LOCATION AND UNDERREPLICATION OF SATELLITE DNA IN *DROSOPHILA MELANOGASTER*

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ABSTRACT

The two light nuclear satellites ($\rho_{CsCl} = 1.672$ and $\rho_{CsCl} = 1.687$) have been quantified in DNA isolated from the larvel imaginal discs and brains of *Drosophila melanogaster* with the genotypes X/O, X/X and X/Y. By comparing the results from these different genotypes, the amounts of the two satellites in the X and Y chromosomes and in the autosomes have been determined. The lightest satellite is not located to any appreciable extent in the X chromosome. The heterochromatic regions are not completely filled by these satellites.—Satellite DNA has also been quantified in DNA isolated from adults containing different genotypes. The two satellites are underreplicated to different extents. The apparent amount of underreplication for one of the satellites is different in different parts of the genome.

FOR *Drosophila melanogaster*, *in situ* hybridization of the satellite DNA sequences to salivary gland chromosomes (GALL, COHEN and POLAN 1971; PEACOCK *et al.* 1973; GOLDRING, BRUTLAG and PEACOCK 1975) and analytical centrifugation of DNA isolated from salivary glands (GALL, COHEN and POLAN 1971; LEE and THOMAS 1973) have been used to show that the satellite DNA sequences are located in the chromocentric heterochromatin and are severely underreplicated during polytenization. PEACOCK *et al.* (1973) have noted the correspondence between the total amount of heterochromatin and the total amount of highly repetitious DNA, most of which is satellite DNA, and BRUTLAG and PEACOCK (1975) have suggested that the location and amount of satellite DNA is important in homologous chromosome recognition. BLUMENFELD and FORREST (1972) reported that the three satellites of *D. virilis* are underreplicated to different extents in adult DNA, and they speculated that for satellite DNA unusual conformations imposed by repetitive base sequences might be signals for underreplication. An important question which arises when considering these phenomena is how the individual satellite DNA are quantitatively distributed among the individual chromosomes. Our efforts have been to quantify the amounts of satellite DNA in the chromosomes of *D. melanogaster* to see if their distribution and patterns of underreplication are compatible with these ideas.

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We have used the analytical ultracentrifuge to determine the amount of satellite DNA in the DNA of *D. melanogaster* mutants of different genotypes that are deficient or duplicated for specific chromosomes or chromosomal segments, and by calculating differences, we have obtained the apparent satellite compositions of the additional chromosomes or chromosomal segments. We have quantified satellite I at $\rho = 1.672$ g/ml in neutral CsCl gradients and satellites II + III, the two satellites at $\rho = 1.687$ g/ml in neutral CsCl gradients (ENDOW, POLAN and GALL 1975). The DNA isolated from larval imaginal discs and brains with the ring glands removed is expected to contain the same satellite composition as DNA found in mitotic chromosomes (ENDOW and GALL 1975). The satellite compositions of such larval DNA from types X/X, X/Y and X/O have been determined. The DNA of adults has an average satellite composition of the diploid, polytene and polyploid cells in the adult fly (BLUMENFELD and FORREST 1972; SCHWEBER 1974; ENDOW and GALL 1975). The satellite compositions of such adult DNA from types X/X, X/Y, X/B^sY, X/O, $X/Y/B^sY$, $X,H^-/Y$ (the X chromosome is deficient for most of its heterochromatin) and $X,2H/Y$ (the X chromosome is duplicated for most of its heterochromatin) have been determined.

From these data we have calculated (1) the amount of satellite I and satellites II + III in mitotic chromosomes and in the DNA of adult flies, and (2) the underreplication of satellite I and satellites II + III in the DNA of adults. These calculations indicate that satellite I is not located in the X chromosome to any measurable extent, but is located in the Y chromosome and somewhere in the autosomes; satellites II + III on the other hand are located in the X chromosome, the Y chromosome and the autosomes. The heterochromatic regions are approximately equally filled and must contain a substantial amount of nonsatellite DNA. Regarding underreplication, the satellites behave differently: satellite I appears to be underreplicated to the same extent throughout the genome, whereas satellites II + III appear to be underreplicated to a different extent in the adult Y DNA than in other parts of the genome.

MATERIALS AND METHODS

Drosophila melanogaster *stocks*

All stocks were first isogenized for chromosomes *1*, *2*, *3* and *4*, using standard techniques. Flies heterozygous for complete inversions of the second chromosome ($In(2LR)SM1$, al^2 Cy cn^2 sp^2) and of the third chromosome ($In(3LR)Ubx^{130}$, Ubx^{130} e^s) and carrying the desired sex chromosome were crossed with wild-type flies. In the first generation, flies were selected carrying both the dominant markers associated with the autosomal inversions (Cy and Ubx). Such flies were crossed with the wild type and, from the progeny, flies were selected *not* carrying Cy and Ubx, and so were expected to be homozygous for the wild-type second and third autosomes. These flies were crossed for several more generations with wild type so as to minimize variations in the chromosome *4* composition.

Attached-X females [$\overline{XX} = C(1)RM$, y^2 $su(w^a)w^a$] were crossed with wild type males to obtain X/O male progeny. Larvae from the cross were karyotyped, and only X/O, $\overline{XX}/Y$, and $\overline{XX}/X$ metaphases were observed. As an additional control, a random sample of the presumed X/O males (about 15% of the total) was crossed to virgin females, and no progeny were observed in each experiment.

$X/X/B^SY$ females were originally selected as occasional nondisjunctions from a cross between wild type females and $X+/B^SY$ males. The B^SY used in these experiments is the B^SY containing both Y^L and Y^S and the small attached euchromatic region carrying the B^S locus.

$X/Y/B^SY$ males were obtained by crossing $X/X/B^SY$ females and $X(y, cv)/Y$ males; $y+ cv+ B^S$ males were selected from the progeny. The expected $X/Y/B^SY$ karyotype of such males was confirmed in brain squashes and by a genetic test in which the cross with wild-type females produced fertile B^S+ sons, confirming the presence of the unmarked Y chromosome.

For the study of the $In(1)sc^{4L}sc^{8R}$ chromosome, two different stocks were used. One was obtained from the Pasadena (Cal Tech) collection $[In(1)sc^{4L}sc^{8R}, y]$ and the other from the Oak Ridge National Laboratory collection $[In(1)sc^{4L}sc^{8R}, y\ cv\ f]$. A B^SY chromosome was introduced into both stocks when they were received to insure that no unmarked Y chromosomes accumulated in the stocks. The satellite content was studied in $In(1)sc^{4L}sc^{8R}/Y$ males. Females heterozygous for the $In(1)sc^{4L}sc^{8R}$ and a normal X chromosome were obtained by crossing homozygous $In(1)sc^{4L}sc^{8R}/In(1)sc^{4L}sc^{8R}/B^SY$ females and wild-type males. These females were then crossed with wild-type males. From the F_2, $In(1)sc^{4L}sc^{8R}/Y$ males were selected as $y\ cv/B^S+$ or $y\ cv\ f/B^S+$ males. As a control, the X/Y males were selected as $y+\ cv+/B^S+$ or $y+\ cv+\ f+/B^S+$ males.

For the study of X chromosome heterochromatin duplication, the $In(1)sc^{S1L}sc^{4R}, sc^{S1}\ v\ B$ chromosome originating from the Oak Ridge National Laboratory was used. Females $[In(1)sc^{S1L}sc^{4R}, sc^{S1}\ v\ B/In(1)sc^{S1L}sc^{4R}, sc^{S1}\ v\ B/B^SY]$ were crossed to wild-type males and the $v\ B$ male progeny were selected.

Isolation of DNA from larval imaginal discs and brains

Larvae were raised on standard corn medium in half-pint bottles. As the larvae left the medium to begin puparium formation, they were collected and separated by sex. Wild-type X/X and X/Y larva and X/O larva from the cross $\overline{XX} \times X/Y$ were selected in this manner. The larvae were washed in Robb's solution (ROBB 1969) and the brain-imaginal disc complex was isolated and fixed in 70% ethanol. Care was taken to identify and remove the ring gland from each brain imaginal disc complex. Twenty to twenty-five of the complexes were washed in 0.05 M $CaCl_2$, 0.05 M NaCl, 0.001 M Tris, pH 7.3, and then digested in fresh buffer for 30 minutes at 37° with 200 µg/ml α-amylase (Calbiochem) and 500 µg/ml ribonuclease (Calbiochem). The tissues were then transferred to 0.2 ml of 0.2 M EDTA, pH 9.5, 1 mg/ml nuclease-free pronase (Calbiochem) and 0.8% sodium dodecyl sarcosinate (Sarkosyl NL-97, Geigy) and digested for 20 hours at 50°. At 1 hour and at 3 hours after the addition of the pronase and detergent, the lysate was dispersed by 10 slow passages through a 22 gauge syringe needle. The lysate was then dialyzed against 0.01 M Tris, pH 8.0, 0.001 M EDTA for 2 days with 3 changes of the dialysis buffer. After two rapid passages through a 27 gauge needle, the volume was increased to 0.43 ml by the addition of 0.01 M Tris, pH 8.0, 0.001 M EDTA, and 0.4 µg of *M. lysodeikticus* DNA and 0.56 g CsCl (Harshaw) were added. The density was then adjusted to 1.695 g/ml for analysis in the analytical ultracentrifuge. The centrifugation was performed at 26° in order to effect a clear separation between the lightest satellite and residual polysaccharide, which is lighter than DNA under these conditions.

Isolation of DNA from flies

0.3–0.5 g of etherized flies were homogenized by 20 strokes of a loose-fitting Dounce homogenizer in 40 ml of 1/6 TKMS at 4°. (TKMS is 0.10 M Tris, pH 7.8, 0.25 M KCl, 0.005 M Mg acetate, and 0.35 M sucrose.) The homogenate was filtered through one layer of 43 µ mesh nylon cloth and centrifuged at 1000 × g for 15 minutes to produce a crude nuclear pellet. We have found that shorter centrifugation than this results in a nuclear pellet that contains relatively smaller amounts of satellite DNA. The nuclear pellet was resuspended in 4 ml of 0.2 M EDTA, pH 9.5 containing 100 µg/ml ribonuclease (Calbiochem) and homogenized in a loose Dounce homogenizer to disperse the pellet. After 30 minutes at 50°, the solution was made 1% in sodium dodecyl sarcosinate (Sarkosyl NL-97, Geigy) and 1 mg/ml in nuclease free pronase (Calbiochem). At this time and again after two hours, the lysate was homogenized with a loose Dounce homoge-

nized in order to disperse visible aggregates. The homogenizations were essential for producing DNA which contained reproducible satellite compositions. After 20 hours at 50°, the volume of the solution was increased to 5.5 ml with 0.2 M EDTA, and 3.37 g of Cs_2SO_4 (Research Organic Inorganic Chemical Corp.) was added. This solution was centrifuged at 40,000 RPM for 48 hours in a type 65 rotor (Beckman). The tube was punctured about 3 mm above the cloudy polysaccharide band, which is denser than the DNA in Cs_2SO_4, and the viscous DNA fractions were collected and dialyzed against 0.01 M Tris, pH 8.0, 0.001 M EDTA.

Typically about 70 μl of this DNA solution containing 2 to 2.5 μg of DNA were diluted to a volume of 150 μl with water and 0.45 ml of saturated CsCl solution and 0.5 μg of *M. lysodeiktichs* DNA were added. The density of this mixture was adjusted to 1.695 g/ml for analysis in the analytical ultracentrifuge.

Determination of the satellite compositions

The DNA solutions were centrifuged for 24 hours at 42,000 RPM in the Spinco Model E Analytical Ultracentrifuge in 2° double-sector titanium centerpieces and AN-F rotor.

The cells were analyzed using the Spinco photoelectric scanner. Absorbance profiles were recorded at full-scale equals 1 A_{260} unit to determine the ratio of the area of the *M. lysodeikticus* DNA peak to the area of *D. melanogaster* main band. The wavelength of the ultraviolet light
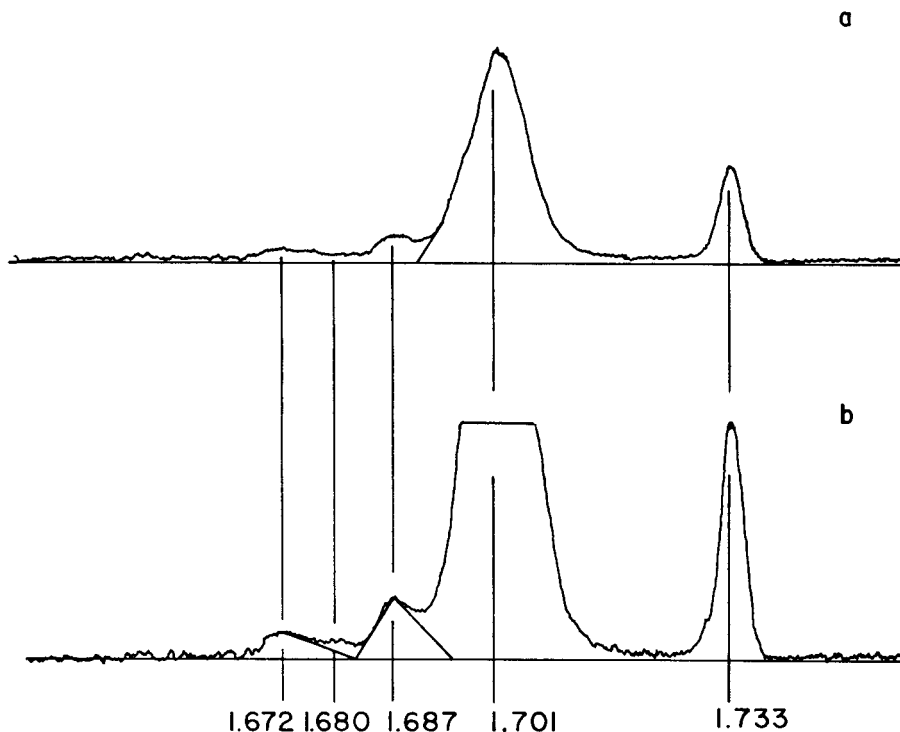

FIGURE 1.—Analytical centrifugation of *D. melanogaster* DNA. About 2.5 μg of DNA from *D. melanogaster* and 0.5 μg of DNA from *M. lysodeikticus* were mixed and brought to a density of 1.695 g/ml with CsCl. After 24 hours at 42,000 RPM scans were taken to quantify the amount of satellite DNA. In these profiles, the *D. melanogaster* DNA isolated from 18 day old X/Y adults contained 2.96% satellite I ($\rho = 1.672$ g/ml) and 5.73% Satellites II + III ($\rho = 1.687$ g/ml). The boundaries of the main band and satellite areas have been drawn according to the rules described in MATERIALS AND METHODS. (a) Absorbance profile at full scale equals 1 A_{260} unit. (b) Absorbance profile at full scale equals 0.5 A_{260} units.

(265–280 nm) was adjusted to keep the maximum absorbance less than 1 OD. Then absorbance profiles were recorded at full-scale equals 0.5 A_{260} units to determine the ratio of the areas of the *D. melanogaster* satellite bands and the area of the *M. lysodeikticus* band. A scan of the cell at 300 nm was also made to detect irregularities in the base line and the areas were corrected for these irregularities. The response of the photodetector is linear under these conditions, and the recorder is electronically calibrated to insure that no distortion is imposed on the photo-detector current.

Rules were established for deciding on each profile how the main band area extends into the satellite area and how the satellite areas extend into the mitochondrial area. The first of these rules was that half of the absorbance at the minimum between satellite II +III and main band belonged to satellites II + III and half belonged to the main band. The main band boundary was defined by drawing a tangent line from this point to the main band envelope (Figure 1a), and the satellites II+III boundary was defined by drawing a line from this point to the maximum of satellites II + III (Figure 1b). This method of separating the overlapping envelopes of satellites II + III and main band is very similar to the best method found by PRASHAD and CUTLER (1976) for determining the amount of mouse satellite in total mouse DNA. The second rule was that the absorbance at the minimum between mitochondrial DNA and satellite II + III belonged to satellites II + III, and a tangent time was drawn from this point to the envelope of satellites II + III (Figure 1b). The satellites II + III peak usually looked quite symmetrical when constructed in this way. The third rule was that one half the absorbance at the minimum between satellite I and mitochondrial DNA belonged to satellite I, and a line was drawn to the maximum of satellite I to complete the boundary (Figure 1b). The satellite I peak usually looked skewed toward heavier densities. Each sample in the analytical ultracentrifuge was scanned twice at the low magnification and three times at the high magnification. Each scan was analyzed separately, and the results were averaged to reduce the chance of errors when determining where each boundary should be drawn.

Areas were measured either by cutting out and weighing the paper or by using an electronic planimeter (Numonics). Given the number of repetitive measurements we have made, both methods give the same average areas. However, the electron planimeter is more precise due to the difficulty of weighing accurately the small pieces of paper. From these data the ratio of the the area of each of the two satellite bands to the total area of all *D. melanogaster* DNA bands was calculated.

<div align="center">RESULTS</div>

DNA isolated using the EDTA, pronase, sarkosyl technique, either from larval discs and brains or from entire adults of *D. melanogaster*, shows a characteristic absorbance profile when centrifuged in neutral CsCl (Figure 1). Three light satellites are distinguishable. Satellite I (ENDOW, POLAN and GALL 1975) at $\rho = 1.672$ g/ml has been identified as a nuclear satellite (BLUMENFELD and FORREST 1971; LEE and THOMAS 1973; PEACOCK *et al.* 1973). The middle satellite ($\rho = 1.680$ g/ml) has been identified as mitochondrial DNA (POLAN *et al.* 1973; BULTMANN and LAIRD 1973; PEACOCK *et al.* 1973). The satellite II + III (ENDOW, POLAN and GALL 1975) at $\rho = 1.687$ g/ml has been identified as a complex of two nuclear satellites of about equal amounts (GALL, COHEN and POLAN 1971; PEACOCK *et al.* 1973; LEE and THOMAS 1973).

To test whether there might be DNA not deproteinated during the EDTA, pronase, sarkosyl treatment, in two experiments lysates prepared in the usual way were dialyzed into Tris EDTA buffer before Cs_2SO_4 was added. At the end of the Cs_2SO_4 preparative centrifugation, the tubes were dripped and the optical density of each fraction was measured. A considerable amount of material was

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TABLE 1

Satellite composition of DNA from larval imaginal discs and brains

Type	n	N.F.	Mean ± SEM		Normalized Mean ± SEM	
			I	II + III	I	II + III
X/Y	19	1.00	4.4 ± 0.11	7.1 ± 0.14	4.4 ± 0.11	7.1 ± 0.14
X/X	17	1.00	2.3 ± 0.09	7.1 ± 0.14	2.3 ± 0.09	7.1 ± 0.14
X/O	10	0.88	2.8 ± 0.16	6.0 ± 0.18	2.5 ± 0.16	5.3 ± 0.18

present at the top of the gradient well above the viscous DNA band. However, when this material from the top of the gradient was dialyzed into SSC, repronased and examined in the analytical ultracentrifuge, it was found to contain less than 5% of the DNA seen in the larger Cs_2SO_4 DNA band and, furthermore, this DNA contained the normal composition of main band and satellite DNA.

In Table 1, the satellite compositions are listed for DNA isolated from the imaginal discs and brains of larvae which possessed the indicated genotype. In order to compare the composition of DNA from larvae possessing different numbers of chromosomes, we have used a normalization factor (N.F.) which corrects for the different amounts of DNA present in the different genotypes (RITOSSA and SCALA 1969; TARTOF 1971). This normalization is necessary because changes in the total amount of DNA in the genome directly affect the apparent amount of a satellite. For instance, deletion of the Y chromosome from the normal male complement results in a 12% reduction in the total amount of DNA. Without normalization the satellite present in the autosomes and X chromosomes would then represent a larger percent of the total DNA. These normalization factors have been calculated from the photometric measurements of RUDKIN (1967, 1969). n is the number of preparations of each type. SEM is the standard error of the mean.

A comparison of the compositions is presented in Table 2. The amounts of satellite in the X and Y chromosomes are calculated by finding the difference between the composition of X/X and X/O DNA and X/Y and X/O DNA. The amounts are calculated for the autosomes as the difference, X/Y −X −Y. The most striking feature is the distribution of satellite I. 1.9% of the total DNA is the portion of satellite I located in the Y chromosome and 2.5% of the total DNA is the portion of satellite I located somewhere in the autosomes. There is no measurable amount of satellite I in the X chromosome. Satellites II + III, on

TABLE 2

Satellite composition of the chromosomes from larval imaginal discs and brains

Difference	I	II + III
X/X—X/O = X	−0.2 ± 0.18	1.8 ± 0.22
X/Y—X/O = Y	1.9 ± 0.19	1.8 ± 0.22
X/Y—X—Y = autosomes	2.5 ± 0.22	3.5 ± 0.34

the other hand, are located throughout the genome. The X and Y chromosomes each contain satellites II + III, amounting in each chromosome to 1.8% of the total DNA. 3.5% of the total DNA is the portion of satellites II + III which is located in the autosomes.

DNA from flies

Using adults, we have been able to study a larger number of different mutants, since the adult phenotype can be used to reveal the genotype. Table 3 contains the data we have accumulated on the satellite composition of DNA isolated from adults having the indicated genotypes.

It must be noted that despite the care we have taken to eliminate loss of DNA during the isolation there is still significant heterogeneity in satellite composition among different preparations of the same DNA type. For the DNA isolated from adults, it is possible to analyze each preparation repeatedly. Two measurements on each preparation typically were performed and the uncertainty is usually ± 0.15. This uncertainty can be reduced by an additional measurement. However, the range in values of satellite composition is many times this uncertainty. For instance, the values for the amount of satellites II + III in the 17 preparations of adult XY DNA range from 5.2 to 6.6%. This must surely be due to real differences between the different DNA preparations. The uncertainty of the mean of the 17 different DNA preparations reflects this heterogeneity and does not result from the imprecision of the assay technique. We suspect heterogeneity also for the DNA prepared from larval imaginal discs and brains, and again the uncertainty is due largely to heterogeneity between the samples and not to imprecision of the assay technique.

The satellite composition of DNA isolated from males increases with an increase in the number of Y chromosomes in the genome. In Figure 2, satellite composition is plotted against the number of Y chromosomes. The amount of

TABLE 3

Satellite composition of DNA isolated from adults possessing the indicated genotypes

Type	n	N.F.	Mean ± SEM		Normalized Mean ± SEM	
			I	II + III	I	II + III
X/Y	17	1.00	3.1 ± 0.07	5.9 ± 0.12	3.1 ± 0.07	5.9 ± 0.12
X/X	6	1.00	1.6 ± 0.10	5.2 ± 0.16	1.6 ± 0.10	5.2 ± 0.16
X/O	10	0.88	1.8 ± 0.06	5.7 ± 0.10	1.6 ± 0.06	5.0 ± 0.10
X/B^SY	2	1.00	3.5 ± 0.10	6.0 ± 0.40	3.5 ± 0.10	6.0 ± 0.40
$X/Y/B^SY$	3	1.13	3.6 ± 0.18	6.1 ± 0.26	4.1 ± 0.18	6.9 ± 0.26
$X,H^-/Y^*$	3	0.97	3.2 ± 0.10	4.9 ± 0.13	3.1 ± 0.10	4.8 ± 0.13
$X,2H/Y$†	2	1.04	3.3 ± 0.10	6.6 ± 0.39	3.4 ± 0.10	6.9 ± 0.39

* $X,H^-/Y$ is $In(1)$ sc^{4L} sc^{8R}/Y. There was no significant difference in the amount of satellite DNA between the two stocks originally received from the Pasadena (Cal Tech) collection and the Oak Ridge National Laboratory collection, so the results have been combined. The satellite composition of the control flies for this cross was equal to that of the wild type.

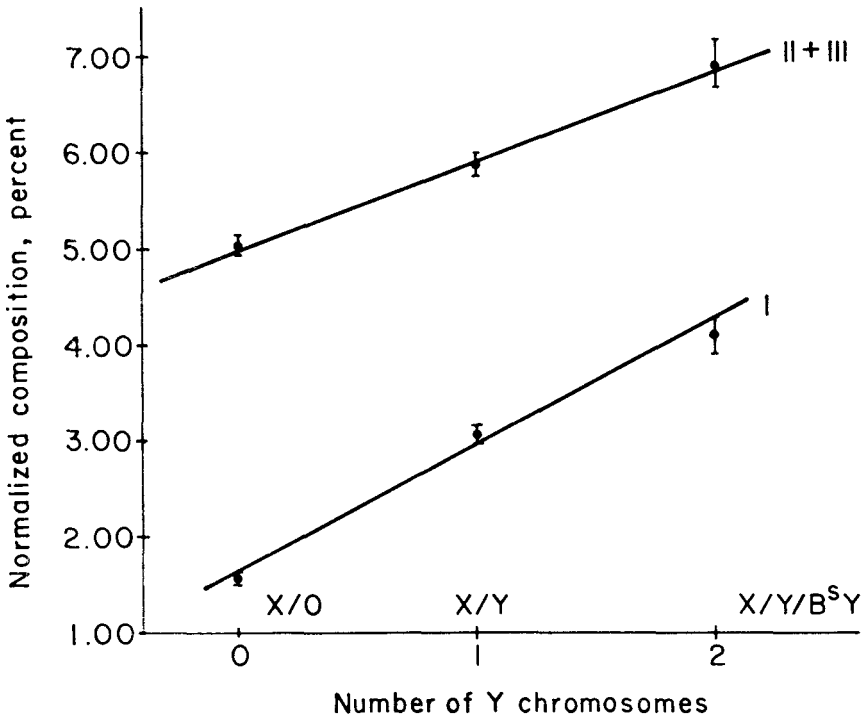† $X,2H/Y$ is $In(1)$ sc^{S1L} sc^{4R}/Y.

FIGURE 2.—Amount of satellite DNA *vs.* number of Y chromosomes in the adult types X/O, X/Y, $X/Y/B^sY$. The normalized amount of satellite I and satellites II + III (as percent of total DNA) is plotted against the number of Y chromosomes in the genome. The error bars indicate standard errors of the mean.

each satellite is approximately a linear function of the Y chromosome content. The B^sY, which is one of the Y chromosomes in the type X-Y/B^sY, has been tested in X/B^sY and contains a normal amount of satellites II + III and a somewhat higher amount of satellite I, but we have not detected any extra amount of satellite I in the type $X/Y/B^sY$. This is probably due to the small number of tests we have performed on the types X/B^sY and $X/Y/B^sY$. From the slope of the straight lines in Figure 2, the amount of satellite I in the Y DNA in the adult is 1.3% of the total DNA, and the amount of satellites II + III in the Y DNA in the adult is 0.9% of the total DNA.

The situation for the X chromosome is more complicated. The difference between the compositions of X/X DNA and X/O DNA indicates a zero amount of satellite I and close to zero amount of satellites II + III: $5.2 \pm 0.16 - 5.0 \pm 0.10 = 0.2 \pm 0.19$. The zero amount of satellite I is an expected result, since the X chromosome in the larval imaginal discs and brains contained no satellite I. The nearly zero amount of satellites II + III is unexpected, since, in the larval imaginal discs and brains, the X chromosomes had as much satellites II + III as the Y chromosome. If there were an equivalent amount of underreplication, we would expect the difference between the composition of X/X DNA and X/O

DNA to be almost 1% in satellites II + III. Given the uncertainty in these measurements and the number of measurements, we can use a t test to estimate that the probability is less than 0.01 that we would not detect this difference. At present, we can offer no explanation for the low satellite content of the X/X adult.

As an alternative, we chose to study the apparent composition of the X heterochromatin in the X/Y male adult. To do this we have quantified the DNA isolated from $In(1)$ sc^{4L} sc^{8R}/Y and $In(1)$ sc^{1L} sc^{4R}/Y. These are mutant chromosomal phenotypes which possess the deletion or the duplication of two thirds of the X heterochromatin delimited by the breakpoints in the sc^4 and sc^8 (or sc^{S1}) inversions. Figure 3 shows satellite compositions in adults plotted against the amount of X heterochromatin. The apparent amount of satellite I is nearly zero again as expected if the X chromosome contains none of this satellite. However, now the amounts of satellites II + III do show an increase with addition of X heterochromatin to the genome. The slope of the straight line drawn to these data indicates that 1.1% of the total DNA is satellites II + III located in this X heterochromatic region. We will show in the discussion that there is probably some satellites II + III in the rest of the X heterochromatin as well.
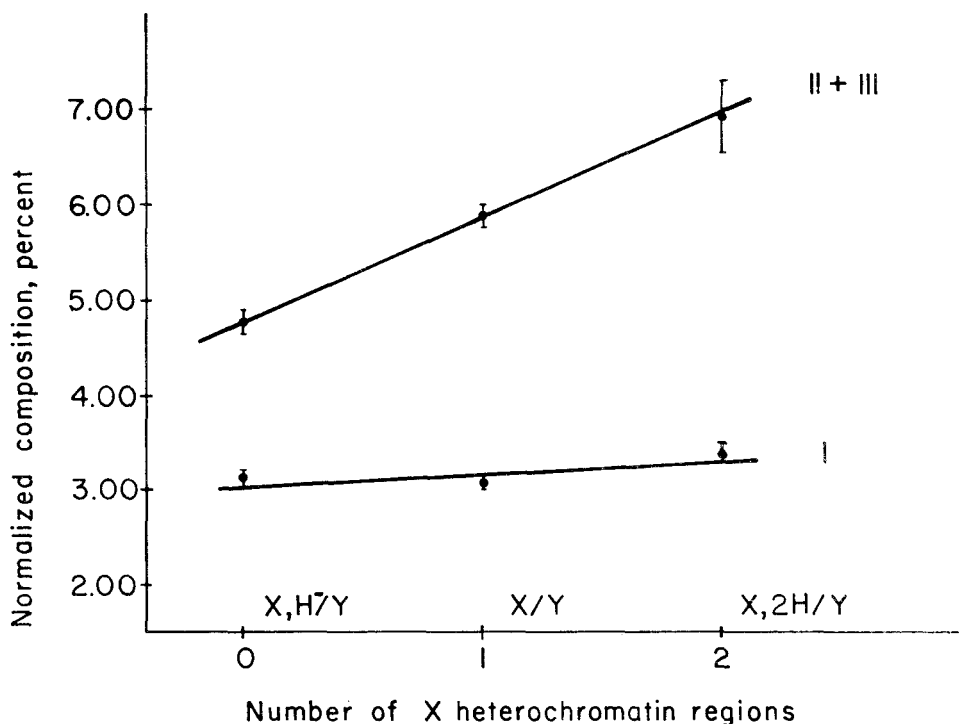

FIGURE 3.—Amount of satellite DNA *vs.* number of X heterochromatin regions in the adult types $X,H^-/Y$, X/Y, and $X,2H/Y$. X,H^- is the X chromosome deficient in two thirds of its heterochromatin and $X,2H$ is the X chromosome duplicated in the same heterochromatic region. Again the error bars indicate standard errors of the mean.

<div align="center">DISCUSSION</div>

The amounts of satellite DNA in the wild types detected during this experiment are similar to those reported previously. The amounts of satellite DNA averaged from female X/X and male X/Y larval imaginal discs and brains are $3.4 \pm 0.14\%$ for satellite I and $7.1 \pm 0.19\%$ for satellites II + III. GALL, COHEN and POLAN (1971) reported about 8% for satellites II + III from larval imaginal discs and brains. PEACOCK et al. (1973) reported that DNA from 8-hour embryos contains about 7.8% of satellites II + III and contains 5.2% of satellite I by yield and 3.8% by saturation hybridization. BLUMENFELD and FORREST (1971) reported satellite I was $3.8 \pm 0.6\%$ in embryo DNA.

ENDOW and GALL (1975), using D. virilis, compared the amount of satellite found in DNA from adult brains, which by several criteria contain diploid cells, to the amount of satellite found in larval imaginal discs and brains (GALL, COHEN and POLAN 1971) and found smaller amounts of satellite in the latter tissue. They felt that this difference might be due to the inclusion of the highly polytenized ring gland during the isolation of the larval imaginal discs and brains. We have taken care to discard the ring gland from our preparations and expect the tissue to contain mainly diploid cells; however, we have not been successful in obtaining DNA from brains of adult D. melanogaster to see if there is any difference between their satellite composition and the composition we have determined for the larval imaginal discs and brains.

Our determination that satellite I is located in the Y chromosome and in some part of the autosomes confirms the report of BLUMENFELD and FORREST (1971), who found a correlation between the number of Y chromosomes in the genome and the amount of this light satellite. They also reported that DNA from the type X/O contained a measurable amount of the satellite. PEACOCK et al. (1973) found satellite I by in situ hybridization to be in the chromocenter of the second chromosome in larval salivary gland chromosomes. GOLDRING, BRUTLAG and PEACOCK (1975) also found satellite I hybridized to the chromocenter of the second chromosome in larval salivary gland chromosomes and showed isopycnic centrifugation profiles of DNA from X/Y and X/O larval imaginal discs and brains which supported the idea that satellite I was in the Y chromosomes and in some other location.

Satellites II + III are located throughout the genome. The X and Y chromosomes contain about equal amounts of satellites II + III. We have not determined the distribution of either satellite II or satellite III separately, but from other reports it is likely each of the satellites II and III are in both the X chromosome and the Y chromosome. First, GALL, COHEN and POLAN (1971) and PEACOCK et al. (1973) both have reported that satellite II and satellite III are each about 4% of the total DNA. Therefore, even if the X or the Y chromosome contained exclusively one of these two satellites, there would be the same satellite in other parts of the genome. Second, GOLDRING, BRUTLAG and PEACOCK (1975) hybridized satellite II to arrested tissue culture cells and found satellite II present in all chromosomes. Third, PERREAULT, KAUFMANN and GAY (1973) used in vitro

hybridization to show sequence homogeneity between the highly repetitious DNA located in the X and Y chromosomes.

Although meiotic crossing over does not occur in male *Drosophila melanogaster*, recombinant chromosomes involving X–Y exchanges are regularly recovered. Such exchanges imply homology (LINDSLEY 1955). Moreover, COOPER (1964) showed that in spermatogenesis the conjunctive competence is not uniformly distributed throughout heterochromatic X and Y, but is a property of localized cohesive elements, or collochores. LINDSLEY and SANDLER (1958) have also demonstrated that control of sex chromosome disjunction during spermatogenesis is a function surely attributable to heterochromatin. BRUTLAG and PEACOCK (1975), based on the sequence analysis of the satellites and the fact that satellite I is located in two chromosomes (GOLDRING, BRUTLAG and PEACOCK 1975), suggested that satellite DNA may play an important role in processes requiring recognition of homologous chromosomes. This seems an unlikely function for satellite I DNA in the Y chromosome, since satellite I is not present in the X chromosome. However, the components of satellites II + III located in the X chromosome and Y chromosome are better prospects for mediating the function BRUTLAG and PEACOCK have suggested. Indeed, the blocks of satellites II + III could well be the localized cohesive elements described by COOPER (1964).

From the amounts of satellite DNA in the chromosomes and knowledge of the size of the heterochromatic regions (COOPER 1950, 1959), we can estimate how much of the heterochromatin is filled by satellite DNA (Table 4). The calculations indicate that each of heterochromatic regions is about one-third filled by satellites I + II + III. Two other cryptic nuclear satellite DNA components of *D. melanogaster* have been reported. One satellite (2% of the total embryo DNA) at $\rho = 1.701$ g/ml in neutral CsCl (PEACOCK *et al.* 1973) has not been characterized or located in the chromosomes. Satellite IV (ENDOW, POLAN and GALL (1975) at $\rho = 1.705$ g/ml (BLUMENFELD and FORREST 1972; PEACOCK *et al.* 1973) amounts to 5% of embryo DNA and has been located in ten chromosomal regions by *in situ* hybridization to mitotic chromosomes by PEACOCK and STEFFENSEN (1975). Four of the sites are in the Y chromosome; one site is in the X heterochromatin, and six sites are in the autosomal heterochromatin. If we assume that each of the sites is equally occupied, we can estimate that the Y chromosome, the X heterochromatin and the autosomal heterochromatin are filled about 48%, 38% and 62%, respectively by satellites I + II + III + IV.

It is clear that the satellite DNA sequences are not homogeneously distributed

TABLE 4

Amounts of satellite DNA I + II + III in the heterochromatic regions of D. melanogaster

Heterochromatic region	Heterochromatin (% of total DNA)	Satellite DNA (% of total DNA) I	II + III	Sat. DNA ——— Het. DNA
Y	11	1.9	1.8	34%
X heterochromatin	5.5	0	1.8	33%
Centromeric heterochromatin of autosomes	14.7	2.5	3.5	41%

among the heterochromatic regions. For instance, the X heterochromatin contains mostly satellites II + III, whereas the Y chromosome contains all of the satellites. There must be regions of nonsatellite DNA in the heterochromatin. This implies that heterochromatin is regionally differentiated with respect to the type of material it contains. HOLMQUIST (1975) has used fluorescent staining techniques to reveal the location of AT rich sequences in the Y chromosome of *D. melanogaster* and has suggested that the brightest fluorescing bands are the positions of the very AT-rich satellite I in *D. melanogaster*. His photographs show eleven fluorescing bands separated by nonfluorescing regions. The fluorescing bands correspond in number and location to the heterochromatic blocks described by COOPER (1959). HOLMQUIST's observations, together with our data, suggest that the satellite DNA sequences are acting as centers of heterochromatinization and the heterochromatinization spreads to include adjacent nonsatellite DNA. This would explain why the heterochromatic regions in *D. melanogaster* are occupied by nearly constant fractions of satellite DNA.

BLUMENFELD and FORREST (1972) have shown that DNA isolated from adults contains about one half the amount of satellite I as found in the DNA from embryos. The relative amount of an underreplicated DNA component from mixed diploid and polytene or polyploid cells, as is the case in the adult fly (ENDOW and GALL 1975), depends on the fraction of polytene or polyploid cells, their average level of ploidy and the extent to which the component is excluded during each replication. BLUMENFELD and FORREST (1972) concluded that the one-half reduction in satellite I could be the result of 1% of the cells attaining a ploidy level of 128C, if the satellite DNA were not replicated at all during each doubling.

We can calculate the underreplication of the satellite I and satellites II + III in the DNA from adults relative to DNA from larval diploid tissue. For the average composition of the types X/X and X/Y, satellite I is reduced by a factor of 0.70 and satellites II + III by a factor of 0.78. If the compositions are calculated as percentages of main band DNA, as suggested by ENDOW and GALL (1975), instead of percentages of total DNA, the reductions are 0.68 and 0.76, respectively. This result is similar to the situation found in *D. virilis* in which there are three satellites underreplicated to different extents in the adult (BLUM-

TABLE 5

Underreplication of satellite DNA in the adult chromosomes

Chromosome	Satellite DNA (% of total DNA)				Fraction replicated	
	Adult		Larval diploid			
	I	II + III	I	II + III	I	II + III
Y	1.3	0.9	1.9	1.8	0.72	0.50
X^*	0.0	1.1	0.0	1.8	—	0.61
Autosomes	1.8	3.9	2.5	3.5	0.72	1.11

* The X heterochromatin studied in the adult is the region delimited by the breakpoints in the inversion X chromosomes *In(1) sc⁴* and *In(1) sc⁸*. This is about 2/3 of the X heterochromatin. The X heterochromatin studied in the larval diploid tissues is the entire X heterochromatin.

ENFELD and FORREST 1972; SCHWEBER 1974; ENDOW and GALL 1975). From this result BLUMENFELD and FORREST (1972) speculated that the particular molecular conformation imposed by the sequence was being recognized by the elements controlling replication. This would mean a particular satellite would be under-replicated to the same extent everywhere in the genome.

Table 5 shows the apparent satellite compositions of the *X*, *Y*, and autosomal DNA in the adult cells, in the DNA from larval diploid tissue, and the calculation of the amount of replication of the satellite DNA in each chromosome. Satellite I is underreplicated to the same extent in the *Y* chromosome as in its autosomal location. Satellites II + III, however, are apparently replicated to much different extents in the *Y* chromosome, the *X* chromosome, and the autosomes. The calculation indicates an overreplication of satellites II + III DNA relative to nonsatellite DNA in the autosomes during the formation of adult cells. Such an overreplication of satellite DNA is known to occur in the larval ovaries and to a lesser extent in adult ovaries in *D. virilis* (ENDOW and GALL 1975).

One obvious difficulty with our data is that we have not quantified the entire *X* adult heterochromatin. For instance, if there were 50% more satellites II + III in the rest of the adult *X* heterochromatin, which is entirely reasonable, the fraction of satellites II + III replicated in the adult increases to 0.90 of the diploid amount, and because we are determining compositions by calculating differences, the fraction of satellites II + III replicated in the autosomes decreases to 0.94 of the diploid amount.

If this is the only change needed in Table 5, we are still left with evidence that satellites II + III are underreplicated to different extents in different heterochromatic regions. In fact, the differences are so large that we can conclude that satellite II and satellite III each must be underreplicated to different extents in different heterochromatic regions. Control of replication, therefore, is not in the satellite sequences themselves, but in some adjacent or intercalated sequence which directs underreplication of the satellite sequence.

There is another phenomenon we should consider when contemplating the satellite DNA compositions of the DNA isolated from adults having different chromosomal phenotypes. TARTOF (1971) has shown that in adult *D. melanogaster* the amount of DNA coding for ribosomal RNA in the type *X/O* and the type *X, NO⁻/X* is increased well above the amount expected. SPEAR and GALL (1974) have shown in larva that this increase is due to disproportionate replication of the rDNA in cells which undergo polytenization. PROCUNIER and TARTOF (1975) have shown that the DNA coding for 5S RNA increases its amount when opposite a deficiency for the 5S gene site. If similar compensation were occurring for satellites II + III, DNA from the type *X/O* and the type *X,H⁻/Y* would contain more satellites II + III than would be expected. We would, therefore, calculate too small a value for the amount of satellites II + III DNA in a normal *Y* chromosome and a normal *X* chromosome in the adult *X/Y*, and by difference, too large an amount in the rest of the adult chromosomes. This would explain why satellites II + III are apparently more underreplicated in the adult *Y* chromosome and adult *X* chromosome than in the rest of the adult chro-

mosomes. Our data for the amount of satellites II + III in $X/Y/B^sY$ and $X,2H/Y$ indicate an unusual aspect of this postulated compensation phenomenon. Since the differences between the composition of $X/Y/B^sY$ and X/Y are the same as between X/Y and X/O and the differences between the compositions of $X,2H/Y$ and X/Y are the same as between X/Y and $X/H^-/Y$, there would have to be downward compensation for the amount of satellites II + III in the DNA of adult types $X/Y/B^sY$ and $X,2H/Y$. This would be unusual because downward compensation for the amount of rDNA in $\overline{XY}/Y$ (and $\overline{XX}/Y$) does not occur (TARTOF 1973); downward compensation for the amount of rDNA in $X,2H/Y$ does not occur (RITOSSA and SPIEGELMAN 1965), nor does downward compensation for the amount of 5S DNA in adults containing a duplication of the 5S gene site occur (PROCUNIER and TARTOF 1975). It is a combination of both upward and downward balancing of the satellites II + III amount in adults that would not allow an unambiguous demonstration of compensation in the same way that compensation for rDNA or 5S DNA has been demonstrated.

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