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Cytogenetic studies of *Haplopappus gracilis* in both callus and suspension cell cultures

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Summary. Investigations have been carried out on karyotype change in both callus and suspension cell cultures of *Haplopappus gracilis* (2n=4). It has been found that polyploidization arises directly in culture to give up to six times the normal diploid chromosome number in some cultures. In polyploid cultures, both chromosome loss and chromosome rearrangements occur to give rise to aneuploid karyotypes displaying chromosomes which differ in morphology from the diploid set. Whole or partial chromosome loss has been observed in the form of lagging chromosomes and chromosome bridges at anaphase, and micronuclei, ring chromosomes and chromosome fragments at other stages in mitosis. C-banded preparations have confirmed the occurrence of chromosomal rearrangements. Comparative investigations suggest that (i) more polyploidy occurs in callus cultures than in suspension cell cultures, and (ii) the presence of cytokinin (kinetin) in the culture medium may reduce the extent of karyotype change.

Key words: *Haplopappus gracilis* – C-banding – Karyotype instability – Callus culture – Suspension culture

Introduction

It is well-established that karyotype changes, including both numerical and structural alterations, commonly occur in cells when they are removed from the whole plant and grown in a tissue culture environment (Sunderland 1973; Bayliss 1980). Such change at the genetic level may lead to the phenomenon of somaclonal variation in regenerated plants (Larkin and Scowcroft 1981), or may indeed inhibit the morphogenic potential of cells in tissue culture (Gaponenko et al. 1988). It has recently also been shown that karyotype abnormalities may lead to a reduced capacity for protoplast division in culture (Hahne and Hoffmann 1986).

The techniques of cell culture, protoplast culture and plant regeneration from such cultures are of central importance in the genetic manipulation of plants for plant improvement. An understanding of the dynamics and extent of chromosome change in cell culture is thus important in determining the systems to be used for such manipulation studies. Additionally, the elucidation of patterns of karyotype change in culture, and the effects of such parameters as culture conditions, interval between subculturing and the levels of plant growth substances will be useful in furthering our knowledge of plant genetic systems.

Much of the work on chromosome instability in plant tissue culture has been performed on plant species with many small, morphologically similar chromosomes, making analysis difficult to perform. The current study utilizes the species *Haplopappus gracilis* (2n=4) and catalogues chromosome change in both callus and suspension cell cultures, and also includes some analysis using C-banding techniques.

Materials and methods

Whole plant material

Seeds of *Haplopappus gracilis* (kindly supplied by R. C. Jackson) were germinated on wet filter paper at 22 °C in the light, and plants were then maintained in a glasshouse with a diurnal temperature range of 15 °C–24 °C. Flowering occurred on a 10-h day, 14-h night regime.

Plant cell culture

Callus cultures of *Haplopappus gracilis* were grown on B5 medium (Gamborg and Eveleigh 1968), supplemented with

1 mg L^{-1} 2,4-D (B5-1) or 2 mg L^{-1} 2,4-D (B5-2) or on Murashige and Skoog medium (1962) supplemented with 2 mg L^{-1} 2,4-D and 0.05 mg L^{-1} kinetin (M&S). Subculturing onto fresh medium was performed every 28 days. All callus cultures were incubated in the dark at 26 °C.

Suspension cell cultures were initiated by placing callus tissue into liquid B5-1 medium and were incubated in the dark at 25 °C on an orbital shaker at 150 rpm. Subculturing was performed every 14 days by placing 10 ml of the old culture into 50 ml of fresh medium.

Five different cell lines of *Haplopappus gracilis* were used in this study, as defined in Table 1.

Orcein staining of chromosomes

Live plant material, either from cell cultures or root tips, was stained directly on a microscope slide using Lactopropionic Orcein (1% Orcein in 1:1 lactic acid:propionic acid), and gently heated by passing through a bunsen flame several times. After a few minutes, the stain was replaced with 1:1 lacto:propionic acid and the cells were tapped and squashed under a coverslip ready for immediate observation under the microscope. Permanent slides were prepared by removal of the coverslip after freezing on dry ice, air drying and mounting in Gurr's XAM neutral mountant.

C-banding

Both cultured cells and root tips were incubated in 0.1% colchicine for 3 h prior to fixation in 3:1 ethanol:acetic acid. After at least 24 h in fixative, cells were softened in 45% acetic acid for 1 h before being tapped out gently onto subbed microscope slides and squashed under a coverslip. The coverslips were removed after freezing on dry ice and the slides were washed in distilled water and left to dry for at least 24 h.

Table 1. Characteristics of *H. gracilis* cell lines

Cell line	Culture type	Medium
HA-1	Suspension	B5 with 1 mg L^{-1} 2,4-D (B5-1)
HA-2	Suspension	B5 with 1 mg L^{-1} 2,4-D (B5-1)
HA-3	Callus	Murashige and Skoog with 2 mg L^{-1} 2,4-D and 0.05 mg L^{-1} kinetin (M & S)
HA-4	Callus	B5 with 1 mg L^{-1} 2,4-D (B5-1)
HA-5	Callus	B5 with 2 mg L^{-1} 2,4-D (B5-2)

In order to achieve C-banding, slides were treated with 0.2 *M* HCl for 20 min, then washed in distilled water prior to incubation in a saturated barium hydroxide solution for 2–5 min at 45 °C. Immediately after removal from the barium hydroxide solution, slides were rinsed in 0.2 *M* HCl followed by distilled water, and then left in 2 × SSC for 60 min at 65 °C. After a final wash in distilled water, the slides were stained in 10% Giemsa in phosphate buffer (pH 6.8) for 5–10 min. All slides were permanently mounted in Gurr's XAM neutral mountant.

Results

The diploid karyotype of *H. gracilis* consists of 2 pairs of chromosomes. (i) One pair of large (approximately 8 μ at metaphase) submetacentric chromosomes, termed the A chromosomes. (ii) One pair of smaller (approximately 6 μ at metaphase) acrocentric chromosomes, having a secondary constriction and a single satellite on the small arm, known as the B chromosomes. (Note that these are not B chromosomes in the classic sense, i.e. they are not supernumary).

When Jackson (1957) first described the karyotype of this species, this was the only plant species known to have such a low chromosome number. The plant material used in this project was all derived from a variety of *H. gracilis* which shows heteromorphism in the short arm of chromosome B, one chromosome having a double satellite, whilst its homologue retains the single satellited short arm. This variety, first described by Jackson (1963), is represented in Fig. 1.

The HA-1 culture was maintained in liquid suspension culture for the duration of this study. During a 4-day subculture regime, the original normal 4-chromosome diploid complement of *H. gracilis* was virtually lost from the culture. After 2 months of culture, 30% of the population had a tetraploid karyotype, with 70% remaining diploid (Fig. 2a), and after a further 3 months in culture, 85% of the population had a 6-chromosome aneuploid karyotype (Fig. 2b). Following a change to a 7-day transfer regime, the cells predominantly retained a 6-chromosome karyotype after 3 months, although 10% of the population had 12 chromosomes per cell and there

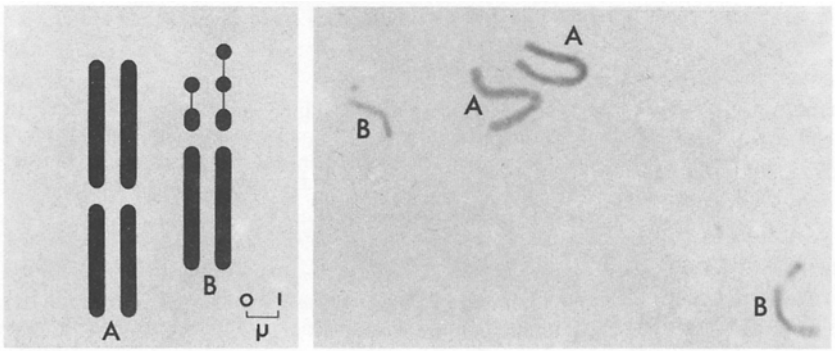

Fig. 1. The normal diploid karyotype of *Haplopappus gracilis*, as seen in idiogram form and in a metaphase plate from an Orcein-stained root tip preparation of the plant

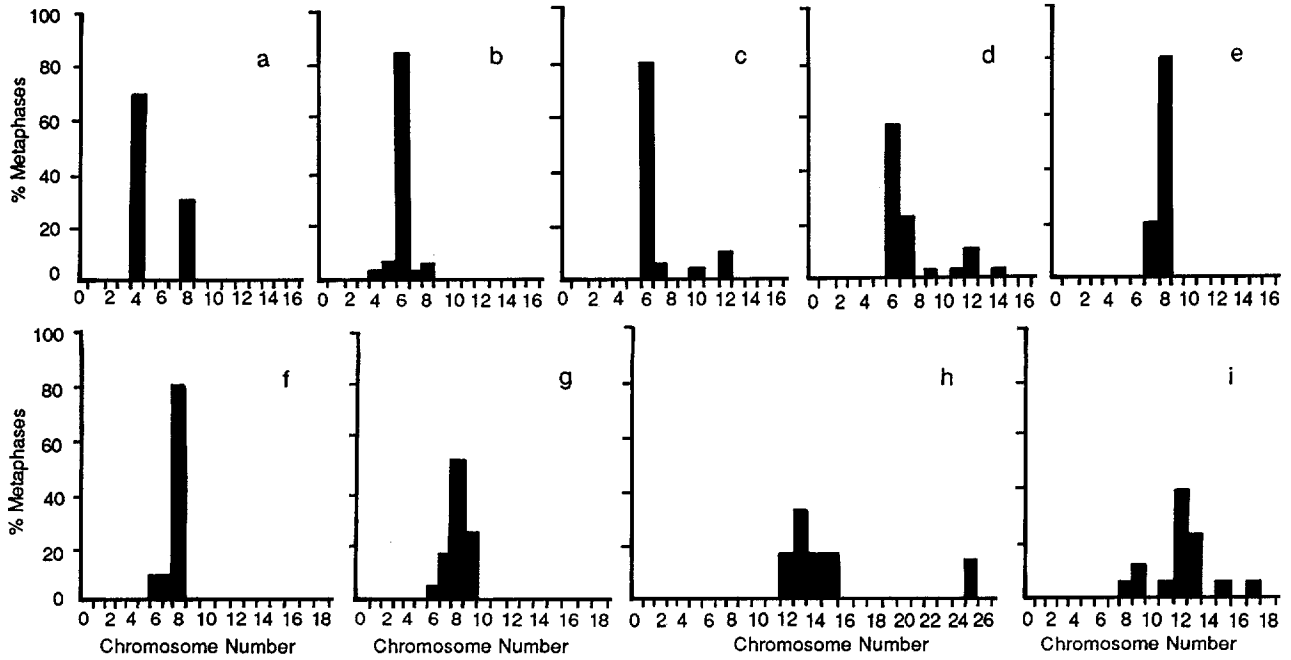

Fig. 2a–i. The frequency distribution of chromosome numbers observed in dividing cells of HA-1 suspension culture after: **a** 3 months in culture on a 4-day subculture regime; **b** 5 months in culture on a 4-day subculture regime; **c** 11 months in culture on a 7-day subculture regime; **d** 18 months in culture on a 7-day transfer regime; **e** 42 months in culture on a 7-day transfer regime; **f** HA-2 suspension culture after 4 months in culture; **g** HA-3 callus culture; **h** HA-4 callus culture; **i** HA-5 callus culture

were a few cells with 8 or 11 chromosomes (Fig. 2c). Seven months later, although 57% of the population had 6 chromosomes per cell, a considerable proportion (12%) now had a 7-chromosome karyotype and 12% of the cell population had 12 chromosomes per cell (Fig. 2d). Two years later, the 6-chromosome karyotype had been lost from the culture and an 8-chromosome karyotype represented the major chromosome complement in the dividing population (75%), with the remaining cells having a 7-chromosome aneuploid karyotype (Fig. 2e).

The chromosomal make-up of the 7 and 8 chromosome cells represented in this last culture were studied in more detail. Observations on gross morphology of the chromosomes of several cells revealed that the 8-chromosome karyotype was not a normal tetraploid complement, but comprised the same 8-derived chromosome types in all cells with this chromosome number (Fig. 3a). Chromosomes 1, 2, 3 and 4 of these sets, which are submetacentric, appear to be derived from the A chromosome of the normal *H. gracilis* complement, although both chromosomes 3 and 4 seem to be considerably shorter than chromosome A. On the other hand, chromosomes 5, 6, 7 and 8 of the 8-chromosome sets, which are all acrocentric, more closely resemble chromosome B of the normal diploid karyotype of *H. gracilis*. Chromosomes 7 and 8 have satellited short arms with chromosome 7 possibly showing the double satellite of the normal B chromosome. Chromosome 7 has an extended long arm as compared with the normal B chromosome, whereas chromosomes 4 and 6 seem to have an extended short arm.

Two examples of 7 chromosome complements are shown in Fig. 3b. Chromosomes 1, 2, 3 and 4 more closely resemble chromosome A of the diploid *H. gracilis* complement, whereas chromosomes 5, 6 and 7 are more likely derived from the B chromosome. Chromosome 6 retains the double satellite whilst a single satellite is retained on chromosome 7.

Giemsa C-banding was attempted on this culture, to try and describe the 7 and 8 chromosome complements more fully. Comparison is made with the normal diploid root tip complement of *H. gracilis*.

C-banded metaphase complements of *H. gracilis* root tip shows some consistent patterns of differential staining along the chromosomes (Fig. 4a). On chromosome A, two interstitial bands are observed on the shorter arm and dark C-banded blocks are seen on the short arm of chromosome B. There may also be a band close to the centromere on the longer arm of this chromosome. Heteromorphism of the short arm of chromosome B may occur but is not obvious in these preparations. The C-band patterns described are similar to those observed by Tanaka and Taniguchi (1975) in this species.

A C-banded preparation of the HA-1 suspension culture is presented in Fig. 4b. This C-banding technique

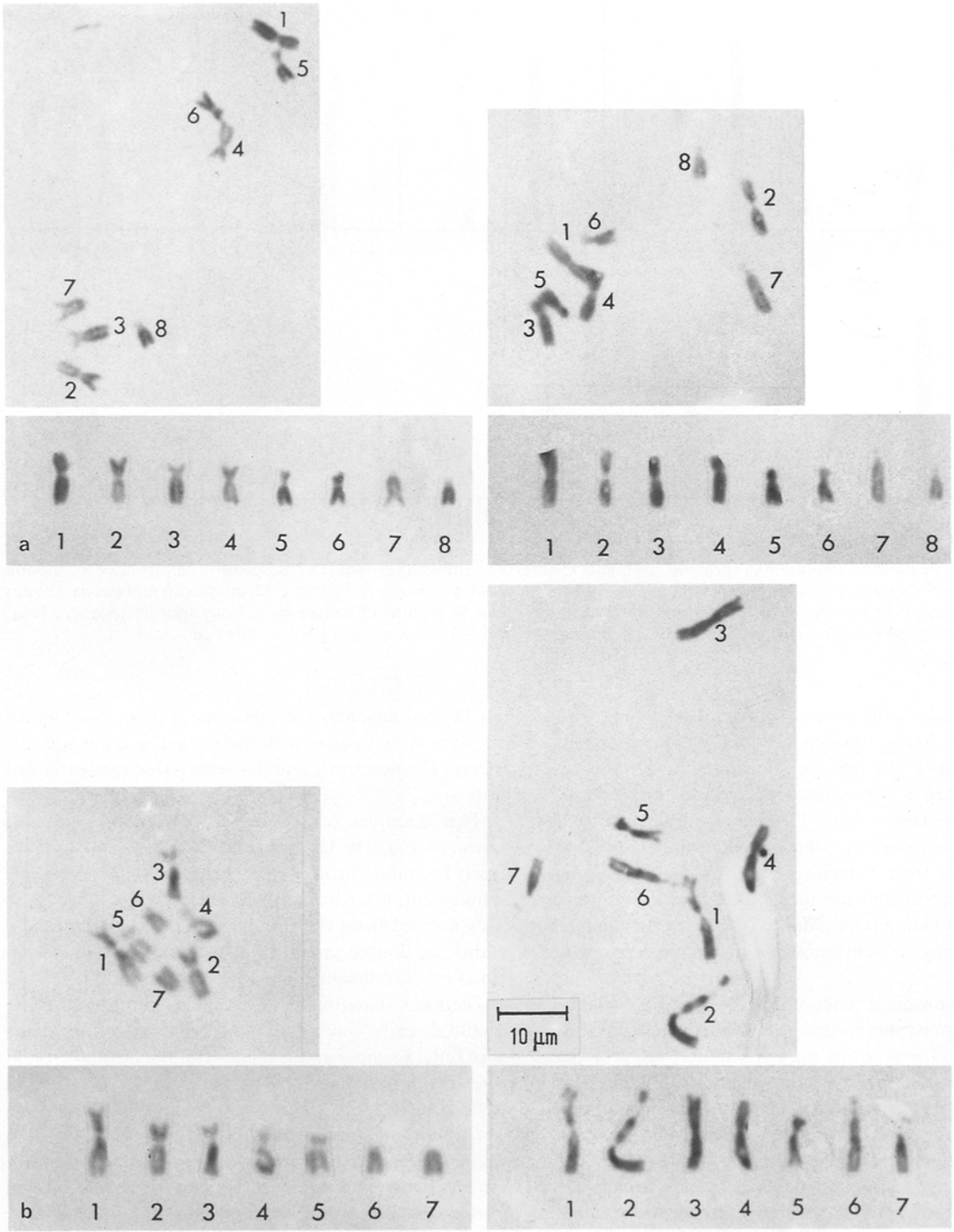

Fig. 3a and b. Metaphase plates of HA-1 suspension culture, showing: **a** two 8-chromosome complements; **b** two 7-chromosome complements

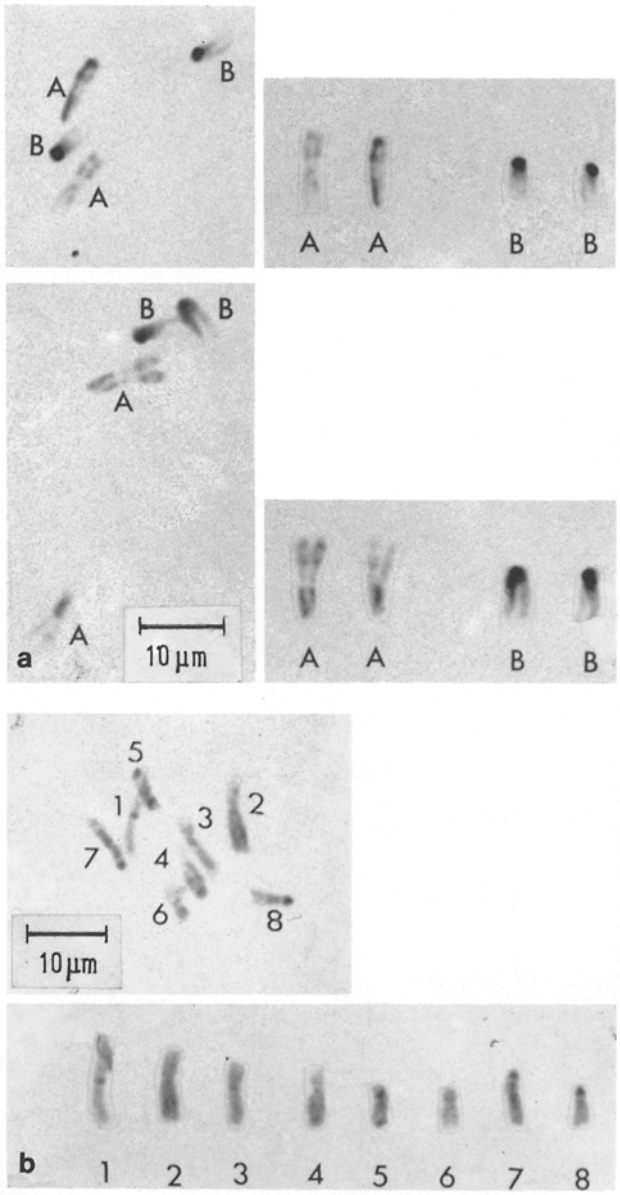

Fig. 4. **a** Two C-banded metaphases from root tip preparations of *H. gracilis*. **b** A C-banded 8-chromosome set from HA-1 suspension culture

was not very successful on these cultured cells, mainly because the chromosomes were difficult to remove from the cells and remained within the cell wall. However, some bands were clearly seen in all C-banded metaphase cells analysed, namely: (i) the dark band on the short arm of chromosome 8; (ii) the dark band on the short arm of chromosome 7; (iii) the band, close to the centromere, on the shorter arm of chromosome 5. Other bands, which appear in some cells included: (i) a centromeric band on chromosome 1; (ii) a band, close to the centromere, on the shorter arm of chromosome 4; (iii) a band, close to the centromere, on the shorter arm of chromosome 3; (iv) a telomeric block on the longer arm of chromosome 5.

The HA-2 culture was initiated from a callus which was predominantly tetraploid. After 4 months of culture, a study of chromosome number in the population revealed that although the dividing cells predominantly retained an 8-chromosome complement, 18% of the dividing cell population had either a 7-chromosome or 6-chromosome set (Fig. 2f). Examples of all 3 karyotypes are shown in Fig. 5. It can be seen that the 8-chromosome karyotype is not a normal tetraploid complement, which would be composed of 4 large submetacentric chromosomes (the A chromosome) and 4 small, satellited, acrocentric chromosomes (the B chromosome), but rather consists of 5 larger chromosomes and only 3 smaller, satellited chromosomes (Fig. 5a). The 'extra' large chromosome may have arisen by an unequal translocation between an A chromosome (presumably chromosome 5 of the derived set) and a B chromosome (presumably chromosome 6 of the derived set). The 7-chromosome complement (Fig. 5b) consists of 5 larger submetacentric chromosomes and only 2 small, acrocentric satellited chromosomes. The heteromorphism of the short satellited arms is clearly observed, whilst this was not so obvious in the 8 chromosome set. The 6-chromosome karyotype is composed of 4 larger, and 2 smaller chromosomes (Fig. 5c).

Many abnormalities of mitosis were observed in this culture at this time, including chromosome bridges, lagging chromosomes at anaphase and micronuclei (Fig. 6).

Analysis of the HA-3, HA-4, HA-5 cell lines was restricted to callus cultures and allowed the effect of three different media (M&S, B5-1, and B5-2, as described in the 'Materials and methods' section) to be observed. HA-1 and HA-2 were both maintained throughout on B5-1. The modal chromosome number of HA-3 in M&S medium was 8 (52%) with chromosome complements of 6 (5%), 7 (17%) and 9 (26%) also being represented (Fig. 2g). The 8-chromosome complement is seen in Fig. 7a and is clearly not a normal tetraploid complement, although there are 4 submetacentric chromosomes which probably derive from chromosome A of the normal diploid karyotype and 4 acrocentric-type chromosomes presumably derived from chromosome B. Chromosomes 1, 2 and 3 have an extended short arm compared to chromosome A, whereas chromosome 5 has an extended long arm compared with chromosome B. Cells with a 9-chromosome complement in this population have 5 A-type chromosomes and 4 B-type chromosomes, whereas cells with a 7-chromosome complement have 4 A-type chromosomes and 3 B-type chromosomes.

The majority of cells of HA-4 show a chromosome number of between 12 and 15 chromosomes (Fig. 2h). Some cells with a chromosome number of 25 were also observed in this population. Examples of a 13-chromosome complement and a 25-chromosome complement from this culture are seen in Fig. 7b and c. These aneu-

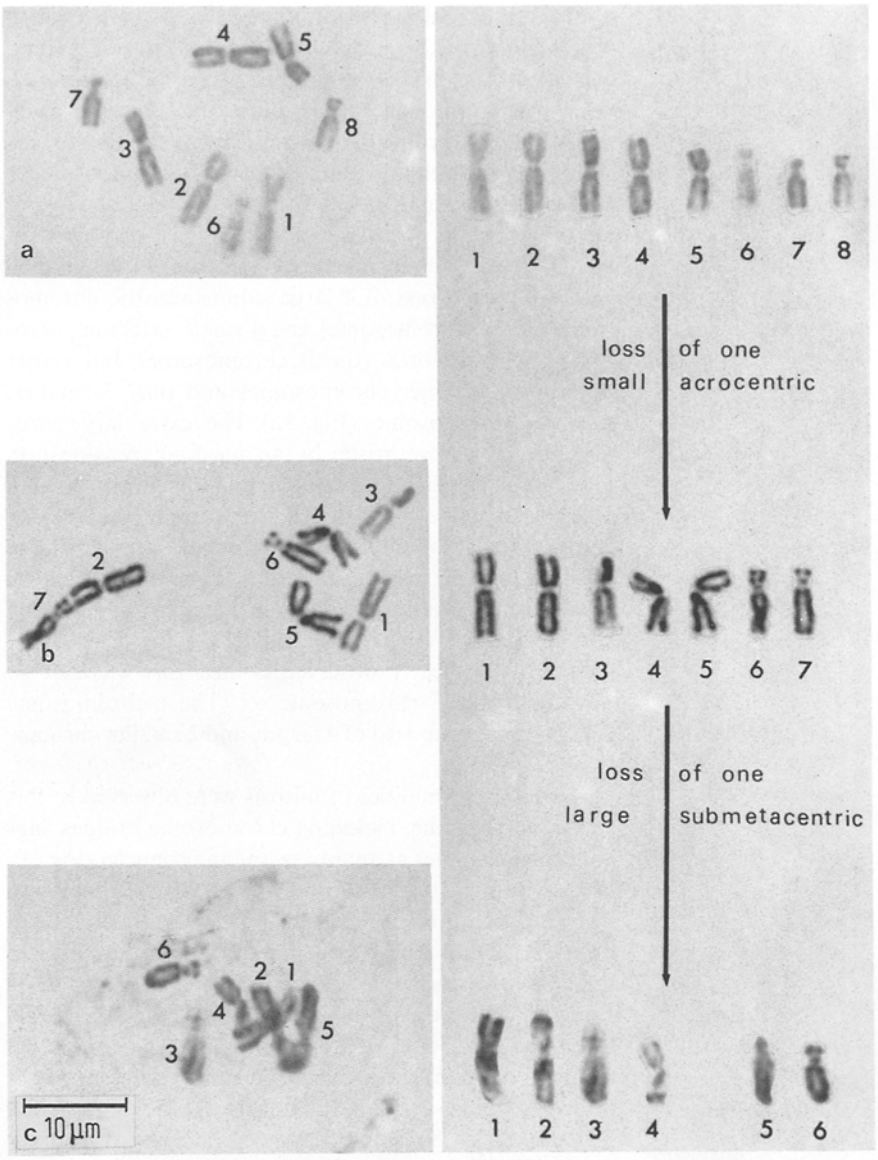

Fig. 5a–c. Orcein stained preparations of metaphase cells of HA-2 suspension culture showing: **a** an 8-chromosome set; **b** a 7-chromosome set; **c** a 6-chromosome set. The possible pathway of chromosome reduction is indicated

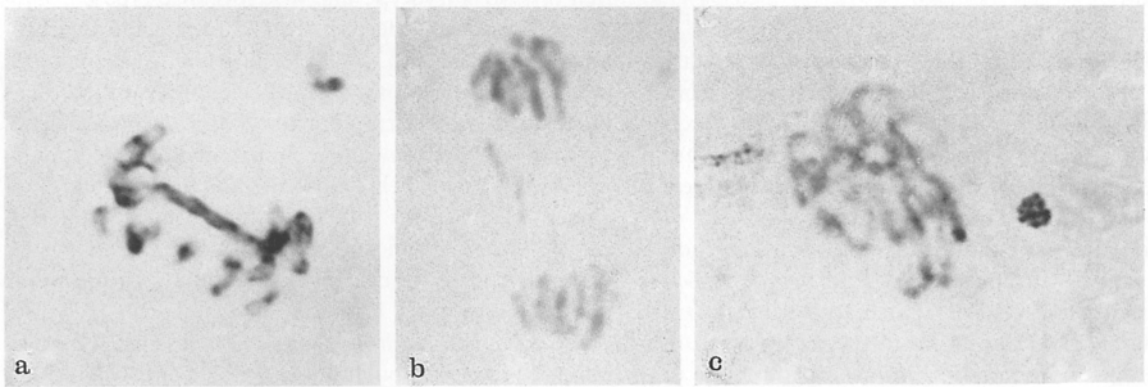

Fig. 6a–c. Abnormalities of division in the HA-2 suspension culture: **a** a chromosome bridge; **b** lagging chromosomes at anaphase; **c** a micronucleus

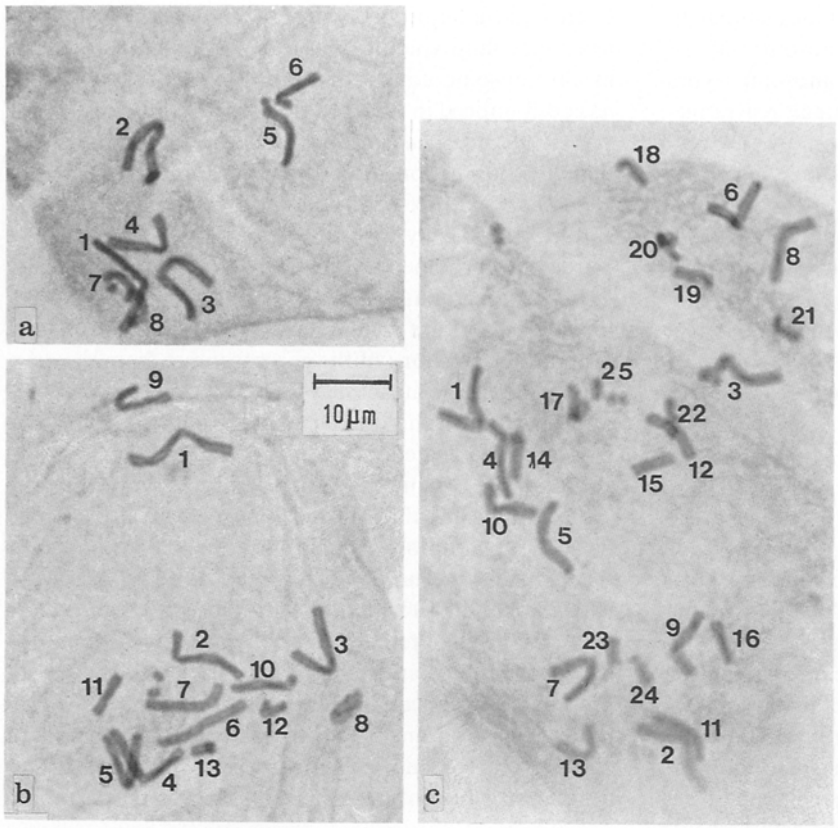

Fig. 7a–c. Orcein-stained preparations of metaphase cells showing: **a** an 8-chromosome set from the HA-3 cell line; **b** a 13-chromosome set from the HA-4 cell line; **c** a 25-chromosome set from the HA-4 cell line

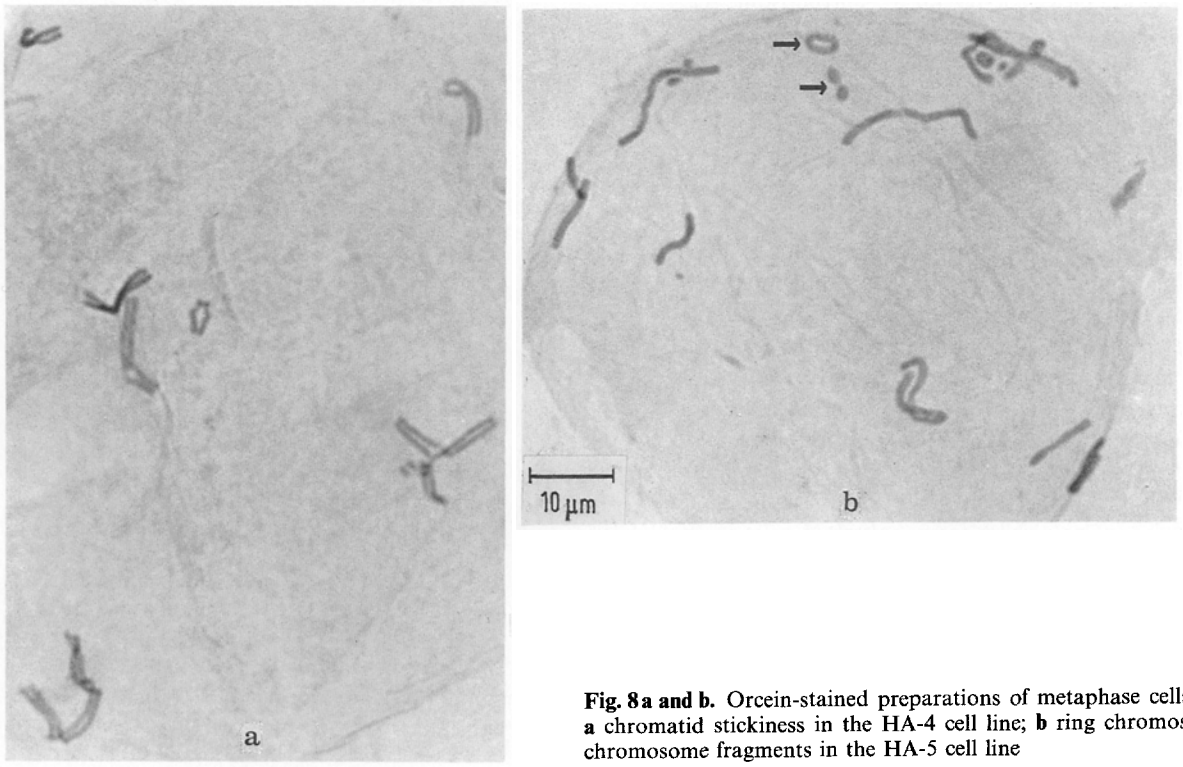

Fig. 8a and b. Orcein-stained preparations of metaphase cells showing: **a** chromatid stickiness in the HA-4 cell line; **b** ring chromosomes and chromosome fragments in the HA-5 cell line

ploid karyotypes include some chromosomes similar in morphology to both the A and B chromosomes of the normal diploid, but also include chromosome types which must have arisen via chromosomal rearrangements. Cells of HA-5 have a range in chromosome number from 8 to 17, with the majority of cells having either a 12-chromosome karyotype (40%) or a 13-chromosome karyotype (24%) (Fig. 2i). Chromosomal rearrangements have also clearly occurred in the generation of these karyotypes.

In both the HA-4 and HA-5 cell lines, there was direct evidence of chromosomal abnormalities, in the form of ring chromosomes, chromosome fragments and chromatid stickiness at mitosis (Fig. 8).

Discussion

The pathway of karyotypic evolution in the HA-1 suspension cell culture seems to have been initiated by the appearance of tetraploidy. A 6-chromosome aneuploid karyotype later dominated in this culture throughout the time that a 4-day transfer program was retained. Following the change to a 7-day transfer program, karyotypic alteration again occurred with 12-chromosome sets plus a few 11- or 8-chromosome sets arising in the population, followed at a later data by a 7-chromosome complement. After 2 years of the 7-day regime, an 8-chromosome karyotype (which was not a normal tetraploid) formed the major chromosome complement in the culture, with a smaller percentage (25%) having a 7-chromosome set.

It can be suggested then that a scheme for karyotypic evolution involves initial chromosome doubling followed by chromosome loss and rearrangements. Chromosomal rearrangements are certainly included in this scheme, since the final 8- and 7-chromosome complements both show deviations from the basic diploid chromosome types. It is not known whether the 6-chromosome set, which previously dominated the culture, included structural chromosome change. Singh (1975) previously observed a 6-chromosome karyotype as the major complement in a suspension cell culture, and in this case one obvious rearrangement was noted. Several authors have supported the view that evolution of the karyotype towards aneuploidy in *H. gracilis* cell culture begins with tetraploidization (Shamina 1966; Singh 1975; Singh and Harvey 1975) and that this chromosome doubling occurs in vitro rather than arising from polyploid cells in the original explant. This view is supported by the appearance of 12-chromosome sets in an HA-1 culture which was previously dominated by a 6-chromosome karyotype.

The fact that karyotypic change, from the 6-chromosome set, occurred in the culture following the change from a 4-day to a 7-day transfer regime is not surprising. Even slight alterations in culture conditions, such as altered inoculum volume, may cause a directional change in chromosome constitution (Kaziwara 1954). Also, it has been noticed in *Haplopappus gracilis* (Singh and Harvey 1975) and *Daucus carota* (Bayliss 1975) cell cultures that a prolongation of stationary phase causes the increased appearance of tetraploids. The change to a 7-day culture period may then be responsible for the shift towards chromosome doubling.

C-banding analysis on the 8-chromosome set in the final HA-1 culture is summarized in Fig. 9, along with proposed derivations of the chromosomes from the original A and B chromosomes of the root tip. Both chromosomes 7 and 8 of this complement are derived from chromosome B, indicated by the darkly staining short arm. In addition, the C-band block on the short arm of chromosome 5 indicates that this chromosome is also derived from the B chromosome. Chromosome 6 does not show this C-block so clearly, but this may be due to problems with the banding technique. Chromosomes 1 and 2 appear to be unaltered examples of chromosome A, whereas chromosomes 3 and 4 have both lost chromatin, presumably in unequal exchanges, producing the larger arm of chromosome 8 and the extended short arms of chromosomes 5 and 6. With improved banding procedures, derivation of culture chromosomes may be more

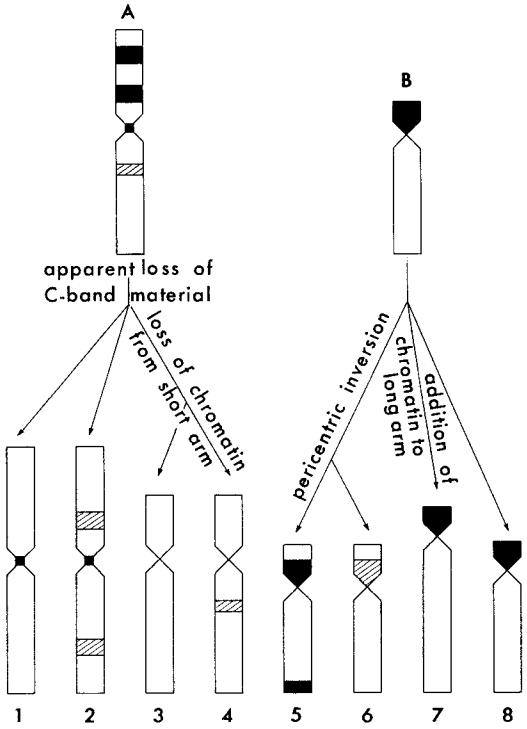

Fig. 9. Schemes for the likely origin of each chromosome of the 8-chromosome set of HA-1 suspension culture from the normal, diploid 2-chromosome types of *H. gracilis* root tip

easily recognized. At any rate, few changes appear to have taken place, and close to tetraploid DNA content may remain.

The HA-2 suspension cell culture was initiated with an 8-chromosome set. Although this was not a normal tetraploid complement, it seems that only one rearrangement has occurred, and it may be assumed that a DNA content close to normal tetraploid remains. Over a 6-month period of monitoring this culture, 7- and 6-chromosome sets appeared, and mitotic abnormalities including lagging chromosomes at anaphase, chromosome bridges and micronuclei were observed. The observations in this culture thus indicate that: (i) chromosomes can indeed be lost from a tetraploid set to give hypotetraploid aneuploids, (ii) such loss may be achieved through the abnormalities of division noticed in this culture.

In the formation of the 6- and 7-chromosome complements, the acrocentric B-type chromosomes seemed to be preferentially lost. A similar observation was made in *H. gracilis* (Singh and Harvey 1975) and *Vicia hajastana* (Singh et al. 1972) cell cultures, where the largest chromosome was represented most frequently in aneuploid karyotypes. However, it appears that 2 of the satellited chromosomes are always retained in the 6- and 7-chromosome sets of HA-2, and this was also the case with the 7- and 8-chromosome complements of the HA-1 culture. It is known that the NOR region is on the small arm of the B chromosome, so that this region is being retained in its original form and number in both these cultures.

Investigations on the HA-3, HA-4 and HA-5 cell lines suggest that cells grown in long-term callus culture may be more susceptible to chromosome change than cells in suspension culture. This is evidenced by the large numbers of chromosomes present, particularly in the HA-4 and HA-5 cell lines. These modal chromosome numbers of 12 and 13 have probably arisen by at least two rounds of polyploidization. It is likely that the increased level of polyploidization in callus is related to the population kinetics of these cultures, including the slower growth rate as compared with suspension cultures, the likelihood that the division process is of longer duration and the probability that cells remain in stationary phase for longer periods before subculture. Direct evidence of polyploidization is seen in the HA-4 line, where a 25-chromosome complement was observed in a culture with the majority of cells having between 12 and 15 chromosomes.

The HA-3 cell line has a modal chromosome number of 8, which indicates a lesser tendency for polyploidization in M&S medium compared with either B5-1 or B5-2 medium. It is likely then that the presence of kinetin in the M&S medium has affected the level of polyploidization in this callus culture. Since cytokinins are implicated in the stimulation of cell division, this may explain the reduced polyploidization in this situation. It would clearly be of interest to investigate this phenomenon in more detail and, in particular, to establish the cell population kinetics in callus as compared with suspension culture, and any variation in kinetics related to the kinds and levels of plant growth substances in the media. It was again noticed in the HA-3 culture that larger metacentric type chromosomes were preferentially retained in altered karyotypes.

Chromosomal rearrangements are clearly involved in the process of karyotype evolution in HA-3, HA-4 and HA-5, since the morphology of the chromosomes in all cell lines differs from the original A and B chromosomes of normal diploid cells of *H. gracilis*. Other unusual chromosome structures, including both ring chromosomes and chromosome fragments, tend to support the view that considerable sub-chromosome level change occurs in these callus cultures. The phenomenon of chromosome stickiness, as seen in these cultures, was also previously observed by Gaponenko et al. (1988) in cultured barley cells.

By using a plant species with extremely low chromosome number, it has been possible to accurately monitor the chromosomal characteristics of the derived cell cultures of this species. By the additional use of the Giemsa C-banding technique which has previously only been used in three other tissue culture systems (Papes et al. 1978; Wochok et al. 1980; Ashmore and Gould 1981), it has also been possible to suggest the nature of any chromosomal rearrangements occurring in the HA-1 culture.

Significant deviations from the original diploid karyotype occur in all cell cultures analysed, and a common pattern of karyotype change has emerged. This involves chromosome doubling and subsequent chromosome loss from the tetraploid set to give rise to aneuploid karyotypes. It should be noted that the 8-chromosome complement of the final HA-1 culture may be considered as an "aneuploid" since it probably arose from a 12-chromosome set.

The pattern of tetraploidization followed by loss to give aneuploidy is a common pathway of chromosomal evolution in both animal cell cultures and in other plant systems (Hsu 1959; Bayliss and Gould 1974).

It is suggested that the aneuploid complements arise from a tetraploid set, since extensive chromosomal change is more likely to be tolerated by a polyploid cell, as this leads to less genic imbalance than in a diploid cell. It has previously been proposed that such chromosome loss is associated with the selection in culture for fast-dividing cells (Karp et al. 1987). Also, for purely mechanical reasons, chromosomal loss may be expected to occur in a tetraploid. Harris (1971) has shown, in pig kidney cell cultures, that polyploidized cells have unaltered dimensional properties as compared with the diploid, so that the surface-to-volume ratio must decline. For a doubling of volume with no change in proportions, the ex-

pected increase in the area per cell is only 1.59 times. It may be that the spindle apparatus of the tetraploid cell is unable to cope with the doubled chromosome number, given that the spindle plate area may only be increased by a factor of 1.59. Thus, competition for attachment of centromeres to the spindle at metaphase may occur, leading to the exclusion of some chromosomes from the telophase nuclei. Terzi (1972) has noticed a positive selection for cells with a diploid centromere number. Added to this is the suggestion that metacentric-type chromosomes may compete more effectively for spindle space than acrocentric type chromosomes, since there is an increased proportion of metacentric chromosomes in the HA-1, HA-2 and HA-3 genomes. Preferential retention of metacentric-type chromosomes has previously been observed in tumorous *Crepis capillaris* (Sacristan and Wendt-Gallitelli 1973; Ashmore and Gould 1981) and in some animal tumours (Hsu 1959; Muldal et al. 1971). It may be that there is an unbalanced drag on acrocentric chromosomes which could cause their preferential loss at anaphase. It has generally been observed that small chromosomes function at anaphase with fewer kinetochore microtobules (kMT's) than large chromosomes (Fuge 1978), and also Moens (1979) has noticed that chromosomes which form as a result of Robertsonian fusion initiate a far larger number of kMT's than telocentrics. Such differences in numbers of kMT's could create a situation where large metacentric chromosomes move more effectively on the spindle and are, therefore, preferentially retained in a competitive situation.

In summary, this study has confirmed that polyploidization commonly arises in *H. gracilis* cultures and that this process may be encouraged by an extended stationary phase in a particular culture regime. This process may thus occur more readily in callus culture rather than suspension culture due to the slower growth rates in the former culture type. Once polyploid cells are present in culture, chromosome loss and rearrangements are more likely to occur, giving rise to aneuploid karyotypes in these cultures. Preferential retention of larger metacentric-type chromosomes may occur during this process, and it may be that chromatin is translocated to become attached to such larger chromosomes during this time. Certainly, observations of Kovacs (1985) indicated that where chromosome reduction occurred in culture, larger chromosomes were observed at the resultant lower ploidy levels than at higher ploidy levels. Satellited, small chromosomes were however retained during karyotype change, presumably due to the location of the NOR regions within the satellite area. C-banding analysis has highlighted the kind of rearrangements that occur, and suggests that both translocations and inversions have occurred in the derivation of the 8-chromosome complement of the HA-1 suspension culture, and also allows definition of the sites of these changes. Finally, it has been noted that the presence of kinetin in the medium may reduce the extent of initial polyploidization in culture.

In terms of practices that might be adopted to reduce karyotype change in culture where this is an undesirable effect, the following observations may be useful: (i) a greater level of polyploidization occurred in callus culture than in suspension cell culture. This is probably related to the slower growth rate in callus culture, (ii) larger chromosome numbers were noted in the presence of media containing 2,4-D as the sole growth substance. Addition of kinetin in the medium may inhibit polyploidization.

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