>
<tr>
<td><em>H. vulgare</em> x <em>S. africanum</em></td>
<td>22, 33</td>
<td>aneusomaty</td>
<td>-</td>
</tr>
<tr>
<td><em>H. chilense</em> x <em>S. cereale</em></td>
<td>10</td>
<td>Probably aneusomaty affecting <em>Secale</em></td>
<td><em>Secale</em> suppressed</td>
</tr>
<tr>
<td><em>H. chilense</em> x <em>S. africanum</em></td>
<td>11</td>
<td>most have aneusomaty</td>
<td><em>Secale</em> suppressed</td>
</tr>
<tr>
<td><em>H. chilense</em> x <em>S. montanum</em></td>
<td>11</td>
<td>most have aneusomaty</td>
<td><em>Secale</em> suppressed</td>
</tr>
<tr>
<td><em>H. jubatum</em> x <em>S. africanum</em></td>
<td>11</td>
<td>most have aneusomaty</td>
<td><em>Secale</em> suppressed</td>
</tr>
<tr>
<td><em>H. pusillum</em> x <em>Triticum aestivum</em></td>
<td>14</td>
<td><em>Psathyrostachys</em> eliminated</td>
<td>-</td>
</tr>
<tr>
<td><em>H. parodii</em> x <em>S. cereale</em></td>
<td>9</td>
<td>Aneusomaty, probably affecting <em>Secale</em></td>
<td><em>Secale</em> suppressed</td>
</tr>
<tr>
<td><em>H. lechleri</em> x <em>S. cereale</em></td>
<td>19</td>
<td>aneusomaty</td>
<td>all active</td>
</tr>
<tr>
<td><em>H. vulgare</em> x <em>T. turgidum</em></td>
<td>8</td>
<td>aneusomaty</td>
<td>-</td>
</tr>
<tr>
<td>(<em>H. vulgare</em> x <em>T. aestivum</em>) x <em>S. cereale</em></td>
<td>8</td>
<td>aneusomaty</td>
<td>-</td>
</tr>
<tr>
<td>Cross</td>
<td>Ref.</td>
<td>Nature of Instability</td>
<td>NOR</td>
</tr>
<tr>
<td>-------</td>
<td>------</td>
<td>-----------------------</td>
<td>-----</td>
</tr>
<tr>
<td>H. bulbosum × T. aestivum</td>
<td>2</td>
<td>some elimination</td>
<td>-</td>
</tr>
<tr>
<td>H. vulgare × Elymus arenarius</td>
<td>1</td>
<td>Hordeum eliminated</td>
<td>Hordeum suppressed</td>
</tr>
<tr>
<td>Agropyron × Elymus</td>
<td>20</td>
<td>aneusomaty</td>
<td>-</td>
</tr>
<tr>
<td>Aegilops triuncialis × T. aestivum</td>
<td>23</td>
<td>aneusomaty</td>
<td>-</td>
</tr>
<tr>
<td>A. crassa × H. bulbosum</td>
<td>27</td>
<td>Hordeum eliminated</td>
<td>-</td>
</tr>
<tr>
<td>A. ovata × T. timopheevi</td>
<td>26</td>
<td>PMC aneusomaty</td>
<td>-</td>
</tr>
<tr>
<td>A. ovata × T. dicoccum</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A. ovata × T. boeticum</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A. ovata × T. earl,icicum</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A. cylindrica × T. durum</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A. cylindrica × T. dicoccum</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A. caudata × T. dicoccum</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A. caudata × T. turgidum</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A. caudata × T. durum</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A. caudata × T. timopheevi</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>T. dicoccoides × A. umbellulata</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>T. timopheevi × A. bicornis</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>T. aestivum × (T. dicoccoides × A. umbellulata)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>T. dicoccoides × A. umbellulata × T. dicoccoides × A. caudata</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Fig. 8.2 References

11. Finch and Bennett (1981 b) 29. Subrahmanyan (1979)
16. Lange (1971 a, b) 34. Von Bothmer et al. (1981)
present it was the mouse rRNA synthesis that was totally suppressed.

The chromosome elimination found in many interspecific crosses in the genus *Hordeum* was discussed in section 8.2. Any hypothesis concerning the causes of such genome elimination must explain the consistency of the eliminating potential of a species and the increase in eliminating potential with polyploidy. Differential amphiplasty shows a similar consistency with one species being dominant in many combinations (Wallace and Langridge 1971). In addition, as differential amphiplasty depends on the presence of the dominant NOR (Bicudo and Richardson 1977), the presence of extra NOR(s) in a polyploid would be likely to increase differential amphiplasty. Differential amphiplasty, therefore, may fit the characteristics required for a cause of genome elimination in *Hordeum* hybrids.

It is certain that there is a link between differential amphiplasty and chromosome instability. What remains unclear is the nature of this link. Three alternatives are possible: i) chromosome instability causes differential amphiplasty; ii) differential amphiplasty causes chromosome instability; or iii) some other factor causes both chromosome instability and differential amphiplasty.

The first alternative is that chromosome instability *per se* causes differential amphiplasty. This can be dispensed with because differential amphiplasty occurs in euploid cells and has been shown to be due to the presence of the dominant NOR.

The second alternative, that differential amphiplasty causes
chromosome instability is possible. As described in Chapter 7, the function of the nucleolus is largely ribosome synthesis. Other functions are possible, of which mRNA processing seems the most likely. If the nucleolus in some way affects the functioning of other parts of the same genome but not different genomes in the same cell, then weak (or deficient) centromeres in one genome are possible. This could explain why, in a hybrid, the genome with suppressed NORs also tends to suffer from chromosome instability.

The third alternative is that some other factor causes both differential amphiplasty and chromosome instability. This seems to be at least as likely as the second alternative, given above, but it remains an unsatisfactory hypothesis unless this other factor can be identified. There are two other cytological features which seem to be correlated with chromosome instability and differential amphiplasty, namely mean cell doubling time and spatial separation of genomes.

Three examples have been given where a cell cycle disparity in a hybrid correlates with the expected direction of chromosome instability. If many more of the combinations of species which give chromosome instability in the hybrid were analysed to determine mean cell doubling times, then the relationships of cell cycle time, differential amphiplasty and chromosome instability would be clearer. It is possible that a disparity of cell cycle times of genomes in a hybrid would lead to NORs of one genome reactivating first after division which allowed them to become dominant. However,
some indications that cell cycle disparity may not be an important cause of chromosome instability have already been discussed.

The spatial separation of genomes in a hybrid has been noted by Finch and Bennett (1981 a), (1981 b), (1983). They have proposed that the centromeres of one genome become less clearly defined, possibly via DNA methylation, which causes that genome to be peripherally disposed at metaphase and subject to chromosome elimination. DNA methylation was proposed as a mechanism because such methylation has recently been shown to be associated with differential amphiplasty (Flavell et al. 1983). It is always difficult to distinguish between cause and effect in such cases. The centromeres of the eliminated genome, for example, may be weak because they are peripheral, or may be peripheral because they are weak. If it is true that DNA methylation causes both differential amphiplasty and chromosome instability, then the problem simply shifts to one of determining what controls methylation in a hybrid. These suggestions on the interactions between chromosome instability and differential amphiplasty are summarised in fig. 8.3.

The value of a discussion of theories to explain chromosome instability lies in the possibility of testing the theories experimentally. Four possible candidates for the cause of chromosome instability have been discussed, namely differential amphiplasty, cell cycle disparity, chromosome disposition and DNA methylation. Possible experimental approaches to investigate each one will now be outlined.
Possible causes (left) and consequences (right) of various cytological phenomena found in interspecific hybrids.

a) differential amphiplasty $\rightarrow$ chromosome instability

b) i) cell cycle disparity $\rightarrow$ differential amphiplasty $\rightarrow$ chromosome instability

ii) cell cycle disparity $\rightarrow$ differential amphiplasty $\rightarrow$ chromosome instability

$\rightarrow$ centromere strength $\rightarrow$ chromosome instability

c) chromosome disposition $\rightarrow$ differential amphiplasty

d) ? $\rightarrow$ centromere strength $\rightarrow$ chromosome disposition $\rightarrow$ chromosome instability

If differential amphiplasty causes chromosome instability, then modifying the dominance of nucleolar organisers would affect chromosome instability. Using a NOR-dominant parental line with a deleted or reduced NOR would be likely to reactivate the suppressed NOR and, if differential amphiplasty causes chromosome instability, reverse the direction of chromosome elimination. Chromosome fragments carrying a foreign NOR are known to cause differential amphiplasty (Bicudo and Richardson 1977). The incorporation of a dominant NOR-carrying fragment would be predicted to affect the direction of chromosome elimination.
If cell cycle disparities between genomes in a hybrid are responsible for chromosome instability and differential amphiplasty, then further study of cell cycle times of parents of hybrids should unearth more correlations. Selecting genotypes with more similar cell cycle times should show decreases in chromosome instability and differential amphiplasty. A study of differential amphiplasty in embryos from crosses of barley and rye with similar and dissimilar cell cycle times was started, following the work of Forster and Dale (1983 a), (1983 b), but no conclusive results were obtained so this work has not been presented here.

If chromosome disposition has a role in chromosome instability and differential amphiplasty, then the same pattern of spatial separation of genomes should be apparent in other hybrids showing chromosome instability.

Methylation of DNA bases may be involved in chromosome elimination, as proposed by Finch and Bennett (1983). It is important to test this. Methylation can be detected by molecular techniques. It is also possible that methylated DNA is antigenically different from non-methylated DNA, so opening up the possibility of detecting methylated sites by immuno-fluorescent techniques. An alternative approach would be the use of methylation suppressors, if they should become available. If methylation could be experimentally suppressed, then, according to Finch and Bennett's model, both chromosome instability and differential amphiplasty would disappear.
8.5 General conclusions

Interspecific or intergeneric hybrids have several interesting features, many of them unique to hybrids, which may have a bearing on the sterility or inviability of the plants. Several of these features of hybrids are poorly understood and require further investigation.

This investigation into hybrids between barley and rye set out to assess the impact of these features on vegetative and reproductive development.

The extent of chromosome instability in barley x rye hybrids has now been documented. Instability has been shown to occur in all meristems studied. Pollen mother cells had a greater variation in chromosome number than root-tip meristem cells. A new acrocentric chromosome, derived from the rye NOR chromosome by deletion of most of its long arm, was much more variable than any other chromosome. Apart from this acrocentric chromosome, chromosome gain was as frequent as chromosome loss. A mechanism for this was proposed. Chromosome instability is important in the vegetative and reproductive development of the hybrid in several ways. It was suggested that chromosome instability causes: the poor root growth seen in these hybrids; morphological aberrations such as split spikes; variegation of leaf tissue; general reduction of vigour; and a potential reduction of fertility through aneuploidy.

Differential amphiplasty was also investigated. It was found
that barley NORs functioned normally but rye NORs were almost
totally suppressed. The effect on the nucleoli was investigated and,
surprisingly, the total nucleolar size was greater than in either
parent. Differential amphiplasty did not, therefore, appear to reduce
ribosome output in barley x rye hybrids. Other new facts about
nucleolar function uncovered by this work were that nucleolar size
correlates well with the degree of fusion of nucleoli and that the
total surface area, and not the total volume, of the nucleoli in a cell
is conserved on fusion. The effect of differential amphiplasty on
barley x rye hybrids must remain obscure because the functions of
the nucleolus, other than the production and assembly of ribosomes,
are not known. The observations reported confirm the correlation
between the direction of chromosome instability and the direction
of nucleolar dominance.

Reproductive development and meiosis have also been
investigated in barley x rye hybrids. Different genotypes were
sterile for different reasons. One group of hybrids (cytotypes A
to D) were totally pistilloid and all pistils were internally abnormal
so that no meiocytes were formed at all. Those hybrids that
developed meiocytes displayed meiotic problems typical of wide
hybrids. One chromosome pair which was present in these
aneuploid hybrids rarely formed a bivalent, however, which indicates
that the amphiploid may not be fertile either.

Barley x rye hybrids, in common with several other wide
hybrids, are difficult to successfully treat with colchicine to obtain
amphiploids. The reasons for this were investigated. Hybrid cells
do not respond to colchicine differently from parental cells. It was
suggested that low vigour, caused, for example, by chromosome
instability, was the major reason for the difficulties in obtaining the
amphiploid through chromosome doubling.

Existing theories to account for chromosome instability have
been shown to be unsatisfactory: there are no existing theories to
explain differential amphiplasty. In this last chapter, alternative
explanations for chromosome instability have been made. The
possible interactions between chromosome instability and differential
amphiplasty have been discussed. These alternative explanations
are an essential precursor to the experimental approaches outlined
for further studies of the causes and correlations of these phenomena.

The experimental approaches outlined in this chapter will help
the understanding of these phenomena. This may enable the control
or avoidance of the harmful consequences of hybridity in future, thus
allowing practical use to be made of hybrids such as that between
barley and rye.
Glossary

Allosyndesis: pairing between homoeologous chromosomes.

Amphiplasty: morphological changes (particularly in size) in chromosomes following interspecific hybridisation.

Amphiploid: polyploid composed of more than one genome.

Anatropous: rotated (ovule) through 180°, so that micropyle points to placenta.

Androgenic progeny: progeny derived from a male haploid nucleus.

Aneuploidy: the (stable) loss or gain of one or more chromosomes from the euploid complement.

Aneusomaty: variation in chromosome number within an individual.

Autosyndesis: pairing between homologous chromosomes derived from the same parent.

Bitegmic: possessing two integuments.

Differential amphiplasty: changes in individual chromosomes following interspecific hybridisation. Usually refers to the NOR-bearing chromosome and indicates the suppression of a NOR in a hybrid.

Dihaploid: possessing two, distinct haploid chromosome sets.

Heterochromatin: chromatin which remains condensed throughout interphase and is generally composed of repeated sequences of DNA.
<table>
<thead>
<tr>
<th>Term</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heterokaryon</td>
<td>A bi- or multinucleate cell whose nuclei are genetically different.</td>
</tr>
<tr>
<td>Hyperaneuploid (Hypo-)</td>
<td>Of an individual or cell with more (or less) than the euploid chromosome number.</td>
</tr>
<tr>
<td>Hypermodal* (Hypo-)</td>
<td>Of a cell in an aneusomatic meristem with more (or less) than the modal chromosome number.</td>
</tr>
<tr>
<td>NOR</td>
<td>A nucleolus organiser region. A region of active rRNA genes, often called a secondary constriction and delimited by a distal satellite.</td>
</tr>
<tr>
<td>Pistillody</td>
<td>Possessing stamens converted to pistils.</td>
</tr>
<tr>
<td>Polysomaty</td>
<td>Containing diploid and polyploid cells side by side.</td>
</tr>
<tr>
<td>Pseudochias mata</td>
<td>Chiasma-like connections between two homologous or non-homologous chromosomes at meiosis.</td>
</tr>
</tbody>
</table>

*I am grateful to Dr. A. F. Dyer for suggesting these terms.
Appendix 1  Giemsa C-banded karyotype of barley
cv. Sundance published by
Linde-Laursen 1978 a

1  2  3  4  5  6  7
Appendix 2  Correspondence between published karyotypes, linkage
groups and wheat homoeology

a)  Barley

<table>
<thead>
<tr>
<th>Chromosome number or linkage group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ramsay (fig. 5.10)</td>
</tr>
<tr>
<td>Linde-Laursen (1975 et. seq.)</td>
</tr>
<tr>
<td>Noda and Kasha (1978 a)</td>
</tr>
<tr>
<td>Vosa (1976)</td>
</tr>
<tr>
<td>Proposed revised linkage groups</td>
</tr>
<tr>
<td>Proposed wheat homoeology</td>
</tr>
</tbody>
</table>

b)  Rye

<table>
<thead>
<tr>
<th>Chromosome number or linkage group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ramsay (fig. 5.11)</td>
</tr>
<tr>
<td>Zeller et al. (1977)</td>
</tr>
<tr>
<td>Lelley et al. (1978)</td>
</tr>
<tr>
<td>Gill and Kimber (1974)</td>
</tr>
<tr>
<td>Bennett et al. (1977)</td>
</tr>
<tr>
<td>Vosa (1974)</td>
</tr>
<tr>
<td>de Vries and Sybenga (1976)</td>
</tr>
<tr>
<td>Sarma and Natarajan (1973)</td>
</tr>
</tbody>
</table>

1. The same as Giraldez et al. (1979) and probably
   Singh and Robellen (1975).
2. Based on Darvey and Gustafson (1975).
3. The letter R indicates an attempt at homoeology
   with wheat.
Nucleolar organiser suppression in barley × rye hybrids

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In many intergeneric and interspecific hybrids, only one of the contributing genomes forms nucleoli. This phenomenon has been widely reported but rarely studied in any detail.

Hybrids of Hordeum vulgare L. × Secale cereale L. from two different sources have been used in this study. Both hybrids are aneuploid and have an extra rye chromosome with a nucleolus organising region (NOR).

<table>
<thead>
<tr>
<th>Hybrid No.</th>
<th>Source</th>
<th>Chrm. No.</th>
<th>Deviation from n + n</th>
</tr>
</thead>
<tbody>
<tr>
<td>20 × R</td>
<td>A. Kruse, Riso, Denmark</td>
<td>15</td>
<td>Extra 1R</td>
</tr>
<tr>
<td>11a</td>
<td>Cooper et al (1978), Edinb.</td>
<td>14</td>
<td>Extra 1R, no barley 2 deletion on barley 7</td>
</tr>
</tbody>
</table>

Cells were stained in AgNO₃ (modified Hizume et al. 1980) to show active NORs and to stain interphase nucleoli.

Nucleolus counts suggest that NORs are suppressed: more than two major nucleoli are never found. In both hybrids, barley NORs (on chromosomes 6 and 7) are stained whereas the rye NORs (on chromosome 1R) are stained weakly or not at all (fig. 1). Surprisingly, data on nucleolar dimensions (fig. 2) show that the nucleoli of the hybrid are much larger than those of either parent. In addition, the data agree with Sacristan-Garante et al. (1974) in that, as nucleoli fuse, the total surface area remains relatively stable but the total volume increases.

References


Das, N. K. 1962 - Synthetic capacities of chromosome fragments correlated with their ability to maintain nucleolar material. J. Cell. Biol. 15, 121-130.


Duncan, R. E. 1945 - Production of variable aneuploid numbers of chromosomes within the root-tips of Paphiopedium wardii Amer. J. Bot. 32, 506-509.


Fiskesjo, G. 1974 - Two types of constitutive heterochromatin made visible in Allium by a rapid C-banding method. Hereditas 78, 153-156.


Honjo, T., and Reeder, R. H. 1973 - Preferential transcription of *Xenopus laevis* ribosomal RNA in interspecies hybrids between *X. laevis* and *X. mulleri*. J. Mol. Biol. 80, 217-228.


Kruse, A. 1976 - Reciprocal hybrids between the genera *Hordeum*, *Secale* and *Triticum*. Hereditas 84, 244.


Levan, A. 1942 - Studies on the meiotic mechanism of haploid rye. Hereditas 28, 177-211.


Martini, G., O'Dell, M., and Flavell, R. B. 1982 - Partial inactivation of wheat nucleolus organisers by the nucleolus organiser chromosomes from *Aegilops umbellulata*. Chromosoma 84, 687-700.


Östergren, G. 1954 - Polyploids and aneuploids of *Crepis capillaris* produced by treatment with nitrous oxide. Genetica 27, 54-64.


Pilch, J. 1978 - Cytological and morphological characteristics of primary trisomics in rye (*Secale cereale* L.) Part II. Genetica Polonica 19, 137-152.

Pohlendt, G. 1959 - Variabilität der chromosomenzahlen und andere kernpathologien in *Aegilops triuncialis* x *Triticum aestivum* bastarden. Zeit. fur Vererbungslehre 89, 170-188.


Scoles, G. J. and Kaltsikes, P. J. 1974 - The cytology and cytogenetics of triticale. Z. Pflanzenzuchtg. 73, 13-43.


Thompson, M. M. 1962 - Cytogenetics of Rubus III. Meiotic instability in some higher polyploids. Amer. J. Bot. 49, 575-582.


Verma, R.S. 1980 - The duration of G1, S, G2 and mitosis at four different temperatures in Zea mays L. as measured with 3H-thymidine. Cytologia 45, 327-333.


Vosa, C. G. 1974 - The basic karyotype of rye (Secale cereale) analysed with Giemsa and fluorescence methods. Heredity 33, 403-408.


Weimark, A. 1975 - Heterochromatin polymorphism in the rye karyotype as detected by the Giemsa C-banding technique. Hereditas 79, 293-300.


Wojciechowska, B. 1978 - Hybrid between *Hordeum jubatum* L. x *Secale cereale* L. and its backcross generations with rye. I. Morphology, fertility and chromosome number of F₁ and BC₁ hybrids. *Genetica Polonica* 19, 265.


