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DIFFERENT KINDS OF HETEROCHROMATIN IN HIGHER PLANT CHROMOSOMES

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SUMMARY

After the use of different Giemsa staining techniques, variations in chromosome banding patterns have often been observed in animal chromosomes. Such staining differences are usually interpreted to indicate that there is more than one type of heterochromatin in many animal chromosomes. Using two differential Giemsa staining techniques we have found different staining patterns in the chromosomes of two higher plants, *Allium cepa* and *Ornithogalum virens*. Furthermore, pericentric heterochromatin that occurs so commonly in animal chromosomes was specifically Giemsa stained in *O. virens*. These results suggest the basic similarity of higher plant and animal chromosomes.

INTRODUCTION

Subsequent to Pardue & Gall's (1970) differential Giemsa staining of mouse chromosomes, a multitude of other Giemsa staining techniques have been developed (see Comings, Avelino, Okada & Wyandt, 1973, for review). All of these techniques are usually considered to stain constitutive heterochromatin. However, because different techniques may yield different patterns of chromosome staining, it has been suggested that there is more than one kind of constitutive heterochromatin in animal chromosomes (Bianchi & Ayres, 1971 *a*, *b*; Drets & Shaw, 1971; Gagne, Tanguay & Laberge, 1971; Cooper & Hsu, 1972; Gropp & Natarajan, 1972; Schnedl & Schnedl, 1972; Comings *et al.* 1973).

So far most differential Giemsa staining has been performed on animal chromosomes. Although Vosa & Marchi (1972), Schweizer (1973), Natarajan & Natarajan (1972), and Stack & Clarke (1973 *a*, *b*) have demonstrated differential Giemsa staining of plant chromosomes, information concerning different kinds of heterochromatin in plants is meagre at best. Schweizer (1973) reported a variation in the chromosome banding of a dicotyledon, *Vicia faba*. Here, by varying the incubation time of chromosomes in hot buffer, certain bands either appeared or disappeared. We have found a comparable, even more striking, change of banding patterns in two monocotyledons, *Allium cepa* and *Ornithogalum virens*.

METHODS

Roots of *Allium cepa* and *Ornithogalum virens* were grown in either water or soil until the roots had reached approximately 3 cm in length. At this point root tips were removed and fixed in acetic ethanol (3 parts ethanol: 1 part glacial acetic acid) for 1 h at room temperature. Meristem cells were picked out of the roots into 45 % acetic acid on a slide and squashed.

Immature *O. virens* anthers (less than 0·20 mm in length and when all divisions are mitotic) were dissected from buds and also fixed in acetic ethanol for 1 h. Following fixation anthers were placed in 0·2 N HCl at room temperature for 30 min before squashing in 45 % acetic acid. In both methods the coverslips were removed by the dry-ice method, and the slides were allowed to air dry.

Root tip and anther squashes were subjected to 2 alternative treatments prior to Giemsa staining. The slides were either (1) placed in 90 °C potassium phosphate buffer (0·12 M, pH 6·8) for 10 min followed by Giemsa staining in 9 parts phosphate buffer (pH 6·8, 0·12 M): 1 part Giemsa stock stain (Harleco) for 1–20 min or (2) treated essentially by the method of Sumner, Evans & Buckland (1971). In the latter technique the slides were placed in a saturated solution of barium hydroxide in distilled water at room temperature for 10 min, followed by three 5-min washes in distilled water. The slides were then incubated for 1–24 h in pH 6·8, 0·12 M phosphate buffer at 60 °C prior to the same Giemsa staining as in method (1). In both methods the Giemsa stain was washed off with distilled water and the slides were air dried and mounted in Euparal.

RESULTS

Squashes of both *O. virens* and *A. cepa* that have been subjected to 90 °C phosphate buffer have very similar patterns of staining. Nucleolus organizer heterochromatin and what appear to be centromeres stain deeply during metaphase through anaphase (Figs. 1, 2, 5, 6). Centromeres become stainable only during mid- to late prophase (Fig. 4), whereas nucleolus organizer heterochromatin remains visible, associated with the nucleolus throughout interphase (Fig. 3) (a more extensive treatment of this technique and its significance is in preparation). In contrast, squashes treated with barium hydroxide show a very different pattern of staining. In the chromosomes of *A. cepa*, centromeres are not visible, while telomeres of various sizes and nucleolus organizer heterochromatin stain darkly throughout the mitotic cycle (Figs. 7, 8). In the chromosomes of *O. virens*, pericentromeric heterochromatin, nucleolus organizer heterochromatin, and interstitial bands on 2 pairs of homologues are stained darkly throughout the mitotic cycle (Figs. 9–12). During early to mid-prophase centromeric heterochromatin looks like terminal, darkly stained areas that have approximately the same diameter as the rest of the chromosome (Fig. 9), but by metaphase the centromeric heterochromatin is replaced by dark spots (Fig. 11) comparable to the metaphase centromeres in the 90 °C phosphate buffer treatment. In this case it appears that deeply staining masses of heterochromatin may be reorganized into more compact centromeres. This is supported by observations that after the 90 °C phosphate buffer treatment, centromeres are first recognized as stainable structures during mid- to late prophase (Fig. 4). Luykx (1970) reviewed similar observations at the ultrastructural level. The apparent lack of centromeric heterochromatin in *A. cepa* prophase chromosomes treated with barium hydroxide does not necessarily cast serious doubt on this suggestion, since heterochromatin has commonly been observed in the centromeric region of *A. cepa* chromosomes (Levan, 1945; Vanderlyn, 1948; Sharma, 1951; Matagne, 1968), and our results and those of many others suggest that a specific Giemsa staining procedure does not necessarily reveal all types of heterochromatin.

DISCUSSION

Although plant chromosomes are generally considered to be comparable to animal chromosomes, the long phylogenetic separation of higher plants and animals could have been accompanied by basic changes in the structure of plant and animal chromosomes. Since animal chromosomes have typically been found to have pericentromeric heterochromatin (Arrighi & Hsu, 1971; Bianchi & Ayres, 1971 *a*, *b*; Bianchi, Sweet & Ayres, 1971; Comings, 1971; Hsu & Arrighi, 1971; MacGregor & Kezer, 1971; Yunis & Yasmineh, 1971; Bradshaw & Hsu, 1972; Comings *et al.* 1973; Gallagher, Hewitt & Gibson, 1973 [although exceptions have been reported – Schnedl, 1972; Sinha, Kakati & Pathak, 1972]), it is not surprising that when Vosa & Marchi (1972) found that pericentromeric heterochromatin was apparently lacking in the chromosomes of seven plant species, they termed this observation their '...most interesting finding'. Subsequently, Stack & Clarke (1973 *a*) reported pericentromeric heterochromatin in the dicotyledon *Plantago ovata*, and pericentromeric heterochromatin is reported here in *O. virens*. Apparently pericentromeric heterochromatin which is so common in higher animals also occurs in plants, but how widely is yet to be determined. Furthermore, Schweizer's (1973) and our alterations of plant chromosome banding patterns by the use of different banding techniques suggest that comparable states of heterochromatin occur in both plant and animal chromosomes.

Considering the presence of pericentromeric heterochromatin and different types of heterochromatin (according to stainability) in both plant and animal chromosomes, one must conclude that thus far differential Giemsa staining of chromosomes supports the concept of the basic similarity of higher plant and animal chromosomes.

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Fig. 1. 90 °C phosphate buffer-treated metaphase chromosomes from a root tip of *A. cepa*. Paired centromeres are stained on each chromosome, and 2 large and 2 small nucleolus organizers (arrows) are stained on 2 pairs of presumably homologous chromosomes. × 1000.

Fig. 2. 90 °C phosphate buffer-treated anaphase chromosomes from an *A. cepa* root tip. Centromeres appear as dark dots at the flexures of the chromosomes that are oriented toward the poles (*p*). × 1500.

Fig. 3. 90 °C phosphate buffer-treated interphase nucleus from a root tip of *A. cepa*. The 2 large nucleolus organizers are visible as masses of heterochromatin to either side of the nucleolus (arrows). × 1000.

Fig. 4. 90 °C phosphate buffer-treated, mid-prophase chromosomes from a root tip of *O. virens*. Near-terminal centromeres are indistinct, but apparently beginning to form (arrow). × 1120.

Fig. 5. 90 °C phosphate buffer-treated metaphase chromosomes from an anther of *O. virens*. Terminal pairs of centromeres can be seen on 4 of the 6 chromosomes. The centromeres of 2 homologous chromosomes are obscured by heavily stained, terminal nucleolus organizer heterochromatin (arrows). × 1120.

Fig. 6. 90 °C phosphate buffer-treated anaphase chromosomes from an *O. virens* root tip. The terminal centromeres and nucleolus organizer heterochromatin (arrows) are directed toward the poles. One nucleolus organizer-bearing chromosome has been lost from the group of chromosomes on the left. × 1120.

Fig. 7. Barium hydroxide-treated metaphase chromosomes from an *A. cepa* root tip. All chromosomes have differentially stained telomeres of various sizes (compare Fig. 1). × 1120.

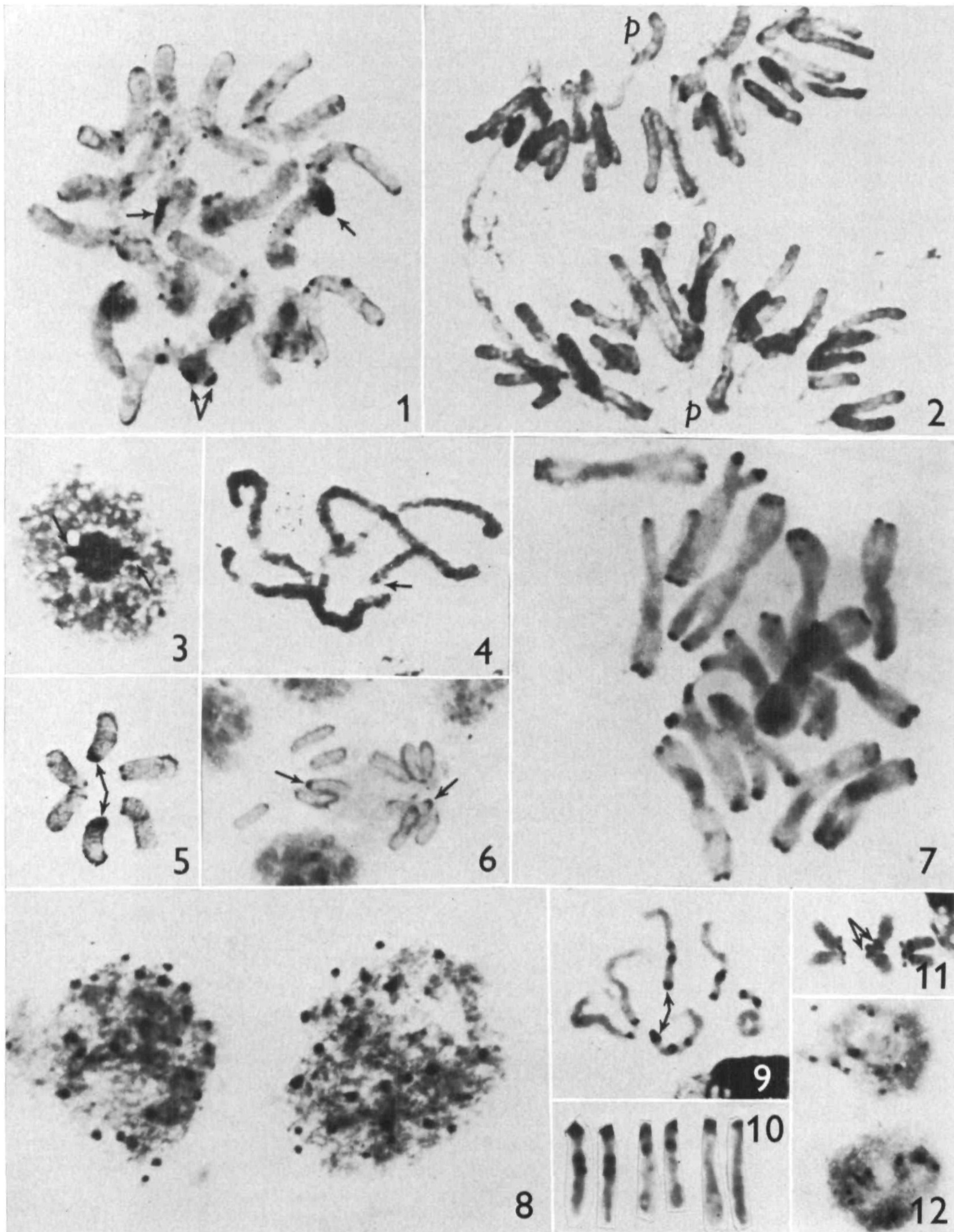
Fig. 8. Two barium hydroxide-treated interphase nuclei from an *A. cepa* root tip. Chromosome telomeres remain heterochromatic and stain differentially. × 1120.

Fig. 9. Barium hydroxide-treated, mid-prophase chromosomes from an *O. virens* anther. Centric ends of all chromosomes stain deeply, including nucleolus organizer heterochromatin (arrows). In a particularly simple manner each homologous chromosome is identifiable by interstitial bands. One pair (left) has no interstitial bands, the second pair (centre) has 2 interstitial bands, and the third pair (right) has one interstitial band. × 1000.

Fig. 10. Idiogram of barium hydroxide-treated anaphase chromosomes from an *O. virens* anther. Centromeric heterochromatin is oriented toward the top of the figure. × 2100.

Fig. 11. Barium hydroxide-treated metaphase chromosomes from an *O. virens* anther. Centric end heterochromatin (see Figs. 9, 10) has condensed to form pairs of centromeres that are obscured in one pair of homologues by nucleolus organizer heterochromatin (arrows). The homologous chromosomes bearing nucleolus organizer heterochromatin have 2 interstitial bands during prophase (see Figs. 9, 10) that fuse to form a large, indistinct dark band during metaphase. Because the banding patterns allow unambiguous identification of homologous chromosomes, one can ascertain with certainty that these chromosomes are homologously paired. × 1000.

Fig. 12. Barium hydroxide-treated interphase nuclei from an *O. virens* anther. Portions of chromosomes that stain differently remain heterochromatic and continue to stain differentially during interphase. × 1000.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