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# Banding techniques for *Drosophila willistoni* mitotic chromosomes**.**

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Studies on the heterochromatic regions of chromosomes have become more and more interesting because it has been discovered that heterochromatin is not "junk" DNA but an essential element of the eukaryotic genome, with possible structural and/or functional relevance (Pardue and Henning, 1990; Henning, 1999). For example, changes in the heterochromatin could influence the expression of genes by position-effect variegation (PEV - reviewed in Zhimulev, 1998). The chromosomes of *Drosophila willistoni* were first described by Metz (1916) and later by Dobzhansky (1950) according to whom, this species presents three pairs of mitotic chromosomes, one pair metacentric, one pair acrocentric, and the metacentric sexual pair (X and Y). Chromosome pair II is a little larger than the X- chromosome, while chromosome pair III is acrocentric. In order to investigate the heterochromatic regions of *D. willistoni* metaphasic chromosomes, we applied banding techniques based on the restriction endonucleases *Alu*I and *Hae*III as well as C-banding techniques. To our knowledge this is the first time that this combination of techniques has been applied to these organisms.

The brain ganglia of *Drosophila willistoni* third instar larvae were processed according to Imai *et al.* (1988). C- banding was carried out by the method of Sumner (1972), except that incubation was in BaOH solution for 30 seconds. After these procedures the slides were rinsed in distilled water, submitted to incubation in double-strength 2×SSC solution for one hour, rinsed again in distilled water and stained with 5% buffered Giemsa for 10 minutes. In order to induce banding by the restriction endonucleases *Alu*I and *Hae*III we applied the method of Mezzanote (1986) using 10 or 20 units of each enzyme per slide. Before submitting the slides to enzymatic digestion, the chromosomes were Giemsa stained, photographed, and the stain removed by immersion in glacial acetic acid for 10 minutes. The final staining of the slides was made with 4% Giemsa (buffered at pH 6.8) for 10 minutes. Negative controls were made using identical methods except that the restriction endonucleases were omitted.

The banding patterns obtained by *Alu*I, *Hae*III and C-banding in the mitotic chromosomes of *D. willistoni* were compared with the patterns induced by Giemsa staining (Figure 1). Giemsa staining produced heavy bands in the centromeric and pericentromeric regions of chromosome II and the X chromosome, as well as one band on the centromere of chromosome III, and a stained block outside the centromeric region. The Y chromosome appeared to be totally heterochromatic.

As far as could be seen by C-banding techniques the constitutive heterochromatin in the chromosomes is localised in the centromeric regions of chromosome pairs II and III, and the X-chromosome. The Giemsa stained blocks in the pericentromeric region of chromosomes II, III and X disappeared after C-banding, demonstrating that they are not formed from constitutive heterochromatin. The Y chromosome, that appeared totally heterochromatic with Giemsa staining, presented an intensely stained band in the centromeric region after C-banding and was sub-metacentric (Figure 2). This finding is different from that described by Dobzhansky (1950) who classified the Y chromosome as metacentric.

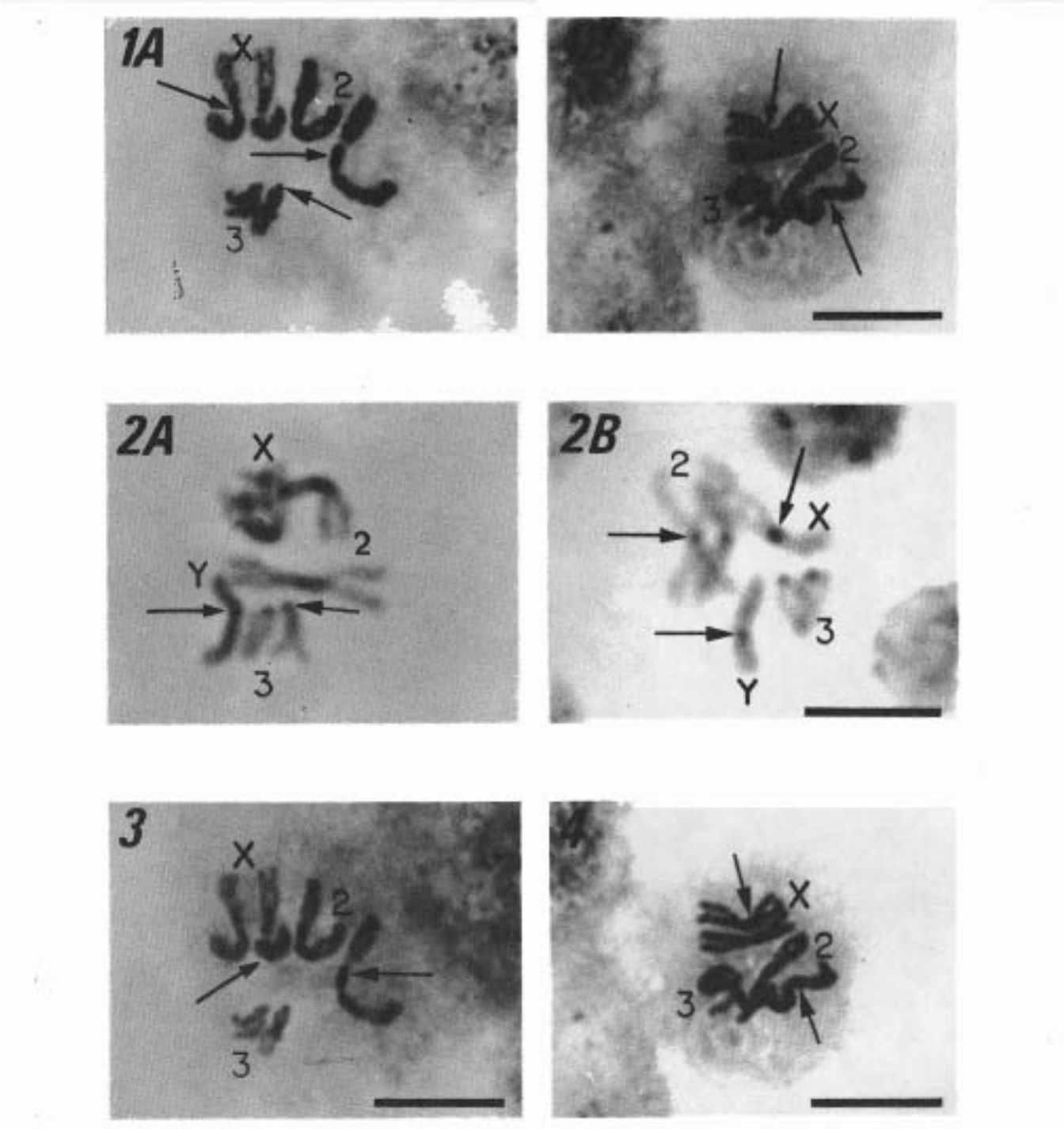


Figure 1. Mitotic chromosomes of third instar larvae brain ganglia of *Drosophila willistoni*. **1A** and **1B**) Giemsa stained. **2A** and **2B**) C- banded slides, with arrows indicating the regions of constitutive heterochromatin, and the arms of Y chromosome with different lenghts. **3**) Banding with *Alu*I in the same nucleus stained with Giemsa in Figure **1A**. **4**) Banding with *Hae*III in the same nucleus stained with Giemsa in Figure **1B**. Arrows indicate labels in the centromeric and pericentromeric regions. Bar = 10 μm.

The restriction endonucleases did not attack the centromeric nor the pericentromeric regions of the heterochromatin, and they did not modify the Giemsa staining pattern (Figures 3 and 4). Strong digestion of the euchromatic regions of the chromosomes, especially in chromosome III, was detected after the enzyme treatments, but this did not provide any additional information regarding differences in the constitutive heterochromatin of these regions. We also tested higher concentrations of both enzymes (up to 22 units per slide), but the results were the same as those obtained with 10 units per slide. The packaging of the DNA of the mitotic chromosomes is so tight that it makes enzyme penetration difficult (Dolfini, 1990). When compared with C-induced banding, restriction enzyme banding did not reveal any new banding patterns for the different classes of heterochromatin in the metaphase chromosomes of *D. willistoni.* This has also been observed for *D. nasuta* (Tiwari and Lakhotia, 1991), in contrast to what happens in human and other mammalian chromosomes (Bianchi *et al.,* 1985).

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