Localization of Constitutive Heterochromatin in the Chromosomes of *Drosophila virilis*

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Biochemical investigations have shown that constitutive heterochromatin is composed primarily of highly repetitive DNA termed satellite DNA (Yunis and Yasmineh 1970, 1971a, 1971b, Arrighi *et al.* 1970). Recent advances in cytological techniques have allowed the visualization of these heterochromatic regions of metaphase chromosomes (Caspersson *et al.* 1969, Arrighi and Hsu 1971). Such techniques have facilitated the study of the distribution of heterochromatin in metaphase chromosomes when both euchromatin and heterochromatin are fully condensed and differentiation of chromosomes into euchromatic and heterochromatic parts disappears.

Heitz (1934) reported that large amounts of heterochromatin were present in the chromosomes of *Drosophila virilis*. Through studies of the mitotic cycle, Makino (1940) described the distribution of heterochromatin in the chromosomes of *D. virilis*. More recently Adkisson *et al.* (1971) used quinacrine mustard to investigate the heterochromatin of *D. virilis* chromosomes and found large heterochromatic regions in every chromosome. Gall *et al.* (1971) and Schweber (1974) have reported that satellite DNA comprises over 40 percent of the total DNA of the diploid cells of *D. virilis*. This represents one of the largest amounts of satellite DNA reported for any organism.

This study involved the use of the C-banding technique of Hsu (1971) to determine the distribution of constitutive heterochromatin in the neuroblast chromosomes of *D. virilis*.

Materials and methods

The ganglia of third instar larvae of *D. virilis* (University of Texas stock 1801.1) were dissected in physiological saline and then immediately transferred to a hypotonic solution consisting of 1.0% sodium citrate for 10 minutes. Some of the larvae had been previously fed on a yeast-sugar 0.05% colchicine solution in order to increase the yield of metaphase plates (Lewis and Riles 1960). After treatment in the hypotonic solution, the ganglia were fixed in 50.0% acetic acid for 10 minutes and then squashed on slides which had been subbed in a solution consisting of 0.1% gelatin and 0.01% chromium potassium sulfate. In order to remove the coverslips, the preparations were frozen on dry ice and the coverslips were flipped off with a scalpel. The chromosomes were stained for constitutive heterochromatin according

to the procedure of Hsu (1971).

Results and discussion

The proximal halves of the autosomes, with the exception of the smallest pair, were shown to be heterochromatic (Fig. 1). By following the chromosomes of *D. virilis* through the division cycle, Makino (1940) has also reported that the autosomes are differentiated into a proximal heterochromatic portion and a distal euchromatic region. He indicated that a portion of the dot chromosomes was heterochromatic. Using quinacrine mustard, Adkisson *et al.* (1971) also found that the proximal regions of the rod-shaped autosomes were heterochromatic. In addition, they indicated that the dot chromosomes were moderately fluorescent and thus heterochromatic. However, in this study the tiny dot chromosomes in all cells observed showed no detectable heterochromatic regions but instead stained lightly like the euchromatic regions of the other autosomes (Fig. 2).

Figs. 1-2. 1, colchicine treated chromosomes of *Drosophila virilis* showing distribution of constitutive heterochromatin. 2, metaphase chromosomes of *Drosophila virilis* without colchicine treatment. Dot chromosomes are indicated by an arrow.

The proximal region of the X-chromosome is heterochromatic but is divided into three darkly-stained regions and two small lighter-stained areas in less condensed X-chromosomes (Fig. 2). Hsu (1971) also described such light-staining segments in the proximal heterochromatin of the X-chromosome of *D. melanogaster*.

The Y-chromosome stained darkly and appeared to be entirely heterochromatic as has been reported by Makino (1940) and Adkisson *et al.* (1971).

The large amount of detectable constitutive heterochromatin in the chromosomes of *D. virilis* corresponded with the reports (Gall *et al.* 1971, Schweber 1974) of a very high proportion of satellite DNA in the diploid cells of this organism.

Summary

The proximal regions of the rod-shaped autosomes and the X-chromosome of *Drosophila virilis* were shown to be heterochromatic. The Y-chromosome

appeared to be entirely heterochromatic while the tiny dot chromosomes showed no detectable heterochromatic regions.

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