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Satellite DNA of *Drosophila nasuta nasuta* and *D. n. albomicana*: Localization in Polytene and Metaphase Chromosomes

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Abstract. The DNA from the two *Drosophila nasuta* races, *D. n. nasuta* and *D. n. albomicana* was investigated by CsCl density gradient centrifugation. *D. n. nasuta* has one major AT-rich satellite DNA sequence with a density of 1.664 g/cm^3, while *D. n. albomicana* has at least three satellites with densities of 1.674 g/cm^3, 1.665 g/cm^3 and 1.661 g/cm^3. The isolated satellite sequences hybridize in situ to all heterochromatic regions of all metaphase chromosomes of both races. In polytene chromosomes the satellite sequences hybridize exclusively to the chromocenter. All chromosomal regions hybridizing with the satellites show also bright quinacrine fluorescence.

Introduction

Drosophila nasuta nasuta (2n=8) and *D. n. albomicana* (2n=6) are morphologically identical and totally cross fertile. Hence they are treated as chromosomal races, belonging to the *nasuta* subgroup of the *immigrans* species group of *Drosophila* (Nirmala and Krishnamurthy, 1972; Ranganath and Krishnamurthy, 1981; Ranganath and Hägele, 1981a). The cytological analysis of the metaphase and polytene chromosomes of both *Drosophila* races shows some interesting aspects. Between homologous metaphase chromosomes of these races, striking length differences exist. In polytene chromosomes, however, such differences cannot be observed. Measurements of the C-band areas in metaphase chromosomes of both *Drosophila* forms have shown that the length differences mentioned above are only due to different sizes of the heterochromatin regions (Ranganath and Hägele, 1981b, 1982). The chromosomes which contain more, or respectively less heterochromatin than their homologues from the other race are alternatingly distributed in *D. n. nasuta* and *D. n. albomicana*, so that the total heterochromatin content of the two races differs only by about 3%.

In general, heterochromatic regions are the sites of satellite DNA fractions (see reviews Peacock et al., 1978; Appels and Peacock, 1978; John and Miklos, 1979; Brutlag, 1980).

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The gross differences in the heterochromatin distribution in metaphase chromosomes of *D. n. nasuta* and *D. n. albomicana* have prompted us to investigate their satellite DNAs and to localize these DNAs in their chromosomes. The results of these experiments are presented in this paper.

Material and Methods

The *Drosophila* races used were *D. nasuta nasuta* (Mysore, India) and *D. nasuta albomicana* (Okinawa, University Texas Collection, 3045.11). Reciprocal crosses were made to obtain F_1 hybrids. The stocks were reared at 17° C on wheat cream agar medium seeded with yeast.

Chromosome Preparation and Staining. Metaphase chromosomes of neural ganglia and salivary gland chromosomes were obtained from third instar larvae. The chromosomes were fixed in alcohol acetic acid (3:1) and squashed in 50% acetic acid. Preparations were stored in 100% alcohol. Chromosomes were stained with 0.4% quinacrine-dihydrochloride in a 0.15 M citric acid phosphate buffer solution (pH 5.5) for fluorescence microscopy (Hägele, 1980) or with 10% Giemsa solution (pH 7.0) after developing the autoradiographs (Hägele and Kalisch, 1980).

DNA-Extraction and Ultracentrifugation. DNA from isolated larval brains (100 per extraction) was extracted from total homogenized tissue as described (Schmidt, 1977). In brief the nuclei were lysed by adding SDS, NaCl and Na_2EDTA to final concentrations of 1% w/v SDS, 0.15 M NaCl and 10 mM Na_2EDTA. The mixture was incubated for 5 min at 65° C and then digested with proteinase K (0.5 mg/ml) at 37° C overnight. The mixture was then treated with chloroform-iso-amylalcohol (24:1) three times and the DNA precipitated with two volumes of ethanol. The DNA was purified from contaminating RNA by digestion with RNase T_1 and RNase A followed by proteinase K digestion and chloroform-iso-amylalcohol treatment. Finally, the DNA was dissolved in TE-buffer (10 mM Tris-1 mM EDTA, pH 8.0).

For analytical CsCl density gradient centrifugation 3–10 µg of brain DNA (30–100 brains) were dissolved in CsCl (density = 1.690 g/cm^3) buffered with TE, and 1–2 µg of *Micrococcus lysodeicticus* DNA was included as density standard. The centrifugation was carried out at 44,000 rpm, 20° C, in a Beckmann model E centrifuge. The densities were calculated relative to the *M. lysodeicticus* DNA according to the method of Szybalski[1] (1968).

The satellite DNA fractions were separated preparatively using the method of Manuelidis (1977), as described previously (Schmidt, 1980).

Nick-Translation and in situ Hybridization. The isolated satellite DNA fractions were purified from Hoechst 33258 dye by extraction with iso-propanol saturated with CsCl, dialysed against 0.3 M NaCl-0.1 M Tris pH 8.0–10 mM Na_2EDTA and then precipitated with two volumes of ethanol, dissolved in TE and used for nick-translation essentially as described by MacGregor and Mizuno (1976), using all four dNTP labelled with tritium.

This nick-translated DNA was used for in situ hybridization with polytene and metaphase chromosomes. Prior to in situ hybridization the slides were treated with aceticanhydrid as described by Hayashi et al. (1978). The hybridization was carried out as described by Singh et al. (1977), using 3 × SSC as hybridization buffer and 60° C as hybridization temperature. The hybridization was allowed to proceed for 3–4 h, then stopped by washing in a large volume of 2 × SSC at 55° C for 1 h. The slides were stripped with Kodak AR 10 stripping film and exposed at 4° C for 10 to 30 days.

[1] This method is based on empirical data and may lead to deviating densities if compared to other calculation methods (Schmidt, 1980)

Results and Discussion

Analytical CsCl Density Gradient Centrifugation

The DNA extracted from the larval brains were analysed by CsCl density gradient centrifugation. The buoyant density profiles of *D. n. albomicana* and *D. n. nasuta* DNAs are shown in Figure 1.

D. n. albomicana DNA is separated in at least four different DNA fractions during neutral CsCl density gradient centrifugation, the main band DNA (1.692 g/cm^3) and three AT-rich satellite fractions, designated as I (1.674 g/cm^3), II (1.665 g/cm^3) and III (1.661 g/cm^3). The sallites I-III comprise to about 28 to 30% of total DNA extracted from mainly diploid larval brains. A fourth peak is observed regularly, however, in varying relative amounts with a calculated density of 1.650 g/cm^3. The nature of this peak is not yet clarified. On the contrary, the analytical CsCl density profile of *D. n. nasuta* brain DNA reveals only one well separated AT-rich satellite DNA peak (1.664 g/cm^3) and a small GC-rich shoulder with a calculated density of 1.702 g/cm^3. The AT-rich satellite DNA peak of *D. n. nasuta* comprise only 7 to 8% of the total DNA.

The comparison of the two DNA profiles of *D. n. nasuta* and *D. n. albomicana* obtained after analytical centrifugation reveals major differences in both the

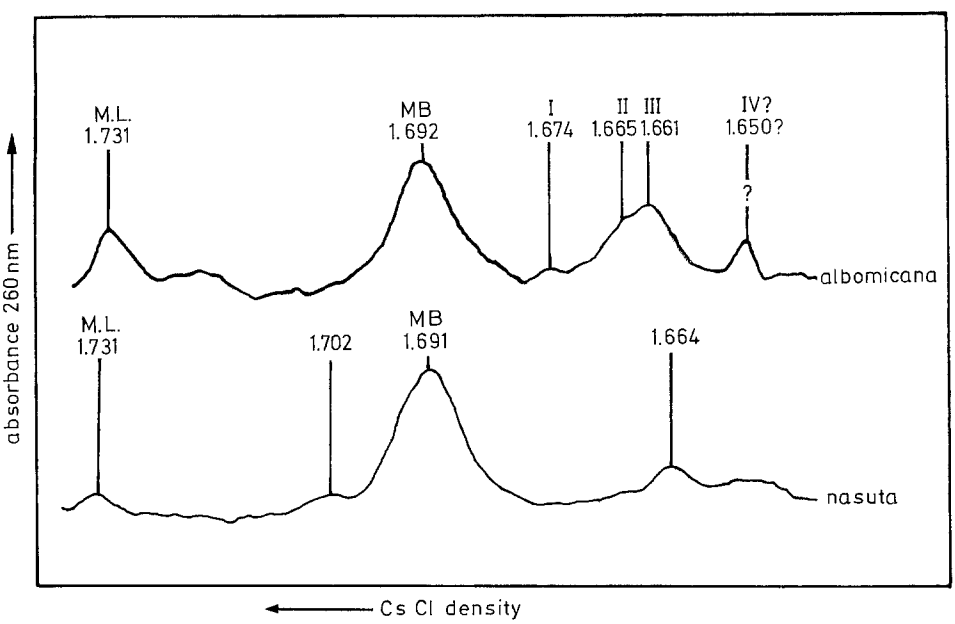

Fig. 1. The CsCl density profiles of *D. n. albomicana* and of *D. n. nasuta* brain DNA are shown. The densities of the various DNA fractions were calculated relatively to *Micrococcus lysodeicticus* (M.L.) DNA according to the method of Szybalski (1968). All density values are means from three independent runs of three different DNA preparations from each species. The three determinations gave nearly identical values, so a standard deviation can be omitted. *MB* main band DNA, I–IV *D. n. albomicana* satellite DNA. The nature of peak IV is unclear, because it was present in different amounts in the different preparations and because of its exceptional low density. *D. n. nasuta* DNA shows only one AT-rich satellite DNA, but it is not clear to which of the *albomicana* satellite DNA it corresponds

quantity as well as the number of satellite DNAs. In spite of these gross differences in satellite DNAs, *D. n. albomicana* and *D. n. nasuta* are completely cross fertile (Ranganath, 1978). These results correspond to and confirm the findings obtained from the *Drosophila virilis* group wherein the viability and the fertility of hybrids do not depend on similarities or differences in the satellite content of parental species (Gall and Atherton, 1974; John and Miklos, 1979).

In *D. n. albomicana* there is a good correlation between the percentage of heterochromatin as measured from C-banding (Ranganath and Hägele, 1981b; 1982) and the percentage of satellite DNA in its genome. On the other hand, in *D. n. nasuta*, the satellite DNA content only corresponds to about one fourth of that expected from the extent of C-band areas. The perusal of literature on other *Drosophila* species, like *D. melanogaster, D. simulans, D. virilis, D. texana, D. hydei* and *D. ezoana* (Hennig et al. 1970; Gall et al., 1971; Travaglini et al., 1972; Peacock et al., 1973; Gall and Atherton, 1974) shows that there is no stringent correlation between the percentage of satellite DNA and the content of constitutive heterochromatin as defined by C-banding.

Preparative Isolation and in situ Localization of Satellite DNA Sequences

The satellite sequences from both *D. n. nasuta* and from *D. n. albomicana* were isolated from preparative CsCl density gradients using the AT-specific fluorescent dye Hoechst 33258 as complexing agent (Manuelidis, 1977). The satellite bands were removed from the gradients without separating the different satellites of *D. n. albomicana* from one another, labelled radioactively with tritium by nick-translation, and used for in situ hybridization to polytene and metaphase chromosomes of F_1 hybrids of *D. n. nasuta* × *D. n. albomicana*. The results are shown in Figs. 2 and 3.

Fig. 2a and 2b shows the hybridization results obtained with polytene chromosomes. *D. n. nasuta* AT-rich satellite DNA (Fig. 2a) as well as *D. n. albomicana* satellite DNA (pooled satellites I-III) (Fig. 2b) hybridize readily to the chromocenter in the polytene chromosomes of the F_1 hybrids. There is essentially no labelling observed in the euchromatic arms of the chromosomes, even after long exposure times (30 days), so that one can assume that most if not all satellite sequences in both races are located within the heterochromatin of the chromocenter. The polytene chromosome hybridization pattern, however, does not allow localization of satellite sequences to individual chromosomes. Therefore we tried to localize the satellite sequence also in the metaphase chromosomes of *D. n. nasuta* × *D. n. albomicana* F_1 hybrids.

The results of in situ hybridization to metaphase chromosomes are shown in Fig. 3a, b (*nasuta* satellite) and 3c, d (*albomicana* satellites). The hybridization obtained with *both* satellite sequences clearly shows labelling of *all* heterochromatic regions of *all* chromosomes. Thus *nasuta* satellite DNA hybridizes to *nasuta* and *albomicana* heterochromatin and in turn, *albomicana* satellite hybridizes to *albomicana* and *nasuta* heterochromatin. Between homologous chromosomes of these races, those with larger C-band areas show more grains than their counterparts. Thus there is very good correlation between the cytological localization of constitutive heterochromatin and the presence of satellite se-

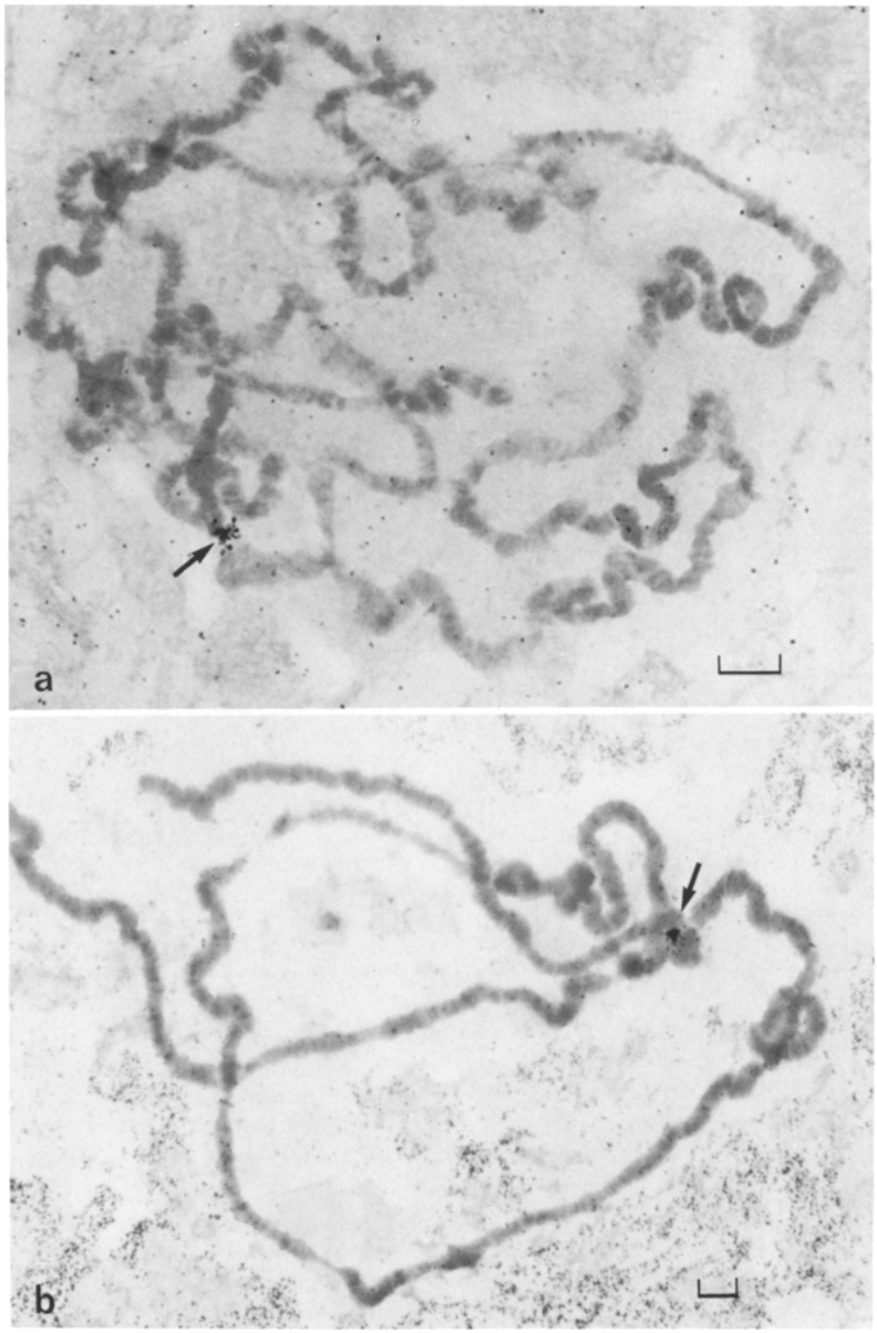

Fig. 2a, b. Polytene chromosomes of the F_1 hybrids of *D. n. nasuta* and *D. n. albomicana* after in situ hybridization with **(a)** *D. n. nasuta* satellite DNA and **(b)** *D. n. albomicana* satellite DNA. *Arrows* point to the labelled chromocenters. Bars in all Figs. represent 10 μm

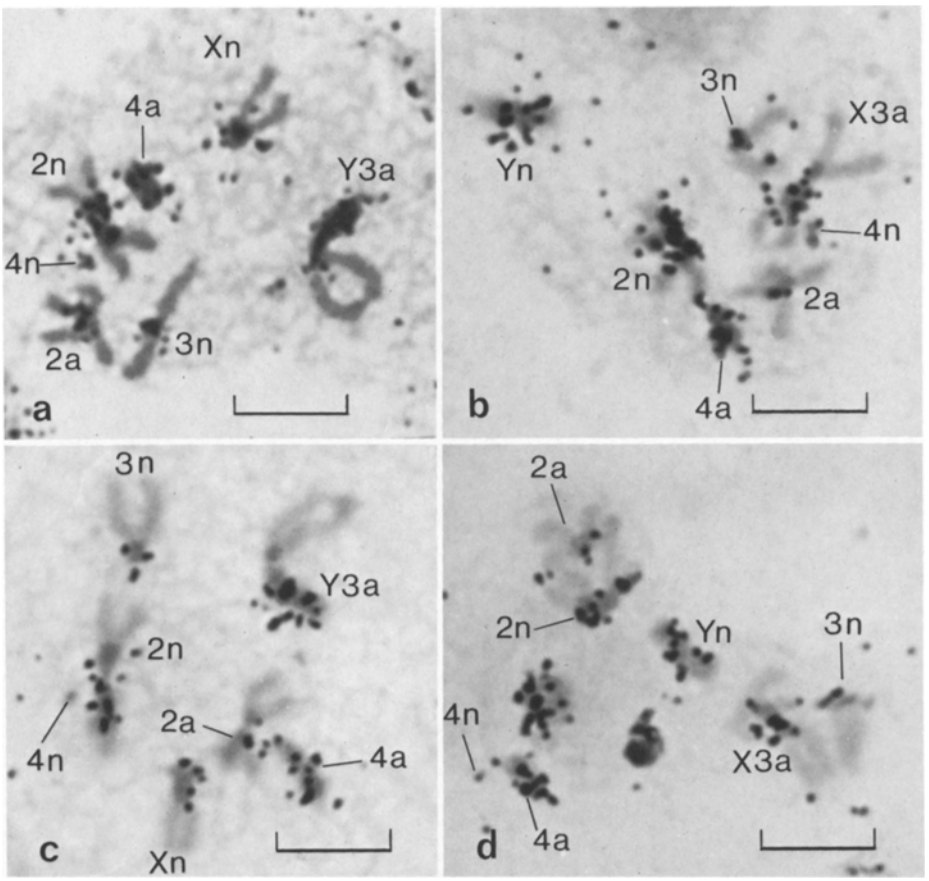

Fig. 3a–d. Male metaphase chromosome complements of the F_1 hybrids of *D. n. nasuta* and *D. n. albomicana* after in situ hybridization with (**a, b**) *D. n. nasuta* satellite DNA and (**c, d**) *D. n. albomicana* satellite DNA. The chromosomes are designated to indicate the race to which they belong: $n = D.\ n.\ nasuta$, $a = D.\ n.\ albomicana$. Further details on the karyotype in the preceding paper by Ranganath and Hägele. (1982)

quences. There is again no hybridization found over euchromatic arms of the chromosomes as already observed with polytene chromosomes. In contrast to the uniform labelling pattern of the heterochromatin area of all other chromosomes, the labelling pattern of the completely heterochromatic Y chromosome of *D. n. nasuta* is differentiated. Compared to its homologous part in *D. n. albomicana*, which is completely heterochromatic and labelled along its total length, the Y chromosome of *D. n. nasuta* has always fewer grains and most of them are confined to the proximal half of its long arm (Fig. 3b, d). This indicates that only the proximal half of the long arm of the Y chromosome of *D. n. nasuta* should contain highly AT-rich satellite sequences.

Quinacrine Fluorescence

Also cytologically the AT-richness of heterochromatin in both *Drosophila* races can well be demonstrated by its bright quinacrine fluorescence (Fig. 4a, b).

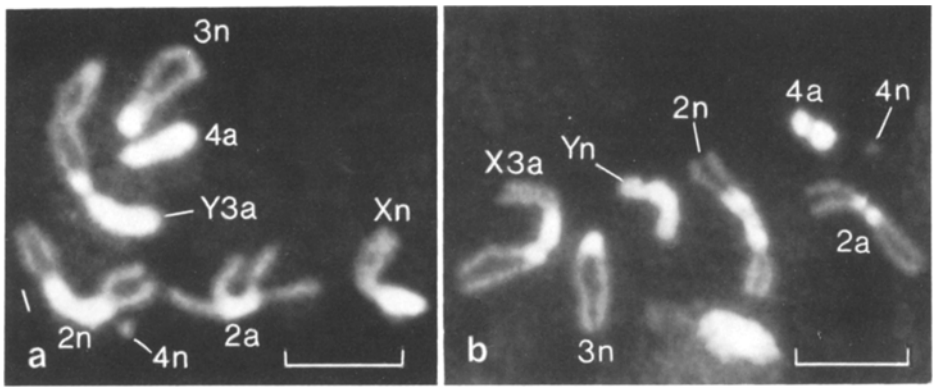

Fig. 4a, b. Male metaphase chromosome complements of the F_1 hybrids of *D. n. nasuta* and *D. n. albomicana* after quinacrine staining: *n* = *D. n. nasuta* chromosomes, *a* = *D. n. albomicana* chromosomes. Further details on the karyotype in the preceding paper by Ranganath and Hägele (1982)

In all metaphase chromosomes, the regions of bright fluorescence coincide with the heterochromatin regions identified by C-banding (Ranganath and Hägele, 1981, 1982). These heterochromatin regions fluoresce uniformly in all chromosomes with the exception of the Y chromosome of *D. n. nasuta*. As already mentioned this chromosome appears totally heterochromatic, however, only the proximal half of its long arm fluoresces as bright as the heterochromatin of other chromosomes. The remaining Y chromosome regions are always comparatively less stained but clearly stronger than euchromatin. Thus, in the Y chromosome of *D. n. nasuta* there is a good correspondence between the in situ hybridization sites and its region of bright fluorescence. This is also true for the heterochromatic areas of the other metaphase chromosomes of the races under study.

The above discussed in situ hybridization results suggest that there are homologous satellite DNA sequences in the heterochromatin areas of the chromosomes of *D. n. nasuta* and *D. n. albomicana*. The satellite DNAs of the chromosomal races under study should differ quantitatively rather than in quality. The results also indicate that an amplification of satellite DNA sequences has occurred in the genome of *D. n. albomicana*. Pending the future results of filter hybridization experiments and sequence analysis of these DNAs, the present findings are in agreement with the library hypothesis of Fry and Salser (1977).

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