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The Chromosomes of Two Drosophila Races: *D. nasuta nasuta* and *D. nasuta albomicana*

II. Differences Between Their Microchromosomes

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Abstract. The microchromosomes of the totally cross fertile *Drosophila* races, *D. nasuta nasuta* and *D. nasuta albomicana* have been studied in metaphase and polytene nuclei. In metaphase the microchromosome of *D. n. albomicana* is nearly five times longer than the homologous chromosome in *D. n. nasuta*. As shown by C-banding these length differences are mainly due to a massive addition of heterochromatin to the *D. n. albomicana* chromosome. In polytene nuclei these striking heterochromatin differences between the microchromosomes of the two *Drosophila* races cannot be observed. Analysis of the polytene banding pattern shows that the microchromosomes of both races differ by an inversion and by a duplication, present only in *D. n. albomicana*. The location and orientation of the duplicated regions in *D. n. albomicana* leads to a specific loop like chromosome configuration. On the basis of these differences within the *Drosophila* races studied it is assumed that the karyotype of *D. n. albomicana* is a more recent evolutionary product.

Introduction

In *Drosophila* the micro – or dot chromosomes have essential genetic functions in spite of their small sizes and their paucity of genetic material. This has been demonstrated by studies done under different genetic aspects. Genetic interactions between the heterochromatin of the microchromosomes and the regulation of X chromosomal genes were described by Judd (1955), Baker and Spofford (1959) and Kaufmann and Gay (1969). More recently, Bicudo et al. (1977) reported about interchromosomal effects on ribosomal gene regulation between the microchromosomes and the X chromosome. There is evidence of the presence of female determiners on the microchromosomes (Fung and Gowen, 1960), and a phylogenetic relationship between the X chromosome and the microchromosomes has been postulated on the basis of genetic and cytological studies (review see Hochman, 1976)

Variations in the location and/or the amount of heterochromatin in the microchromosomes of *Drosophila* species are common features (Yoon and

Richardson, 1978). In the two *Drosophila* races, *D. n. nasuta* and *D. n. albomicana,* heterochromatin as well as euchromatin differences exist between their microchromosomes. In this report we present our findings on these differences which have been studied on mitotic and polytene chromosomes of both *Drosophila* races and their hybrids as well.

Material and Methods

The *Drosophila* races studied were *D. nasuta nasuta* (2n=8) (Mysore, India) and *D. nasuta albomicana* (2n=6) (Okinawa, University Texas collection, 3054.11). The F_1 hybrids were obtained by making reciprocal crosses of these races. The stocks were maintained at 17° C on wheat cream agar medium seeded with yeast.

Neural ganglia from third instar larvae were pretreated according to Wilson et al. (1969), fixed in alcohol acetic acid (3:1) and squashed in 50% acetic acid. Slides were stored in 100% alcohol for a minimum of 15 days at −20° C. For C-banding the method of Sumner (1972) was modified. Air dried slides were treated with satured BaOH for 8 to 10 min, washed in distilled water, incubated in 2×SSC for 90 min at 60° C, rinsed in distilled water and again air dried. Staining was performed in a 10% Giemsa solution in 0.025 M phosphat buffer (pH 7.0) for 10 min (Hägele, 1977). The slides were then differentiated in buffer solution, rinsed in distilled water and air dried.

Dissected salivary glands from late third instar larvae were fixed in 50% acetic acid and then conventional orcein stained squash preparation were made. Analysis of the banding pattern of the microchromosomes was done in the parental races as well as in their F_1 hybrids.

Results and Discussion

For the direct comparison of the microchromosomes of both *Drosophila* races metaphase plates of the F_1 hybrids (2n=7) were chosen. The microchromosome of *D. n. albomicana* is nearly 5 times longer than its homologue from *D. n. nasuta.* After C-banding the microchromosome of *D. n. albomicana* shows longitudinal differentiation. It has three blocks of C-band positive regions interrupted by two small C-band negative sections (Fig. 1a). The largest C-band is located at its distal end. In the typical microchromosome of *D. n. nasuta* such a differentiation cannot be seen after C-banding. In cases in which this chromosome has a stretched appearance it is possible to recognize that about one half of it consists of C-band positive material, the remaining part being C-band negative (Fig. 1a). As shown by C-banding the differences in size between the microchromosomes of *D. n. nasuta* and *D. n. alnomicana* are mainly due to their differences in heterochromatin content. In both *Drosophila* races the amount of heterochromatin is nearly the same, but in *D. n. albomicana* the microchromosomes contribute nearly 25% to the total C-band heterochromatin, whereas in *D. n. nasuta* only about 2% of the total heterochromatin comes from the microchromosomes (Ranganath and Hägele, 1982).

The above mentioned striking heterochromatin differences between the microchromosomes of both *Drosophila* races, as seen in metaphase, cannot be observed in polytene nuclei (Fig. 1b, c). Especially in the *D. n. albomicana* polytene microchromosomes, regions with bands of heterochromatic nature are underrepresented in comparison to the heterochromatin sections of metaphase chromosomes. This suggests that most of the heterochromatin belongs to the α-heterochromatin type (Heitz, 1934) which is not replicated during polyteniza-

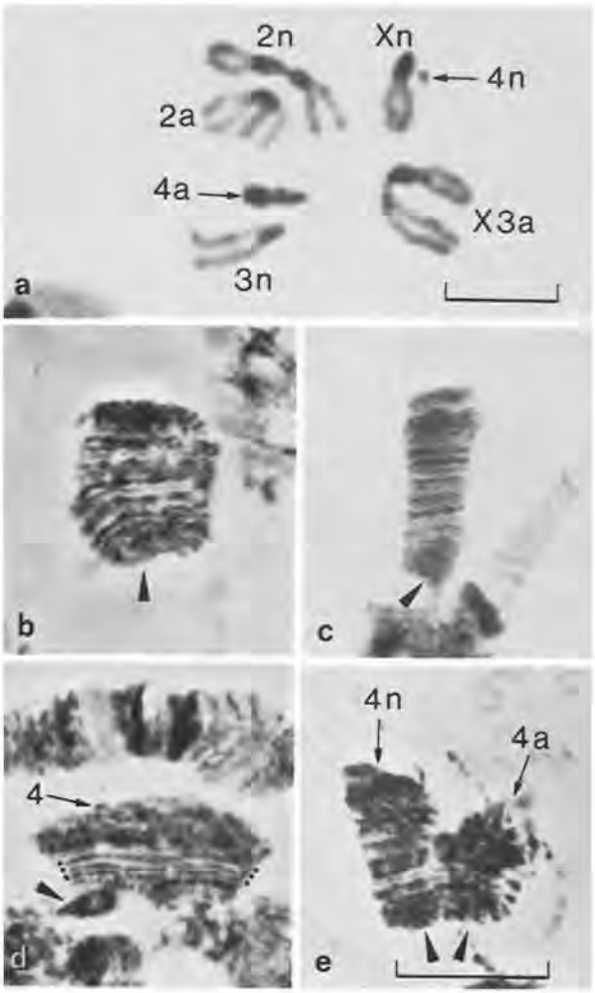

Fig. 1a–e. a C-banded metaphase chromosomes of the F_1 hybrids of *D. n. nasuta* × *D. n. albomicana*. The chromosomes are designated to indicate the race to which they belong. n = *D. n. nasuta*, a = *D. n. albomicana*. *Arrows* point to the microchromosomes. **b–e** Polytene microchromosomes of **b, d** *D. n. albomicana*, **c** *D. n. nasuta* and **e** the F_1 hybrid. Note the differences in length and diameter between the *D. n. nasuta* and *D. n. albomicana* chromosomes. Arrow heads point to the position of the centromeres. In **d** the pairing of the duplicated regions in *D. n. albomicana* is recognizable *(dotted lines)*. The arrow designates the apex of the chromosomal loop, where the bands bend down. *Arrow head* = α-heterochromatin. The bars represent 10 μm, Figs. **b–e** with same magnification

tion (Rudkin, 1969). Between the polytene microchromosomes of *D. n. nasuta* and *D. n. albomicana* there exist, however, differences concerning the chromosome configuration, the banding pattern, and the number of bands. In contrast with the conditions in metaphase, in polytene nuclei the *D. n. albomicana* microchromosome appears always shorter than the homologous *D. n. nasuta* chromosome and it has about twice the diameter than the other chromosomes within

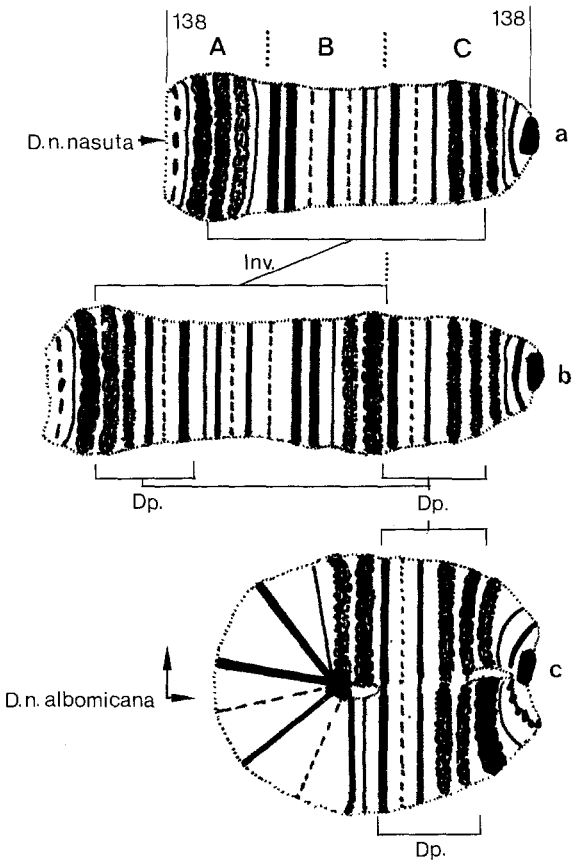

Fig. 2a–c. Chromosome maps of **a** the *D. n. nasuta* and **b, c** the *D. n. albomicana* microchromosomes *Inv:* inversion, *Dp:* duplication. In **b** for simplicity the *D. n. albomicana* chromosome is drawn linearly. This linear arrangement could never be observed, instead of this the loop configuration **c** is common. Brackets in **c** indicate the paired duplicated region

the same nucleus or the *D. n. nasuta* microchromosome (Fig. 1b–e). The analysis of the banding pattern shows that the *D. n. nasuta* chromosome has 21 bands and the *D. n. albomicana* chromosome 26 bands. Besides this the chromosomes differ by a simple inversion comprising 15 bands (Fig. 2a, b). The difference in the number of bands between the microchromosomes is due to a duplication in *D. n. albomicana* including a region of 5 bands (Fig. 2b). One of the duplicate sections is located close to the distal chromosome end whereas the other duplication is located near the proximal end. The two duplicated regions are orientated to one another in an inverted sense. In polytene nuclei these duplicated regions are paired (Figs. 1d, 2c). The result of this pairing is a loop like chromosome configuration (Fig. 2c). This loop formation is the reason why the *D. n. albomicana* polytene microchromosome appears shorter than the homologous chromosome of *D. n. nasuta*. Within the small loop homologous as well as non homolo-

gous bands are tightly lying side by side and, therefore, the microchromosome diameter in *D. n. albomicana* seems to be twice that of the other chromosomes.

In the F_1 hybrids of the two *Drosophila* races all homologous polytene chromosomes of *D. n. nasuta* and *D. n. albomicana* pair intimately with the exception of the microchromosomes. In the hybrids, the *D. n. albomicana* microchromosome also forms a loop and sometimes a few homologous bands at the basis of the *D. n. nasuta* and the *D. n. albomicana* chromosome are losely attached (Fig. 1e). In most cases, however, the inversion differences and the loop structure seem to prevent pairing of the microchromosomes of the two races.

As already mentioned, in the *D. n. albomicana* C-banded metaphase microchromosomes the two euchromatic regions are separated by an intercalary C-band. Because in polytene microchromosomes of *D. n. albomicana* those regions which are duplicated and which look like euchromatin, are located at opposite sites near both chromosome ends, it can be assumed that these duplications are separated from one another by this heterochromatin area seen as intercalary C-band in metaphases. This C-band area could be represented in the polytene microchromosomes by two large bands which are located near the mid of the chromosome and which have a somewhat dispersed appearance.

In Hawaiian Drosophilidae, species with heterochromatically enlarged microchromosomes are the most recently derived forms and they occupy terminal phylogenetic positions (Yoon and Richardson, 1978). In view of this and other evidences (Ranganath and Hägele, 1981; Hägele and Ranganath, in press), we propose that of the two chromosomal races under study, *D. n. albomicana* is a recent evolutionary product. In *D. n. albomicana,* during the karyotypic evolution a massive addition of heterochromatin along with an euchromatin duplication in its microchromosomes has occurred.

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