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The satellite DNAs of *Drosophila simulans*

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SUMMARY

The resolution of antibiotic-CsCl gradients enabled an examination of the satellite DNAs in the nuclear DNA of *Drosophila simulans*. Of the eight distinct satellite DNAs which were detected, four band at almost the same buoyant density in CsCl but can be resolved in netropsin sulphate-CsCl gradients. Each consists of a repeated sequence which, in five of the satellites, is shown to be arranged in tandem for long regions of the chromosomal DNA. One satellite (1·697 g/ml in CsCl) contains repeated sequences interspersed with other sequences. The satellite DNAs were compared with the satellite DNAs known to be present in the sibling species, *D. melanogaster*. The two species have different overall complements of satellite DNAs, but one satellite (1·672 g/ml) may be identical.

1. INTRODUCTION

A characteristic of possibly all eukaryotic genomes is the presence of highly repeated DNA sequences which often band as satellite peaks in CsCl gradients. A satellite DNA consists of a short nucleotide sequence of sometimes ten or less base pairs repeated tandemly many thousands of times. Previous reports on the type and amount of satellite DNAs in different species suggested that satellite sequences are not conserved during evolution (Walker, 1968; Hennig & Walker, 1970), and that even closely related species contain different types and amounts of satellite DNAs (Hennig, Hennig & Stein, 1970; Mazrimas & Hatch, 1972; Sutton & McCallum, 1972; Gall & Atherton, 1974). Species specificity of satellite DNAs implies evolutionary instability of the sequences. It would follow that the nucleotide sequences of the repeats are not maintained during evolution because of an absence of selection, and therefore if satellite DNA has a function then the sequence of the repeating unit could not be important. Sequence heterogeneity in guinea pig and mouse satellite DNAs has been interpreted as evidence for the accumulation of many mutations in the basic repeating sequence (Southern, 1970, 1975), thereby supporting the notion that satellite DNA sequences evolve as rapidly as evolution permits and are simply excess DNA.

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A large amount of information is now available for five satellite DNAs in *D. melanogaster* (Peacock *et al.* 1973; Endow, Polan & Gall, 1975; Peacock *et al.* 1977*a*, *b*). In order to examine the apparent species specificity of satellite DNAs more closely, a study of the satellite DNAs in *D. simulans* was undertaken. *D. simulans* is one of the six species in the *melanogaster* subgroup (Lemeunier & Ashburner, 1976) and is a sibling species to *D. melanogaster*. These two species probably diverged about a million years ago (Bock, personal communication) and will form F_1 hybrid progeny, although both sexes of the F_1 are sterile. This paper describes procedures designed to detect highly repeated sequences in *D. simulans* which are present in large enough amounts to be seen as satellite bands by optical methods. *D. simulans* and *D. melanogaster* differ in most of their major satellite DNAs, confirming similar reports in species comparisons, but at least one satellite may be identical in both species as judged by several parameters.

2. MATERIALS AND METHODS

The stock of *D. simulans* was obtained from Dr S. Barker, University of Sydney, and was marked with the body mutant *ebony* and eye mutant *scarlet*. Population cages of the flies were kept at 22 °C and eggs were laid on grape juice-agar trays supplemented with a thick yeast paste.

(i) *DNA isolation*

Embryos, of average age 12 h, were washed from grape trays with 0·7 % NaCl, 0·01 % Triton X-100 and were dechorionated with a 5× dilution of sodium hypochlorite solution (BDH) for 2 min. The embryos were washed with the NaCl, Triton X-100 solution, resuspended in a 3:1 ethanol:ether mixture and frozen in liquid nitrogen. DNA was prepared according to Brutlag *et al.* (1977). 120 ml of packed embryos yielded 20–30 mg of DNA, and mitochondrial DNA, which bands in the 1·680 g/ml region (Dennis & Peacock, 1975), could not be detected optically in the preparations.

(ii) *Preparative ultracentrifugation*

The DNA was mixed with actinomycin D (a gift of Merck, Sharp and Dohme; stock solution 1 mg/ml in TE buffer (TE is 10 mM-Tris-HCl, pH 8·4, 1 mM-EDTA)) at a ratio of 0·3 mg actinomycin D per mg of DNA, and CsCl was added to give a density of 1·660 g/ml. Up to 3 mg of DNA were centrifuged in a Ti60 tube (Beckman) at 40000 rev/min, 20 °C for 60 h. The tube was punctured at the bottom and the optical density of each fraction (18 drops) was measured at 260 nm. Appropriate fractions were pooled, actinomycin D was removed from the DNA in CsCl by six extractions with an isopropanol solution saturated with a saturated aqueous solution of NaCl, and the DNA was dialysed against TE buffer.

The netropsin sulphate-CsCl preparative gradients were made equimolar with

respect to netropsin sulphate (a kind gift from Dr H. Thrum; stock solution, 200 μg/ml in TE buffer) and DNA phosphate, and CsCl was added to give a density of 1·640 g/ml. The solution was centrifuged in a Ti50 rotor at 38000 rev/min, 20 °C, 60 h, and 8 drop fractions were collected from the bottom of the tube. Netropsin sulphate was removed from the DNA in CsCl by extracting six times with an equal volume of an isopropanol solution saturated with a saturated aqueous solution of NaCl, which was also 4 M-$NaCCl_3$ COO, pH 7·0. The CCl_3COO^- anion is a powerful chaotropic agent (Hamaguchi & Geiduschek, 1962), and the extraction was done at 0–4 °C as a precaution against the denaturation of AT-rich DNA.

(iii) *Analytical ultracentrifugation*

Analytical ultracentrifugation was carried out in a Beckman Model E equipped with a photoscanner at 44000 rev/min, 25 °C, 20 h, using the AN–F rotor with double sector cells. Sheared and renatured DNA was centrifuged for at least 36 h before scanning. The CsCl gradient analysis with native DNA was as above except in a photographic Beckman Model E at 44770 rev/min with single sector cells; negatives were scanned on a Joyce-Loebl microdensitometer. About 2·5 μg of DNA in TE buffer were loaded per cell and CsCl (Merck, suprapure) was added to a density of 1·700 g/ml except for the netropsin sulphate-CsCl samples, which were made to 1·640 g/ml. Twenty microlitres of the stock solution of netropsin sulphate were added to the DNA solution in the netropsin sulphate-CsCl analysis. Buoyant densities for neutral CsCl gradients were calculated using the marker DNA *Micrococcus luteus* (1 μg), taken to be 1·731 g/ml relative to *E. coli* DNA at 1·710 g/ml (Szybalski & Szybalski, 1971) and using the β factor of $1{\cdot}190 \times 10^{-9}$ (Ifft, Voet & Vinograd, 1961). Netropsin sulphate-CsCl buoyant densities were calculated using the isoconcentration point, measured by refractive index at 25 °C, and the same β value.

(iv) *Denaturation and renaturation of DNA*

The DNA solution in TE buffer was made to 0·2 M-NaCl and denatured by heating to 100 °C for 3 min. Renaturation was to a C_0t value of 1 by incubation for 16 h at 60 °C. All highly repeated and some or all intermediate repeated DNA would have renatured by this time. For the sheared and renatured analysis, the DNA solution was first sonicated with a Branson B-12 Sonifier at maximum power ($\sim$75 W) for 3×30 s to give a fragment length of approximately 400 base pairs.

(v) *Thermal dissociation*

The thermal dissociation of the DNA in TE buffer was monitored in a Gilford 2000 recording spectrophotometer. The temperature was raised at 0·5 °C/min with a programmed Haake waterbath, and the optical density at 260 nm and temperature were recorded with time. The initial DNA concentration was from 12–15 μg/ml

in TE buffer, and no correction was made for thermal expansion of the solvent in the determination of the T_m (mean melting temperature).

3. RESULTS

Rae (1972) reported two distinct satellite DNAs in a CsCl gradient of *D. simulans* DNA at about 1·672 g/ml and 1·686 g/ml, as well as shoulders (at 1·694 g/ml and 1·707 g/ml) to the main band. Only one distinct satellite, at 1·672 g/ml, is visible in the present analysis (Fig. 1) although some samples showed a shoulder at 1·686 g/ml to the main band.

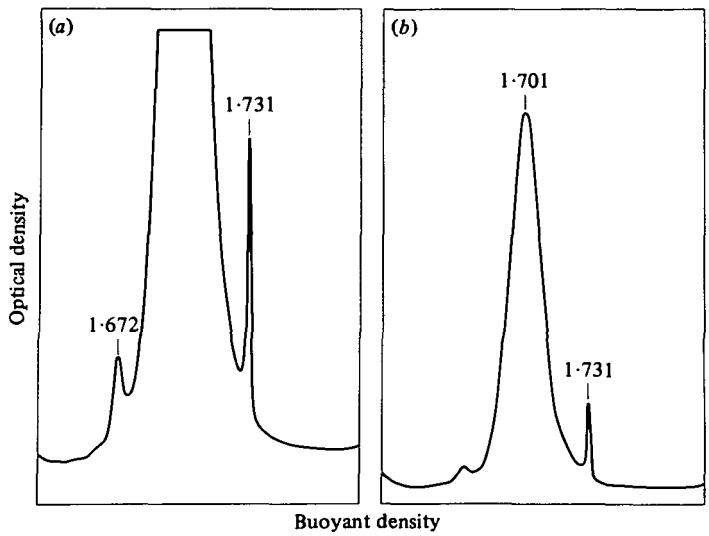

Fig. 1. Analytical ultracentrifugation of *D. simulans* nuclear DNA in CsCl. 15 μg of DNA were centrifuged for 60 h in CsCl and scanned at (*a*) 265 nm or (*b*) 292 nm, where the absorption of the DNA is decreased. The marker at 1·731 g/ml is *M. luteus* DNA.

To obtain better resolution, total *D. simulans* nuclear DNA was dispersed in an actinomycin D-CsCl gradient (Peacock *et al.* 1973). Actinomycin D has a GpC binding preference (Gellert *et al.* 1965; Sobell & Jain, 1972), and main-band DNA binds more of the antibiotic than the satellite DNAs, which tend to be AT-rich in *Drosophila*. An actinomycin D-CsCl gradient of *D. simulans* nuclear DNA was cut into 15 fractions around any satellite peaks (Fig. 2), and the corresponding fractions of six gradients pooled. Actinomycin D was removed and the gradient fractions examined analytically in five ways. Satellite DNAs have been named according to their neutral CsCl buoyant density.

(i) *Native DNA in CsCl*

Two satellite DNAs are present in the first fraction of the actinomycin D-CsCl gradient (Fig. 3, fraction 1). Their buoyant densities are 1·695 g/ml and 1·707 g/ml. As the fractions further across the gradient are examined, satellites at 1·672 g/ml (fractions 3–6) and 1·686 g/ml (fractions 5–7) are seen. Apart from main-band DNA (1·701 g/ml), no other buoyant density species are apparent, although the persistence of DNA banding at about 1·694 g/ml from fractions 4–7 may indicate

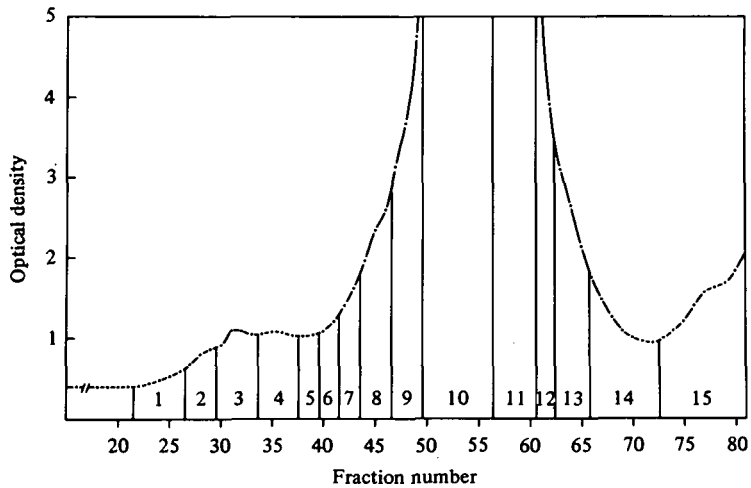

Fig. 2. A preparative actinomycin D-CsCl gradient of *D. simulans* nuclear DNA. The gradient was collected from the bottom of the tube, and the optical density of individual fractions measured at 260 nm. The DNA was pooled to form 15 fractions as indicated by the bars.

another satellite DNA in this region, having a buoyant density similar to the 1·695 g/ml satellite. Fractions 12–14 contain main-band DNA heavier than average and fraction 15 may be DNA resulting from gradient tailing. The neutral CsCl analysis of native DNA following actinomycin D-CsCl centrifugation has thus revealed four satellite DNAs in *D. simulans*: 1·672 g/ml, 1·686 g/ml, 1·695 g/ml, and 1·707 g/ml.

(ii) *Native DNA in CsCl plus netropsin sulphate*

Unlike actinomycin D, which disperses DNA in a GC-rich dimension, the antibiotic netropsin sulphate has an AT base preference (Zimmer, 1975) and separates out each DNA fraction in a second dimension. Identification of satellite DNAs in the netropsin sulphate-CsCl analytical scans was achieved by running a preparative netropsin sulphate-CsCl gradient, removing the netropsin sulphate from the DNA, and rerunning the DNA back in CsCl.

Although only two satellites, 1·695 g/ml and 1·707 g/ml, were found in fraction 1

by CsCl alone (Fig. 3), netropsin sulphate-CsCl separates out three satellite DNAs (Fig. 4). Two satellites have an almost identical neutral CsCl buoyant density with the less dense netropsin sulphate satellite (1·605 g/ml) banding at 1·695 g/ml in CsCl, and the more dense satellite (1·663 g/ml) banding at 1·696 g/ml in CsCl. The

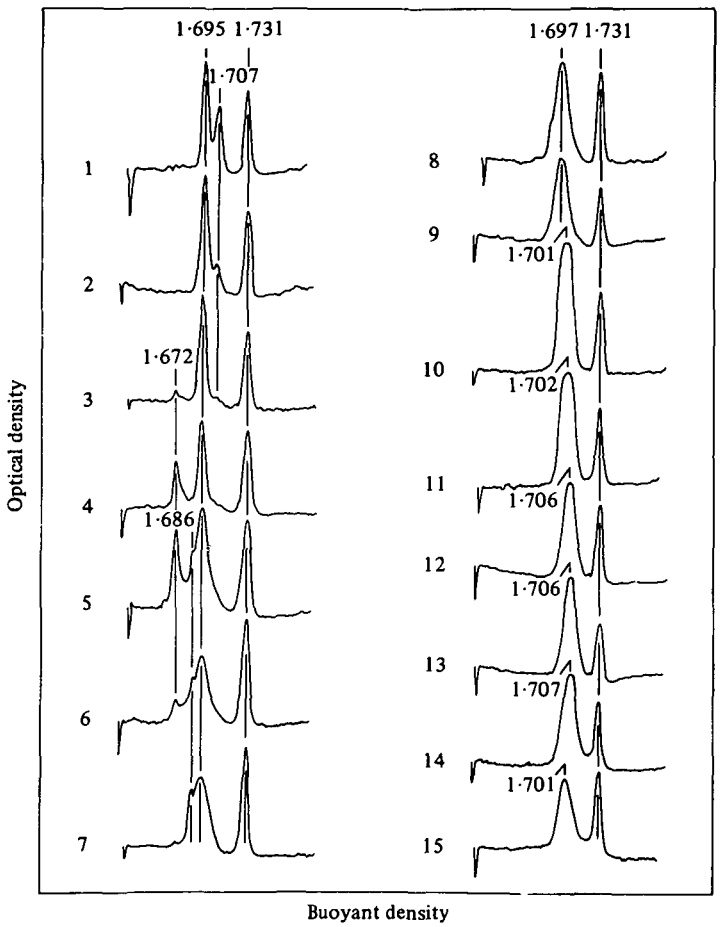

Fig. 3. The CsCl profiles (native DNA) of the 15 actinomycin D-CsCl fractions (see Fig. 2) in the analytical ultracentrifuge, following removal of the actinomycin D. The marker at 1·731 g/ml is *M. luteus* DNA.

third satellite is the 1·707 g/ml satellite. Fractions 3 and 4 show a satellite less dense than the 1·695 g/ml satellite, the 1·672 g/ml satellite. Two additional satellites appear in fractions 4–6. One satellite is 1·686 g/ml and the other is a sixth major satellite at 1·694 g/ml (slightly more dense than 1·686 g/ml in netropsin sulphate-CsCl). The third peak in fraction 8 has a neutral CsCl buoyant density of 1·697 g/ml and contains repeated sequences (see next section).

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Netropsin sulphate-CsCl gradients have thus resolved two more satellites from those observed using CsCl alone, the 1·694 g/ml and 1·696 g/ml satellites. None of the 1·694 g/ml, 1·695 g/ml, or 1·696 g/ml satellites can be distinguished from each other by CsCl centrifugation and band as one peak.

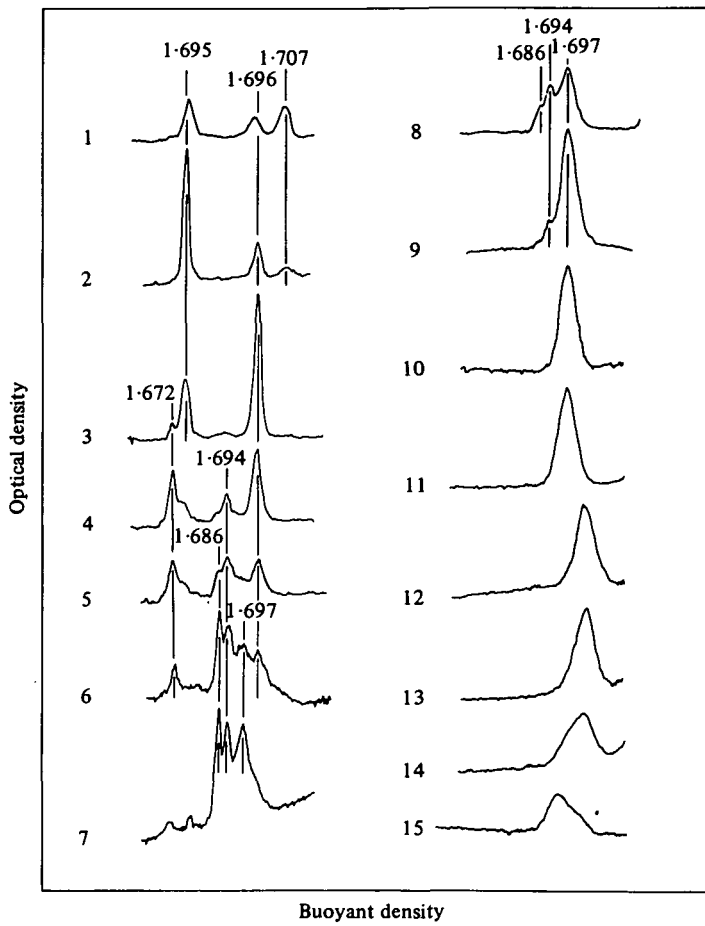

Fig. 4. The netropsin sulphate-CsCl profiles (native DNA) of the 15 actinomycin D-CsCl fractions in the analytical ultracentrifuge, following the removal of the actinomycin D. The buoyant densities shown are CsCl values and not the netropsin sulphate-CsCl values, which increase from 1·589 g/ml (1·672 g/ml satellite) to 1·683 g/ml (1·707 g/ml satellite).

(iii) *Renatured DNA in CsCl*

The renaturation properties of the DNA fractions were examined to determine whether the satellite DNAs are composed of repeated sequences, and to resolve additional satellite DNAs co-banding with main band (non-repeated) DNA.

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Selected DNA fractions were denatured and renatured to a C_0t value of 1, permitting only the renaturation of repeated DNA.

Fraction 1 when renatured shows two peaks at 1·695 g/ml and 1·707 g/ml (Fig. 5), as in the native DNA analysis (Fig. 3), but the relative proportions of the two peaks are changed. The renatured buoyant density of purified 1·696 g/ml satellite in CsCl is 1·699 g/ml, and renatured 1·696 g/ml DNA would be banding in between

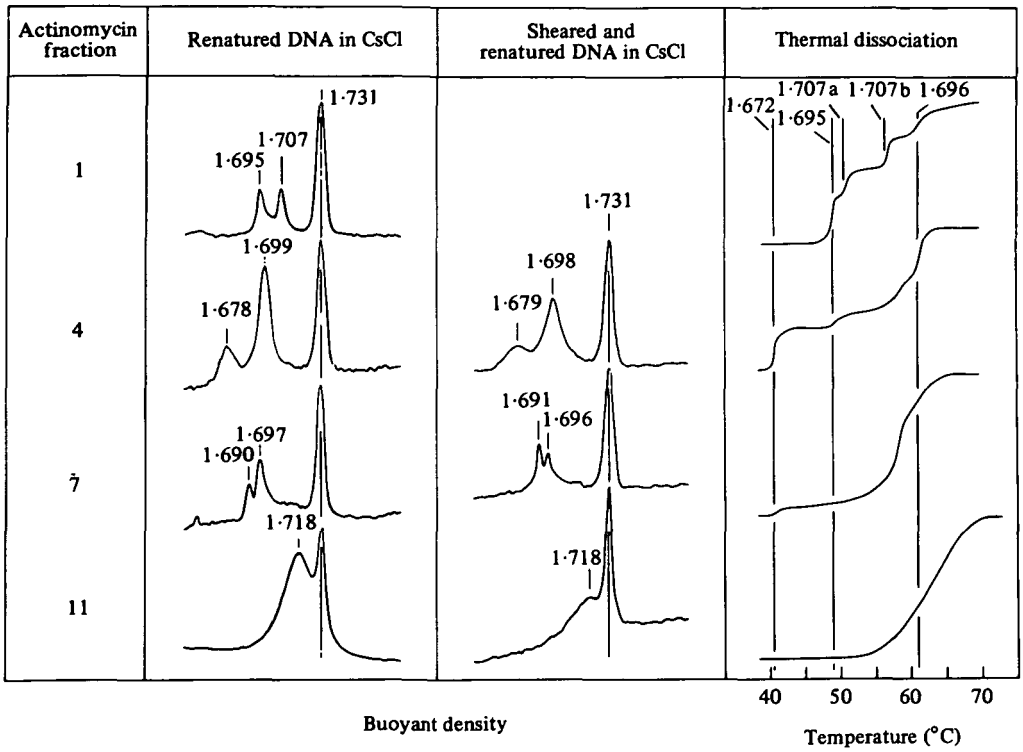

Fig. 5. The behaviour of actinomycin D-CsCl fractions 1, 4, 7 and 11, when denatured and renatured to $C_0t = 1$ at 60 °C in 0·2 M-NaCl, in CsCl (first panel); or first sheared before denaturation and renaturation (second panel); or when melted in a recording spectrophotometer (third panel). The marker at 1·731 g/ml is *M. luteus* DNA.

the two peaks seen in fraction 1 – renatured. Fraction 4 – renatured also shows two peaks as in the native DNA scan. The minor peak is 1·672 g/ml DNA which renatured to 1·678 g/ml in this experiment, but renaturation of 1·672 g/ml DNA at T_m −25 °C (40 °C) results in a buoyant density of 1·673 g/ml (Lohe, 1981). The major peak in fraction 4 must contain three renatured satellite DNAs (see Fig. 4), and no definite conclusions can be made from these data as to their renatured buoyant densities. Fraction 7 – renatured shows two peaks, the less dense peak at 1·690 g/ml representing renatured 1·686 g/ml satellite and the denser peak, at

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1·697 g/ml, containing a major amount of the 1·694 g/ml satellite. The renatured buoyant density of the purified 1·694 g/ml satellite is 1·696 g/ml.

Although the CsCl and netropsin sulphate-CsCl data combined suggest that fraction 8 contains mostly main-band DNA with some of the 1·686 g/ml and 1·694 g/ml satellites, this fraction contains only a small amount of main-band DNA. The remainder is a cryptic satellite DNA. Denaturation and renaturation of fraction 8 DNA reveals a seventh major satellite with a renatured buoyant

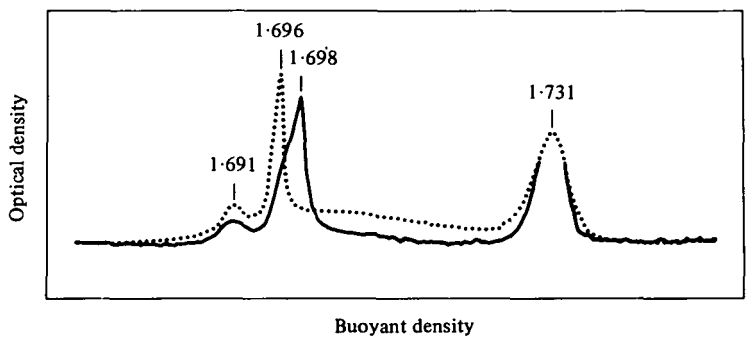

Fig. 6. Analytical ultracentrifugation of fraction 8 DNA when denatured and renatured to $C_0t = 1$, in CsCl (——). The same DNA when sheared and then denatured and renatured to $C_0t = 1$ (. . . .). A small amount of renatured 1·686 g/ml satellite is banding at 1·691 g/ml. The marker at 1·731 g/ml is *M. luteus* DNA.

density of 1·698 g/ml (Fig. 6). It has a native CsCl buoyant density of 1·697 g/ml (Fig. 3, fraction 8). Fraction 9 – renatured also shows the 1·698 g/ml peak but most of the DNA bands broadly around 1·710 g/ml (data not shown) and is presumably unique sequence DNA from the main-band which has not renatured under these conditions.

No rapidly renaturing species are seen from fractions 10–15 and the DNA profile shows a broad peak at a buoyant density greater than 1·715 g/ml. This behaviour is characteristic of single stranded DNA and is consistent with the DNA being largely or entirely of unique sequence.

(iv) *Sheared and renatured DNA in CsCl*

The repeating unit of satellite DNAs is tandemly repeated in long arrays (Waring & Britten, 1966; Bond *et al.* 1967; Gall & Atherton, 1974; Goldring, Brutlag & Peacock, 1974; Brutlag *et al.* 1977), and even with sheared DNA the repeating unit is often much shorter than the fragment size. However, if the fragment size is shorter than the repeating unit, as could be the case for a satellite DNA interspersed with other sequences, the buoyant density profiles of sheared and renatured DNA compared to unsheared and renatured DNA may be different.

It is not yet known whether the 1·707 g/ml satellite is shear sensitive. All other

satellite DNAs are shear insensitive, except for the 1·697 g/ml satellite. Upon shearing fraction 8 DNA followed by denaturation and renaturation, the major peak previously at 1·698 g/ml is replaced by a hypersharp peak at 1·696 g/ml (Fig. 6). Some of the DNA in the sheared and renatured 1·697 g/ml satellite bands broadly, mostly at higher densities, while other sequences in this satellite now band at 1·696 g/ml. Although fraction 8 DNA also contains some 1·694 g/ml satellite, analysis of purified 1·694 g/ml DNA shows that its banding profile is unaffected by shearing before renaturation. Differences in the profiles of renatured compared to sheared and renatured DNA in fraction 7 of Fig. 5 are also attributable to the shear sensitivity of the 1·697 g/ml satellite.

(v) *Thermal dissociation*

Thermal dissociation has detected an eighth major satellite DNA in *D. simulans*. Four components are evident from the melt of fraction 1 (Fig. 5) and two of these components, which are approximately equal in amount, correspond to the 1·707 g/ml satellite. Therefore, 1·707 g/ml DNA consists of two distinct satellite DNAs which co-band, 1·707a melting at T_m = 51·0 °C, and 1·707b (T_m = 57·0 °C).

Table 1. *Characteristics of the satellite DNAs in* D. simulans

Buoyant density in CsCl (g/ml) Native DNA	Buoyant density in CsCl (g/ml) Renatured DNA	Buoyant density in netropsin-CsCl (g/ml)	T_m (°C) native DNA (TE buffer)
1·672	1·673	1·589	40·0
1·686	1·690	1·627	58·0
1·694	1·696	1·634	58·0
1·695	1·695	1·605	49·5
1·696	1·699	1·663	61·5
1·697	1·698	1·646	57·0
1·707a	1·707	1·683	51·0
1·707b	1·707	1·683	57·0

The T_m values for the eight satellite DNAs in TE buffer are listed in Table 1. Six of the satellite DNAs could be associated with a distinct melt transition by comparison of the amount of the component in a melt with the amount of a satellite DNA from the centrifugation data. The T_m values of the 1·694 g/ml and 1·696 g/ml satellites were determined from melting the purified DNA. In contrast to the satellite fractions, main-band DNA fractions melt over a much broader temperature range (Fig. 5, fraction 11), as would be expected for more heterogeneous DNA sequences. The T_m of successive fractions across the main band increases from T_m = 59·3 °C for fraction 9 to T_m = 66·8 °C for fraction 14.

4. DISCUSSION

The power of antibiotic-CsCl resolution of satellite DNAs combined with analysis of consecutive gradient fractions has been clearly shown with *D. simulans* nuclear DNA. Whereas only one distinct satellite peak was detected in CsCl profiles, there are at least eight major satellite DNAs present in the genome. The use of two antibiotics with different binding specificities has also provided greater resolution. For example, a puzzling aspect of the CsCl analysis of the actinomycin D-CsCl gradient (Fig. 3) was that the 1·695 g/ml satellite peak at the bottom of the gradient (fraction 1) did not decrease in amount as fractions toward main-band DNA were examined (fraction 9). One possible explanation was that other satellite DNAs of similar CsCl buoyant densities were banding in these regions, and this was shown to be the case. The cryptic satellites are the 1·696 g/ml, 1·694 g/ml, and 1·697 g/ml satellites, in order of their increasing actinomycin D binding affinity. The discrimination of the antibiotics is also shown with the 1·695 g/ml and 1·696 g/ml satellites, which band as one in CsCl gradients and close to each other in actinomycin D-CsCl, but are widely separated in netropsin sulphate-CsCl (Table 1). A careful examination of the analytical ultracentrifugation scans shows two minor satellite DNAs appearing as small peaks. Both peaks are present in at least two fractions, suggesting that they are not artifactual. One minor satellite bands at 1·664 g/ml, on the light side of 1·672 g/ml (Fig. 3, fraction 5) and is less dense than 1·672 g/ml in netropsin sulphate-CsCl gradients (Fig. 4, fractions 4 and 5). The other minor satellite bands in between 1·672 g/ml and 1·686 g/ml in netropsin sulphate-CsCl (Fig. 4, fractions 6 and 7). This description of the satellite DNAs in *D. simulans* has been confirmed by Barnes, Webb & Dover (1978), who examined total DNA in actinomycin D-CsCl and distamycin A-CsCl gradients.

Each of the satellites consists of repeated sequences as demonstrated by the renaturation data. The sheared and renatured DNA experiments show that except for the 1·697 g/ml satellite, where satellite repeats are interspersed with other sequences, the repeat units are arranged tandemly in the chromosomal DNA. The satellite T_m values vary from 40 °C (1·672 g/ml satellite) to 61·5 °C (1·696 g/ml satellite) and therefore the base compositions of the satellites differ greatly.

A comparison of the *D. simulans* satellite DNAs with those of *D. melanogaster* shows that the satellite complements differ markedly. There have been no 1·694 g/ml, 1·695 g/ml, 1·696 g/ml or 1·707b g/ml satellites reported in *D. melanogaster* (Peacock *et al.* 1977*b*) and no 1·688 g/ml or 1·690 g/ml satellites were detectable in *D. simulans*. Although both species have 1·686 g/ml satellites with similar renatured buoyant densities, *D. simulans* 1·686 g/ml DNA bands as one peak at 1·748 g/ml in alkaline CsCl (Lohe, 1977) in contrast to *D. melanogaster* 1·686 g/ml DNA, which separates into two peaks at 1·714 g/ml and 1·769 g/ml (Peacock *et al.* 1973). Therefore the 1·686 g/ml satellites differ as well.

However, not all of the satellite DNAs may be different in the sibling species. The 1·672 g/ml satellites have similar native and renatured buoyant densities, and the netropsin sulphate-CsCl and actinomycin D-CsCl densities also correspond

closely. The T_ms of native 1·672 g/ml DNAs are similar as are the buoyant densities of single strands in alkaline CsCl (Peacock *et al.* 1973; Lohe, 1981). In addition, both species contain satellites at 1·697 g/ml and these are the only satellites where shearing before renaturation alters the buoyant density profile of renatured DNA (Fig. 6 and Peacock *et al.* 1977*a*). The *D. simulans* 1·707a g/ml satellite also has similar antibiotic-CsCl buoyant densities and T_m to the 1·705 g/ml satellite in *D. melanogaster*. Although the CsCl buoyant densities for native DNA are slightly different (1·707 g/ml and 1·705 g/ml), the value of 1·707 g/ml may be affected by the 1·707b g/ml satellite banding in the same peak.

Therefore, in general the type and amount of satellite DNAs differ between these two closely related species, confirming other findings of satellite DNA species specificity (Walker, 1968; Hennig & Walker, 1970; Hennig *et al.* 1970; Mazrimas & Hatch, 1972). It was concluded from these earlier results that speciation could result in rapid molecular alterations in satellite DNA sequences (Southern, 1970; Walker, 1971; Flamm, 1972), but an inconsistency with this conclusion in the present comparison of *D. simulans* and *D. melanogaster* is the similarity of at least the 1·672 g/ml satellites. Another interpretation consistent with the data is that many of the satellite DNAs of one species are still present in the other species but in quantities too low to be detected as satellite peaks in CsCl gradients. Salser *et al.* (1976) have proposed that rodents and other mammals may share a common 'library' of satellite DNA sequences, and that the observed species specificity of satellite DNAs may reflect only quantitative changes for a given sequence. The 'library' hypothesis may be considered to apply to *D. simulans* and *D. melanogaster* when it has been shown that satellite sequences are identical in these species.

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