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# Mapping Simple Repeated DNA Sequences in Heterochromatin of *Drosophila melanogaster*

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## ABSTRACT

Heterochromatin in Drosophila has unusual genetic, cytological and molecular properties. Highly repeated DNA sequences (satellites) are the principal component of heterochromatin. Using probes from cloned satellites, we have constructed a chromosome map of 10 highly repeated, simple DNA sequences in heterochromatin of mitotic chromosomes of *Drosophila melanogaster*. Despite extensive sequence homology among some satellites, chromosomal locations could be distinguished by stringent *in situ* hybridizations for each satellite. Only two of the localizations previously determined using gradient-purified bulk satellite probes are correct. Eight new satellite localizations are presented, providing a megabase-level chromosome map of one-quarter of the genome. Five major satellites each exhibit a multichromosome distribution, and five minor satellites hybridize to single sites on the *Y* chromosome. Satellites closely related in sequence are often located near one another on the same chromosome. About 80% of *Y* chromosome DNA is composed of nine simple repeated sequences, in particular $(AAGAC)_n$ (8 Mb), $(AAGAG)_n$ (7 Mb) and $(AATAT)_n$ (6 Mb). Similarly, more than 70% of the DNA in chromosome *2* heterochromatin is composed of five simple repeated sequences. We have also generated a high resolution map of satellites in chromosome *2* heterochromatin, using a series of translocation chromosomes whose breakpoints in heterochromatin were ordered by N-banding. Finally, staining and banding patterns of heterochromatic regions are correlated with the locations of specific repeated DNA sequences. The basis for the cytochemical heterogeneity in banding appears to depend exclusively on the different satellite DNAs present in heterochromatin.

ALTHOUGH constitutive heterochromatin often comprises a large proportion of the genome of higher eukaryotes, its biological function is enigmatic. In *Drosophila melanogaster*, approximately 30% of the genome is composed of heterochromatin. Heterochromatic regions of mitotic chromosomes include the proximal half of the *X*, the entire *Y*, one-quarter of the pericentromeric region of each major autosome, and three-quarters of the dot-like chromosome *4* (HEITZ 1933; KAUFMANN 1934; HANNAH 1951). Unlike the euchromatin, heterochromatin remains condensed throughout the cell cycle, and sister chromatids remain closely associated in late prophase. Heterochromatic regions of chromosomes also replicate later in the cell cycle than euchromatin (BARRIGOZZI *et al.* 1966), undergo little or no meiotic recombination relative to euchromatin (MULLER and PAINTER 1932; ROBERTS 1965) and are, in general, highly underrepresented in polytene nuclei (RUDKIN 1969; GALL 1973).

In *D. melanogaster*, heterochromatin, in proportion to its length, contains only a small number of genetic functions relative to euchromatin (see HILLIKER, APPELS and SCHALET 1980). However, genetic functions other than the centromere have been definitively assigned to heterochromatic regions of all chromosomes except chromosome *4*. The *Y* chromosome carries no loci essential for viability since *XO* males are fully viable although completely sterile (BRIDGES 1916). Six genes essential for fertility in males are present on the *Y* chromosome, four on the long arm (*YL*) and two on the short arm (*YS*) (BROSSEAU 1960; KENNISON 1981; HAZELRIGG, FORNILI and KAUFMAN 1982; GATTI and PIMPINELLI 1983). Approximately 30 genes essential for viability are present in the heterochromatin of chromosomes *2* and *3* (HILLIKER and HOLM 1975; HILLIKER 1976; MARCHANT and HOLM 1989a,b; see also HILLIKER and SHARP 1988). The density of vital genes in chromosome *2* heterochromatin is estimated at about 1% the density of vital genes in euchromatin (HILLIKER 1976).

Four abundant satellite DNAs band at 1.672, 1.686, 1.688 and 1.705 g/ml in cesium chloride buoyant density gradients of nuclear DNA from *D. melanogaster* (GALL, COHEN and POLAN 1971; PEACOCK *et al.* 1973). The satellites consist of short nucleotide se-

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quences, 5–359 bp in length, are arranged separately in long tandem arrays and comprise the bulk of the DNA in heterochromatin of mitotic chromosomes (PEACOCK *et al.* 1976, 1977; PEACOCK and LOHE 1980). However, the complexity of DNA sequences in heterochromatin is significantly greater than predicted from simply four classes of highly repeated sequences. Molecular cloning of the physically isolated 1.672, 1.686 and 1.705 satellites has resolved each of them into multiple closely related, simple sequences that differ in their reiteration frequency (LOHE and BRUTLAG 1986). Thus, the prominent satellites are only the most abundant of many different repeated sequences. These sequences have characteristic buoyant densities that show overlapping distributions. Three complex and 12 simple satellite repeats have been identified in *D. melanogaster* to date. Eleven simple satellites consist of tandem arrays of a short nucleotide sequence that conforms to the formula $(RRN)_m(RN)_n$, where R is A or G and N is any nucleotide (LOHE and BRUTLAG 1986). Recently, a simple satellite containing 11–12 bp repeated units that do not fit this expression has been described (ABAD *et al.* 1992).

By *in situ* hybridization to mitotic chromosomes, PEACOCK *et al.* (1976, 1977) showed that the major satellites of *D. melanogaster* have a multichromosome distribution and are localized in large blocks. They also presented a composite chromosome map of the satellites. However, for the purpose of precise satellite locations this map is not reliable for two major reasons. First, the probes in these mapping experiments were not cloned but were made from distinct satellites purified in CsCl gradients. Since these preparations are now known to consist of mixtures of different repeated sequences (LOHE and BRUTLAG 1986), the localizations should be considered as composite maps for the several closely related repeats present in the one satellite preparation. Second, different satellite repeats were mapped in independent *in situ* hybridization experiments. One cannot reliably order different satellites within specific blocks of heterochromatin because of the small size of mitotic chromosomes and autoradiographic scatter. Relative localizations must be obtained using cytologically ordered chromosome rearrangements that have breaks in both euchromatin and heterochromatin (STEFFENSEN, APPELS and PEACOCK 1981; HILLIKER and APPELS 1982; BONACCORSI and LOHE 1991; this study).

An unresolved problem is how the genetic functions in heterochromatin relate to the highly repeated satellite sequences, the principal component of DNA in heterochromatin (LOHE and ROBERTS 1988). To begin to relate genetic functions that are identifiable in heterochromatin with specific DNA sequences, we have determined the chromosomal assignments of ten simple satellite repeats in heterochromatin of *D. melanogaster*. Chromosome mapping of heterochromatic sequences must use mitotic rather than polytene chromosomes. Heterochromatin in the larval salivary gland nucleus is exceedingly underreplicated and does not form banded structures. With the exception of minor amounts of one satellite, the simple repeated sequences are absent from the banded euchromatin of polytene chromosomes, as judged by *in situ* hybridization, and localize only within the chromocenter (LOHE and ROBERTS 1988). In this paper, we consider heterochromatin as defined cytologically in mitotic chromosomes and we do not relate satellite localizations in mitotic heterochromatin to the $\alpha$ and $\beta$ heterochromatin visible in the chromocenter of polytene chromosomes.

Chromosomal assignments were carried out by *in situ* hybridization using cloned and sequenced probes (LOHE and BRUTLAG 1986) and stringent hybridization criteria to avoid cross-hybridizations between closely related satellites (LOHE and BRUTLAG 1987a). We have extended this analysis by employing chromosome rearrangements to map satellite blocks to specific heterochromatic segments. The mapping of the *Y* chromosome is presented elsewhere (BONACCORSI and LOHE 1991). Here, we present in detail a refined satellite map of chromosome *2* heterochromatin as well as the overall distribution of these cloned sequences in the genome of *D. melanogaster*.

## MATERIALS AND METHODS

**Satellite probes for *in situ* hybridization:** For quantitations of *in situ* hybridizations, probes were single-stranded transcribed RNA copies from cloned satellite segments (LOHE and BRUTLAG 1986) subcloned into the SP6 polymerase vectors pSP64 or pSP65. Single-stranded RNA probes were used for satellite localizations and quantitations, although double-stranded DNA probes were also suitable for localizations. Single-stranded RNA was used to avoid self-hybridization of the highly repeated probe during hybridization, to enable RNase digestion of excess $^3$H probe following hybridization, and because parameters in quantitations of *in situ* hybridizations have been determined with RNA probes (SZABO *et al.* 1977). For more detailed localizations, *in situ* hybridizations were carried out using biotinylated DNA probes (LANGER, WALDROP and WARD 1981).

Probes were synthesized from plasmid clones containing a single satellite sequence repeated in a tandem array (LOHE and BRUTLAG 1986), except for plasmids 1.672-181 and 1.672-453 which each contain a small amount of moderately repeated DNA linked to the satellite array. Plasmid 1.672-181 is a subclone of 1.672-1, which contains a 181-bp tandem array of AATAC repeats adjacent to 448 bp of a *297* mobile element (LOHE and BRUTLAG 1987b). An *Eco*RI fragment from 1.672-1 containing the complete satellite array flanked by 68 bp of mobile element DNA was subcloned into the pSP65 vector, to produce plasmid 1.672-181. Plasmid 1.672-453 contains 479 bp of AATAAAC repeats linked to 84 bp of moderately repeated DNA sequences (LOHE and BRUTLAG 1987b). Plasmid aDm23-24

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contains a single 359-bp repeat unit of the 1.688 satellite (HSIEH and BRUTLAG 1979) and was used as a probe for 359-bp repeats.

*Tritiated RNA probes:* Single strands of simple repeated sequences usually have strong biases in their base composition. To maximize the incorporation of labeled [$^3$H]UTP precursor, care was taken in choosing the vector (pSP64 or pSP65) for subcloning. For example, AAGAG repeats were subcloned into the pSP64 vector to ensure that the purine-rich strand was transcribed for incorporation of a [$^3$H]UTP precursor. No UTP can be incorporated into RNA transcribed from the complementary strand $(TTCTC)_n$. Probe lengths varied from 200–400 bp according to the satellite fragment subcloned.

Plasmid DNA (2.5 μg) containing the satellite insert was linearized with the appropriate restriction endonuclease and the solution was made to a final concentration of 0.5 mM ATP, GTP and CTP, 10 mM dithiothreitol, 40 mM Tris-HCl, pH 7.5, 6 mM $MgCl_2$, 1 unit/μl RNasin (Promega) and 10 μg/ml bovine serum albumin. This solution was added to a tube in which [$^3$H]UTP (25 μCi, Amersham; 43 Ci/mmol) had been dried. The [$^3$H]UTP was resuspended and the reaction initiated by the addition of SP6 RNA polymerase (3.5 units, Bresa). The progress of the reaction was monitored by incorporation of label into trichloroacetic acid-precipitable material. Following incubation at 37° for 1 hr, *Escherichia coli* tRNA was added (60 μg), and the DNA template was digested at 37° for 30 min by addition of DNase I (1 unit/μl; RQ1, Promega). The solution was made to 0.1% sodium dodecyl sulfate and 10 mM EDTA (pH 8), phenol extracted, an equal volume of 4 M ammonium acetate was added, and the RNA was precipitated with 2 volumes of ethanol (2×). The RNA was dissolved in 100 μl of 6 × SSC (1 × SSC is 150 mM NaCl, 15 mM Na-citrate) and stored at −20°. The length of the labeled RNA was monitored by electrophoresis on a 5% acrylamide (1:30, bis:acrylamide), 50% urea gel. The gel was soaked in the fluor Amplify (Amersham) and exposed to X-ray film (XAR-5; Kodak). Probes were kept full length and varied in size from approximately 160 bp (1.686-171) to 460 bp (1.686-198). Hybridization was extremely efficient did not vary significantly between different probes or preparations of the same probe, probably because of the highly reiterated nature of the satellites.

*Biotinylated DNA probes:* Plasmid DNA (0.5 μg) or an *Eco*RI-*Xba*I fragment of plasmid 1.672-563 containing a pure tandem array of $(AATAGAC)_n$ (LOHE and BRUTLAG 1987b) were labeled by nick-translation or random priming using biotinylated 11-dUTP (Bethesda Research Laboratories). Color detection was carried out with the horseradish peroxidase/diaminobenzidine system (Enzo) and the preparation was mounted in Euparal for microscope analysis.

**Stringency of hybridization:** The $T_m$ value for RNA-DNA hybrids of individual satellites can differ significantly from the DNA-DNA value determined for these same probes (see LOHE and BRUTLAG 1987a). Therefore, the $T_m$ value was determined empirically for each satellite probe by filter hybridizations and thermal melting in 3 × SSC-50% formamide (vol/vol) before proceeding with *in situ* hybridizations (see Table 1). Conditions for *in situ* hybridizations, such as temperature, salt and formamide concentrations, were the same as used for the filter hybridizations. Stringent annealing conditions were obtained by carrying out hybridizations 8–15° below the $T_m$ value, and the hybridization temperature was, in general, specific for each class of satellite repeats (Table 1). The temperatures for hybridization of biotinylated DNA probes were lower by about 4° from that used for RNA-DNA hybridizations. The $T_m$ value of biotinylated DNA is reduced compared to non-substituted DNA (LANGER, WALDROP and WARD 1981), and this was verified experimentally for some satellites by filter hybridization and thermal melting.

**Cytology:** Mitotic chromosome preparations of the Oregon R stock and *T(2;3)* translocation chromosomes (HILLIKER and TRUSIS-COULTER 1987) were made as described by HALFER (1981) or GATTI and PIMPINELLI (1983). The locations of some satellites were also examined in a stock marked with the $w^{1118}$ mutation, and these locations were similar to those of the Oregon R stock. Chromosome preparations were N-banded as described in ASHBURNER (1989). Once the heterochromatic positions of the *T(2;3)* breakpoints had been established relative to the five N-banded regions in chromosome *2* heterochromatin, they were not further refined. For illustrative purposes most breakpoints were placed equidistant from flanking N-banded regions, or the euchromatic-heterochromatic junction on the left arm for *T(2;3)46*, except for four *T(2;3)*s that have a different breakpoint in region h37 (see RESULTS). The *T(2;3)* chromosomes were assumed to be reciprocal translocations arising from two break events and rejoining without heterochromatic loss. Translocations were generated with a relatively low X-ray dose (2,000 rad), three or more break events were rare in these experiments (HILLIKER and TRUSIS-COULTER 1987) and genetic analyses showed that no *T(2;3)*s were deficient for genes in chromosome *2* heterochromatin (C. B. SHARP, personal communication). However, we cannot rule out that some *T(2;3)*s were formed by three or more breaks, possibly resulting in loss of heterochromatin.

***In situ* hybridization:** The *in situ* hybridization method is similar to that used by PEACOCK *et al.* (1977), with some modifications. Freshly made, air-dried slides were placed in 0.2 M HCl (37°) for 30 min, rinsed briefly in distilled water, dehydrated in 70% ethanol (2×), 95% ethanol (2×) and air-dried. Labeled satellite RNA (1 × $10^5$ cpm) or biotinylated DNA (10–50 ng) was applied to the slide in 10 μl of 3 × SSC-50% formamide, an 18 × 18-mm coverslip was placed over the solution, and the coverslip was sealed with rubber cement. Since significant cross-hybridization between some satellite sequences can occur at room temperature while a series of slides are being processed (P. ROBERTS and A. LOHE, unpublished), slides were placed at 65° in an air incubator for 15 min prior to hybridization to melt nucleic acid hybrids. To initiate hybridization, slides were incubated for 16 h at a temperature specific for each satellite probe (Table 1). For hybridizations using some biotinylated probes, the hybridization solution was made to 10% dextran sulfate.

Following hybridization, coverslips were removed and residual probe was washed away by incubation in 50 ml of hybridization solution at the hybridization temperature for 15 min (2×). For [$^3$H]RNA probes, slides were washed in 2 × SSC for 15 min, treated with RNase A (2 μg/ml of 2 × SSC, 30 min at room temperature), washed again in 2 × SSC (3×, 15 min each), placed in 70% ethanol, 95% ethanol (2×), and air-dried. Slides were dipped in Ilford K2 emulsion (diluted 1:1 with water) and exposed at 4° in a light-tight box. Exposure times were determined empirically and varied for different probes, from 2 days to several weeks. Slides were developed for 2 min in Kodak Dektol diluted 1:1 with water, washed in water and fixed in Kodak Fixer for 4 min. The slides were stained for 10–30 min with Giemsa (BDH, R66; 5% solution, freshly prepared) in Gurr's phosphate buffer (pH 6.8), destained in 100% methanol, air-dried, and mounted in immersion oil (Zeiss) with a coverslip. Slides were stored indefinitely in this way.

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In some experiments chromosomal DNA was denatured by alkali treatment. This procedure gave more efficient labeling but resulted in variable chromosome morphology, unless air-dried slides were aged for 1–2 weeks before hybridization. Chromosome preparations were treated at 65° in 2 × SSC for 30 min and dehydrated in 70% ethanol (2×) and 95% ethanol (2×). DNA was denatured with 0.07 M NaOH for 2 min, slides were placed in 2 × SSC (3×, 5 min each) and dehydrated in ethanol. Probe was then applied to the slides and hybridization was carried out as described above.

Assignments of satellites to chromosomes were made using $^3$H probes. Accurate assignments were made by examination of typically, 50–100 cells, and hybridization pattern for individual $^3$H probes was determined using slides with different times of autoradiographic exposure. Multiple experiments were carried out for each satellite localization, and the hybridization pattern was the same in different experiments and with different probe preparations. Using $^3$H probes, no attempt was made to localize accurately satellites within heterochromatin. Only gross localizations to chromosomes or the approximate number of sites per chromosome were recorded, even if the cytological quality was favorable. Better resolution was obtained using biotinylated probes, where localizations of multiple sites of hybridization within a chromosome (usually chromosomes *2* and the *Y*) could be made with more confidence. However, we relied on results using translocations of chromosomes *2* and the *Y* to derive a detailed satellite map for these chromosomes.

The use of $^3$H probes in hybridizations permitted quantitations by counting silver grains, in contrast to hybridizations with biotin probes where quantitation was not possible with the color detection system. Short exposures of slides in these experiments were used for quantitations of satellites on different chromosomes.

**Southern hybridization:** To ensure high stringency, Southern hybridizations were carried out at a temperature specific for each satellite probe (LOHE and BRUTLAG 1987a). DNA was isolated from heads of adults since this source does not show significant underrepresentation of satellite DNAs due to underreplication of heterochromatic DNAs in polytene cells, unlike many tissues in adults (LOHE and ROBERTS 1990). The $w^{1118}$ stock was used to prepare male DNA. The stock used to detect the *Y* chromosome in occasional *XXY* females was *6P6/FM7a/B$^S$Y*, and was supplied by S. GOODE.

**Quantitation:** Grain counts of *in situ* hybridizations were carried out on cells where all chromosomes could be identified. Autoradiographs overexposed for abundant sites require extrapolation to estimate grain numbers at these sites, due to coincidence of grains (STEFFENSEN, APPELS and PEACOCK 1981). Therefore, exposures of slides were monitored carefully to avoid saturation of the emulsion at sites abundant in satellite repeats. Consequently, most cells showed labeling at the abundant sites but minor sites were labeled in only a proportion of cells. To obtain significant grain numbers at sites containing a small number of satellite repeats, usually 25–45 nuclei were scored. Some satellites hybridized to more than one site per chromosome. We did not attempt relative grain counts to quantitate different satellite locations on the one chromosome because excellent resolution and cytology is required consistently in autoradiographs to distinguish these sites.

The validity of the quantitative *in situ* hybridization method for RNA-DNA hybridizations has been established by SZABO *et al.* (1977). We have confirmed that quantitative hybridizations are valid for our experimental method using the rDNA. From saturation hybridizations to filter-bound diploid DNA of *D. melanogaster* there are about 1.5 rRNA genes on the *X* chromosome for every copy on the *Y* (SPEAR 1974). Quantitations of *in situ* hybridizations to mitotic chromosomes using an rRNA probe gave a value of 1.6:1 for the *X:Y* chromosomes (LOHE and ROBERTS 1990), in good agreement with ratios from saturation hybridization.

**TABLE 1**

**Mean melting temperatures of satellite RNA-DNA hybrids and hybridization temperatures**

| Satellite clone | Repeating sequence | $T_m$ value of RNA-DNA | Hybridization temperature |
|---|---|---|---|
| 1.672-38 | AATAT | 26 | 18 |
| 1.672-349 | AATAG | 35 | 23 |
| 1.672-181 | AATAC | 36 | 23 |
| 1.686-198 | AAGAC | 53 | 40 |
| 1.705-42 | AAGAG | 59 | 44 |
| 1.705-34 | AAGAGAG | 61 | 48 |
| 1.686-171 | AATAACATAG | 36 | 23 |

In estimating amounts of a satellite per chromosome, it is necessary to take into account differences in the ways the molecular and chromosome data were obtained (see PEACOCK *et al.* 1977). Estimates in kilobases of satellite repeats per haploid genome (LOHE and BRUTLAG 1986) were obtained using DNA isolated from equal numbers of male and female embryos, whereas satellite distributions from *in situ* hybridizations were recorded on a single chromosome basis. The haploid chromosome content in a DNA preparation averages 3/4 *X* chromosome plus 1/4 *Y* chromosome plus 1 each of chromosomes *2*, *3* and *4*. Therefore, kilobase amounts of individual satellite sequences per chromosome were estimated by multiplying the proportions represented by each chromosome in the DNA preparation with the proportions of a particular satellite repeat per chromosome, as determined by quantitative autoradiography (PEACOCK *et al.* 1977). These calculations assume a haploid DNA content of 170 Mb for *D. melanogaster* (RASCH, BARR and RASCH 1971) and that the *X* and *Y* chromosomes each contain 40 Mb of DNA (PEACOCK *et al.* 1977).

## RESULTS

**Stringency of hybridization:** Some simple satellite sequences in *D. melanogaster* differ from each other by only one nucleotide in five (LOHE and BRUTLAG 1986) and cross-hybridize readily under standard conditions of hybridization. The base composition of cloned simple satellite repeats varies from 57% AT content (AAGAGAG repeats) to 100% AT (AATAT repeats), and for this reason each repeat class has a characteristic $T_m$ value (LOHE and BRUTLAG 1987a). To carry out chromosome mapping at high stringency, the hybridization temperature was varied and was usually specific for each satellite sequence (Table 1). Thus, the labeling pattern reflected the true distribution of the satellite repeats under study rather than cross-hybridization. Since molecular cloning has shown that many different repeated sequences are present in a satellite purified by conventional CsCl gradient methods, identification of satellites by their buoyant density value is no longer warranted. In this

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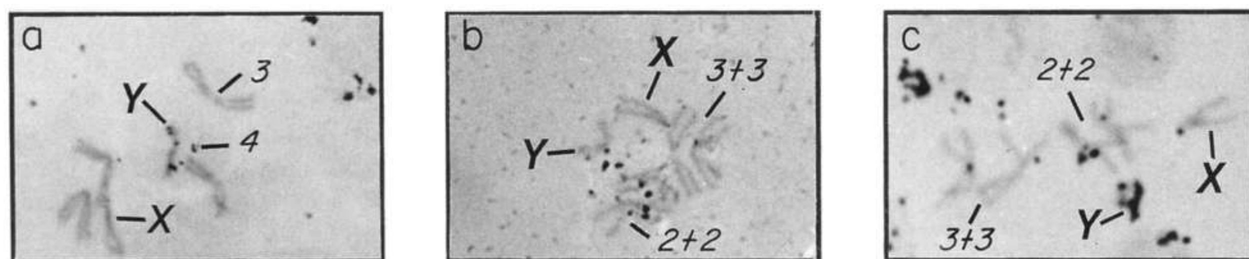


FIGURE 1.—Localizations of simple satellite repeats to mitotic chromosomes of *D. melanogaster* at a gross level, by *in situ* hybridization of $^3$H-labeled satellite. (**a**) Hybridization of AATAT repeats, showing labeling of chromosomes *4* and the *Y*. (**b**) Hybridization of AAGAG repeats. Most of this satellite is located on chromosomes *2* and the *Y*, at multiple sites on both chromosomes. (**c**) Hybridization of AAGAGAG repeats, showing labeling of all major chromosomes but especially chromosomes *2* and the *Y*.

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report, we will refer to cloned simple satellites by the sequence in a 5′ → 3′ direction.

**AATAT repeats:** These repeats are the principal component of the 1.672 satellite and comprise 3% of the genome (PEACOCK *et al.* 1977; LOHE and BRUTLAG 1986). *In situ* hybridization shows that the repeats map primarily to the *Y* chromosome, and to chromosome *4* (Figure 1a). Four distinct sites of labeling on the *Y* were seen in favorable preparations. From grain counts of autoradiographs, labeling of the *Y* and both chromosomes *4* accounts for 85% of the total in male neuroblast cells (Table 2). AATAT repeats are also present in significantly smaller amounts on other chromosomes of the complement. There is one site on chromosome *3*, near the euchromatin-heterochromatin junction, and a minor site is present on the *X* chromosome, near the centromere.

Our localizations and quantitations for AATAT repeats are similar to those reported by STEFFENSEN, APPELS and PEACOCK (1981), using gradient-purified 1.672 satellite as template. These authors also showed that a site of 1.672 satellite sequences lies between the $In(1)sc^{s1}$ and $In(1)sc^{v1}$ breakpoints in the *X* chromosome heterochromatin, an interval that includes the centromere.

**AAGAG and AAGAGAG repeats:** AAGAG repeats are one of the most abundant satellite sequences in *D. melanogaster* and comprise about 6% of the genome (LOHE and BRUTLAG 1986). These repeats are the principal component of the 1.705 satellite. A satellite sequence closely related to AAGAG repeats is the 7-bp sequence AAGAGAG (1.5% of the genome; FRY and BRUTLAG 1979; LOHE and BRUTLAG 1986). Repeats of both 5- and 7-bp sequences band together in CsCl gradient preparations of the 1.705 satellite. There are roughly four 5-bp repeats for every 7-bp repeat in the genome. Clones of about 0.5 kb of these satellite sequences contain exclusively tandem arrays of one or the other sequence, suggesting that the two repeat classes are not interspersed, at least over short distances (LOHE and BRUTLAG 1986).

Previous localizations of 1.705 satellite sequences to mitotic chromosomes employed either polypyrimidine tracts (SEDEROFF, LOWENSTEIN and BIRNBOIM 1975) or total 1.705 satellite DNA (PEACOCK *et al.* 1976, 1977; STEFFENSEN, APPELS and PEACOCK 1981) as probe. These probes therefore contained a mixture of 5- and 7-bp repeats and, possibly, other repeated sequences of similar buoyant density. The use of cloned repeats for *in situ* hybridization permits the relative locations of 5- and 7-bp repeats to be distinguished.

In our initial *in situ* hybridizations, 7-bp AAGAGAG repeats showed a similar chromosomal distribution to 5-bp AAGAG repeats. For this reason, we carried out additional filter hybridizations to investigate whether 5-bp repeats hybridize with 7-bp repeats under conditions of moderate stringency. A tandem array of $(AAGAG)_n$ aligned with the complementary strand of $(AAGAGAG)_n$ shows 17/35 mismatches (49% mismatching). Therefore, at 25° these two repeated sequences would be expected to hybridize to each other poorly if at all, because this temperature is about 35° below the $T_m$ value of the homologous hybrids. However, the two satellite repeats cross-hybridize readily at 25°. The heterologous hybrids are unusually stable and melt with a sharp transition at 46°, in contrast to the homologous hybrids that melt at 59–61° (Table 1). Despite 49% mismatching, the $T_m$ value is lowered by only 13°. Thus, semicomplementary strands of the two polypyrimidine-polypurine satellites can form a more stable configuration than predicted from alignment of complementary strands. Hybridization of these satellite probes was carried out at temperatures close to or above the $T_m$ value of the heterologous hybrids formed between 5- and 7-bp arrays (Table 1).

By *in situ* hybridization, AAGAG repeats are present on all chromosomes (Table 2), but especially chromosomes *2* and the *Y* (Figure 1b). In most cells at least three sites of labeling were visible on the *Y* chromosome, two on the long arm and one on the short arm. Similarly, there are multiple sites on chromosome *2*, although we could not determine their number using a $^3$H probe because of the proximity of sites and autoradiographic scatter. Smaller amounts of (AA-

TABLE 2

**Quantitation of satellite *in situ* hybridizations**

| Satellite (no. of nuclei scored, *n*) | Percent of satellite per chromosome set (grains scored)[a] | | | | | Total grains scored[b] |
|---|---|---|---|---|---|---|
| | *X* | *Y* | *2+2* | *3+3* | *4+4* | |
| $(AATAT)_n$ (*n*=35) | 5 (29) | 44 (281) | 0.3 (2) | 10 (61) | 41 (261) | 634 |
| $(AAGAG)_n$ (*n*=25) | 5 (14) | 33 (86) | 50 (132) | 10 (27) | 2 (4) | 263 |
| $(AAGAGAG)_n$ (*n*=25) | 5 (14) | 31 (94) | 57 (174) | 5 (15) | 4 (11) | 308 |
| $(AAGAC)_n$ (*n*=29) | 0.6 (5) | 69 (527) | 29 (220) | 2 (14) | 0 | 766 |
| $(AATAG)_n$ (*n*=45) | 0.9 (6) | 33 (227) | 43 (299) | 7 (45) | 17 (116) | 693 |
| $(AATAACATAG)_n$ (*n*=8) | 0 | 0 | 54 (13) | 46 (11) | 0 | 24 |

Quantitations were carried out only in cells in which all chromosomes could be identified. Each line in this table represents the pooled data from multiple experiments, multiple probe preparations, and therefore includes multiple slides and exposure times, except for AATAACA-TAG repeats where the data shown represent the pooled grain counts from two slides in one experiment. The results between different slide and probe preparations were always consistent.

[a] A neuroblast cell contains one copy each of the *X* and *Y* chromosomes and a diploid number of autosomes. Values shown represent the sum for a single *X* or *Y* chromosome together with the sum for each pair of autosomes (*2+2,3+3,4+4*).

[b] Grain numbers are not indicative of the relative amount of different satellites in the genome, and depend on factors such as the specific activity of the probe, efficiency of hybridization and exposure time of slides. However, grain counts are an accurate measure of the distribution of a specific satellite among the chromosomes.

$GAG)_n$ are also present on the *X* and chromosomes *3* and *4*, in order of decreasing amount. In the Oregon R strain the predominant site on the *X* chromosome is close to the euchromatic-heterochromatic junction, but some labeling was also seen close to the centromere. Using a biotin-labeled $(AAGAG)_n$ probe, there are 7 sites on the *Y* chromosome, 4–5 sites on chromosome *2* and a single site on each of chromosomes *3* and *4* (see Figure 6a). The site near the euchromatic-heterochromatic junction of the *X* is polymorphic because labeling was seen only at the *X* centromere in a *T(2;3)* stock. Our results with the 5-bp AAGAG repeats are similar to those of SEDEROFF, LOWENSTEIN and BIRNBOIM (1975) who used pyrimidine tracts as probe, and STEFFENSEN, APPELS and PEACOCK (1981) who used gradient-purified 1.705 satellite. A polymorphism for the number of AAGAG repeats on the *X* was also observed by STEFFENSEN, APPELS and PEACOCK (1981) in the *In(1)sc$^8$* chromosome.

Using stringent hybridization criteria, 7-bp AAGAGAG repeats have a chromosomal distribution similar to the 5-bp AAGAG repeat pattern (Table 2). Further, the 7-bp repeats appear to occur only in regions that hybridize the 5-bp repeats. For example, 7-bp repeats are present at several sites on the *Y* chromosome, with the majority at the tips of each arm. The 5-bp repeats localize to similar positions on the *Y* chromosome, although these repeats are more abundant than 7-bp repeats at the site in the middle of *YL*. There are also two major sites of 7-bp repeats on chromosome *2*. As with the 5-bp repeats, smaller amounts of 7-bp repeats are present on the *X* and chromosomes *3* and *4* (Figure 1c). Grains were observed at two sites on the *X* chromosome, at the euchromatic-heterochromatic junction and close to the centromere. The major differences in hybridization patterns of the 5- and 7-bp repeats are, therefore, the localization of 7-bp repeats to a smaller number of sites on chromosomes *2* and the *Y*. Since the 7-bp repeats are fourfold less abundant than 5-bp repeats (LOHE and BRUTLAG 1986), we conclude that there is a lower number of 7-bp repeats on all chromosomes, compared to the 5-bp repeats.

**AAGAC repeats:** These repeats comprise 2.4% of the genome and were cloned from the 1.686 satellite, of which they form only a minor component (LOHE and BRUTLAG 1986). An unusual property is that the majority of AAGAC repeats band in about equal amounts in two distinct regions in CsCl buoyant density gradients, at 1.689 g/ml and 1.701 g/ml (LOHE and BRUTLAG 1986). Most satellite DNAs band as a single symmetrical peak in such gradients. The molecular basis for this property is not understood, but one of the two peaks could result from covalent linkage of AAGAC repeats to other DNA sequence classes.

By *in situ* hybridization, AAGAC repeats map principally to the *Y* chromosome. They are at four sites but are concentrated at the two tips. These repeats are present at one site on chromosome *2* (Figure 2a). Grain counts show that each chromosome *2* contains one-fifth the amount of AAGAC repeats on the *Y*, with even smaller amounts on chromosomes *3* and the *X* (Table 2). The locations of AAGAC repeats on chromosomes *2* and the *Y* are similar to those of AAGAG and AAGAGAG repeats, although again, the patterns can be distinguished by quantitative differences.

Highly purified preparations of the 1.705 satellite (consisting mainly of AAGAG repeats) show, at about 1.701 g/ml, a trailing peak that appears to be enriched for junction molecules (BRUTLAG *et al.* 1977; STEFFENSEN, APPELS and PEACOCK 1981). Because about half of AAGAC repeats also band at 1.701 g/ml, we investigated whether junction molecules, for example con-

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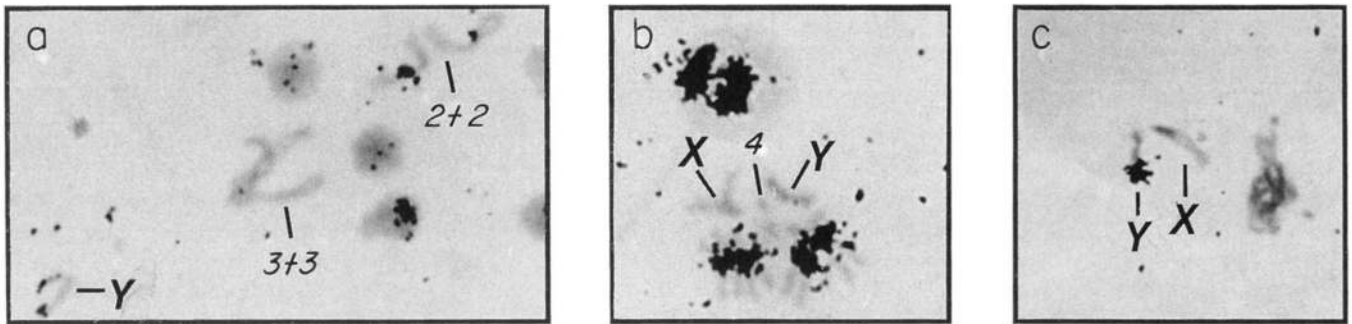


FIGURE 2.—Localizations of simple satellite repeats to mitotic chromosomes of *D. melanogaster* at a gross level, by *in situ* hybridization of $^3$H-labeled satellite. (**a**) Hybridization of AAGAC repeats. Chromosomes *2* and the *Y* show most labeling. There are two major sites near the tips of the *Y* chromosome. (**b**) Hybridization of AATAACATAG repeats. This satellite is located principally on the major autosomes, as shown in this long exposure. (**c**) Hybridization of AATAC repeats. This satellite is present only on the *Y* chromosome, near the tip of *YL*.

taining tandem arrays of both AAGAG and AAGAC repeat types, could be recovered as cloned fragments from DNA in the 1.701 g/ml region.

When purified 1.705 satellite DNA was cloned into a plasmid vector, about 3% (4/137) of recovered colonies hybridized strongly with an $(AAGAG)_n$ probe, a low efficiency characteristic of *D. melanogaster* simple satellites (LOHE and BRUTLAG 1986). Neither the $(AAGAC)_n$ nor $(AATAT)_n$ probes hybridized to any colonies. In contrast, when a trailing fraction of purified 1.705 satellite DNA (the netropsin-1 fraction of BRUTLAG *et al.* 1977) was used as a source for cloning, 12% (23/199) of clones hybridized strongly to the $(AAGAC)_n$ probe and 6% (11/199) to the $(AATAT)_n$ probe. The proportion of clones hybridizing to the $(AAGAG)_n$ probe remained about the same, at 4% (7/199). One clone hybridized to both $(AAGAG)_n$ and $(AAGAC)_n$ probes. These results show that AAGAC repeats are significantly enriched in the trailing fraction of 1.705 satellite (AAGAG) preparations. Since these two classes of repeats co-localize to the same N-banded regions on the *Y* (BONACCORSI and LOHE 1991) but map to distinct sites on chromosome *2* (see below), the source of DNA from the trailing fraction of 1.705 satellite preparations is likely to be the *Y* chromosome.

**The 10-bp repeats:** The 1.686 satellite consists predominantly of 10-bp AATAACATAG repeats (BRUTLAG and PEACOCK 1975; ENDOW, POLAN and GALL 1975) and these represent about 2% of the genome (LOHE and BRUTLAG 1986). *In situ* hybridization with a biotinylated probe shows that 10-bp repeats are, for the most part, present on chromosomes *2* and *3*, at one site in the heterochromatin of each chromosome (see Figure 6b). In slides hybridized with a $^3$H probe and exposed for a short time, grain counts on chromosomes *2* and *3* are similar (Table 2). In another experiment, slides that were overexposed for the major autosomes showed little or no labeling of the sex chromosomes and chromosome *4* (Figure 2b). The restriction of most or all 10-bp repeats to the two major autosomes is a significantly different pattern from that of other simple satellites.

**The 359-bp repeats:** These complex repeats are the major component of the 1.688 satellite (HSIEH and BRUTLAG 1979) and are located in the proximal heterochromatin of the *X* chromosome (D. BRUTLAG, personal communication; HILLIKER and APPELS 1982). By *in situ* hybridization they appear as a major block (Figure 3a) that encompasses about half the *X* heterochromatin.

To determine whether 359-bp repeats are restricted to the *X* chromosome, we repeated *in situ* hybridizations with a $^3$H-labeled probe using long autoradiographic exposures. This verified the location of 359-bp repeats on the *X* but some labeling of both major autosomes was also observed (Figure 3b). Chromosomes *4* and the *Y* were not labeled. The autosomal labeling could result from hybridization to a small number of 359-bp copies or from the closely related 254- or 353-bp repeats, each 80% homologous to 359-bp repeats (CARLSON and BRUTLAG 1979; LOHE and BRUTLAG 1986).

**Chromosomal assignments of satellites minor in abundance:** Five simple repeated sequences band as distinct satellites in CsCl gradients but represent only 0.1–0.5% of the genome (LOHE and BRUTLAG 1986). With repeats of AAAAC, we were unable to obtain a reproducible signal for *in situ* hybridizations after an autoradiographic exposure of one month. Three other minor satellite sequences are present only on the *Y*, each at a single site. Repeats of AATAC mapped close to the tip of *YL* (Figure 2c), repeats of AATAAAC mapped to *YS*, and AATAGAC repeats mapped to the middle of *YL*. Repeats of AATAG are present at a single site near the tip of *YL*, but are also found on other chromosomes (Table 2). We recently extended these results by detailed mapping using *Y* rearrangements (BONACCORSI and LOHE 1991).

**Southern hybridization of satellites on the *Y*:** Male and female DNAs were compared by Southern hybridization to determine whether satellites that map

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**TABLE 3**

**Satellite DNA content (kilobases) per chromosome**

| Satellite | *X* | *Y* | *2* | *3* | *4* |
|---|---|---|---|---|---|
| (AATAT)$_n$ | 600 | 5,800 | 10 | 630 | 2,700 |
| (AATAG)$_n$ | 8.1 | 310 | 200 | 30 | 78 |
| (AATAC)$_n$ | 0 | 3,500 | 0 | 0 | 0 |
| (AAAAC)$_n$ | 0 | 400 | 0 | 0 | 0 |
| (AAGAC)$_n$ | 81 | 8,500 | 1,800 | 110 | 0 |
| (AAGAG)$_n$ | 1,200 | 7,200 | 5,500 | 1,100 | 170 |
| (AATAAAC)$_n$ | 0 | 1,600 | 0 | 0 | 0 |
| (AATAGAC)$_n$ | 0 | 1,600 | 0 | 0 | 0 |
| (AAGAGAG)$_n$ | 270 | 1,800 | 1,700 | 140 | 100 |
| (AATAACATAG)$_n$ | —[a] | —[a] | 1,900 | 1,600 | —[a] |
| 359 bp | 11,000 | —[a] | —[a] | —[a] | —[a] |
| rDNA[b] | 2,900 | 1,800 | 0 | 0 | 0 |
| Sum of DNA above: | 16,059.1 | 32,510 | 11,110 | 3,610 | 3,048 |
| DNA estimated in heterochromatin[c]: | 20,000 | 40,000 | 8,000–15,000 | 13,000–16,000 | 3,000–4,500 |

Kilobase estimates are shown to two significant figures only due to errors in measurement of DNA content of the genome, the DNA content of individual chromosomes, satellite amounts in the genome, from partitioning satellite amounts among chromosomes by quantitative autoradiography, and possible interstrain differences in satellite amounts. Estimated values of four *Y*-specific satellites in the genome are derived from quantitative hybridizations (LOHE and BRUTLAG 1986). These repeated sequences are exclusive to the *Y*, based on Southern hybridizations (Figure 4).

[a] Low amounts of the 10-bp satellite cannot be excluded from the *X*, *Y* or chromsome *4* because of the small number of grains scored (Table 2). However, no significant labeling of these chromosomes was observed in long autoradiographic exposures (Figure 2b). For 359-bp repeats, some labeling of chromosomes *2* and *3* was observed in slides where the *X* chromosome was heavily labeled (see text).

[b] This estimate assumes that each rDNA repeat is 11.7 kb in length, the *X* chromosome contains 250 copies and the *Y* chromosome 150 copies (see LOHE and ROBERTS 1990), even though rDNA copy number may differ between strains.

[c] Estimates for the amount of autosomal heterochromatin are taken from PEACOCK *et al.* (1977). The estimate for the *X* heterochromatin assumes that 50% of the entire chromosome is heterochromatic.

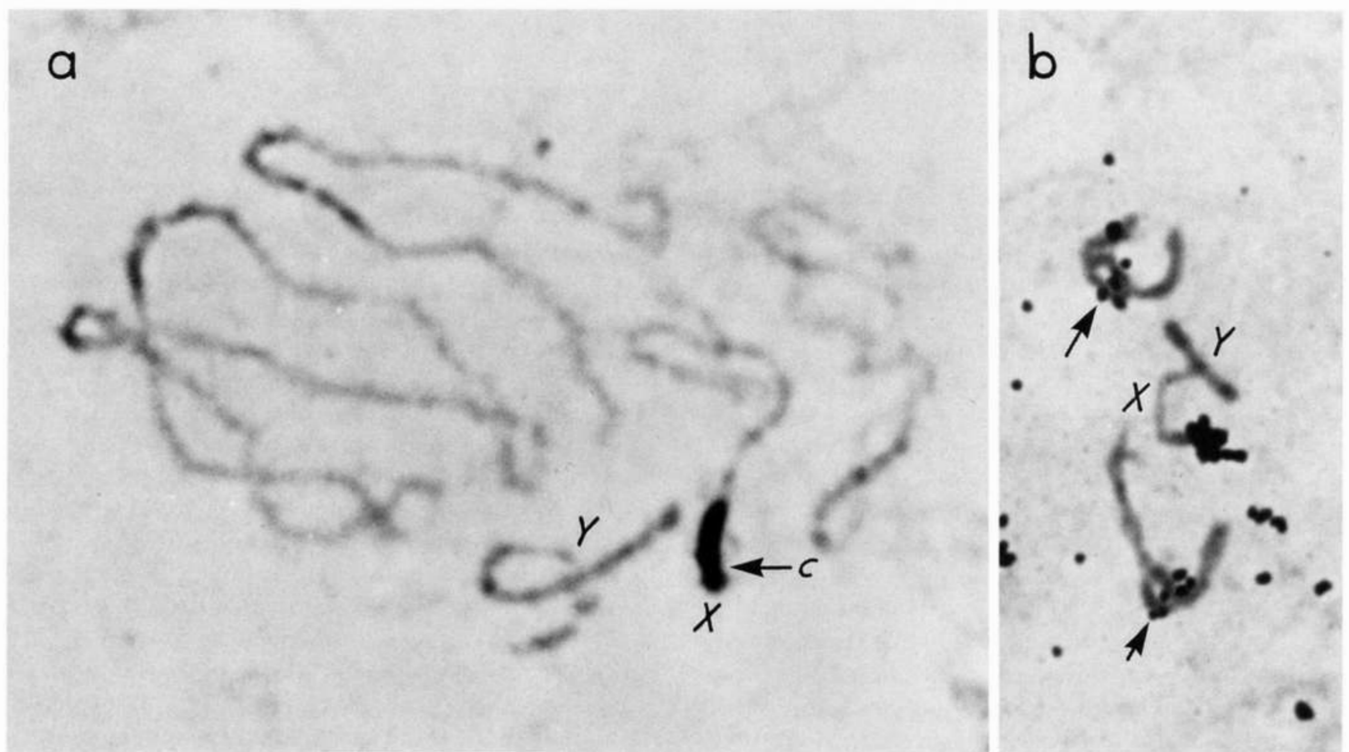


FIGURE 3.—*In situ* hybridization of 359-bp repeats to mitotic chromosomes of *D. melanogaster*. (**a**) Hybridization of a biotin-labeled probe to prometaphase chromosomes, showing heavy labeling of the *X* chromosome. The position of the centromere (C) is shown by the arrow. (**b**) A long exposure of an autoradiograph with a $^3$H probe showing minor labeling of both major autosomes (arrows) which are paired. The *X* chromosome site is saturated with silver grains and the *Y* chromosome is unlabeled.

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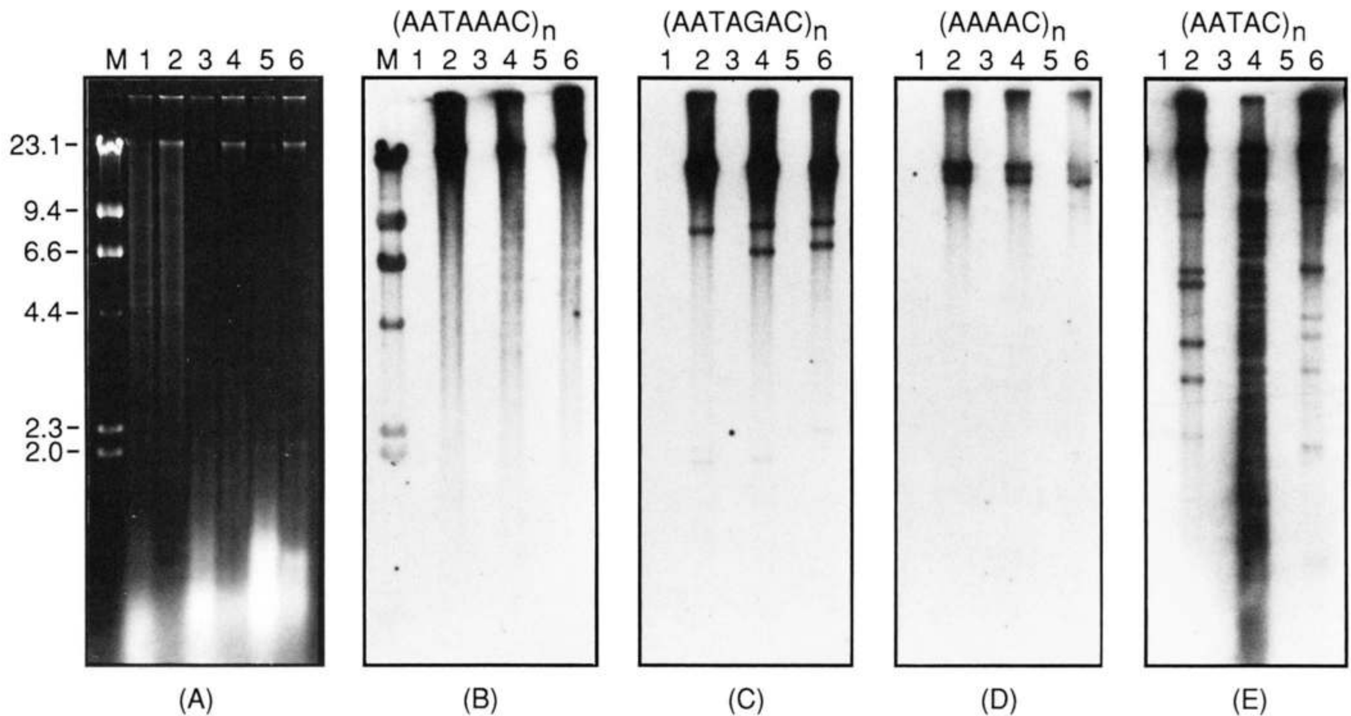


FIGURE 4.—Southern hybridization of *Y*-located satellites to DNA from females and males. (**A**) Ethidium bromide fluorescence of the gel before transfer to a nitrocellulose filter. Note that DNA migrating at limiting mobility into the gel is enriched in lanes containing male DNA. (**B**) The filter was probed with tandem repeats of AATAAAC from plasmid 1.672–453. (**C**) Probe is repeats of AATAGAC from plasmid 1.672–563. (**D**) Probe is repeats of AAAAC from plasmid 1.672-233. (**E**) Probe is repeats of AATAC from plasmid 1.672-181. Lanes 1, female DNA digested with *Hin*dIII; lanes 2, male DNA (*Hin*dIII); lanes 3, female DNA (*Rsa*I); lanes 4, male DNA (*Rsa*I); lanes 5, female DNA (*Hae*III); lanes 6, male DNA (*Hae*III). An amount of DNA equivalent to 10 heads was loaded per track in a 0.5% agarose gel. The marker M is a *Hin*dIII digest of lambda DNA and sizes of the fragments are shown in kilobases.

**TABLE 4**

**Cytology of translocation chromosomes**

| Translocation | Chromosome *2* breakpoint (heterochromatin)[a] | Chromosome *3* breakpoint (euchromatin) | Phenotype[b] | Reference[c] |
|---|---|---|---|---|
| *T(2;3)46* | h35 | 67E | $lt^v$ | 1, 2 |
| *T(2;3)40* | h37 | 95F | $lt^v$ | 1, 2 |
| *T(2;3)73* | h37 | 65F | $lt^v$ | 1, 2 |
| *T(2;3)37* | h37 | 98B | $lt^v$ | 1, 2 |
| *T(2;3)88* | h37 | 98CD | $lt^v$ | 1, 2 |
| *T(2;3)33* | h39 | 91F | wt | 1 |
| *T(2;3)127* | h41 or h42 | 94D | wt | 1 |
| *T(2;3)76* | h44 | 64E | wt | 1 |

[a] The heterochromatin of chromosome *2* has been divided into 12 blocks (h35 to h46, from *2L* to *2R*) based on N-banding and Hoechst 33258 fluorescence (DIMITRI 1991).
[b] Shows variegation for the *light* gene ($lt^v$) or is wild-type (wt) in eye color.
[c] 1, HILLIKER and TRUSIS-COULTER (1987); 2, C. B. SHARP and A. J. HILLIKER (unpublished).

to the *Y* are present exclusively on this chromosome. So that rare *XXY* females arising from nondisjunction might be discovered and removed, males in the stock used to prepare female DNA contained a *Y* chromosome marked with $B^S$. DNA prepared in this way and digested with restriction enzymes migrated principally as a smear. Some DNA entering the gel migrated at limiting mobility, and this DNA was prominent in lanes containing male DNA (Figure 4a). This suggests that the *Y* contains significant amounts of DNA that are spared even by enzymes having a 4-bp recognition sequence. Such a property is characteristic of tandemly repeated DNAs of low sequence complexity.

In Southerns probed with four of the satellites, DNA from males hybridized strongly (Figure 4). Even in long autoradiographic exposures there was no signal from female DNA, except for AATAC repeats where a faint band at 2.4 kb was seen in *Hin*dIII digests (Figure 4E, lane 1). However, the 1.672-181 probe contains 68 bp of a *297* mobile element that adjoins AATAC repeats (LOHE and BRUTLAG 1987b). Since *297* is cleaved by *Hin*dIII to yield a 2.4-kb fragment (INOUE, YUKI and SAIGO 1986), the faint band most likely derives from hybridization to multi-

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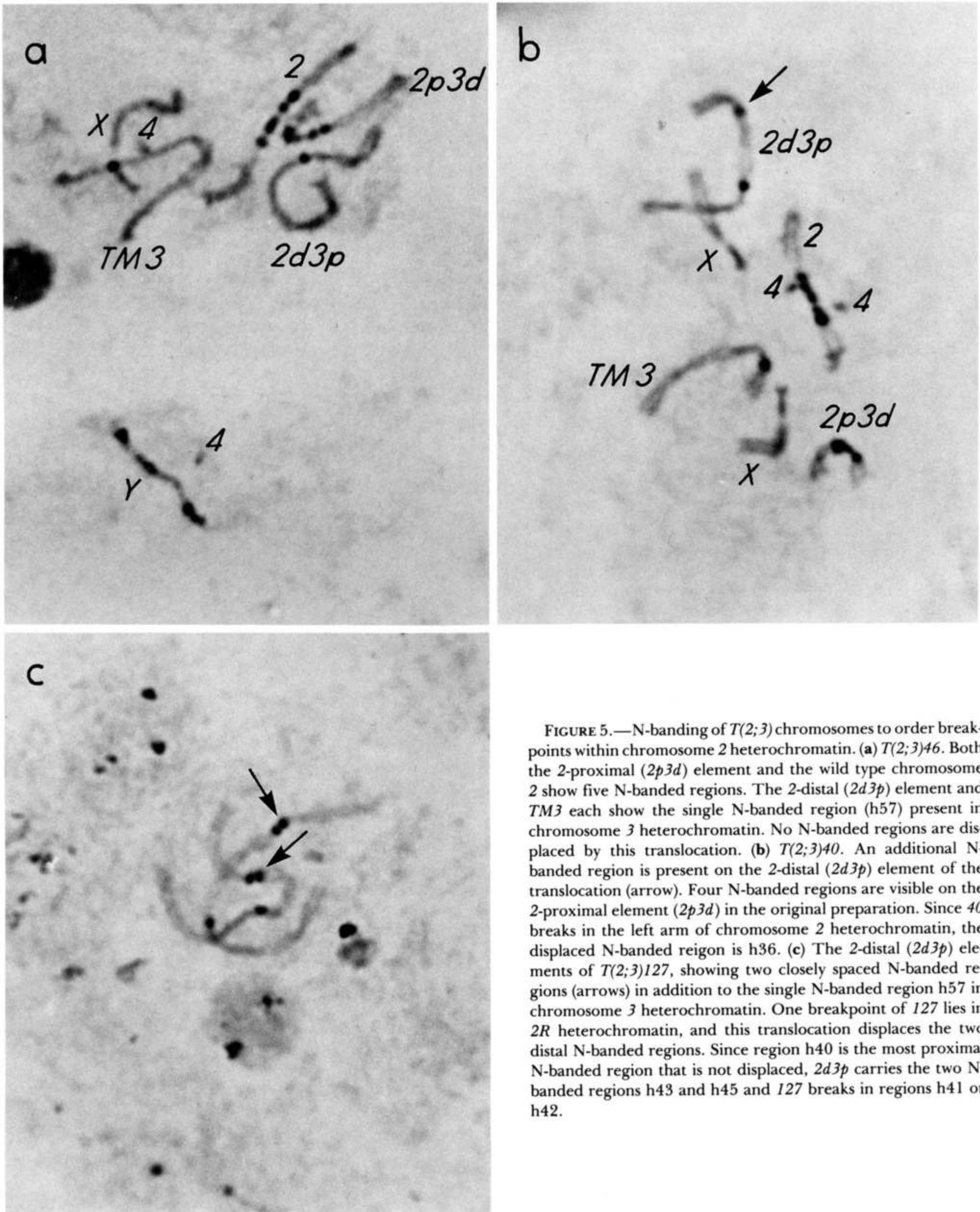


FIGURE 5.—N-banding of *T(2;3)* chromosomes to order breakpoints within chromosome *2* heterochromatin. (**a**) *T(2;3)46*. Both the *2*-proximal (*2p3d*) element and the wild type chromosome *2* show five N-banded regions. The *2*-distal (*2d3p*) element and *TM3* each show the single N-banded region (h57) present in chromosome *3* heterochromatin. No N-banded regions are displaced by this translocation. (**b**) *T(2;3)40*. An additional N-banded region is present on the *2*-distal (*2d3p*) element of the translocation (arrow). Four N-banded regions are visible on the *2*-proximal element (*2p3d*) in the original preparation. Since *40* breaks in the left arm of chromosome *2* heterochromatin, the displaced N-banded reigon is h36. (**c**) The *2*-distal (*2d3p*) elements of *T(2;3)127*, showing two closely spaced N-banded regions (arrows) in addition to the single N-banded region h57 in chromosome *3* heterochromatin. One breakpoint of *127* lies in *2R* heterochromatin, and this translocation displaces the two distal N-banded regions. Since region h40 is the most proximal N-banded region that is not displaced, *2d3p* carries the two N-banded regions h43 and h45 and *127* breaks in regions h41 or h42.

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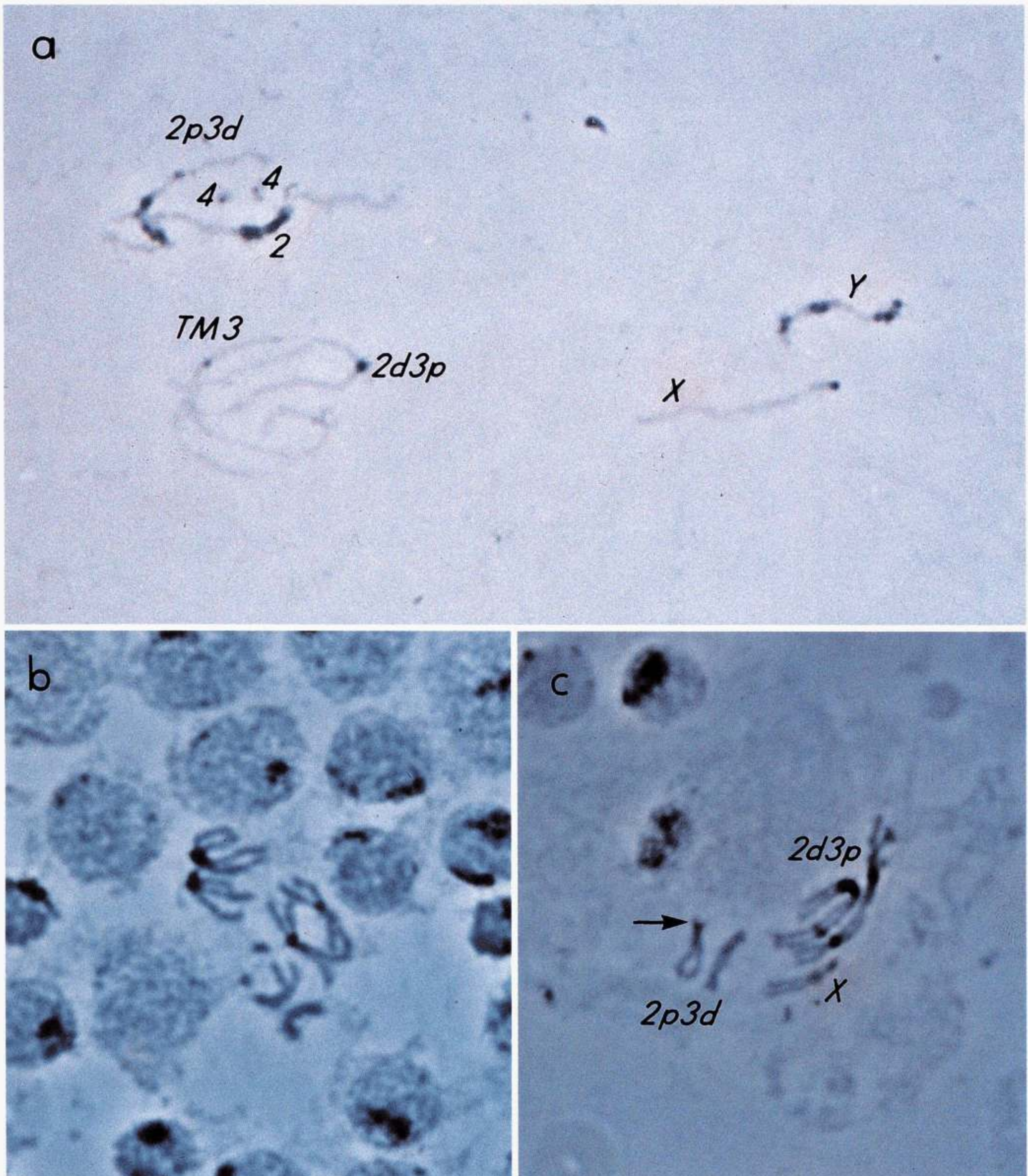


FIGURE 6.—*In situ* hybridizations of biotin-labeled probes to mitotic chromosomes. (**a**) *T(2;3)46* hybridized with 5-bp AAGAG repeats. The *2*-distal (*2d3p*) element of this translocation is labeled at one site in chromosome *3* heterochromatin (region h57), showing that AAGAG repeats are proximal to the breakpoint of *46* on chromosome *2*. Note that the *Y* chromosome is labeled at 7 sites and the labeling at or near the telomeres of the *X* and chromosomes *4*. (**b**) Chromosomes from wild-type *D. melanogaster* hybridized with 10-bp AATAACATAG repeats. Each of the major autosomes carries a single site of labeling. The *X*, *Y* and chromosome *4* are unlabeled. (**c**) *T(2;3)88* hybridized with 10-bp AATAACATAG repeats. The translocation is homozygous for both elements. Some labeling is visible at the breakpoint of one of the *2*-proximal elements (*2p3d*) (arrow). The *2*-distal element (*2d3p*) is heavily labeled at two sites. One site is normally present in chromosome *3* heterochromatin (region h48). This suggests that the chromosome *2* breakpoint of *88* in region h37 lies near the proximal end of a block of 10-bp repeats. One sex chromosome is missing from this cell.

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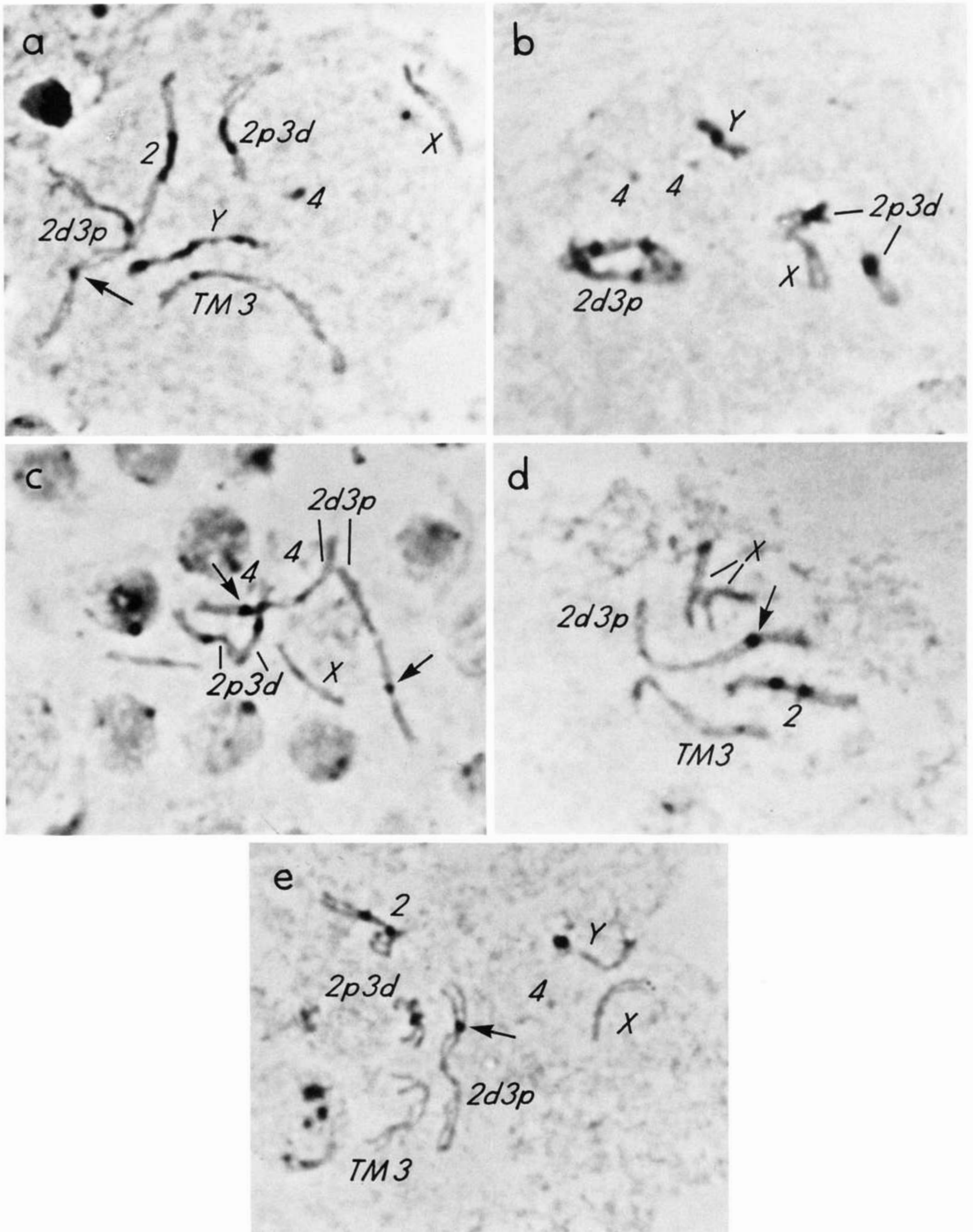


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FIGURE 7.—*In situ* hybridizations of biotin-labeled 5-bp (AAGAG)$_n$ or 7-bp (AAGAGAG)$_n$ probes to *T(2;3)* chromosomes. For ease of comparison between these closely related repeats, the uppermost figures on the left (**a** and **c**) show hybridizations to *T(2;3)40* and the right

ple genomic copies of *297*. When the blot was reprobed with 1.672-614, a plasmid also containing AATAC repeats but linked to other simple repeats (LOHE and BRUTLAG 1987b), the 2.4-kb band was not seen (data not shown). Southern hybridization also showed AAAAC repeats are restricted to the *Y*. These repeats are present in at least two blocks because two high molecular weight fragments were seen with each of three restriction enzymes (Figure 4D). Therefore, at the level of Southern hybridization, repeats of four minor satellites appear to be specific to the *Y*.

An unexpected result was that in digestions with *Rsa*I, AATAC repeats hybridized to multiple bands less than 20 kb in length (Figure 4E, lane 4), in contrast to *Hin*dIII and *Hae*III digestions. This hybridization is not due to the small amount of the *297* element present in the 1.672-181 probe, since the same result was obtained with plasmid 1.672-614, also containing AATAC repeats. The recognition sequence of *Rsa*I is GTAC, only one nucleotide different from 4 bp of the AATAC repeat unit. Because a single A → G transition can create an *Rsa*I site in an AATAC repeat, the hybridization to multiple bands probably reflects heterogeneity in AATAC arrays. Although this result appears to suggest that AATAC repeats are highly heterogeneous, sequencing of 592 bp from four AATAC clones showed only 2% sequence alteration (LOHE and BRUTLAG 1987b). The heterogeneity in AATAC arrays is apparent because of the short repeat unit and high copy number, estimated to be 700,000 in males (Table 4).

**Quantitation of satellite repeats on a single chromosome basis:** Estimates of satellite DNA content in the genome have been significantly improved by the availability of cloned probes (LOHE and BRUTLAG 1986) and the use of stringent, sequence-specific hybridization conditions (LOHE and BRUTLAG 1987a). For example, using bulk-isolated 1.686 satellite a value of 3.3% of the genome was obtained (PEACOCK *et al.* 1977). However, quantitation of 10-bp repeats using a cloned probe and high stringency hybridizations gave a value of 2.1%. The 50% excess in estimation using bulk-isolated 1.686 satellite may have resulted from inclusion in satellite preparations of other abundant repeated DNA classes (such as rDNA) that co-band in trace amounts, or from cross hybridization between related sequence classes.

We have used these more accurate values to estimate the content of individual repeat types per chromosome (Table 3). Satellite content per chromosome was calculated by partitioning the genomic amount of a satellite (LOHE and BRUTLAG 1986) according to grain counts from autoradiographs (Table 2). These results show that the heterochromatin of each chromosome is characterized by distinctive combinations of sequence types and amounts. The 12 repeated sequences compose the majority of the DNA in heterochromatin of each chromosome, except for chromosome *3*, where six satellites account for roughly 25% of the heterochromatic DNA. It should be noted that the kilobase values in Table 3 were obtained from combining several different estimates, each associated with an error, and are therefore approximate. For example, some chromosomes appear to lack specific satellites but significant numbers of repeats may have gone unnoticed. For two different probes the minimum size of a satellite array detected by autoradiography is about 10 kb (Table 3), representing 2,000 copies of a 5-bp repeat, but the error associated with these estimates is uncertain due to possible nonlinearity in the autoradiographic response for low copy number of repeats.

Although three satellites are considered minor in a quantitative sense, they are still present in megabase amounts on the *Y* chromosome, as are many of the abundant satellites (Table 3). The reason for this paradox is that the *Y* chromosome contributes only one-quarter as much as the autosomes to the embryonic DNA pool used for quantitations. Further, the "minor" satellites on the *Y* are concentrated at single locations, at least as seen by *in situ* hybridization. Four of the abundant satellites are also present in megabase amounts on the *Y*. From Table 3, nine simple repeated sequences total about 31 Mb, or approximately 80%, of the estimated DNA content of the *Y*. Therefore, the *Y* chromosome is composed principally of a limited number of simple satellite DNA sequences.

**Higher resolution mapping of satellites in chromosome *2* heterochromatin:** Chromosome *2* contains large regions of pericentric heterochromatin on both arms. Although *in situ* hybridization to wild-type chromosomes has identified the chromosome locations of satellites (Table 2), it does not indicate their relative order. Satellites were mapped in independent exper-

panel (**b** and **d**) to *T(2;3)76*. (**a**) *T(2;3)40* hybridized with AAGAG repeats. A single site of labeling is evident on the *2*-distal element (*2d3p*; arrow) in addition to the site always present in chromosome *3* heterochromatin. (**b**) *T(2;3)76* hybridized with AAGAG repeats. The translocations are homozygous in this individual. Both *2*-distal elements (*2d3p*) are labeled at two sites, one site resulting from labeling of translocated heterochromatin from chromosome *2*. (**c**) *T(2;3)40* hybridized with AAGAGAG repeats. The translocation is homozygous in this individual. Each *2*-distal element (*2d3p*) shows a single site of labeling (arrows) and each *2*-proximal element (*2p3d*) is also labeled, at one site. (**d**) *T(2;3)76* hybridized with AAGAGAG repeats. The *2*-distal element (*2d3p*) shows labeling at one site (arrow). The *2*-proximal element is missing from this cell. (**e**) *T(2;3)33* hybridized with AAGAGAG repeats. Although most of the *2R* heterochromatin, including three N-banded regions, is located on the *2*-distal element of this translocation only one site of labeling is visible (*2d3p*; arrow). The *2*-proximal element (*2p3d*) shows a single site of labeling. Also note the major site of labeling near the tip of *YL*, and some labeling at the tip of *YS*.

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iments using different chromosome preparations. However, chromosome inversions have improved the resolution in mapping ribosomal DNA sequences and several CsCl-purified satellites in *X* chromosome heterochromatin (STEFFENSEN, APPELS and PEACOCK 1981; HILLIKER and APPELS 1982). We have extended this approach to map more precisely five of the simple satellites in chromosome *2* heterochromatin (Table 3) using *T(2;3)* chromosomes constructed by HILLIKER and TRUSIS-COULTER (1987).

The heterochromatic breakpoints of the translocations provide an internal point of reference for comparisons in different satellite hybridization experiments, enabling satellite locations to be determined relative to each other. It is essential that the heterochromatic breakpoints of the rearrangement chromosomes have first been ordered cytologically. Translocations used for mapping were reciprocal and were generated by simple two-break events without identifiable loss of DNA. To avoid possible misinterpretation from satellite hybridization to chromosome *3* heterochromatin, *T(2;3)*s chosen for mapping had chromosome *3* breakpoints in euchromatin, well separated from heterochromatin. The resolution of such mapping depends on the number of breakpoints available in a chromosome region, and elongated chromosomes are not required. It is only necessary to determine which elements of the translocation hybridize with the satellite probe.

Estimates of the DNA content of chromosome *2* heterochromatin vary from 8–15 Mb (PEACOCK *et al.* 1977), but our quantitations of five simple satellites present on this chromosome are 11 Mb (Table 3). Since these five satellites do not account for all of the DNA in chromosome *2* heterochromatin (see below), we have used the upper estimate of 15 Mb in our calculations.

**Ordering heterochromatic breakpoints of *T(2;3)*s:** The heterochromatin of chromosome *2* contains 12 regions distinguishable by their response to different chromosome banding methods. There are five well spaced N-banded regions, one on the left arm, one at the centromere, and three on the right arm (PIMPINELLI, SANTINI and GATTI 1976; DIMITRI 1991). Seven other regions in chromosome *2* heterochromatin show a variable response to Hoechst 33258 fluorescence. Some regions fluoresce brightly (h37 and h39) or moderately (h44), and other regions are dull (h35, h41 and h46) or do not fluoresce at all (h42). The N-banding procedure was chosen to order the *T(2;3)* breakpoints since the five N-banded regions on chromosome *2* are well spaced and are usually separated by only one other heterochromatic region that stains differently (DIMITRI 1991). Chromosome *3* carries a single N-banded region h57, allowing the ready identification of the distal chromosome *2* element of the translocation. The position of the heterochromatic breakpoint in the left or right arm of chromosome *2* has been established by genetic and polytene chromosome analyses (HILLIKER and TRUSIS-COULTER 1987).

From a series of *T(2;3)* chromosomes examined by N-banding, eight were selected for *in situ* hybridizations with satellite probes (Table 4). Seven of the eight translocations carried one or at most two additional N-banded regions on the element carrying the distal section of chromosome *2*. *T(2;3)33* carried three additional N-banded regions on this element. Figure 5 shows some representative translocations that were mapped by N-banding. The heterochromatic breakpoint of each translocation is separated by an N-banded region except for four translocations that break in region h37. This region fluoresces brightly with Hoechst and lies between two N-banded regions, h36 and h38. However, the heterochromatic breakpoints of the four *T(2;3)*s are different from each other (see below).

**Mapping AAGAG and AAGAGAG repeats using *T(2;3)*s:** The 5-bp AAGAG repeats are abundant on chromosome *2*, but chromosome *3* contains only about one-fifth the number of copies of $(AAGAG)_n$ compared to chromosome *2* (Table 3). In hybridizations to prometaphase chromosomes 4–5 sites of labeling were visible on chromosome *2* and 1 site on chromosome *3* (Figure 6a). Each of the translocations examined showed additional labeling of chromosome *2* material appended to chromosome *3*, with the exception of *T(2;3)46*. This translocation breaks in distal *2L* heterochromatin (region h35) and leaves the five N-banded regions intact (Figure 5a). For example, both the distal and proximal elements of *T(2;3)40* were labeled, the distal element at one site (Figure 7a). This localizes some AAGAG repeats to the interval between the *46* and *40* breakpoints, regions h35–h36. These repeats are also present in each of the intervals defined by four additional translocation breakpoints, from *T(2;3)88* in region h37 on *2L* through the heterochromatin distal to *T(2;3)76* on *2R*, regions h37–h46 (Figure 7b).

One interpretation of these results is that AAGAG repeats are dispersed along most of chromosome *2* heterochromatin. However, in hybridizations to prometaphase chromosomes 4–5 distinct sites of hybridization were seen on chromosome *2* (Figure 6a). This suggests that the resolution of mapping $(AAGAG)_n$ with rearrangement chromosomes is not sufficient to allow more precise localizations, due to the number and proximity of AAGAG blocks. We note that each of the five breakpoint intervals that contains AAGAG repeats also contains an N-banded region (h36, h38, h40, h43 and h45; see Figure 9) and suggest that these

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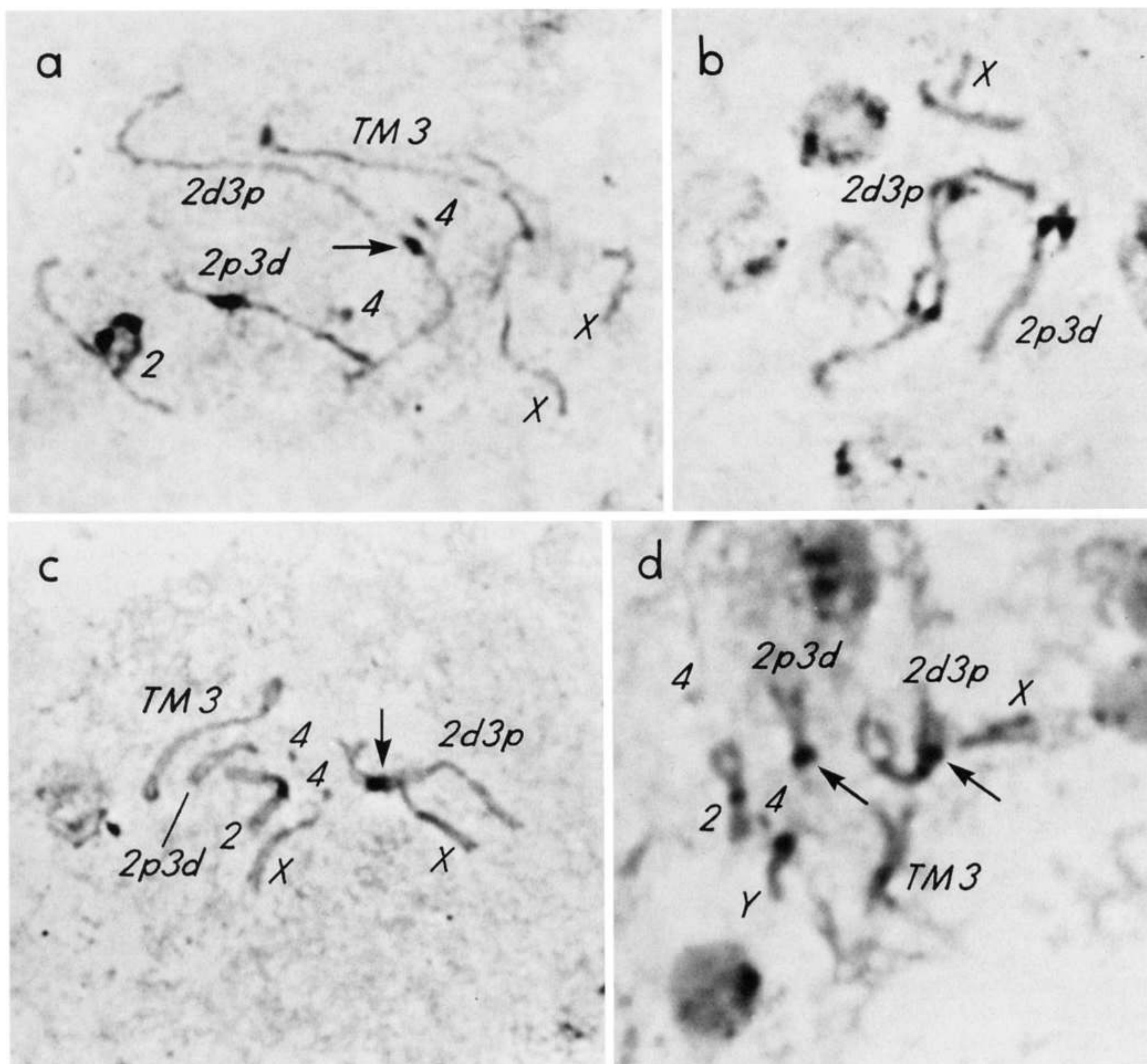


FIGURE 8.—(**a**) *T(2;3)88* hybridized with 7-bp AAGAGAG repeats. The *2*-distal element (*2d3p*) shows a single site of labeling (arrow), confirming the result shown in Figure 7c that one site of these repeats is present in *2L* heterochromatin. In these extended chromosomes, the site of 7-bp repeats on *2R* in wild-type chromosome (*2*) and the *2*-proximal element (*2p3d*) appears as two subsegments. (**b**) *T(2;3)76* hybridized with AAGAC repeats. The translocation is homozygous in this cell with homologous chromosomes showing somatic pairing. Both elements of the translocation are labeled, suggesting that the breakpoint of *76* lies within a block of AAGAC repeats. An additional site of labeling can be seen on the *2*-distal element (*2d3p*) from a site in the heterochromatin of chromosome *3*. (**c**) *T(2;3)37* hybridized with AATAG repeats. The *2*-proximal element (*2p3d*) is unlabeled showing that the single site of $(AATAG)_n$ is distal to the breakpoint of *37* in *2L* heterochromatin. The *2*-distal element (*2d3p*) is labeled (arrow). (**d**) *T(2;3)40* hybridized with AATAG repeats. Both the *2*-proximal element (*2p3d*) and *2*-distal element (*2d3p*) are labeled (arrows), suggesting that *40* breaks within a block of $(AATAG)_n$ in region h37. A minor site of labeling in chromosome *3* heterochromatin can also be seen immediately proximal to the labeling on the *2d3p* element and on *TM3*. The *Y* chromosome is labeled at a single site near the tip.

repeats are located specifically in N-banded regions (see below).

The 7-bp AAGAGAG repeats were mapped to two distal sites in chromosome *2* heterochromatin, one on each arm. One interval containing these repeats is distal to the breakpoint of *88* on *2L* because the *2*-distal element was labeled (Figure 8a). This site was further mapped to the interval between the breakpoints of *46* and *40* (regions h35–h36), since 7-bp repeats hybridized to the *2*-distal element of *40* (Figure 7c). The other interval is in regions h44–h46, distal to the breakpoint of *76* on the right arm. The *2*-proximal element of *76* showed one site of labeling on *2L* and no labeling near the *2R* breakpoint. The *2*-distal element contains one N-banded region (h45) from chromosome *2* and was labeled at a single site

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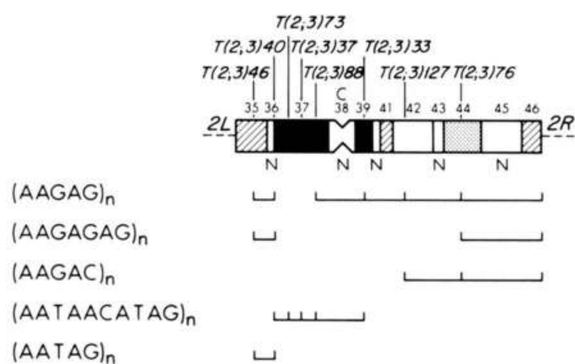


FIGURE 9.—Localizations of five simple satellite DNAs to chromosome *2* heterochromatin of *D. melanogaster*. Positions of *T(2;3)* breakpoints identified by N-banding are shown above a schematic representation of chromosome *2* heterochromatin stained with Hoechst 33258 (DIMITRI 1991). Regions of heterochromatin that can be identified by a characteristic response to staining or banding are numbered (h35–h46). Closed (black) segments indicate bright fluorescence, the stippled segment (region h44) moderate fluorescence, hatched segments dull fluorescence, and open (white) segments indicate no fluorescence. C, centromere; N, N-banded regions; *2L*, left arm of chromosome *2* and *2R*, right arm. Euchromatin (thin line) extends distally from the left and right arms. Breakpoints used in each localization are shown as short vertical lines distributed along each horizontal line. A horizontal line shows that a satellite is present somewhere within a breakpoint interval but does not imply that the satellite maps throughout the interval. Similarly, the localization of more than one class of satellite repeats to the same interval does not necessarily indicate interspersion of satellite repeats. The apparent overlap of satellites in chromosome *2* heterochromatin may have been resolved if more translocation breakpoints were available. Satellite positions can be further refined by correlating the characteristic staining and banding patterns of heterochromatin with the sequence class of satellite mapping to that location (see text).

(Figure 7d). These results suggest that 7-bp repeats are absent from proximal *2R* heterochromatin, including the two N-banded regions h40 and h43. The labeling pattern of *33* confirmed this result since the *2*-distal element also showed a single site of labeling (Figure 7e) even though this element contains most of the *2R* heterochromatin, including three N-banded regions. In extended chromosomes the site of labeling on *2R* appeared as two subsegments in close proximity (Figure 8a). Each of the two intervals that hybridize 7-bp repeats also contains an N-banded region, h36 on *2L* and h45 on *2R* (Figure 9).

**Mapping AAGAC repeats using *T(2;3)*s:** These repeats are located at a single site on the right arm of chromosome *2* (see above). They hybridized to the *2*-distal element of *T(2;3)127* and are therefore present somewhere in regions h42-h46 (Figure 9). They are also present on both the *2*-proximal and *2*-distal elements of *76*, close to the breakpoints (Figure 8b). Therefore, *76* appears to break within a block of AAGAC repeats. Since the breakpoint of *76* lies in region h44 (Table 4), this suggests that AAGAC repeats are located only in region h44. Although these mapping results do not exclude the presence of AAGAC repeats from regions h42, h43, h45 and h46, the characteristic staining properties of region h44 and the absence of other regions staining similarly in chromosome *2* heterochromatin (DIMITRI 1991) suggest that AAGAC repeats are confined to region h44. From quantitations there are 1.8 Mb of AAGAC repeats on chromosome *2* (Table 3). This represents 12% of the DNA in chromosome *2* heterochromatin. Since region h44 represents 13% of the cytological length of chromosome *2* heterochromatin (DIMITRI 1991) these data suggest that AAGAC repeats are the principal component of the DNA in region h44.

**Mapping 10-bp repeats using *T(2;3)*s:** AATAACATAG repeats also localize to a single site in chromosome *2* heterochromatin (Figure 6b), but on the left arm. There was a site of hybridization near the translocation breakpoint on both proximal and distal elements of the three translocations *T(2;3)37*, *73* and *88*, suggesting that the breakpoints lie within a block of 10-bp repeats. The three translocations each have a breakpoint in region h37 (Table 4). Only a small amount of labeling remained on the *2*-proximal ele-

TABLE 5

**Rearrangements with breakpoints in known satellite sequences**

| Rearrangement | Heterochromatic breakpoint | Chromosome | Satellite disrupted | Reference[a] |
|---|---|---|---|---|
| *In(1)sc*$^{8}$ | h32 | *X* | 359 bp | 1 |
| *T(Y;2)R50* | h1/h2 | *Y* | $(AATAT)_n$ | 2 |
| *T(X;Y)F12* | h15 | *Y* | $(AATAT)_n$ | 2 |
| *T(Y;3)B115* | h15 | *Y* | $(AATAT)_n$ | 2 |
| *T(Y;2)D19* | h3 | *Y* | $(AAGAG)_n$ | 2 |
| *T(Y;2)A162* | h21 | *Y* | $(AAGAG)_n$ | 2 |
| *T(2;3)40* | h37 | *2* | $(AATAG)_n$ | 3 |
| *T(2;3)73* | h37 | *2* | $(AATAACATAG)_n$ | 3 |
| *T(2;3)37* | h37 | *2* | $(AATAACATAG)_n$ | 3 |
| *T(2;3)88* | h37 | *2* | $(AATAACATAG)_n$ | 3 |
| *T(2;3)76* | h44 | *2* | $(AAGAC)_n$ | 3 |

[a] 1, HILLIKER and APPELS (1982); PIMPINELLI *et al.* (1985); 2, BONACCORSI and LOHE (1991); 3, this study.

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**TABLE 6**

**Staining and banding response of repeated DNA sequences**

| Satellite | Location in CsCl gradient (g/ml)[a] | Percent AT rich | Staining and banding response[b] | | |
|---|---|---|---|---|---|
| | | | Quinacrine | Hoechst | N-banding |
| $(AATAT)_n$ | 1.672 | 100 | Bright | Bright | None |
| $(AATAAAC)_n$ | 1.669 | 86 | Bright | Bright | None |
| $(AATAG)_n$ | 1.693 | 80 | —[c] | —[c] | Weak[d] |
| $(AATAC)_n$ | 1.680 | 80 | Bright | Bright | None |
| $(AATAACATAG)_n$ | 1.686 | 80 | Moderate | Bright | None |
| $(AATAGAC)_n$ | 1.688 | 71 | —[e] | —[e] | —[e] |
| 359 bp | 1.688 | 69 | Moderate | Bright | None |
| rDNA | 1.697 | 66[f] | Dull[g] | Dull[g] | None |
| 240-bp Responder[h] | — | 65 | Dull | Bright | None |
| $(AAGAC)_n$ | 1.689, 1.701 | 60 | None | Moderate | None |
| $(AAGAG)_n$ | 1.705 | 60 | None | None | Positive |
| $(AAGAGAG)_n$ | 1.705 | 57 | None | None | Positive |

[a] Taken from LOHE and BRUTLAG (1986).
[b] See text for references.
[c] It is difficult to make conclusions about the response of AATAG repeats to quinacrine and Hoechst staining. Significant amounts of these repeats are present at two sites in the genome. The site on the *Y* chromosome is adjacent to AATAT and AATAC repeats, and the site on chromosome *2* is adjacent to large numbers of AATAACATAG repeats. Each of these neighboring simple repeat types fluoresces brightly with Hoechst.
[d] Based on results of N-banding in *D. simulans* (PIMPINELLI, SANTINI and GATTI 1976) and the abundance of $(AATAG)_n$ and absence of $(AAGAG)_n$ on chromosome *4* of *D. simulans* (LOHE and ROBERTS 1988).
[e] AATAGAC repeats map to an interval of the *Y* chromosome that is also abundant in AAGAG and AAGAC repeats, making it difficult to conclude about the response of AATAGAC repeats to staining or banding.
[f] Reference: TAUTZ *et al.* (1988).
[g] The rDNA on the *X* and *Y* chromosomes shows a nucleolar constriction and may be relatively decondensed.
[h] Reference: WU *et al.* (1988).

ment of *88* (Figure 6c). *T(2;3)40* also breaks in region h37 but the breakpoint is apparently even more distal because the *2*-distal element was not labeled. We consistently saw a pattern of hybridization which suggested that the distal to proximal order of the breakpoints is *40*, *73*, *37* and *88* (Figure 9).

Region h37 is Hoechst-bright and comprises 18% of the cytological length of chromosome *2* heterochromatin (DIMITRI 1991). The 10-bp repeats represent 13% of the DNA in chromosome *2* heterochromatin (Table 3), suggesting that these repeats are the major component of the DNA in region h37. In agreement with this conclusion is the finding that region h37 is the only block in *2L* heterochromatin to fluoresce brightly with Hoechst (PIMPINELLI and DIMITRI 1989). Hoechst binds preferentially to DNA that is AT rich, and the AT content of 10-bp repeats is 80%.

The 10-bp repeats are also present at one major site in the heterochromatin of chromosome *3* (Figure 6b), in an amount approximately equal to that in chromosome *2* (Table 3). Chromosome *3* contains a single major Hoechst-bright block, region h48 (see ASHBURNER 1989), suggesting that h48 is the other major location of 10-bp repeats in the genome.

**Mapping AATAG repeats using *T(2;3)*s:** In wild-type chromosomes AATAG repeats hybridized mainly to two sites, one on the *Y* and one in chromosome *2* heterochromatin. The amount of AATAG repeats is only about 200 kb on chromosome *2* (Table 3). *In situ* hybridization to *37* shows that AATAG repeats are distal to the chromosome *2* breakpoint (Figure 8c). These repeats were mapped to the interval between the breakpoints of *46* and *40*. In heavily exposed slides hybridization was observed on both the *2*-distal and *2*-proximal elements of *40* (Figure 8d), suggesting that *40* breaks within the block of AATAG repeats. The breakpoint of *40* lies in the distal segment of region h37 because *40* does not disrupt the large block of 10-bp AATAACATAG repeats that lies proximally. Since the AT content of AATAG is 80%, these repeats probably fluoresce brightly with Hoechst. The simplest interpretation of these results is that AATAG repeats lie at the distal edge of the brightly fluorescent block in h37, a region composed predominantly of AATAACATAG repeats.

**Chromosome banding and satellite DNA sequences:** Heterochromatin of *D. melanogaster* chromosomes has been characterized into 61 distinct regions by staining with quinacrine, Hoechst or N-banding (GATTI and PIMPINELLI 1983; DIMITRI 1991; see ASHBURNER 1989). This detailed resolution has been possible because of the ability to sequentially characterize the same prometaphase chromosomes with Hoechst or quinacrine staining followed by N-banding, and consequently to order accurately the differently responding regions of heterochromatin. It has not been possible to generate a similar high reso-

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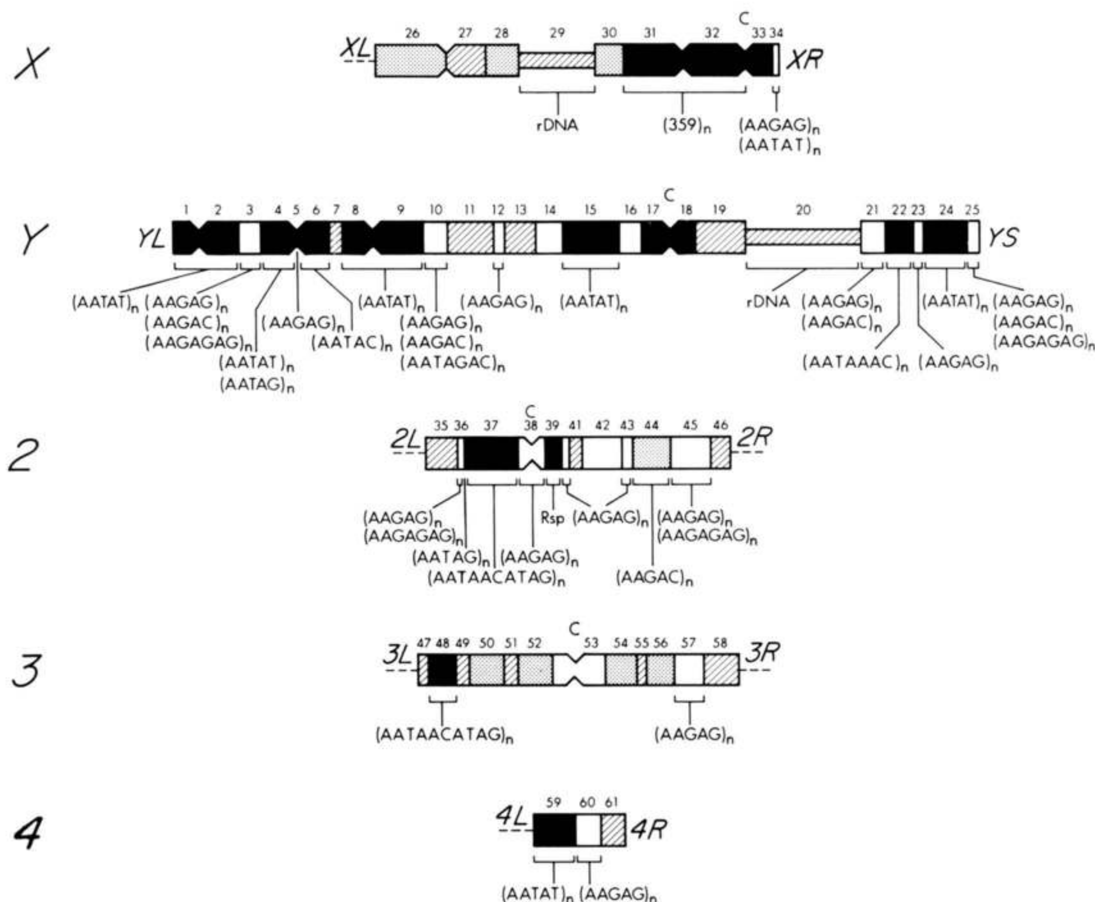


FIGURE 10.—Map of major satellite locations in *D. melanogaster* heterochromatin. Locations are shown relative to heterochromatic regions defined by staining and banding. Satellite repeats shown are the major component of the DNA in the region indicated and other DNA sequences, either repeated or nonrepeated, may also be present within the region. Only regions containing satellite amounts more than about 300 kb (Table 3) are shown. Satellite repeats are shown immediately adjacent to each other for illustrative purposes only. The molecular arrangement at the junctions of neighboring repeat classes is unknown. Only the heterochromatic portions of chromosomes are shown. Euchromatin extends from the ends of chromosomes as indicated by a broken line (- - - - -). C indicates the position of the centromere. Closed (black) segments within the chromosomes indicate bright fluorescence, stippled segments moderate fluorescence, hatched segments dull fluorescence, and open (white) segments indicate no fluorescence [from PIMPINELLI *et al.* (1985) and S. PIMPINELLI *et al.* (personal communication) in ASHBURNER (1989); modified with permission by Cold Spring Harbor Laboratory Press, copyright 1989]. Satellite locations for 359-bp repeats on the *X* chromosome were derived from HILLIKER and APPELS (1982), for the *Y* chromosome from BONACCORSI and LOHE (1991), and for satellites on chromosome *2* from this paper. Other satellite locations for chromosomes *3*, *4* and the *X* were determined by taking into account several results. These were, principally, satellite abundance at a given site from *in situ* hybridizations to wild-type chromosomes (LOHE and ROBERTS 1988; this paper) and the relationships between the staining and banding response of repeated DNA sequences (Table 6), as determined from rearrangements with breakpoints in known satellite sequences (Table 5).

lution map of satellites in heterochromatin from *in situ* hybridization results alone. Even using rearrangement chromosomes and biotin detection, the resolution of satellite localizations is limited by the distance between adjacent breakpoints. Further, sequential *in situ* hybridizations with different satellite probes, or staining and banding followed by *in situ* hybridization, are not technically reliable. The difficulty in providing an accurate satellite map could be overcome if the relationship between cytochemical properties of heterochromatin and satellite content were established. By identifying specifically stained or banded regions of heterochromatin on the cytochemical map with known satellite repeats, the resolution of satellite mapping is greatly increased.

Three criteria were used to correlate the cytochemical composition of heterochromatin with satellite sequences. First, some rearrangements that break within a heterochromatic region characterized by staining or banding also disrupt a satellite array. Ten rearrangements fall into this category (Table 5). Identifying these rearrangements was simplified because the same rearrangement chromosomes were used for satellite mapping as for cytochemical characterizations. From our observations in mapping different satellites to wild-type and rearrangement chromosomes (see above), the most abundant satellite at or near the breakpoint could be identified. Six different satellite sequences were thus tentatively assigned to specifically stained or banded regions of heterochromatin. This interpretation assumes that satellites are, in general, organized in large expanses consisting

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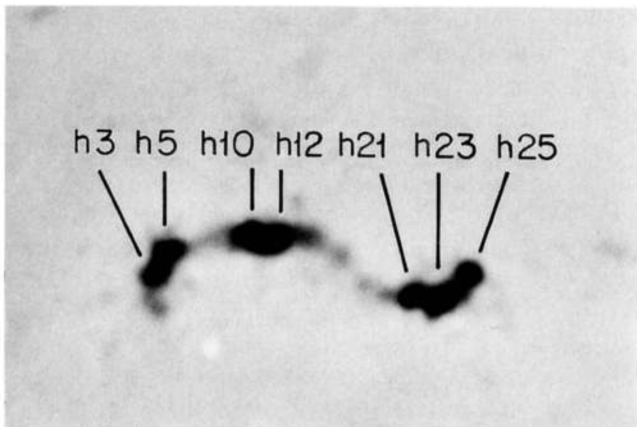


FIGURE 11.—Relationship of N-banded regions with AAGAG satellite repeats on the *Y* chromosome. The seven positions of labeling by biotinylated $(AAGAG)_n$ on the wild-type *Y* chromosome shown in Figure 6a correspond well with seven N-banded regions (h3, h5, h10, h12, h21, h23, h25) of the nine N-banded regions on the *Y*. AAGAG satellite repeats were mapped previously to much larger intervals that include these same N-banded regions, using a $^3$H-$(AAGAG)_n$ probe and *T(Y;A)* chromosomes (BONACCORSI and LOHE 1991).

primarily of one repeated sequence class, as shown for some major satellites (BRUTLAG *et al.* 1977).

Second, the base composition of a satellite should correlate with the staining properties of heterochromatin in that region. Quinacrine and Hoechst bind preferentially to AT rich DNA sequences (see LATT and LANGLOIS 1990). The cytochemical properties of heterochromatin in regions containing known satellites (Table 5) show a good correlation with base composition of these satellites, for the three types of staining and banding responses (Table 6). Five satellites highly AT rich (80–100%) are located in quinacrine- and Hoechst-bright regions. The 359-bp satellite and 240-bp Responder repeats (65–69% AT rich) are present in regions moderate or dull with quinacrine but bright with Hoechst. Neither AAGAG nor AAGAGAG repeats (57–60% AT rich) respond to Hoechst or quinacrine staining but are positive with N-banding. The final criteria in relating cytochemical properties of heterochromatin with satellite sequences was that satellites having multiple chromosome locations should show the same staining or banding properties at the different locations. Apart from minor exceptions (see DISCUSSION), all *in situ* hybridization results for satellites showing multiple sites of hybridization were consistent with the staining and banding responses of heterochromatin at those sites.

Together, these properties have enabled us to assign specific satellite sequences with stained or banded regions of heterochromatin in *D. melanogaster* (Figure 10). For example, the *Y* breakpoint of *T(Y;2)D19* lies within the N-banded region h3, and *D19* disrupts an array of $(AAGAG)_n$ (Table 6; BONACCORSI and LOHE 1991). Further, $(AAGAG)_n$ are present at, or in the vicinity of, 15 of 16 N-banded regions in the genome, suggesting that N-banded regions are usually composed of $(AAGAG)_n$. Thus, the polypurine:polypyrimidine $(AAGAG)_n$ have been assigned specifically to 15 N-banded regions, seven on the *Y*. The imprecise localizations of 5-bp AAGAG repeats to chromosome *2* heterochromatin (Figure 9) can now be refined to the five N-banded regions h36, h38, h40, h43 and h45 (Figure 10). The accuracy in $(AAGAG)_n$ localizations is supported by *in situ* hybridization to the prometaphase *Y* chromosome. Seven distinct sites of labeling were observed, each site corresponding well to the position of an N-banded region (Figure 11).

The polypurine:polypyrimidine 7-bp AAGAGAG repeats localize to two intervals on chromosome *2* (Figure 9) and to two intervals on the *Y* (BONACCORSI and LOHE 1991), each interval also containing an N-banded region. We suggest that tandem arrays of 7-bp repeats are present together with 5-bp arrays in the same N-banded regions, h36 and h45 on chromosome *2* and h3 and h25 on the *Y*. The molecular arrangement of the two repeat types within these regions is unknown. It cannot be shown conclusively that 7-bp repeats alone result in an N-banded region because 7-bp repeats are always present together with 5-bp repeats. However, we have proposed that 7-bp repeats correspond to an N-banded region (Table 6) because AAGAG forms part of each 7-bp sequence and 7-bp repeats are also polypurine:polypyrimidine in structure.

Region h44 on chromosome *2* contains large numbers of AAGAC repeats but is not an N-banded region (Figure 9). Thus, a single nucleotide change in a 5-bp repeated sequence is sufficient to alter the response to N-banding. Similarly, $(AATAC)_n$ stain brightly with Hoechst but $(AAGAC)_n$ stain only moderately (Table 6). AATAT repeats were mapped to six major locations in heterochromatin, five on the *Y*. From considerations above, $(AATAT)_n$ stain as Hoechst-bright and quinacrine-bright regions of heterochromatin. Although also staining as Hoechst-bright, $(AATAAAC)_n$ in region h22 show a lower degree of fluorescence than other Hoechst-bright regions on the *Y* (GATTI and PIMPINELLI 1983), in accord with the relatively lower AT content (86% *vs.* 100%).

The 359-bp repeats have been assigned to the Hoechst-bright regions h31 and h32 of the *X* chromosome. They were not assigned to h33, a region on the short arm of the *X* that stains brightly with Hoechst, because h33 shows different staining properties with distamycin A (see ASHBURNER 1989). However, we note that 359-bp repeats appear to hybridize to the short arm (Figure 3a). Finally, the overall molecular organization of DNA sequences within a region of heterochromatin shown in Figure 10 is unknown. A single satellite assigned to an entire block

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of heterochromatin may represent only the predominant DNA component. Other DNA sequences, either repeated or nonrepeated, could also reside within a region.

## DISCUSSION

**Organization of DNA sequences in heterochromatin:** To better define the organization of the most abundant DNA sequences in heterochromatin of *D. melanogaster*, we have determined the chromosomal locations of ten different simple repeated sequences. Repeats of five satellite sequences (AATAT, AAGAC, AAGAG, AAGAGAG, AATAACATAG) are especially abundant in the genome and form visible peaks in CsCl gradients of total DNA. Each of these satellites has a multichromosome distribution and is organized in a small number of large domains, sometimes in several places on one chromosome. Although satellite domains appear as large blocks by *in situ* hybridization, this does not mean that the arrays exist in uninterrupted long tracts. Other sequences such as the mobile elements *copia* and *297* (CARLSON and BRUTLAG 1978a,b; LOHE and BRUTLAG 1987b) are present at low density in at least some satellite arrays. Further, the abundant satellites are cleaved into many lower molecular weight fragments by most restriction enzymes (A. LOHE, unpublished).

Complex 359-bp repeats (1.688 satellite) are also abundant and are present mainly in the *X* heterochromatin. These repeats lie proximal to the rRNA locus of the *X*, appearing as one site by *in situ* hybridization. This site contains about 11 Mb (30,000 copies) of DNA, roughly half the DNA in the *X* heterochromatin (Table 3). A small number of 359-bp repeats, or repeats with a closely related sequence, are present in the heterochromatin of chromosomes *2* and *3*. Recently, 2–4 copies of a sequence 60–80% similar to the 359-bp repeat have been found at several sites in euchromatin, principally of the *X* chromosome (DIBARTOLOMEIS, TARTOF and JACKSON 1992). When copy numbers are considered, the euchromatic locations of 359-bp related repeats are minor compared to the heterochromatic location on the *X*. But even the limited distribution of a complex satellite repeat in euchromatin is unlike the simple satellites, which are largely absent from euchromatin (LOHE and ROBERTS 1988).

Southern or *in situ* hybridizations of four minor simple repeats (AAAAC, AATAC, AATAAAC, AATAGAC) showed that each is located only on the *Y* chromosome, at one site that is different for each satellite. This restriction to the *Y* is surprising considering the high copy number, approximately 60,000 for AATAG, 320,000 each for AATAAAC and AATAGAC, and 700,000 for AATAC repeats (Table 3). Other simple satellites that are also abundant on the *Y* show a multichromosome distribution. However, a small number of tandem repeats elsewhere in the genome could easily be undetected. For example, eight tandem copies of the AATAC sequence are present near the s38 chorion gene, located in the euchromatin of the *X* chromosome (SPRADLING *et al.* 1987).

Large blocks of simple satellite sequences are found in abundance on both the sex chromosomes and the autosomes. Although several highly repeated sequences appear to be specific to some chromosomes, other repeats are abundant on both sex chromosomes and the autosomes. We conclude that the overall molecular structure of heterochromatin is similar in the sex chromosomes and the autosomes, despite the vastly different genetic and physical properties of the functions contained in sex chromosome and autosomal heterochromatin.

**Detailed mapping of satellites in chromosome *2* heterochromatin:** Using reciprocal translocations to order different satellites, we have constructed a more detailed map of five simple satellites in chromosome *2* heterochromatin (Figure 9). More than one satellite is present in some intervals defined by adjacent breakpoints, giving the impression of interspersion of satellites. A similar result was obtained from detailed mapping of satellites on the *Y* (BONACCORSI and LOHE 1991).

One explanation is that the intervals between translocation breakpoints may be larger than the sizes of satellite blocks because satellites on both the *2* and the *Y* map to one or more discrete sites in prometaphase chromosomes. Chromosome *2* heterochromatin contains about 15 Mb of DNA, and the eight translocations therefore divide the heterochromatin into intervals of average size 2 Mb. For four abundant satellites the size of each block on chromosome *2* averages 1–2 Mb (Table 3). By relating the chromosome banding properties of heterochromatin with satellite sequences, satellites could be localized to specific stained or banded regions (Figure 10), permitting a higher level of resolution.

A pattern emerges from the detailed mapping of 5-bp AAGAG and 7-bp AAGAGAG repeats on chromosomes *2* and the *Y*, the two chromosomes that contain most of these polypurine-polypyrimidine satellites. The 5-bp repeats map to five sites on chromosome *2*, and 7-bp repeats map to two of the same sites (Figure 10). Similarly, 5-bp repeats map to seven distinct sites on the *Y* and 7-bp repeats map to two of the same sites (BONACCORSI and LOHE 1991). It appears that AAGAGAG repeats are always present within or in close proximity to AAGAG repeats, although the converse is not always true. We do not know whether AAGAGAG repeats are interspersed with AAGAG repeats or if the two repeat classes exist

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as separate blocks that are closely juxtaposed.

This co-localization of related 5- and 7-bp repeats on chromosomes *2* and the *Y* differs significantly from the organization of the related AAGAG and AAGAC repeats on the same chromosomes. Both of these 5-bp repeat classes are present in the same three N-banded regions on the *Y* (BONACCORSI and LOHE 1991), but on chromosome *2* $(AAGAC)_n$ localize only to h44, a region that is N-band negative and appears to lack $(AAGAG)_n$. However, h44 is flanked by two N-banded regions that contain $(AAGAG)_n$. The significance of the different organizations of the two repeated sequences on chromosomes *2* and the *Y* is not clear. It could reflect functional properties of these repeats or else may be a consequence of their mode of origin (LOHE and ROBERTS 1988).

None of the cloned satellite repeats presently available mapped to regions h35, h41, h42 and h46. These regions are dull or do not stain with Hoechst and may contain satellite repeats of lower AT content. Region h39 fluoresces brightly with Hoechst and contains 240-bp satellite repeats that correspond to the *Responder* locus (WU *et al.* 1988; PIMPINELLI and DIMITRI 1989). Together, the five simple satellites mapped to chromosome *2* amount to approximately 11 Mb of DNA, representing more than 70% of the DNA in chromosome *2* heterochromatin.

**Relationship between chromosome banding and satellite repeats:** From comparisons between the banding and satellite DNA maps of heterochromatin, many workers have made inferences about the satellite DNA composition of differently stained heterochromatic blocks. For example, we had suggested that AT rich 1.672 satellite sequences are almost exclusively located in Hoechst bright regions and that the relatively GC rich 1.686 and 1.705 satellites are located in N-banded regions on the *Y* (BONACCORSI and LOHE 1991). However, the relationship between banding and satellite DNAs was limited to these gross approximations because *Y* rearrangements that clearly disrupted blocks of known satellites identified only two different classes of satellite repeats (Table 5).

Identification of other chromosome rearrangements that disrupt satellite blocks has identified four additional repeated sequence classes with differently stained regions of heterochromatin (Table 5). In determining this relationship, it was necessary to take into account the abundance of satellites in the region of interest (Table 3). The *in situ* hybridization results of a satellite at different chromosomal locations were also checked to be sure they were consistent with the staining patterns. Together, these results permitted us to correlate the base composition of abundant repeated DNA sequences in *D. melanogaster* with the staining and banding response of different heterochromatic regions (Table 6) and to relate the cytochemical map of heterochromatin in *D. melanogaster* with specific satellite sequences (Figure 10).

Some heterochromatic blocks show identical banding patterns but contain different combinations of satellite repeats. From these results it was concluded that the molecular basis for banding does not depend exclusively on DNA content (BONACCORSI and LOHE 1991). However, this did not take into account the relative amounts of different satellites in a region or that more than one class of satellite repeat can show the same response to staining or banding (Table 6). Our results now suggest that where two or more classes of satellite repeats localize to a single region, the response to staining or banding reflects the cytochemical property of the most abundant satellite.

For example, five different repeated sequences map to the three adjacent regions h4–6 on the *Y* (BONACCORSI and LOHE 1991). Molecular cloning has shown that some repeat classes are interspersed in this region, particularly $(AATAC)_n$, $(AATAT)_n$ and $(AATAG)_n$ arrays (LOHE and BRUTLAG 1987b). From genomic quantitations and *in situ* hybridizations, only two of the five repeated sequences in h4–6 are major in amount, repeats of AATAT and AATAC. Since $(AATAC)_n$ map to region h6, $(AATAT)_n$ have been assigned to region h4 (Figure 10), in accord with their AT richness and Hoechst-bright staining. A low amount of $(AATAG)_n$ is also present in region h4 (Table 3; BONACCORSI and LOHE 1991). Since the AT content of these repeats is 80% they probably stain brightly with Hoechst, together with $(AATAT)_n$. We have assigned $(AAGAG)_n$ to h5 because it is a small N-banded region (see also Figure 11). On the *Y* chromosome $(AAGAC)_n$ are present in four N-banded regions that always contain $(AAGAG)_n$ and sometimes other repeats. On chromosome *2* $(AAGAC)_n$ localize to a site distinct from N-banded regions, suggesting that their apparent N-band positive response on the *Y* is due to interspersion with $(AAGAG)_n$. We conclude that the basis for the cytochemical heterogeneity in banding depends exclusively on the different satellite DNAs present in heterochromatin.

**Cytological length and DNA content:** We have asked whether the lengths of specifically stained or banded regions in prometaphase chromosomes are reliable indicators of the amount of DNA in these regions. Five N-banded regions account for about 30% of the length of chromosome *2* heterochromatin at prometaphase (DIMITRI 1991) but the $(AAGAG)_n$ and $(AAGAGAG)_n$ repeats comprise about 50% of the DNA in chromosome *2* heterochromatin (Table 3). Similarly, three satellites $(AAGAG)_n$, $(AAGAC)_n$ and $(AAGAGAG)_n$ have been mapped to seven N-banded regions on the *Y* chromosome (BONACCORSI and LOHE 1991). The three satellites amount to about 40% of the *Y* DNA (Table 3) but the corresponding N-banded

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regions amount to less than 20% of the cytological length (GATTI and PIMPINELLI 1983). This excludes from length measurements h20, a decondensed region that contains rRNA genes. Thus, from analyses of DNA content of heterochromatin in chromosomes *2* and the *Y*, N-banded regions appear to contain considerably more DNA than expected from their lengths at prometaphase.

In contrast are the AT rich satellites $(AATAT)_n$, $(AATAC)_n$ and $(AATAAAC)_n$ that map to nine Hoechst-bright regions on the *Y* (BONACCORSI and LOHE 1991). These regions account for about 50% of the cytological length of the *Y*, but the three satellites account for only about 25% of the *Y* DNA (Table 3). Similarly, the single Hoechst-bright band on chromosome *2*, region h37, represents 18% of the cytological length of chromosome *2* heterochromatin but contains only 13% of chromosome *2* DNA. Two possible explanations are that DNA in Hoechst-bright regions of heterochromatin is less condensed than DNA in Hoechst-dull regions, or that other repeated sequences are present in Hoechst-bright regions in addition to those mapped using cloned probes.

Since N-banded regions appear to contain more DNA than expected from their lengths as measured at prometaphase, and the GC content of DNA in N-banded regions is higher than DNA in Hoechst-bright regions (Table 6), we suggest that chromatin in N-banded regions is more compact compared to AT-rich, Hoechst-bright regions, at least at the prometaphase stage of the cell cycle. If Hoechst-bright regions contain less DNA that predicted from cytological lengths, as suggested from data presently available, these results caution against directly relating cytological length of heterochromatic regions with DNA content.

**Chromosomal assignments of satellites:** Our chromosomal assignments of satellites differ significantly from the map presented by PEACOCK *et al.* (1976, 1977), particularly for the *Y* chromosome. For example, 359-bp repeats (1.688 satellite) are absent from the *Y*, and 10-bp repeats (1.686 satellite) are not present on the *X* or *Y* chromosomes. Together, the 359- and 10-bp satellite sequences were estimated by PEACOCK *et al.* (1977) to comprise 11 Mb of DNA of the *Y*. An additional 3.4 Mb of DNA on the *Y* was attributed to 1.697 satellite sequences by PEACOCK *et al.* (1977) but in the absence of cloned probes for this satellite, it is difficult to assess the contribution from non-rDNA sequences to the hybridization patterns. One explanation for the erroneous localizations of the 1.686 and 1.688 satellites to the *Y* (PEACOCK *et al.* 1976, 1977) is that the gradient-purified satellite preparations used as probes for *in situ* hybridizations were contaminated by small amounts of other repeated sequences such as rDNA. Cloned satellite probes were not available when these studies were carried out.

We confirmed the results of PEACOCK *et al.* (1976, 1977) that both AATAT and AAGAG repeats are abundant on the *Y*, as well as on other chromosomes. Together with the rDNA, these three repeated sequences account for about 15 Mb of *Y* chromosome DNA (Table 3), less than half of the total DNA of this chromosome. The conclusion that much of the *Y* is composed of highly repeated sequences (PEACOCK *et al.* 1977) is, nevertheless, supported by our results because we found that other satellite sequences, amounting to about 18 Mb of DNA, are abundant on the *Y* (Table 3). Finally, we did not find that all satellites have a multichromosome distribution. Four satellite repeats are restricted to the *Y* (Figure 4).

Our localizations for AATAT and AAGAG repeats with the Oregon R strain are in good agreement with the map of the 1.672 and 1.705 satellites in the Canberra wild-type strain (PEACOCK *et al.* 1976, 1977; STEFFENSEN, APPELS and PEACOCK 1981), both in positions and proportions on chromosomes. The RNA probes used in the original 1.672 and 1.705 mapping experiments were synthesized with only ATP and UTP (1.672) or ATP and GTP (1.705), and this undoubtedly minimized contamination from other repeated sequences in the satellite preparations.

**Structure of the *Y* chromosome:** Nine of eleven satellite sequences cloned in *D. melanogaster* are present on the *Y*, and only the abundant 10- and 359-bp repeats appear to be absent from this chromosome. The satellites on the *Y* are found throughout its length and are usually present in large domains. Repeats of AAGAC (8.5 Mb), AAGAG (7.2 Mb) and AATAT (5.8 Mb) are each located at several sites on the *Y*. These simple satellite repeats amount to about 31 Mb of DNA on the *Y* (Table 3). Also present on the *Y* are about 150 rRNA genes (1.7 Mb including spacer) and approximately 0.4 Mb of 240-bp spacer repeats in addition to the rRNA intergenic spacer repeats (LOHE and ROBERTS 1990). The total amount of simple satellites, rRNA genes and excess spacer DNA is therefore roughly 33 Mb. Assuming the DNA content of the *Y* chromosome is about 40 Mb (PEACOCK *et al.* 1977), then these 10 highly repeated sequences alone account for more than 80% of the total DNA of the *Y* chromosome.

About 7 Mb of *Y* chromosome DNA are not accounted for by cloned satellites and rDNA. When these regions are identified on the cytochemical map of the *Y* (see Figure 10) they show distinctive banding properties (GATTI and PIMPINELLI 1983), suggesting that much of this DNA may also be composed of repeated DNA sequences that have not yet been cloned. The localizations of complex He-T repeats to both arms of the *Y* (DANILEVSKAYA *et al.* 1991) and of the minor dodeca satellite to an autosome and the *Y*

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(ABAD *et al.* 1992) support this interpretation. However, our results do not necessarily imply that all satellites on the *Y* exist as megabases of DNA in uninterrupted tandem arrays, or that this entirely heterochromatic chromosome contains only repeated DNA sequences. A small number of unique, protein-coding sequences or mobile elements may also be present. Consistent with this view is the isolation of temperature-sensitive and EMS-induced mutants of the fertility factor genes (WILLIAMSON 1970, 1972; AYLES *et al.* 1973) and the analysis of testis proteins in various genotypes carrying mutations in these genes (GOLDSTEIN, HARDY and LINDSLEY 1982). Currently, it is difficult to further investigate the long-range organization of DNA from the *Y* because simple satellite repeats are absent from yeast artificial chromosome (YAC) libraries of *D. melanogaster* (HARTL *et al.* 1992).

It has been shown recently that *Drosophila simulans*, the closest known relative of *D. melanogaster*, carries about 3 Mb of complex 240-bp satellite repeats at one site near the tip of *YL* (LOHE and ROBERTS 1990). The 240-bp repeats in this region represent about 10% of the DNA of the *D. simulans Y*. Sequences homologous to the complex *Stellate* repeats on the *Y* of *D. melanogaster* are present on the *Y* chromosomes of both *D. simulans* and *Drosophila mauritiana* (LIVAK 1984). Simple satellites are also present in large amounts on the *Y* chromosomes of *D. simulans* and other species in the *melanogaster* subgroup (LOHE and ROBERTS 1988). Therefore, a general feature of Drosophila *Y* chromosome structure is that simple and sometimes more complex repeated sequences can extend for long regions of this chromosome. We do not know how the unusual molecular structure of the *Y* chromosome relates to its genetic functions in spermatogenesis.

MULLER (1932; MULLER and GERSHENSON 1935) theorized that the *Y* chromosome started as an *X* chromosome derivative carrying a male determining factor. MULLER (1932) hypothesized that, in the absence of crossing over, the *Y* would suffer mutational degeneration, protected from elimination by natural selection by its continual heterozygosity. Our results show that in proportion to their lengths, the *X* and *Y* chromosomes share little sequence homology. The *X* chromosome carries about 11 Mb of 359-bp repeats and these are absent from the *Y*. Similarly, the *Y* carries about 7 Mb of simple satellite repeats that are absent from the *X* (Table 3). Therefore, much of the *Y* chromosome of *D. melanogaster*, rather than a collection of mutationally degenerate structural genes, now appears to be virtually a new construct, the product of amplification of the array of simple sequence DNA considered in this report.

**Juxtaposition of related satellites:** Striking similarities were observed in the chromosomal distributions of some simple satellites. In many cases, the positions of one satellite seemed to be a subset of the locations of another satellite. These results are unlikely to be artifacts of cross-hybridization because we employed stringent annealing conditions, specific for each satellite probe (Table 1), and not all sites were held in common between any two satellites.

Detailed mapping with rearrangement chromosomes suggests that the four closely related satellites AATAG, AAGAG, AAGAC and AAGAGAG are often physically adjacent to each other in heterochromatin. Each of these satellites maps primarily to chromosomes *2* and the *Y*. Major amounts of 5-bp AAGAG repeats are found at five sites on chromosome *2* and at seven sites on the *Y* (Figures 6a and 11; BONACCORSI and LOHE 1991). Repeats of the 7-bp sequence AAGAGAG are also present at two sites on chromosome *2*, in similar positions to two sites of the 5-bp AAGAG repeats, and on the *Y* at terminal locations on each arm, in similar positions to two of the 5-bp sites. Similarly, repeats of AAGAC map to four sites on the *Y*, similar in position to four sites of AAGAG repeats, and to h44 on chromosome *2*, a region flanked by AAGAG repeats. The juxtaposition of AAGAG and AAGAC repeats is also supported by the molecular cloning of a 1.705 satellite fraction (AAGAG repeats) that trails towards a lighter density. A significant proportion of recovered clones from this fraction contained AAGAC repeats.

Most AATAG repeats are present at two sites in the genome, on chromosomes *2* and the *Y*. The chromosome *2* site was mapped in close proximity to AATAACATAG repeats by *in situ* hybridization with *T(2;3)* chromosomes. The resolution in these experiments is not sufficient to determine whether blocks of the two repeat families are immediately adjacent in the chromosomal DNA. However, clone 1.686-201 contains tandem arrays of both AATAG and AATAACATAG repeat types physically linked to each other (LOHE and BRUTLAG 1987b). It is unlikely that this clone represents the single junction between two large tandem arrays of these repeated sequences in region h37 because the clone was found by chance in a small collection of 1.686 satellite clones. 1.686-201 may be representative of the types of junctions between adjacent arrays of satellites, particularly if there is a gradual transition between classes of repeats that is characterized by multiple junctions (LOHE and BRUTLAG 1987b). Similarly, AATAG repeats on the *Y* chromosome were mapped close to tandem arrays of AATAC in region h6 (BONACCORSI and LOHE 1991). The sequences of junction molecules containing physically linked arrays of AATAG and AATAC repeats have been described in clones 1.672–816 and 1.672-573 (LOHE and BRUTLAG 1987b).

From the structure of junction molecules at the

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ends of neighboring satellite arrays, it was found that satellites in adjacent tandem arrays are usually closely related in nucleotide sequence (LOHE and BRUTLAG 1987b). One common class of junction molecules contained simple satellite repeats that differed by one nucleotide in five. In another class, the repeating sequence of one tandem array could be derived from the adjacent array by single events such as deletion of part of the repeat. These patterns in the structure of junctions were interpreted as the products of copying events where one repeat is used as a template for synthesis of the neighboring repeat (see LOHE and BRUTLAG 1987b). The chromosome maps presented here support the hypothesis that satellites closely related in nucleotide sequence originate contiguously by miscopying during DNA amplification.

**Gaps in the satellite map:** The simple satellite repeats mapped here, together with the 359-bp satellite and rRNA genes, constitute about 23% of the genome of *D. melanogaster* (LOHE and BRUTLAG 1986). The amount of heterochromatin in this species is estimated at 30% from cytological measurements (see HEITZ 1933; KAUFMANN 1934). Our map of defined repeated sequences therefore represents a major proportion of DNA sequences in the heterochromatin of *D. melanogaster*. However, sizable regions of DNA in heterochromatin remain unaccounted for by known satellite sequences. The estimated DNA content of chromosome *3* heterochromatin is 13–16 Mb (PEACOCK *et al.* 1977) but the satellites mapped to this chromosome occupy only 3–4 Mb (Table 3). Because of their homogeneous dull staining with Hoechst, these regions may contain GC-rich satellite repeats that have not been cloned. *D. melanogaster* satellites have traditionally been purified in CsCl-antibiotic or CsCl-silver gradients that tend to select for satellites with high AT content (ENDOW, POLAN and GALL 1975; GALL 1973; GALL, COHEN and POLAN 1971; PEACOCK *et al.* 1973).

The satellite map is more complex than expected from a small number of abundant satellites in heterochromatin. Molecular cloning has defined the organization of satellite DNAs of *D. melanogaster* at the kilobase level (LOHE and BRUTLAG 1986). By *in situ* hybridizations to mitotic chromosomes, this paper has revealed the organization of satellites at the megabase level. Additional molecular analyses will be required to determine the organization of heterochromatic DNA sequences between these two levels of resolution.

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## LITERATURE CITED

ABAD, J. P., M. CARMENA, S. BAARS, R. D. C. SAUNDERS, D. M. GLOVER, P. LUDENA, C. SENTIS, C. TYLER-SMITH and A. VILLASANTE, 1992 Dodeca satellite: a conserved G + C-rich satellite from the centromeric heterochromatin of *Drosophila melanogaster*. Proc. Natl. Acad. Sci. USA **89:** 4663–4667.

ASHBURNER, M., 1989 *Drosophila: A Laboratory Handbook*. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.

AYLES, G. B., T. B. SANDERS, B. I. KIEFER and D. T. SUZUKI, 1973 Temperature-sensitive mutations in *Drosophila melanogaster*. XI. Male sterile mutants of the *Y* chromosome. Dev. Biol. **32:** 239–257.

BARIGOZZI, C., S. DOLFINI, M. FRACCARO, G. R. RAIMONDI and L. TIEPOLO, 1966 *In vitro* study of the DNA replication patterns of somatic chromosomes of *Drosophila melanogaster*. Exp. Cell Res. **43:** 231–234.

BONACCORSI, S., and A. LOHE, 1991 Fine mapping of satellite DNA sequences along the *Y* chromosome of *Drosophila melanogaster*: relationships between satellite sequences and fertility factors. Genetics **129:** 177–189.

BRIDGES, C. B., 1916 Non-disjunction as proof of the chromosome theory of heredity. Genetics **1:** 1–52 and 107–163.

BROSSEAU, G. E., 1960 Genetic analysis of the male fertility factors on the *Y* chromosome of *Drosophila melanogaster*. Genetics **45:** 257–274

BRUTLAG, D. L., and W. J. PEACOCK, 1975 Sequences of highly repeated DNA in *Drosophila melanogaster*, pp. 35–46, in *The Eukaryote Chromosome*, edited by W. J. PEACOCK and R. D. BROCK. Australian National University Press, Canberra.

BRUTLAG, D. L., R. APPELS, E. S. DENNIS and W. J. PEACOCK, 1977 Highly repeated DNA in *Drosophila melanogaster*. J. Mol. Biol. **112:** 31–47.

CARLSON, M., and D. BRUTLAG, 1978a One of the *copia* genes is adjacent to satellite DNA in *Drosophila melanogaster*. Cell **15:** 733–742.

CARLSON, M., and D. BRUTLAG, 1978b A gene adjacent to satellite DNA in *Drosophila melanogaster*. Proc. Natl. Acad. Sci. USA **75:** 5898–5902.

CARLSON, M., and D. BRUTLAG, 1979 Different regions of a complex satellite DNA vary in size and sequence of the repeating unit. J. Mol. Biol. **135:** 483–500.

DANILEVSKAYA, O. N., E. V. KURENOVA, M. N. PAVLOVA, D. V. BEBEHOV, A. J. LINK, A. KOGA, A. VELLEK and D. L. HARTL, 1991 He-T family DNA sequences in the *Y* chromosome of *Drosophila melanogaster* share homology with the *X*-linked *Stellate* genes. Chromosoma **100:** 118–124.

DIBARTOLOMEIS, S. M., K. D. TARTOF and F. R. JACKSON, 1992 A superfamily of *Drosophila* satellite related (SR) DNA repeats restricted to the *X* chromosome euchromatin. Nucleic Acids Res. **20:** 1113–1116.

DIMITRI, P., 1991 Cytogenetic analysis of the second chromosome heterochromatin of *Drosophila melanogaster*. Genetics **127:** 553–564.

ENDOW, S. A., M. L. POLAN and J. G. GALL, 1975 Satellite DNA sequences of *Drosophila melanogaster*. J. Mol. Biol. **96:** 665–692.

FRY, K., and D. L. BRUTLAG, 1979 Detection and resolution of closely related satellite DNA sequences by molecular cloning. J. Mol. Biol. **135:** 581–593.

GALL, J. G., 1973 Repetitive DNA in *Drosophila*, pp. 59–74, in *Molecular Cytogenetics*, edited by B. A. HAMKALO and J. PAPACONSTANTINOU. Plenum Press, New York.

GALL, J. G., E. H. COHEN and M. L. POLAN, 1971 Repetitive DNA sequences in *Drosophila*. Chromosoma **33:** 319–344.

GATTI, M., and S. PIMPINELLI, 1983 Cytological and genetic analysis of the *Y* chromosome of *Drosophila melanogaster*. Chromosoma **88:** 349–373.

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GOLDSTEIN, L. S. B., R. W. HARDY and D. L. LINDSLEY, 1982 Structural genes on the *Y* chromosome of *Drosophila melanogaster*. Proc. Natl. Acad. Sci. USA **79:** 7405–7409.

HALFER, C., 1981 Interstrain heterochromatin polymorphisms in *Drosophila melanogaster*. Chromosoma **84:** 195–206.

HANNAH, A., 1951 Localization and function of heterochromatin in *Drosophila melanogaster*. Adv. Genet. **4:** 87–125.

HARTL, D. L., J. W. AJIOKA, H. CAI, A. R. LOHE, E. R. LOZOVSKAYA, D. A. SMOLLER and I. W. DUNCAN, 1992 Towards a *Drosophila* genome map. Trends Genet. **8:** 70–75.

HAZELRIGG, T., P. FORNILI and T. C. KAUFMAN, 1982 A cytogenetic analysis of X-ray induced male steriles on the *Y* chromosome of *Drosophila melanogaster*. Chromosoma **87:** 535–559.

HEITZ, E., 1933 Cytologisch untersuchungen an dipteran. III. Die somatische heteropyknose bei *Drosophila melanogaster* und ihre genetische bedeutung. Z. Zellforsch. **20:** 237–287.

HILLIKER, A. J., 1976 Genetic analysis of the centromeric heterochromatin of chromosome *2* of *Drosophila melanogaster*: deficiency mapping of EMS-induced lethal complementation groups. Genetics **83:** 765–782.

HILLIKER, A. J., and R. APPELS, 1982 Pleiotropic effects associated with the deletion of heterochromatin surrounding rDNA on the *X* chromosome of Drosophila. Chromosoma **86:** 469–490.

HILLIKER, A. J., R. APPELS and A. SCHALET, 1980 The genetic analysis of *D. melanogaster* heterochromatin. Cell **21:** 607–619.

HILLIKER, A. J., and D. HOLM, 1975 Genetic analysis of the proximal region of chromosome *2* of *Drosophila melanogaster*. I. Detachment products of compound autosomes. Genetics **81:** 705–721.

HILLIKER, A. J., and C. B. SHARP, 1988 New perspectives on the genetics and molecular biology of constitutive heterochromatin, pp. 91–115, in *Chromosome Structure and Function*, edited by J. P. GUSTAFSON and R. APPELS. Plenum Press, New York.

HILLIKER, A. J., and S. N. TRUSIS-COULTER, 1987 Analysis of the functional significance of linkage group conservation in Drosophila. Genetics **117:** 233–244.

HSIEH, T-S., and D. BRUTLAG, 1979 Sequence and sequence variation within the 1.688 g/cm$^3$ satellite DNA of *Drosophila melanogaster*. J. Mol. Biol., **135:** 465–481.

INOUE, S., S. YUKI and K. SAIGO, 1986 Complete nucleotide sequence and genome organization of a *Drosophila* transposable genetic element, *297*. Eur. J. Biochem. **154:** 417–425.

KAUFMANN, B. P., 1934 Somatic mitoses of *Drosophila melanogaster*. J. Morphol. **56:** 125–155.

KENNISON, J. A., 1981 The genetic and cytological organization of the *Y* chromosome of *Drosophila melanogaster*. Genetics **98:** 529–548.

LANGER, P. R., A. A. WALDROP and D. C. WARD, 1981 Enzymatic synthesis of biotin-labeled polynucleotides: novel nucleic acid affinity probes. Proc. Natl. Acad. Sci USA **78:** 6633–6637.

LATT, S. A., and R. G. LANGLOIS, 1990 Fluorescent Probes of DNA microstructure and DNA synthesis, pp. 249–290 in *Flow Cytometry and Sorting*, edited by M. R. MELAMED, T. LINDMO and M. L. MENDELSOHN. Wiley-Liss, New York.

LIVAK, K. J., 1984 Organization and mapping of a sequence on the *Drosophila melanogaster X* and *Y* chromosomes that is transcribed during spermatogenesis. Genetics **107:** 611–634.

LOHE, A. R., and D. L. BRUTLAG, 1986 Multiplicity of satellite DNA sequences in *Drosophila melanogaster*. Proc. Natl. Acad. Sci. USA **83:** 696–700.

LOHE, A. R., and D. L. BRUTLAG, 1987a Identical satellite DNA sequences in sibling species of *Drosophila*. J. Mol. Biol. **194:** 161–170.

LOHE, A. R., and D. L. BRUTLAG, 1987b Adjacent satellite DNA segments in *Drosophila melanogaster*: Structure of junctions. J. Mol. Biol. **194:** 171–179.

LOHE, A., and P. ROBERTS, 1988 Evolution of satellite DNA sequences in Drosophila, pp. 148–186, in *Heterochromatin: Molecular and Structural Aspects*, edited by R. VERMA. Cambridge University Press, New York.

LOHE, A. R., and P. A. ROBERTS, 1990 An unusual *Y* chromosome of *Drosophila simulans* carrying amplified rDNA spacer without rRNA genes. Genetics **125:** 399–406.

MARCHANT, G. E., and D. G. HOLM, 1988a Genetic analysis of the heterochromatin of chromosome *3* in *Drosophila melanogaster*. I. Products of compound-autosome detachment. Genetics **120:** 503–517.

MARCHANT, G. E., and D. G. HOLM, 1988b Genetic analysis of the heterochromatin of chromosome *3* in *Drosophila melanogaster*. II. Vital loci identified through EMS mutagenesis. Genetics **120:** 519–532.

MULLER, H. J., 1932 Some genetic aspects of sex. Am. Nat. **66:** 118–132.

MULLER, H. J., and S. M. GERSHENSON, 1935 Inert regions of chromosomes as the temporary products of individual genes. Proc. Natl. Acad. Sci. USA **21:** 69–75.

MULLER, H. J., and T. PAINTER, 1932 The differentiation of the sex chromosomes of Drosophila into genetically active and inert regions. Z. Indukt. Abstammungs. Vererbungsl. **62:** 316–365.

PEACOCK, W. J., and A. R. LOHE, 1980 Repeated sequences in the chromosomes of Drosophila. Symp. R. Entomol. Soc. Lond. **10:** 1–12.

PEACOCK, W. J., D. BRUTLAG, E. GOLDRING, R. APPELS, C. W. HINTON and D. L. LINDSLEY, 1973 The organization of highly repeated DNA sequences in *Drosophila melanogaster* chromosomes. Cold Spring Harbor Symp. Quant. Biol. **38:** 405–416.

PEACOCK, W. J., R. APPELS, P. DUNSMUIR, A. R. LOHE and W. L. GERLACH, 1976 Highly repeated DNA sequences: chromosomal localization and evolutionary conservatism, pp. 494–506, in *International Cell Biology*, edited by B. R. BRINKLEY and K. R. PORTER. Rockefeller University Press, New York.

PEACOCK, W. J., A. R. LOHE, W. L. GERLACH, P. DUNSMUIR, E. S. DENNIS and R. APPELS, 1977 Fine structure and evolution of DNA in heterochromatin. Cold Spring Harbor Symp. Quant. Biol. **42:** 1121–1135.

PIMPINELLI, S., and P. DIMITRI, 1989 Cytogenetic analysis of segregation distortion in *Drosophila melanogaster*: the cytological organization of the Responder (*Rsp*) locus. Genetics **121:** 765–772.

PIMPINELLI, S., G. SANTINI and M. GATTI, 1976 Characterization of Drosophila heterochromatin. II. C- and N-banding. Chromosoma **57:** 377–386.

PIMPINELLI, S., W. SULLIVAN, M. PROUT and L. SANDLER, 1985 On biological functions mapping to the heterochromatin of *Drosophila melanogaster*. Genetics **109:** 701–724.

RASCH, E. M., H. J. BARR and R. W. RASCH, 1971 The DNA content of sperm of *Drosophila melanogaster*. Chromosoma **33:** 1–18.

ROBERTS, P. A. 1965 Difference in the behavior of eu- and heterochromatin: crossing-over. Nature **205:** 725–726.

RUDKIN, G. T., 1969 Non-replicating DNA in Drosophila. Genetics **61:** 227–238.

SEDEROFF, R., L. LOWENSTEIN and H. C. BIRNBOIM, 1975 Polypyrimidine segments in *Drosophila melanogaster* DNA : II. Chromosome location and nucleotide sequence. Cell **5:** 183–194.

SPEAR, B. B., 1974 The genes for ribosomal RNA in diploid and polytene chromosomes of *Drosophila melanogaster*. Chromosoma **48:** 159–179.

SPRADLING, A. C., D. V. DE CICCO, B. T. WAKIMOTO, J. F. LEVINE, L. J. KALFAYAN and L. COOLEY, 1987 Amplification of the *X*-linked Drosophila chorion gene cluster requires a region upstream from the s38 chorion gene. EMBO J. **6:** 1045–1053.

STEFFENSEN, D. M., R. APPELS and W. J. PEACOCK, 1981 The

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distribution of two highly repeated DNA sequences within *Drosophila melanogaster* chromosomes. Chromosoma **82:** 525–541.

SZABO, P., R. ELDER, D. M. STEFFENSEN and O. C. UHLENBECK, 1977 Quantitative *in situ* hybridization of ribosomal RNA species to polytene chromosomes of *Drosophila melanogaster*. J. Mol. Biol. **115:** 539–563.

TAUTZ, D., J. M. HANCOCK, D. A. WEBB, C. TAUTZ and G. A. DOVER, 1988 Complete sequences of the rRNA genes of *Drosophila melanogaster*. Mol. Biol. Evol. **5:** 366–376.

WILLIAMSON, J. H., 1970 Ethyl methanesulfonate-induced mutants in the *Y* chromosome of *Drosophila melanogaster*. Mutat. Res. **10:** 597–605.

WILLIAMSON, J. H., 1972 Allelic complementation between mutants in the fertility factors of the *Y* chromosome in *Drosophila melanogaster*. Mol. Gen. Genet. **119:** 43–47.

WU, C.-I., T. W. LYTTLE, M.-L. WU and G.-F. LIN, 1988 Association between a satellite DNA sequence and the *responder of segregation distorter* in *Drosophila melanogaster*. Cell **54:** 179–189.

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