Arrangement of the highly repeated DNA of Drosophila melanogaster
E. S. Goldring, D. L. Brutlag and W. J. Peacock

The highly repeated DNA of Drosophila melanogaster occurs in a few long stretches (600 kb) in the chromosome complement. Cytological hybridization to polytene chromosomes shows that each of the highly repeated species is localized in the chromocentre and also at a site on the left arm of chromosome 2 (region 2IC-D). The 1.686 and 1.705 density species hybridize to all chromosomes, but the 1.672 species is restricted to chromosome 2 and possibly also to the Y chromosome. Hybridization to mitotic chromosomes from tissue culture cells confirms the centromeric location of these highly repeated DNA species. Analysis of molecules containing the 'ends' of 1.705 sequences indicates that these segments are, in some chromosomes, adjacent to 1.686 segments, and in fewer chromosomes to 1.672 segments. We suggest that each chromosome may have a specific arrangement of highly repeated DNA sequences.

The highly repeated DNA of the Drosophila melanogaster genome can be isolated by buoyant density centrifugation. Four density species, accounting for about 85% of the highly repeated sequences, can be separated as satellite bands distinct from the bulk of nuclear DNA by the successive use of actinomycin-CsCl and metropsin-CsCl gradients. After removal of the antibiotics these satellites have CsCl buoyant densities of 1.672, 1.686, 1.688 and 1.705 g/cc (Peacock et al., 1974). As well as determining the base sequence of the repeating units of the satellite (Brutlag and Peacock, 1975), we have investigated their organization in the genome. In particular we have examined:

(i) the length and number of segments in which each satellite occurs as a 'pure' species;

(ii) the location of each satellite in the chromosome complement; and

(iii) the arrangement of the various satellites relative to each other.

LENGTH OF THE SATELLITE SEGMENTS

Each satellite comprises about 4% of the genome, or approximately 6,000 kilobases (kb); this DNA could be in one long segment
or there could be segments at more than one locus in the chromosomal complement. If the mean chromosomal segment length of a particular satellite were smaller than the mean size of the isolated DNA molecules, then many of the satellite sequences would not band at the satellite density in a gradient. Yields of the 1.705 satellite were equivalent from populations of molecules of 2.4 and 30.0 kb mean size, implying a mean chromosomal segment length greater than 30 kb (Peacock et al., 1974). In both gradients only 5% of the 1.705 DNA remained in the main band region. This suggested that the chromosomal segment length of the 1.705 species was of the order of 20 x 30 kb.

We have extended this analysis using tissue culture cells from which long DNA molecules are readily obtained. An analysis of the relationship between yield and molecular length was possible for the 1.705 and 1.686 satellites because of their positions in actinomycin-CsCl and netropsin-CsCl gradients. In actinomycin-CsCl, the 1.705 satellite is the most dense band and is clearly separated from the next band (1.686 + 1.672) and from the lighter main band region. In netropsin-CsCl the 1.686 DNA is the least dense band. As the length of the molecules increases we can expect that there will be an increase in the proportion of molecules containing more than one sequence type, i.e. different sequences are more likely to be included in any one molecule. Such mixed-sequence molecules will take a position in the gradient dependent upon the relative amounts of each sequence. A density peak, which with short lengths of DNA is pure satellite, could, with long DNA, be composed of different molecule types. In choosing the 1.705 and 1.686 satellites we avoided this form of sequence contamination. When molecule length approaches the segment length of these two satellites, the amounts banding at their characteristic densities will be reduced. We have compared the yields of these satellites with DNA sheared to short lengths (4.5 kb) or isolated as very long molecules (225 kb). The use of two different isotopes enabled this comparison to be made in a single gradient; the details of methods and results are given in Table 1. The experiment showed that when the mean molecule length was 225 kb the 1.705 peak was reduced only 17% relative to the sheared control. This implies that the 1.705 satellite occurs in segments that are in the range of 225 kb to 6 x 225 kb in length. It is difficult to be more precise than this until the sequences neighbouring the 1.705 segments have been defined – the buoyant density shifts of mixed molecules obviously depend upon sequence composition. In another experiment where the molecules were greater than 270 kb, the 1.705 peak was reduced by about 20%.

Both of these experiments suggest that the earlier estimate (Peacock et al., 1974) of segment length of 600 kb is of the right
### TABLE 1

**Satellite recoveries from sheared and unsheared DNA**

**(a) Actinomycin-D-CsCl gradient**

- $^3$H labelled DNA - average length 225 kb
- $^{14}$C labelled DNA - average length 4.5 kb

<table>
<thead>
<tr>
<th></th>
<th>Counts in 1.705 peak</th>
<th>Counts in 1.686 and 1.672 peak</th>
<th>Total counts in gradient</th>
</tr>
</thead>
<tbody>
<tr>
<td>$^3$H</td>
<td>87,671 (3.8%)</td>
<td>123,204 (5.4%)</td>
<td>2.29 x 10^6</td>
</tr>
<tr>
<td>$^{14}$C</td>
<td>4,291 (4.6%)</td>
<td>5,171 (5.5%)</td>
<td>93,277</td>
</tr>
</tbody>
</table>

**(b) Netropsin-CsCl gradient**

- $^3$H labelled DNA - average length 180 kb
- $^{14}$C labelled DNA - average length 4.5 kb

<table>
<thead>
<tr>
<th></th>
<th>Counts in 1.686 peak</th>
<th>Total counts in gradient</th>
</tr>
</thead>
<tbody>
<tr>
<td>$^3$H</td>
<td>34,407 (2.1%)</td>
<td>1.67 x 10^6</td>
</tr>
<tr>
<td>$^{14}$C</td>
<td>2,532 (2.0%)</td>
<td>1.28 x 10^5</td>
</tr>
</tbody>
</table>

Tissue culture cells (line 2, Schneider, 1972) in log phase growth were labelled by the addition of 100 µCi of $^3$H-thymidine (5-Me T, 5Ci/mM), or by the addition of 5 µCi of $^{14}$C-thymidine (62 mCi/mM) for 4 hr. The cells were harvested by centrifugation (2,000 rpm, 10 min), washed in 0.79% NaCl and resuspended in buffer (0.25 M sucrose, 0.03 M Tris, 1 mM EDTA, 2.5 mM CaCl₂ pH 7.5). Sarkosyl was added to 0.5%, and saturated CsCl layered underneath the sample. Actinomycin or netropsin was added to a final concentration of 40 µg/ml, and the density adjusted to 1.66 g/cc for actinomycin-D gradients or 1.62 g/cc for netropsin gradients. The tubes were centrifuged for 60 hr at 39,000 rpm in a #40 rotor (Spinco). Fractions were collected and 50 µ aliquots spotted onto glass fibre discs, dried and counted in a Packard liquid scintillation counter. The two channels were set so that the spillover from the $^3$H channel into the $^{14}$C channel was negligible, and from $^{14}$C into $^3$H, 10%. The ratio of $^3$H to $^{14}$C counts was 10:1. The $^{14}$C-DNA was sheared, prior to CsCl addition, through a 26 gauge needle. Samples were withdrawn and spread immediately for electron microscopy by the method of Davis et al. (1971). A mass average molecular weight was calculated. If $^{14}$C and $^3$H labelled DNAs were sheared together, the proportion of DNA in the 1.705 band was found to be the same for both samples.
order and that there must only be relatively few chromosomal locations of the 1.705 sequences since the total length of 1.705 DNA in the genome is only 6,000 kb. The 1.686 satellite must also occur in very few segments in the genome since 4.5 kb and 180 kb DNA gave the same yields of this species. We are unable to study either the 1.672 or 1.688 satellites by this method; 1.672 is virtually absent from these tissue culture cells and the 1.688 peak, in both

![Figure 1. Cytological hybridization to salivary gland chromosomes.](image)

$^3$H-RNA was synthesized using the heavy strand of the 1.686 satellite as template (after Pardue and Gall, 1970). Chromosomal DNA in salivary gland squashes was denatured in 0.2 N HCl, 30 min at 37°C. $^3$H-RNA (3 x $10^8$ cpn in 3 λ) was hybridized by heating to 90°C for 15 min and then at 66°C for 16 hr. After RNase digestion, slides were dipped in NTB-2 (Kodak) or L-4 (Ilford) emulsion. Exposure was usually 4-7 days and slides were then stained in Giemsa.
actinomycin-CsCl and metropsin-CsCl gradients, is subject to contamination by main band and mixed sequence molecules. Preparative gradients have provided some information about these two satellites; the yields of both were unchanged in gradients of 4.5 kb or 30 kb DNA. Therefore it is probable that all four highly repeated DNA species occur in a limited number of segments in the genome.

LOCATION OF THE SATELLITE SEGMENTS

Cytological hybridization (Pardue and Gall, 1970) confirms that the satellites are present in only a few sites in the chromosome complement. Tritiated RNAs synthesized using the satellite DNAs as templates, when hybridized to the polytene chromosomes in salivary gland cells, localize to the chromocentre (aggregated centromeres) and to region 21C-D on the left arm of chromosome 2 (Figure 1). This could mean that each satellite occurs in the centromeric region of each chromosome. Occasionally, individual chromosomes separate from the chromocentre during the squash procedure, and these cells yield more specific information about the chromosomal location of the satellites. Data from such cells (Figure 2) indicate that the 1.705 and 1.686 species occur on all chromosomes, but that the 1.672 satellite is restricted to chromosome 2.

<table>
<thead>
<tr>
<th>Highly repeated DNA species</th>
<th>Polytene chromosomes from salivary glands</th>
<th>Tissue culture mitotic chromosomes</th>
</tr>
</thead>
</table>
| 1.672                       | 2L X 3L 4 2R 3R                           | Centromeres of all chromosomes  
|                             | 2L X 3L 4 2R 3R                           | one major autosome particularly |
| 1.686                       | 2L X 3L 4 2R 3R                           | Centromeres of all chromosomes  
|                             | 2L X 3L 4 2R 3R                           | one major autosome particularly |
| 1.705                       | 2L X 3L 4 2R 3R                           | Centromeres of all chromosomes  
|                             | 2L X 3L 4 2R 3R                           | less on X                         |

Figure 2. Summary of cytological hybridization results. Diagram of results of cytological hybridization of \(^3\)H-RNAs complementary to the 1.705, 1.686 and 1.672 satellites. The hatched areas indicate labelling. The methods used for salivary gland chromosomes are given in Figure 1, and the methods for mitotic chromosomes in Figure 4.
Although the 1.672 satellite localized to only chromosome 2 in preparations from both male and female larvae, it is possible that it also occurs on the Y chromosome since this chromosome cannot be identified in the chromocentre (Nicoletti and Lindsley, 1960). Blumenfeld and Forrest (1971) have suggested that this satellite does occur on the Y chromosome; they found a correlation between the amount of this satellite and the number of Y chromosomes. We have data consistent with their findings. DNA isolated from brains dissected from X/0 larvae, showed a decreased amount of 1.672 compared with the amount found in X/Y larval brains. Figure 3 shows the comparative recoveries of the satellites in CsCl, actinomycin-CsCl and netropsin-CsCl for DNA from X/0 and X/Y brains, and from tissue culture cells. The tissue culture cells (line 2, Schneider, 1972) contained no Y chromosome and their small yield of the 1.672 satellite provides additional support for a Y chromosome location.

<table>
<thead>
<tr>
<th>DNA isolated from</th>
<th>CsCl</th>
<th>CsCl-Act-D</th>
<th>CsCl-Net</th>
</tr>
</thead>
<tbody>
<tr>
<td>XY larval brains</td>
<td>1.672</td>
<td>1.672 1.686</td>
<td>1.672 1.686</td>
</tr>
<tr>
<td>XO larval brains</td>
<td>1.686</td>
<td>1.705</td>
<td>1.672 1.686</td>
</tr>
<tr>
<td>Line 2 tissue culture</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Figure 3. Relative amounts of the satellites present in DNA extracted from X/0 and X/Y larval brains and from tissue culture cells. Brains were dissected from larvae in *Drosophila* ringer's buffer (Ephrussi–Beadle) and frozen on dry ice. They were lysed in 0.01 M Tris, 1 mM EDTA, pH 8.4, with 1% sarkosyl, and CsCl added to a density of 1.70 g/cc. The lysates were centrifuged for 48 hr at 40,000 rpm. The DNA was then run in an analytical CsCl gradient. Tissue culture cells (line 2, Schneider, 1972) were harvested and lysed with 1% sarkosyl in 0.01 M Tris, 1 mM EDTA, pH 8.4 and processed as above. Actinomycin and netropsin gradients were as described previously (Peacock et al., 1974).
of this highly repeated species. Cytological hybridization to tissue culture metaphase cells (lines 1 and 2, Schneider, 1972) has confirmed the centromeric location of all of the satellites. However these cells, whilst showing the 1.672 species to be predominantly on chromosome 2 and the presumptive Y (in line 1), also showed that it may occur, to a small extent, on chromosome 3 (Figure 4). The small amount of labelling on chromosome 3 may result from other satellites contaminating the 1.672 template, but this is unlikely. If the 1.672 satellite is restricted to chromosomes 2 and Y, in vivo, the possibility arises that the two related sequences of this highly repeated species (Brutlag and Peacock, 1975) are distributed with chromosomal specificity. It will be important to isolate this satellite separately from males and females to determine whether both of the sequence types occur in each sex. The tissue culture cells show the 1.686 and 1.705 satellites to be present on all chromosomes, in differing proportions, with the 1.705 occurring to a limited degree in the X chromosome. The 1.686 species appears to be largely confined to one of the major autosomes.

These results, obtained with tissue culture cells, will have to be confirmed with hybridization to in vivo mitotic cells since the culture lines do not have the standard male and female chromosome complement. Although the chromosomal morphologies are similar to the normal chromosomes, there could have been rearrangements affecting the amounts and distribution of heterochromatin.

ARRANGEMENT OF THE SATELLITE SEGMENTS

Since cytological hybridization has shown each chromosome of the complement to contain two or more species of satellite in its centromeric region we have attempted to determine the arrangement of the satellites with regard to each other, and with respect to the unique and less repeated DNA of the genome. We have examined the 1.705 satellite and attempted to define its neighbouring sequences; 1.705 DNA from an actinomycin-CsCl gradient was rerun in a CsCl gradient following extraction of the actinomycin. This DNA banded in a single peak but showed a pronounced skew on the light side, suggesting that other DNA species were covalently attached to the 1.705 DNA (Figure 5). The peak was divided into three fractions and each was analysed by buoyant density centrifugation, thermal denaturation and by hybridization. The buoyant density profiles (Figure 5) show fraction 1 to be a single symmetrical band of density 1.705, fraction 2 to have a single band with some skew to the light side, and the band in fraction 3 to have a pronounced shoulder at a density of 1.697 g/cc. Since each of the 1.672, 1.686 and 1.705 satellites has a distinctive Tm, different from that of main band DNA, we were able to use thermal denaturation analysis to monitor the sequence composition of the three fractions. Fraction 1 showed a monophasic
Figure 4. Cytological hybridization of $^{3}$H-RNA, complementary to the 1.672 satellite, to mitotic chromosomes from tissue culture cells. Chromosomes were prepared by the addition of colchicine (2.5 x $10^{-6}$ M) to early log phase cultures (lines 1 and 2, Schneider, 1972) for 16 hr. Cells were harvested and resuspended in 0.075 M KCl for 4 min. Cells were fixed, 5 to 30 min, in 3:1 ethanol acetic acid, washed in fresh fixative and resuspended in a small amount of fixative and dropped on to a slide. The chromosomes were stained with 2% orcein for 2 hr prior to cytological hybridization procedures (see Figure 1).

Melt with a $T_m$ identical to that of pure 1.705. Fraction 2 showed a polyphasic melt with transitions corresponding to the $T_m$'s of 1.686 and main band DNA as well as 1.705. Fraction 3 showed these same transitions with an increased amount of main band, but also
showed a small amount of the 1.672 satellite. We were not able to monitor the presence of the 1.688 satellite because its $T_m$ is indistinguishable from that of the 1.705 species.

![Embryo DNA run in Act D-CsCl gradient](image1)

![1.705 region pooled, Act D extracted, run in CsCl preparative gradient](image2)

<table>
<thead>
<tr>
<th>Fraction No.</th>
<th>Analytical CsCl</th>
<th>Composition Hybridization</th>
<th>Thermal denaturation</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1.672 1.686 1.705 MAIN BAND</td>
<td>0 5% 86% 8.8%</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>1.672 1.686 1.705 MAIN BAND</td>
<td>3% 8% 70% 19%</td>
<td>0 11% 76% 17%</td>
</tr>
<tr>
<td>3</td>
<td>1.672 1.686 1.705 MAIN BAND</td>
<td>10% 25% 35% 30%</td>
<td>2.5% 14% 45% 38%</td>
</tr>
</tbody>
</table>

Figure 5. Analyses of DNA species present at the 'ends' of 1.705 molecules. Nuclear DNA from 8 hr embryos was run in actinomycin-CsCl. (a) The 1.705 peak was collected, actinomycin extracted and a second CsCl preparative gradient run. (b) The 1.705 peak was divided into 3 regions as indicated and each fraction analyzed by analytical centrifugation, thermal denaturation and filter hybridization. (c) Thermal denaturation was by the method of Mandel and Marmur (1968). In the hybridization analysis each of the three fractions was fixed to a filter by the method of Gillespie (1968). Small filters were cut and pre-incubated in 0.02% polyvinyl pyrrolidine and 0.02% ficoll. Radioactive DNA was prepared by copying purified species with DNA polymerase in the presence of $^3$H-triphosphates. DNA (40,000 counts/min/μg approximate specific activity) was denatured by heating to 90°C for 2 min. The filters were added and incubated in 2 x SSC, with shaking, carried out at $T_m-25°$ for each of the satellite species over a period of 12 hr. For the main band hybridization, sufficient DNA was added so that hybridization could be carried out to a Cot1/2 = 1,000. Filters were then washed three times in 2 x SSC, dried and counted.
Filter hybridization analysis, using radioactive DNA copies of each of the satellites and of main band, confirmed the thermal denaturation results. The estimate of covalently attached main band sequences could be inflated by a contaminating tail of the very large main band peak in the initial actinomycin gradient, but the 1.686 estimate is free from this error as the second CsCl gradient would have removed any non-covalently attached sequences of this satellite. Covalently attached 1.686 sequences, in molecules which contain the junctions of 1.705 and 1.686 segments, account for approximately 2% of the DNA in the 1.705 peak. Since the average length of the molecules in the gradient is about 15 kb, the junction molecules will contain, on average, 7.5 kb of 1.686. We have estimated the total amount of 1.705 DNA in the genome to be 6,000 kb, so the 2% contamination by 1.686 is consistent with there being a limited number of 1.705-1.686 junctions and with there being a limited number of long segments of the 1.705 sequence. The analysis has shown that the 1.705-1.672 junctions are only one third as frequent as 1.705-1.686 junctions, which is consistent with the 1.672 satellite being restricted to two chromosomes.

DISCUSSION

Our experiments show that the major satellites of Drosophila melanogaster occur in long segments at only a few sites in the genome. The shearing-yield experiments have led to estimates of c.600 kb for the length of uninterrupted stretches of each of the highly repeated DNA species. These estimates contrast with those of Kram et al. (1972) who concluded that the rapidly renaturing sequences of Drosophila occur in stretches of only 2 to 18 kb, interspersed with segments of more complex DNA. In large part their failure to detect long stretches of satellite can be attributed to their method of DNA preparation which results in the loss of most of the satellites (Peacock et al., 1974). The relation of their hydroxylapatite rapidly renaturing (h.a.r.r.) DNA to our nuclear satellites is uncertain. Junctions between satellites and main band DNA could yield molecules corresponding to the h.a.r.r. DNA. We do have some evidence for junctions between the 1.705 satellite and main band sequences but quantitatively these involve only a few per cent of the satellite sequences.

There has been another report of satellite sequences occurring in long stretches. Bond et al. (1967) concluded for the mouse genome that 'nearly all of the satellite sequences do indeed occur in such stretches (60 kb)'. It is likely that in all species in which cytological hybridization has shown a centromeric location of highly repeated DNA, that these sequences occur in long segments.
We pointed out earlier that the restriction of the 1.672 satellite to two chromosomes could mean that each one of its two repeating sequences (Brutlag and Peacock, 1975) occurs on only one chromosome. However, our cytological hybridization data rule out the possibility that each of the major satellite species occurs on only one chromosome. Nevertheless, the *Drosophila* satellites could be distributed in a pattern which is chromosome specific. Both arrangement and length of segments of the different repeating species could distinguish the centromeric heterochromatin of a chromosome from that of all other chromosomes in the complement. Findings of individual satellites occurring on only some members of a chromosome complement (Jones et al., 1973; Jones and Corneo, 1971; Prescott et al., 1973) reflect some chromosomal specificity of highly repeated DNA species. It should be possible in *Drosophila*, by manipulating the chromosome complement, to determine whether a chromosome specific arrangement of the satellites does exist.

A surprising result of the cytological hybridization experiments was the finding that each of the three satellites (1.672, 1.686 and 1.705) hybridized to region 21C-D on chromosome 2. The 1.705 localization to 21C-D has been checked by competition hybridization analysis to be sequence specific and this undoubtedly applies to the other two satellites. We have calculated from the relative number of grains over 21C-D and over the chromocentre that the 1.705 sequences at the 21C-D region account for only 6 kb of the total of 6,000 kb of 1.705 DNA in the genome (Peacock et al., 1974). The calculation is dependent on a number of assumptions; however, the fact remains there is a small representation of each of the three satellites in what may be a single band on chromosome 2. The explanation for this may be trivial; it may be a consequence of two or more inversions which involved break points in the heterochromatin resulting in the transposition of portions of the heterochromatin to 21C-D. On the other hand this locus may have a fundamental role in the establishment and maintenance of satellite sequences. It will be of some interest to examine other *Drosophila* species to determine whether there is a similar band in the complement, whether it is in a homologous position in the complement, and whether it contains each of the centromeric satellite sequences.

We have previously pointed out a correspondence in amount and location of heterochromatin and the satellite sequences (Peacock et al., 1974). However, this may be an oversimplification. The tissue culture chromosomes have shown satellite localization only to the centromeric heterochromatin of the X chromosome and not more generally over the basal heterochromatin of this chromosome. Thus the satellite species may be restricted to block A of the heterochromatin (Cooper,
1959). The nucleolus organizer region, the site of the ribosomal RNA genes, occurs between blocks B and C, yet filter hybridization with radioactive ribosomal RNA has given no evidence for ribosomal cistrons in any of the three purified satellite species. This is consistent with them having a restricted distribution in the basal heterochromatin. We did find an enrichment of ribosomal sequences in the preparation of 1.688 DNA. However, until we have further purified this satellite the significance of this finding is uncertain and may merely represent a co-purification of 1.688 and ribosomal DNA in the actinomycin gradient.

If the satellites are restricted to block A of the X chromosome heterochromatin this may conflict with our suggestion of chromosomal specificity of their arrangement. Chromosomal specificity implies an importance in homologous chromosome recognition, yet Cooper (1964) has shown that block A lacks sites for meiotic X and Y chromosome conjunction in the male. Nevertheless, even if highly repeated DNA does not play this role in meiosis in the male, it may in meiosis in the female, or in other processes in which homologous chromosome recognition is important.

REFERENCES


