CHROMOSOMAL POLYMORPHISMS OF CONSTITUTIVE HETEROCHROMATIN AND INVERSIONS IN DROSOPHILA

VISUT BAIMAI

Department of Biology, Faculty of Science, Mahidol University, Rama 6 Road, Bangkok 4, Thailand

Manuscript received June 17, 1976
Revised copy received September 9, 1976

METHODS

A simple technique for preparing mitotic metaphases from a larval ganglion of Drosophila is described. Parallel examination of polytene and metaphase chromosome groups shows that inversion polymorphism in chromosome 3 of *D. recticilia* from East Maui (Hawaii) manifests a one-to-one correlation with a metaphase karyotype polymorphism due to the presence of an extra heterochromatic portion. These observations are consistent with the previous findings on other species of Hawaiian Drosophila. They strongly support the hypothesis that when one breakpoint of a long inverted segment of a chromosome element occurs in the vicinity of the constitutive heterochromatin, it may exert an effect in eliciting the production of heterochromatic material in the same chromosome.

CHROMOSOMAL polymorphism in the form of presence or absence of extra heterochromatin has long been known to exist in natural populations of eukaryotes, be they plants or animals including man (see reviews by BATTAGLIA 1964; BROWN 1966; WHITE 1973; YUNIS and YASMINEH 1972). Similarly, polymorphism due to chromosomal rearrangements (*e.g.*, inversions) is also believed to be ubiquitous in higher organisms, but can be easily detected only in salivary gland chromosomes of dipterans. Moreover, it has been shown recently that there is a striking correlation between the two forms of polymorphism, based on evidence from parallel studies of polytene and mitotic metaphase chromosomes of certain endemic species of Hawaiian Drosophila (BAIMAI 1975 a, b). The data suggest that a chromosome break occurring in the area of constitutive (centromeric) heterochromatin may evoke an increase of heterochromatic material. The precise mechanism responsible for such an effect is still obscure.

To further investigate this problem, *Drosophila recticilia* from East Maui (State of Hawaii) is especially favorable. A population from Kaupo Gap exhibits a curious polytene inversion polymorphism in chromosome *3* (CARSON and STALKER 1968; CLAYTON, CARSON and SATO 1972). This species, and six others belonging to the same subgroup, have the fixed inversion *3g* relative to the group-standard chromosome *3* of *Drosophila grimshawi*. The chromosome which forms the polymorphism carries two long overlapping inversions superimposed on the *3g* arrangement. These are *3s* and *3v* respectively, each of which occupies more

than 50% of the chromosome element. It is particularly interesting that the proximal breakpoints of both inversions *3g* and *3s* appear to occur in the vicinity of constitutive heterochromatin, possibly within the limits of euchromatin-heterochromatin junction. This report describes the details of this case, which provides additional evidence supporting the proposed hypothesis.

MATERIALS AND METHODS

Samples of *D. recticilia* were collected from Kaupo Gap at about 1160 meters elevation, East Maui, in June 1975. This species specifically breeds, in nature, on the slime fluxes of the endemic tree *Acacia koa*. It is quite difficult to rear in the laboratory due to the fact that the wild-caught females often refuse to lay eggs in the ordinary food vial for some unknown reason. However, about 10% of the isofemale lines did lay eggs and yielded the F_1 larvae which were used for chromsome analyses in this study.

Metaphase karyotypes were prepared from the larval ganglion cells after pretreatment with colcemid, using a modification of the technique described by GUEST and HSU (1973). The simple method used in this study yields a high frequency of metaphase plates with well-spread chromatid arms, which are preferred for chromosomal analysis. Additionally, some mitotic cells at late prophase and/or pro-metaphase stages have also been obtained by this technique, yielding sharp differentiation between the euchromatic and heterochromatic entities of the chromosome complement. This is especially useful for a critical comparison of the lengths of the heterochromatic portions of homologous chromosomes such as those in the present case. The details follow.

The brain ganglia of a third instar larva were dissected in normal saline solution and immediately transferred to a drop of colcemid (10 mg/ml of Colcemid in Hanks' Balanced Salt Solution, Gibco). Meanwhile, the salivary glands of the same larva were used for polytene chromosome preparations in lacto-aceto-orcein in the usual fashion. After 15 minutes in colcemid solution at room temperature, the brain was transferred to a drop of 1% trisodium citrate solution for 10 minutes. Fixation of the brain ganglion was then carried out in a fixative consisting of equal parts of 45% acetic acid and 95% ethanol for at least one minute. Then it was transferred to a drop of 60% acetic acid in a welled slide where the ganglion cells were rapidly dissociated to form a cell suspension, with the help of gentle flushing with a clean Pasteur pipette. Drops of cell suspension were then placed on a clean microscope slide on a warming plate at about 45°. At the time of dropping, the droplet was slowly withdrawn into the pipette leaving a circular trail of monolayered cells, particularly at the edge of the droplet. The cells were heat-dried onto the slide as the fluid was sucked back into the pipette. The slides were stained in 10% Giemsa stock solution in phosphate buffer, pH 7.0, for 30 minutes, rinsed with deionized water, drained and air-dried at room temperature and mounted in DPX.

RESULTS AND DISCUSSION

A total of 84 individual larvae deriving from 12 isofemale lines collected from the wild were examined cytologically, both for metaphase figures and gene arrangement in polytene chromosomes. Results from this parallel analysis reveal that a larva exhibiting the homozygous condition for the chromosome 3 gene order (*3g/3g*) shows a metaphase plate figure consisting of 5 pairs of major chromosomes and one pair of microchromosomes (dots). Of these, 2 pairs are large V-shaped chromosomes. In the case of the female, one of these pairs is formed by the two *X* chromosomes. In the male, the *Y* chromosome is apparently J-shaped; it is almost totally heterochromatic and is readily distinguishable. The remaining chromosomes form two large J-shaped pairs and one small J-shaped

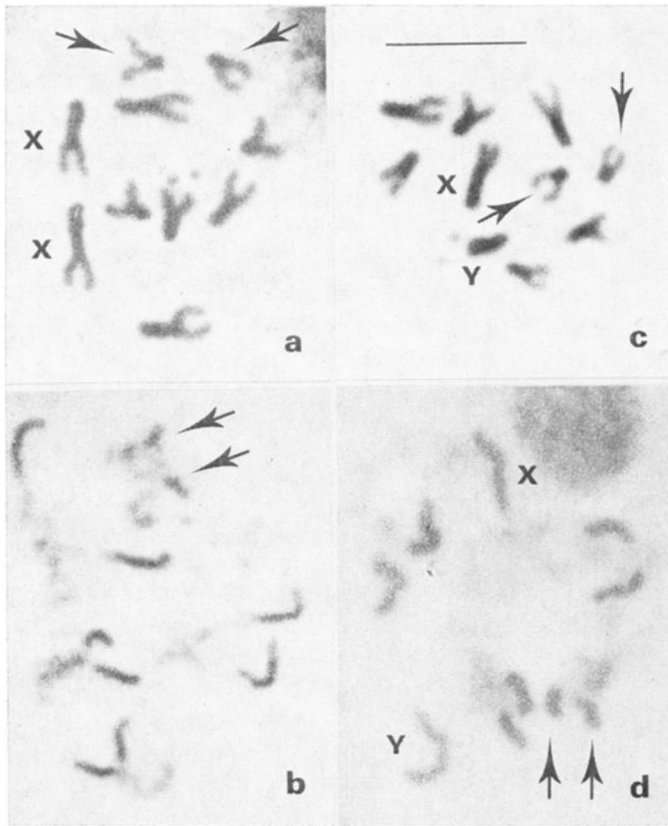

FIGURE 1 a-d.—Photomicrographs of mitotic chromosomes of *D. recticilia*. (a) and (c) show metaphase karyotype of female and male larvae respectively which exhibit homozygous state for chromosome *3g/3g* inversion; (b) and (d) are the corresponding chromosome complement at late prophase taken from the same individual larvae depicting conspicuous constitutive heterochromatic portions of each homologous pair. Chromosome 3 homologues are indicated by arrows in each figure. (The bar represents 10 μ).

pair. The four large J-chromosomes cannot be resolved into two pairs (Fig. 1). The dots are quite conspicuous; this was also noticed by CLAYTON (1969). In all cases, one arm of each of these major chromosomes is obviously heterochromatic, being clearly characterized by a darker staining with Giemsa or orcein in comparison with euchromatic arm (Fig. 1a and c). This interpretation is further confirmed by examination of the chromosome complement at late prophase stage of the same individual larvae, when synapsis of the homologues is still retained and may be seen (Fig. 1b and d). This description of the normal metaphase of this species differs from that of CLAYTON (1968), who described the karyotype as consisting of 5 pairs of rods and 1 pair of dots.

On the other hand, a larva manifesting the heterozygous condition for chromosome *3g/3gsv* complex inversions (Fig. 3) shows polymorphism also in metaphase chromosomes. In a female larva, for example, there is one less small

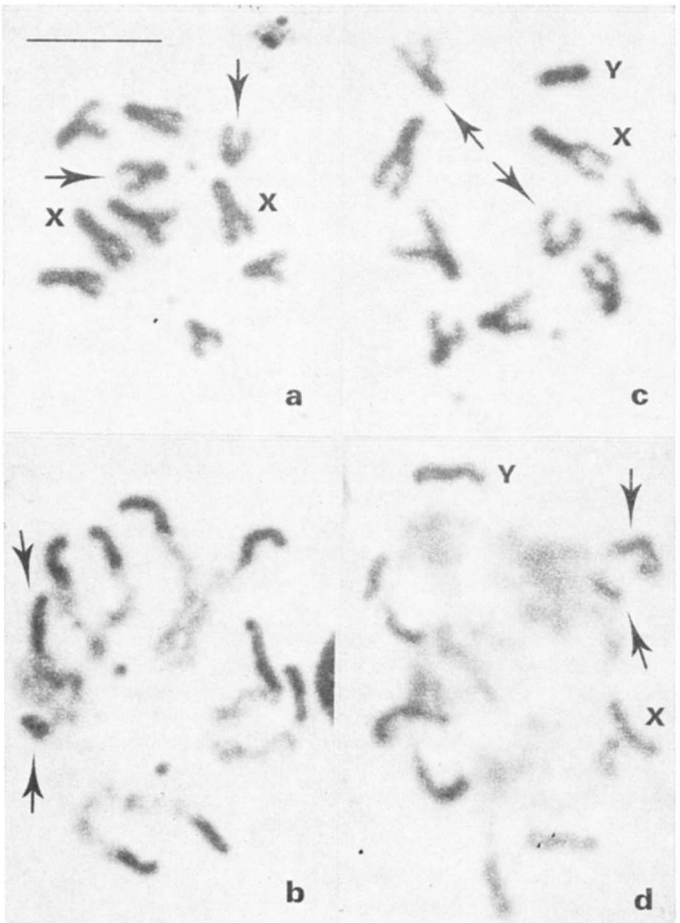

FIGURE 2 a-d.—Photomicrographs of mitotic chromosomes of *D. recticilia* showing heteromorphic homologues of chromosome 3. (a) and (c) show the respective metaphase plate figures of female and male larvae which manifest heterozygous condition for *3g/3gsv* complex inversions; (b) and (d) represent the corresponding chromosome complement at late prophase exhibiting the constitutive heterochromatic arms of each chromosome homologues. The heteromorphic pairs are indicated by arrows in each figure. (The bar represents 10 μ).

J-shaped and one more (*i.e.*, 5) large J-shaped chromosomes (Fig. 2a) when compared with the normal metaphase karyotype described above. These heteromorphic chromosomes are presumably the homologues, one of which contains a more extensive extra heterochromatic portion than the other, transforming it into a larger J-shaped chromosome. A similar situation has been observed in male larvae (Fig. 2c). Moreover, conclusive evidence for the differences in heterochromatin content of the chromosome pairs is best seen in the mitotic cycle during late prophase or pro-metaphase stages (Fig. 2b and d, arrows). However, neither the homozygous condition for the *3gsv/3gsv* complex inversions nor the homozygous pair for the particularly large J-chromosomes has been encountered

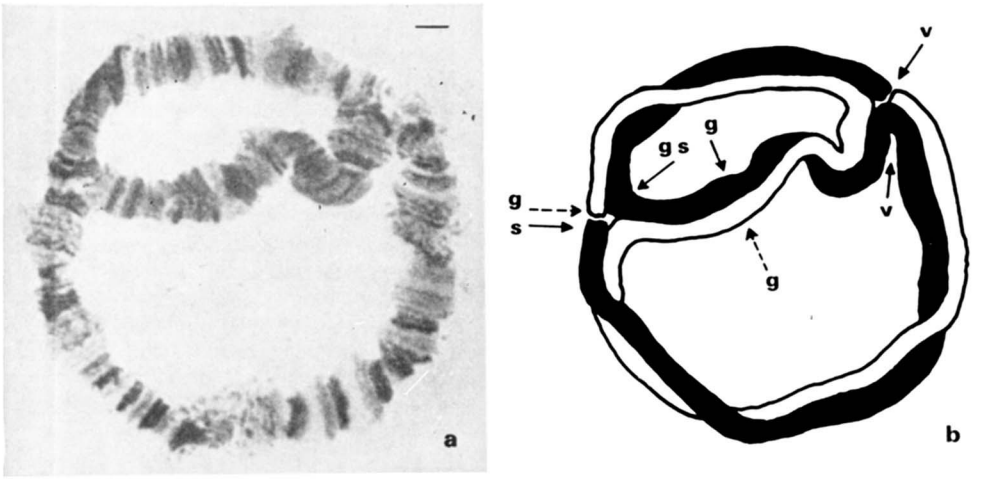

FIGURE 3 a-b.—(a) Salivary gland chromosome *3* showing heterozygous condition for *3g/3gsv* overlapping complex inversions. (b) Diagrammatic interpretation of the heterozygous inversions shown in (a). The chromosome with *3g* gene order is denoted by an open line. The broken arrows represent the break points. Black line represents the *3gsv* gene sequence. Break points of the complex rearrangements are indicated by the solid arrows along the chromosome element. (The bar represents 10 μ).

in the materials under investigation. Nevertheless, homozygosity for the complex inversions was found in earlier studies of material from which metaphases were not prepared in the manner described in this paper. (CLAYTON, CARSON and SATO 1972; CARSON, personal communication). In August 1971, polytene chromosome smears were prepared from 50 larvae taken directly from a slime flux of *Acacia koa* found at Kaupo Gap, Maui. Eighteen were homozygous for *3g/3g* and 6 were homozygous for *3gsv/3gsv*; the remainder were heterozygotes. It is noteworthy that the number of inversion homozygotes *3gsv/3gsv* is close to that expected on the basis of the Hardy-Weinberg equilibrium. Without exception, the large J-shaped chromosome occurs only in the larvae possessing the *3g/3gsv* complex inversions (Table 1). It is, therefore, concluded that the large J-shaped chromosome with the extra heterochromatin is actually the chromosome *3* loaded with

TABLE 1

Condition of mitotic metaphase configurations and salivary chromosome gene arrangements in chromosome 3 *of the same individual larvae of* D. recticilia *from Kaupo Gap, East Maui (Hawaii)*

Polytene gene sequence	Metaphase configurations	No. of larvae	
		Female	Male
3g/3g	Homozygous pair of J-shaped chromosomes with short heterochromatic arms	33	31
3g/3gsv	Heteromorphic pair of J-shaped chromosomes; one with a short arm and one with a longer arm of heterochromatin	8	12
		41	43

the complex gene order. Thus, the results of these observations are in good accord with the earlier findings in *D. disjuncta* and *D. formella* (BAIMAI, 1975a, b).

The parallelism of chromosomal polymorphisms observed in *D. recticilia* may be equally well interpreted in terms of an effect of a chromosome break occuring in the area of constitutive heterochromatin, as has been previously hypothesized, if it is assumed that the hypothetical chromosome *3* with the standard gene sequence has only a small amount of heterochromatin (Fig. 4). Chromosome *3g* inversion covers about 45% of the chromosome arm with the proximal breakpoint occurring in the constitutive heterochromatin region. Should there have been interference in the chromosome part concerned, it could have caused additional production of heterochromatic DNA by some unknown mechanism. There is good evidence indicating that the pericentromeric heterochromatin is rich in highly repetitive DNA (FLAMM 1972). As a corollary, a considerable amount of extra heterochromatin could have been accumulated into the hypothetical ancestral rod chromosome and have been maintained as a small J-shaped chromosome (Fig. 1a-d, arrows) in the natural populations of this species.

Furthermore, the gene rearrangement involving the paracentric inversion *3s* extends over 60% of the chromosome element, with the proximal break also occurring in the vicinity of constitutive heterochromatin. This point of breakage could have further caused an additional regulation for more heterochromatic material in the affected area of the chromosome. Consequently, still more extra heterochromatin could have arisen. Thus, the small J-shaped chromosome in-

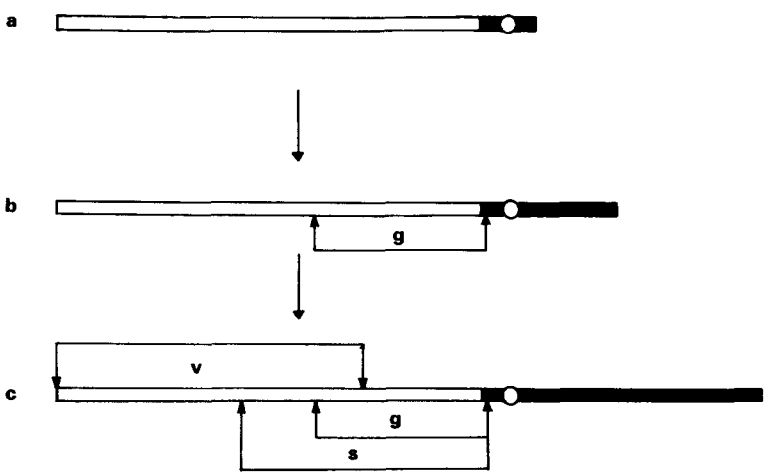

FIGURE 4 a-c.—Schematic representation of the possible pathways of the relationship between chromosomal inversions and extra heterochromatic material through which a chromosome *3* with a different amount of heterochromatin content could have arisen. (a) Hypothetical diagram of chromosome *3* of standard gene order with small amount of constitutive heterochromatin. (b) shows the points of breakage of chromosome *3g* gene arrangement with a considerable amount of extra heterochromatin forming a small J-shaped chromosome. (c) shows the break points of *3gsv* overlapping complex inversions with the addition of large amount of extra heterochromatin forming a large J-shaped chromosome.

volved could have been transformed into the large J-shaped chromosome. This event could probably be followed by chromosome *3v* inversion, which occupies about 70% of the chromosome arm with the distal break-point occurring at the tip of the chromosome. Thus, the entire length of the chromosome is tied up in the *3gsv* complex rearrangements (see Figs. 3 and 4). It is assumed that this *3v* inversion alone has no effect on heterochromatin production. The opposing argument as to loss instead of gain of heterochromatin due to a chromosome-break effect has been ruled out by the fact that inversion *3g* is the ancestral gene order within *D. recticila*. Indeed, this gene order is fixed in this species and in other closely related species. The rest of the inversions are thus unique to *D. recticilia*. According to the phylogenetic tree of the *Hawaiiensis*-complex subgroup, based on the evidence of extensive cytological investigations by Carson and his colleagues (CARSON and STALKER 1968; CLAYTON, CARSON and SATO 1972), inversions *3s* and *3v*, in the order of occurrence, were more recently evolved in this species and have been maintained as chromosomal polymorphisms in the Kaupo Gap population, whereas other known localities lack these complex gene sequences. Therefore, acquisition of extra heterochromatin accompanying the development of certain inverted sequences of the chromosome is a more likely explanation for the parallel chromosomal polymorphisms described in the present study (see Fig. 4).

Inversions which show a clear relationship with increased constitutive heterochromatin are generally those which have a major portion of the chromosome arm inverted. In *D. formella*, for instance, chromosome $4k^s$ inversion involves more than 50% of the polytene chromosome element. Chromosome $4vg^sh^si^s$ complex inversions found in *D. disjuncta* also cover more than one-half of the chromsome length (BAIMAI, 1975 a, b). This is also true in the present observations in *D. recticilia*. Thus, the evidence so far seems to suggest a significant role for certain long inverted segments of a chromosome in relation to the increase in the amount of constitutive heterochromatin.

Polymorphism of constitutive heterochromatin is known to occur in natural populations of higher organisms in the forms of (i) supernumerary *B*-chromosomes (BATTAGLIA 1964; JOHN and HEWITT 1966; NUR 1969; SOUTHERN 1970; WESTERMAN and FONTANA 1973) and (ii) extra heterochromatic portions either in one or more chromosome complements (BAIMAI 1969; PATHAK, HSU and ARRIGHI 1973) or in the area of centromeric heterochromatin as detected by C-banding technique (CRAIG-HOLMES and SHAW 1971; FOREJT 1973; YUNIS and YASMINEH 1971; YOSIDA and SAGAI 1975). CRAIG-HOLMES, MOORE and SHAW (1975) have recently suggested that variation in centromeric heterochromatin in certain chromosomes in the human may result from unequal somatic crossing over involving the heterochromatin of the homologues. However, it is still a mystery how polymorphism of constitutive heterochromatin became so widespread in plants and animals. It appears that the phenomenon might reflect an adaptive advantage for the chromosome(s), or the cell as a whole, which carry extra heterochromatin, perhaps similar to the situation of chromosomal inversion polymorphisms as well established in Drosophila (DA CUNHA 1955; DOBZHANSKY

1970). Thus a long chromosomal inversion with one breakpoint occurring in the neighbourhood of constitutive heterochromatin could account, in part, for heterochromatin polymorphism in certain species of Hawaiian Drosophila. This could be a common phenomenon in various species of higher organisms including man.

I am grateful to PROF. H. L. CARSON for his helpful advise during the course of this work and for providing unpublished data on inversion frequencies. This investigation was carried out at Department of Genetics, University of Hawaii, and was supported by NSF Grant 27586 and by a General Research Support Grant to the John A. Burns School of Medicine from the Public Health Service.

LITERATURE CITED

BAIMAI, V., 1969 Karyotype variation in *Drosophila birchii*. Chromosoma (Berl.) **27**: 381–394. ——, 1975a Heterochromatin and multiple inversions in a Drosophila chromosome. Can. J. Genet. Cytol. **17**: 15–20. ——, 1975b The relationship between metaphase heterochromatin and polytene inversions in Drosophila. Experientia **31**: 779–781.

BATTAGLIA, E., 1964 Cytogenetics of B-chromosomes. Caryologia **17**: 245–299.

BROWN, S. W., 1966 Heterochromatin. Science **151**: 417–425.

CARSON, H. L. and H. D. STALKER, 1968 Polytene chromosome relationships in Hawaiian species of Drosophila. I. The *D. grimshawi* subgroup. Univ. Texas Publ. **6818**: 335–354.

CLAYTON, F. E., 1968 Metaphase configurations in species of the Hawaiian Drosophilidae. Univ. Texas Publ. **6818**: 263–278. ——, 1969 Variations in metaphase chromosomes of Hawaiian Drosophilidae. Univ. Texas Publ. **6918**: 95–110.

CLAYTON, F. E., H. L. CARSON and J. E. SATO, 1972 Polytene chromosome relationships in Hawaiian species of Drosophila. VI. Supplementary data on metaphases and gene sequences. Univ. Texas Publ. **7213**: 163–177.

CRAIG-HOLMES, A. P. and M. W. SHAW, 1971 Polymorphism of human constitutive heterochromatin. Science **174**: 702–704.

CRAIG-HOLMES, A. P., F. B. MOORE and M. W. SHAW, 1975 Polymorphism of human C-band heterochromatin. II. Family studies with suggestive evidence for somatic crossing over. Am. J. Human Genet. **27**: 178–189.

DA CUNHA, A. B., 1955 Chromosomal polymorphism in the Diptera. Advan. Genet. **7**: 93–138.

DOBZHANSKY, TH., 1970 *Genetics of the evolutionary process*. Columbia University Press, New York.

FLAMM, W. G., 1972 Highly repetitive sequences of DNA in chromosomes. Int. Rev. Cytol. **32**: 1–51.

FOREJT, J., 1973 Centromeric heterochromatin polymorphism in the house mouse. Chromosoma (Berl.) **43**: 187–201.

GUEST, W. C. and T. C. HSU, 1973 A new technique for preparing Drosophila neuroblast chromosomes. Drosophila Inform. Serv. **50**: 193.

JOHN, B. and G. M. HEWITT, 1966 A polymorphism for heterochromatic supernumerary segments in *Chorthippus parallelus*. Chromosoma (Berl.) **18**: 254–271.

NUR, U., 1969 Mitotic instability leading to an accumulation of B-chromosomes in grasshoppers. Chromosoma (Berl.) **27**: 1–19.

PATHAK, S., T. C. HSU, and F. E. ARRIGHI, 1973 Chromosomes of *Peromyscus* (Rodentia, Cricetidae). IV. The role of heterochromatin in karyotypic evolution. Cytogenet. Cell Genet. **12**: 315–326.

SOUTHERN, D. I., 1970 Polymorphism involving heterochromatic segments in *Metrioptera brachyptera*. Chromosoma (Berl.) **30**: 154–168.

WESTERMAN, M. and P. G. FONTANA, 1973 Polymorphisms for extra heterochromatin in *Phaulacridium marginale*. Heredity **31**: 223–229.

WHITE, M. J. D., 1973 *Animal cytology and evolution*. 3rd ed. Cambridge Univ. Press, London.

YOSIDA, T. H. and T. SAGAI, 1975 Variation of C-bands in the chromosomes of several subspecies of *Rattus rattus*. Chromosoma (Berl.) **50**: 283–300.

YUNIS, J. J. and W. G. YASMINEH, 1971 Heterochromatin, satellite DNA, and cell function. Science **174**: 1200–1209. ——, 1972 Model for mammalian constitutive heterochromatin. Advan. Cell Mol. Biol. **2**: 1–46.

Corresponding editor: G. LEFEVRE