CHARACTERIZATION OF THE DNA IN *DROSOPHILA MELANOGASTER*[1]

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ABSTRACT

DNA has been quantitatively extracted from *Drosophila melanogaster* at various stages of embryonic development and analyzed by isopycnic centrifugation in CsCl and by fractionation on methylated albumin columns. The DNA is composed of three main classes of DNA, as defined by their buoyant density, ρ, in CsCl: a bulk DNA, $\rho = 1.699$ g cm^{-3}, and two satellite DNAs, $\rho = 1.685$ g cm^{-3} and $\rho = 1.669$ g cm^{-3}. These three types of DNA persist throughout the development of the insect. In the unfertilized egg, 80% of the total DNA consists of the satellite DNAs; this amount decreases to 18% during the first three hours after fertilization and then remains constant through embryogenesis. There is a concomitant increase of the satellite DNA's with the bulk DNA after blastoderm formation.

IN 1956 we reported (SCHULTZ 1956) that the unfertilized eggs of *Drosophila melanogaster* contained approximately 500 times the DNA present in a haploid nucleus. In order to understand the significance of this result, we initiated studies in 1964 to characterize the DNA of *D. melanogaster* by means of analytical isopycnic centrifugation in CsCl, a technique that had just become available to us. Some of the results of these studies were reported in abstract form (TRAVAGLINI, PETROVIC and SCHULTZ 1968), but a detailed account has not been published. In this and the following two papers, we describe an extensive analysis of three main classes of DNA in Drosophila. These DNA's, as defined by their buoyant density, ρ, in CsCl consist of: a bulk DNA ($\rho = 1.699$ g cm^{-3}) and two satellite DNA's ($\rho = 1.685$ g cm^{-3} and $\rho = 1.669$ g cm^{-3}). The least dense of these satellites has been shown to be a poly dAT (FANSLER *et al.* 1970). In the present paper, we will demonstrate the presence of these three classes of DNA in *D. melanogaster* and their persistence throughout the embryogenesis of the insect.

MATERIALS AND METHODS

D. melanogaster *stocks:* Oregon R-S, Oregon RM, Canton S, Swedish C, Lausanne and *C(1)RM,w^{48h}/In(1)EN,Y^{S}X·Y^{L}* stocks have been maintained on a cornmeal-agar medium [to 7.6 liters of water is added 44 g agar, 200 g dry yeast, 1200 g cornmeal, 1440 ml of black strap

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molasses and 16.8 g of methyl parasept (dissolved in ethanol)] for several years at The Institute for Cancer Research. The Oregon R-S strain has been inbred by brother-sister matings for over 100 generations. For mass matings, 12 males and 10 females were placed in a half-pint bottle containing cornmeal-agar medium seeded with live yeast. One tray containing 30 bottles yields approximately 10,000 flies at the end of a 2-week period at 25°C. Flies were utilized within a week of eclosion. To obtain unfertilized eggs, virgin females were mated to $X/0$ males. Such $X/0$ males are produced by mating X/X females to attached XY *[In(1)EN,$Y^{S}X{\cdot}Y^{L}$]* males.

Egg collections: Approximately 10 g of 3-day-old flies (8500) were put in a widemouth gallon jar into which a tray of molasses-agar [420 ml molasses, 3.5 g methyl parasept (dissolved in alcohol), 2800 ml water and 70 g of Bacto agar] containing 6 (2 cm diam.) lumps of yeast had been placed. The jar was plugged with a cotton stopper and incubated at 25°C, and the females, if undisturbed, deposit their eggs in the clumps of yeast on the molasses-agar trays. Trays were changed at 3-hr intervals and the eggs were either harvested immediately or the tray was placed in a humidor at 25°C and the eggs aged for varying periods of time, so that the maximum age of the embryos was 3-, 5-, 8- or 18-hr. The eggs were stored in a 3:1 ethanol-ether mixture at —30°C until analyzed.

DNA extraction: The eggs were separated from the ethanol-ether by a low speed centrifugation at 4°C. The DNA was extracted from eggs or adults by means of the CsCl equilibrium gradient technique (TRAVAGLINI and MELONI 1962). The eggs (2 ml per 10 ml CsCl) were homogenized in 4 M CsCl ($\rho = 1.40$ g cm^{-3}) with a Potter-Elvehjem glass homogenizer at 4°C. The homogenate was placed in a Spinco Type 50 rotor and spun at 40,000 rpm for 20 hr. At equilibrium, the pellet contains the total nucleic acids and polysaccharides of the egg. This pellet was dissolved in a minimal amount of 1 × SSC (0.15 M NaCl, .015 M Na citrate) usually 9 ml of 1 × SSC for a pellet from 2 ml eggs, and then digested with the following enzymes successively for 1 hr each at 37°C: 0.01 volume of alpha amylase (10 mg/ml) (Worthington Biochemical Corp.); 0.1 volume of ribonuclease A (0.1 mg/ml) (Sigma, 5 × crystallized) heated to 100°C for 10′ to destroy any possible contaminating deoxyribonuclease activity; and finally, 0.01 volume of pre-digested (10 min at 37°C) pronase (50 μg/ml) (Calbiochem, Grade B). Insoluble materials in the digest were removed by low speed centrifugation and the DNA was then pelleted by centrifugation (Spinco Type 50 rotor at 40,000 rpm for 1 hr at 4°C). The DNA was dissolved in a minimal amount of 1 × SSC and stored at —30°C until analyzed.

Determination of nucleic acids: For quantitative analysis of the nucleic acids, samples of 5,000 eggs or embryos were homogenized in 4 M CsCl and centrifuged at 40,000 rpm for 20 hr in a Spinco type 50 rotor. The number of eggs in each sample was determined by using an electronic egg counter which had a counting error of less than ± 3%. The nucleic acid pellet obtained after extraction was solubilized in 1 ml of distilled water, treated with 0.01 volume of alpha amylase (10 mg/ml) (Worthington Biochemical Corp.) for 5 min at 25°C and centrifuged 10 min at 2000 *g* at 4°C to remove the flocculent polysaccharides.

To determine the amount of RNA present in the pellet, 0.02 ml aliquots were analyzed by total ultraviolet absorption at 260 mμ [ε(P) = 8,900] and by the orcinol reaction for purine ribose (MEJBAUM 1939). The two methods gave the same results for several samples and we felt confident that the ultraviolet absorption at 260 mμ could be used as a measure of the total RNA present in the sample, after the DNA contribution to the UV absorption was subtracted.

The sample was assayed for DNA by a modification of the diphenylamine test for DNA (PATTERSON and DACKERMAN 1952) and the Hoff-Jorgensen bioassay for DNA using the bacterium *Lactobacillus acidophilus* R-26 (MILLER 1958). The latter assay was five times more sensitive than the diphenylamine test for DNA. Both methods yielded the same value of DNA/egg from aliquots of a given sample, for example: diphenylamine (4 samples) 0.48 ± .04 mμg/egg; Hoff-Jorgensen assay (10 samples) 0.45 ± .04 mμg/egg. The Hoff-Jorgensen assay was used routinely for measuring the DNA (where sample sizes were small), i.e., unfertilized eggs and 0–3-hr embryos.

Methylated albumin column chromatography: Methylated albumin-kieselguhr (MAK) columns were prepared as described by MANDELL and HERSHEY (1960); 250 μg of DNA diluted to 40 μg/ml with 0.3 × SP (1 × SP = 1 M NaCl, 0.05 M PO_4 buffer pH 6.7) were applied to a

column (2.5 cm diam.) packed with 1 ml of MAK. The nucleic acids were eluted step-wise by increasing salt concentrations (0.3, 0.4, 0.5, 0.6, 0.7, 0.8 and 0.9 × SP) as described by SUEOKA and CHENG (1962a). The eluates from each salt concentration step were centrifuged at 40,000 rpm for 1 hr in a Spinco type 50 rotor. Each pellet was taken up in a minimal amount of 1 × SSC and stored at −30°C for further analysis.

Analytical ultracentrifugation: Density gradient centrifugations were performed in a Spinco Model E analytical ultracentrifuge equipped with a UV monochromator. Four cells with 12 mm 4° Kel F centerpieces each containing 0.65 ml of sample were run simultaneously in an Anal-F rotor at 44,770 rpm for 20 hr. Photographs using Ilford lantern plates (6.5 × 9 cm) were made with an alternating aperture mask at 265 mμ. Samples containing approximately 20 μg of nucleic acid were dissolved in 0.7 ml of a concentrated CsCl solution with a final density of 1.700 g cm^{-3}, as determined by a refractometer. *Pseudomonas fluorens* DNA, with a density of 1.721 g cm^{-3} as determined by SCHILDKRAUT, MARMUR and DOTY (1962), was used as a marker. The photographic plates were scanned on a Jarrold-Asch densitometer and the buoyant density of the DNA was calculated as described by VINOGRAD and HEARST (1962). Assuming that the DNA molecules of a given buoyant density are homogeneous and distributed evenly about a mode, i.e., Gaussian distribution, the amount of DNA in each case can be resolved by planimetry.

The change in density of DNA upon denaturation was studied by equilibrium centrifugation. DNA at a concentration of 20 μg/ml was heated at 100°C for 10 min in 1 × SSC (0.15 M NaCl, 0.015 M Na citrate), cooled quickly in an ice bath, the density adjusted to 1.700 g cm^{-3} with CsCl and centrifuged to equilibrium. The resultant buoyant densities are a measure of the homogeneity of the DNA's base composition.

RESULTS

DNA's from 18-hr embryos: Since 18-hr Drosophila embryos are easily obtained in large quantities, we chose to use the DNA extracted from this material for initial study. When the total DNA from these embryos is fractionated by density gradient centrifugation, the result seen in Figure 1 is obtained. The DNA separates into three classes, each of characteristic density: a main band DNA, M-DNA, $\rho = 1.699$ g cm^{-3} and two satellite DNA's, Class II, with $\rho = 1.685$ g cm^{-3}, and Class I, with $\rho = 1.669$ g cm^{-3}. The relative proportion of each density class is given in Table 1 for several wild-type strains that have been examined. When the areas under these curves are measured, the major peak, M-DNA, comprises 86% of the total DNA; the DNA of Class II, 11%; and the least dense DNA, Class I, 4%. The M-DNA is the most heterogeneous, i.e., the mean deviation of the Gaussian distribution of molecules from band center is .006 g cm^{-3} as compared to .004 g cm^{-3} for Class II DNA and .003 g cm^{-3} for Class I DNA.

The base composition of each class of DNA can be calculated as percent GC from its buoyant density in CsCl when this density is within the range 1.68–1.73 g cm^{-3} (SCHILDKRAUT *et al.* 1962). The M-DNA and Class II DNA's fall in this range and their base compositions have been calculated (Table 2). M-DNA has a base composition of 39.8% GC which is in close agreement with the base composition data for DNA extracted from nuclear pellets obtained from adult *Drosophila melanogaster* (HASTINGS and KIRBY 1966; RITOSSA *et al.* 1966). The Class II DNA has a base composition of 25.5% GC which is lower than that reported for the nuclear DNA's of higher organisms, but is similar to certain mitochondrial DNA's. such as that of yeast. The least abundant DNA, Class I, has a density which is less than that of the alternating poly dAT synthesized *de novo* by the *E.*

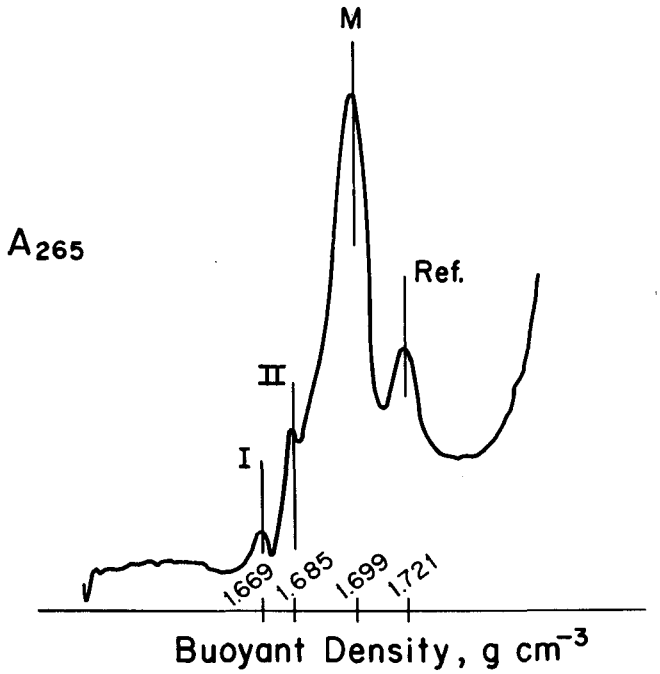

FIGURE 1.—A CsCl density gradient profile of the DNA from *D. melanogasetr.* 20 μgs of Drosophila DNA from pre-larval embryos and 3 μg of a reference DNA (*P. fluorens* DNA, $\rho = 1.721$ g cm^{-3}) were dissolved in 0.7 ml of CsCl, $\rho = 1.700$ g cm^{-3} and centrifuged to equilibrium, 20 hr at 44,770 RPM in a Spinco Model E analytical ultracentrifuge. The gradient was scanned on a recording densitometer and the buoyant densities of the DNAs calculated as described by VINOGRAD and HEARST (1962). The Roman numerals refer to the density classes of DNA, Class I $\rho = 1.669$ g cm^{-3}, Class II $\rho = 1.685$ g cm^{-3} and Class M $\rho = 1.699$ g cm^{-3}.

TABLE 1

Distribution of DNA's in 18-hr embryos of several wild-type strains of D. melanogaster

D. melanogaster wild-type strain	Percent DNA in each class		
	M	II	I
Oregon R-S	86.0	11.0	3.8
Canton S	81.0	13.0	6.0
Lausanne	83.0	12.0	5.0
Oregon R-M	85.0	10.0	5.0
Swedish C	81.0	13.0	6.0

The total DNA from the 18-hr embryos of each strain was run in a CsCl density gradient (see Figure 1). The DNA separated into three density classes: a main band DNA, M-DNA, $\rho = 1.699$ g cm^{-3}, and two satellite DNA's, Class II, $\rho = 1.685$ g cm^{-3} and Class I, $\rho = 1.669$ g cm^{-3}. The area under each peak was measured with a planimeter and expressed as the percent of the total DNA per embryo. The means and standard errors, S.E., for 10 samples of the Oregon R-S strain of embryo is: Class M DNA, 86 ± 6%; Class II, 11 ± 3%; and Class I, 3.8 ± 0.7%.

TABLE 2

Physical characteristics of D. melanogaster *DNA classes*

DNA	ρ g cm^{-3}	$\Delta\rho$ (denaturation) g cm^{-3}		Percent GC (Buoyant density)	Percent GC (Chemical analysis)
		Heat	Alkali pH 10.5		
Class M	1.699	+ 0.018	+ 0.045	39.8	40.0 (a)
Class II	1.685	+ 0.006	+ 0.036	25.5	—
Class I	1.669	0	+ 0.025	—	2.0 (b)
de novo d(A-T)	1.672	0	—	0	0
crab dAT	1.676	0	—	—	3.0 (c)

The classes of DNA are those of *D. melanogaster*, the *de novo* dAT is that synthesized by SCHACHMAN *et al.* (1960) and the crab dAT is the *C. borealis* dAT discovered by SUEOKA (1962b). The density, ρ, is the buoyant density of each DNA in CsCl. The percent GC is calculated from the DNA buoyant density using the formula of SCHILDKRAUT *et al.* (1962). The letters after the chemical analyses refer to: a) HASTINGS and KIRBY (1966), RITOSSA *et al.* (1966); b) FANSLER *et al.* (1971); c) SWARTZ *et al.* (1962).

coli DNA polymerase (WELLS and BLAIR 1967). In other studies, we have compared the density of the Class I DNA to that of the poly dAT or to the crab dAT extracted by SUEOKA and CHENG (1962b) which contains approximately 3% GC (Table 2). We obtain, using *P. fluorens* DNA as a marker ($\rho = 1.721$ g cm^{-3}), a density of 1.669 g cm^{-3} for Class I DNA as compared to a density of 1.671 g cm^{-3} for poly dAT and 1.676 g cm^{-3} for crab dAT. We have also replicated *D. melanogaster* dAT with *E. coli* DNA polymerase and analyzed its base composition and nearest neighbor frequencies (FANSLER *et al.* 1970). By this method, the Drosophila dAT satellite contains 83% alternating dAT (dA·T), 15% apposed dAT (dA·dT) and 2% dG·dC.

When DNA is denatured by heating at 100°C, rapidly diluted and cooled at 0°C and then analyzed on a CsCl gradient, the increase in density as ascertained by comparison of the relative peaks in a similar gradient containing the undenatured DNA is 0.018 g cm^{-3} for the M-DNA and 0.006 g cm^{-3} for the Class II DNA; the ρ of the Class I DNA does not change (Table 2). The density shift for renatured M-DNA is what is expected for a heterogeneous DNA. The fact that the Class II DNA density shift is less than that for M-DNA suggests that this DNA undergoes intrastrand reassociation. Class I DNA behaves like the synthetic poly dAT and the crab dAT inasmuch as it exhibits no increase in density after renaturation, suggesting essentially complete intrastrand reassociation as would be expected for dAT.

These classes of DNA can also be distinguished by alkaline denaturation; pH 10.5 increases the ρ of M-DNA by 0.045 g cm^{-3}, Class II DNA by 0.036 g cm^{-3}, and Class I DNA by 0.025 g cm^{-3} (Table 2). This agrees with VINOGRAD's *et al.* observation (1963) that density changes on alkaline titration increase with rising GC content until the pH exceeds 11, where the DNA becomes single-stranded and the density shift becomes independent of base composition. This density shift as a function of GC base composition has been attributed to the secondary structure imposed on the DNA by the G and C bases.

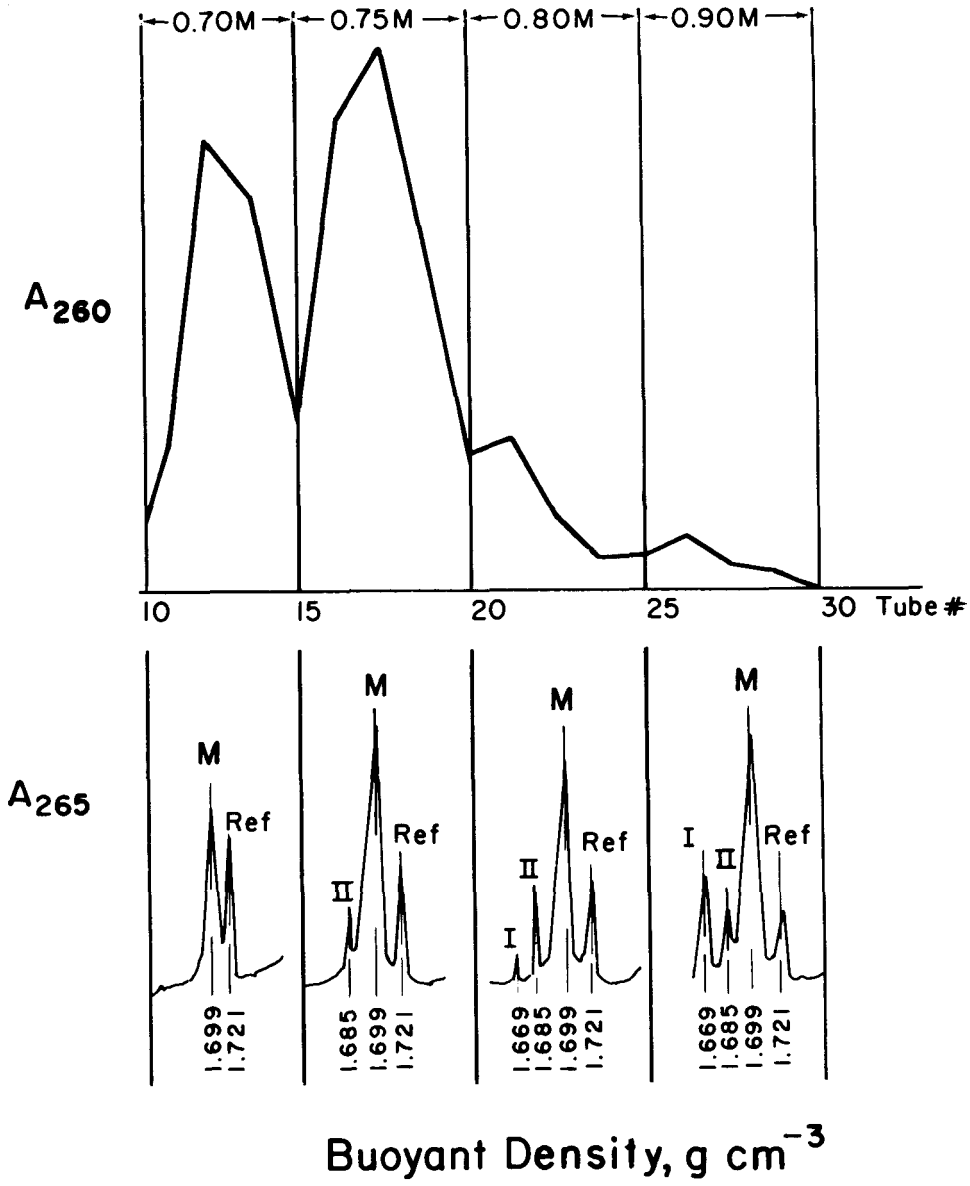

FIGURE 2.—Fractionation of the DNA from *D. melanogaster*. Above: a MAK column chromatogram of the total Drosophila DNA from pre-larval embryos. 250 μg of DNA were applied to a MAK column and eluted stepwise by increasing NaCl concentrations. Below: CsCl density equilibrium patterns for the nucleic acids obtained from the MAK eluates at each salt concentration. The density profiles were obtained as described in Figure 1.

Because of the novelty of the extraction method and the anomalous low density of the Class I DNA, it was necessary to verify that the buoyant density difference did reflect differences in the base composition of the DNA components.

TABLE 3

Amounts of RNA and DNA in D. melanogaster *embryos*

Age of embryos / Stage of development	Number of samples	RNA (μg/embryo ± S.E.)	DNA (mμg/embryo ± S.E.)
Unfertilized egg (from sterile mating)	14	.076 ± .001	0.45 ± 0.04
0–3 hr (Blastoderm)	9	.073 ± .003	1.5 ± 0.7
2–5 hr (Early gastrula)	4	.070 ± .003	2.0 ± 0.8
5–8 hr (Post-gastrula)	5	.079 ± .006	12.4 ± 6.3
15–18 hr (Pre-larva)	6	.083 ± .004	23.7 ± 6.4

SUEOKA and CHENG (1962a) have shown that on MAK columns, DNA with greater GC content is eluted at lower salt concentration. When the Drosophila DNA's were applied to a MAK column, the chromatogram shown in Figure 2 was obtained. The lower part of the figure shows an equilibrium buoyant density analysis for each major peak of the chromatogram. The Drosophila DNA's are eluted in order of decreasing GC content. Only M-DNA is eluted at 0.7 M NaCl (the same salt concentration as that reported for *B. subtilis* DNA which has a similar base composition). At 0.75 M NaCl, Class II DNA begins to elute while most of Class I DNA is not eluted until a concentration of 0.9 M NaCl is reached.

DNA's present during embryogenesis: The unfertilized egg contains 0.45 mμg (Table 3) or 2500 times more DNA than can be accounted for by one haploid nucleus; this value is based on the value of 0.18×10^{-12} gm DNA/haploid nucleus, as determined by RASCH, BARR and RASCH (1971). The various classes of DNA present in unfertilized eggs are shown in Figure 3. From these data we calculated the percent DNA in each class (Table 4). Twenty percent of this is M-DNA or an amount equivalent to that contained in 250 diploid nuclei. If the M-DNA is nuclear, this may mean that unfertilized eggs still contain high molecular weight

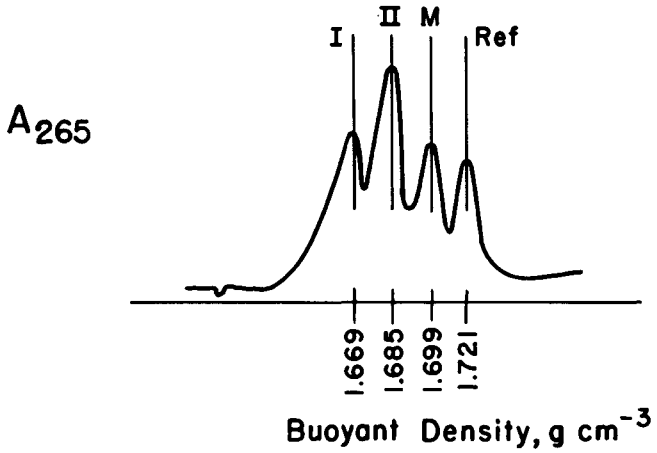

FIGURE 3.—A CsCl density gradient profile of the DNA from unfertilized eggs of *D. melanogaster*. The methods are those described in Figure 1.

TABLE 4

Distribution of DNA's in D. melanogaster *during embryogenesis*

Stages of development	Number of samples	Percent DNA in each class			Ratio II/I
		M	II	I	
Unfertilized egg	1	20.0	50.0	30.0	1.7
Blastoderm	2	81.0	13.2	5.4	2.4
Gastrula	6	85.0	11.0	4.2	2.6
Post-gastrula	7	87.0	10.0	4.7	2.1
Pre-larva	10	86.0	11.0	3.8	2.9

The three classes of DNA from embryos of *D. melanogaster* were separated on CsCl density gradients and their relative amounts calculated as described in Table 1.

DNA contributed by the polyploid nurse cell nuclei during oogenesis. The remaining 80% of the DNA present is in the form of Class II and Class I DNA's. It is clear that the satellites, compared to the M-DNA, are in large excess in the unfertilized egg.

The actual values of DNA per egg or embryo (Table 3) are lower than those reported elsewhere in the literature (MOHAN and RITOSSA 1970; TSIEN and WATTIAUX 1971). Other methods of extracting DNA's do not remove impurities such as pigments, etc., which result in higher DNA values when assayed by ultraviolet, colorimetric and fluorimetric analyses. The variations in the DNA content per egg at various stages of development probably reflect the variation in staging eggs during early embryogenesis rather than errors of the analytical methods. In spite of the large error in staging the embryos, there is a constant increase in the amount of total DNA during embryogenesis, although the amount of RNA per embryo remains relatively constant.

If the average age of 0–3 hr embryos is considered to be 1½ hr, the amount of DNA is higher than that expected on the basis of the number of nuclei present in such embryos. At this stage, the embryo should contain approximately 256 nuclei or .092 mμg DNA per embryo; the actual value is 1.50 mμg DNA. This surplus cannot solely be attributed to the Class I and Class II components as will be seen below. If 2% of the female flies held their fertilized eggs (Drosophila females do this under certain conditions, i.e., crowding, etc.) for as much as 3 hr, this excess of DNA would be accounted for. If this type of analysis is carried through to the 2–5 hr embryos, the value of the DNA per embryo is approximately correct. The values of the DNA per embryo at 5–8 hr and at 15–18 hr are closer to the expected value for the nuclear DNA in these embryos because the rate of nuclear division slows down markedly.

From the results of CsCl equilibrium gradients run with DNA extracted from embryos at various stages of development, the distribution and amount of each type of DNA at any one stage can be estimated. As can be seen in Table 4, the initial value of 20% for the M-DNA in the unfertilized egg increases upon fertilization to 81% at blastoderm and to 86% in the pre-larval stage. In the meantime, the ratio of the Class II DNA to the Class I DNA remains constant throughout

TABLE 5

The relative rates of increase in amounts of DNA's in D. melanogaster *during embryogenesis*

Stages of development	mμg of each class of DNA/embryo		
	M	II	I
Unfertilized egg	0.09	0.23	0.14
Blastoderm	1.22	0.22	0.08
Gastrula	1.70	0.22	0.08
Post-gastrula	10.79	1.24	0.62
Pre-larva	20.38	2.60	0.96

The amount of each class of DNA was calculated using the data from Tables 3 and 4.

embryogenesis (Table 4), although the actual amount of these satellites increases at the same rate as the M-DNA after gastrulation (Table 5). This ratio also remains constant in the adult fly: the amount of DNA per female fly is 0.07 ± .01 μg and for the male 0.05 ± .01 μg; both types of satellite are present in about the same amount in either sex; DNA II is approximately 5%, and DNA I, 3% of the total DNA. Again the ratio of DNA II/DNA I is approximately 2:1.

Recently, BLUMENFELD and FORREST (1971) have suggested that the concentration of dAT in *D. melanogaster* embryos can be correlated with the *Y* chromosome dosage in the genome of these embryos. Our own data (Table 4) indicate that in the unfertilized wild-type egg where no *Y* chromosome is present, 30% of the total DNA is dAT; upon fertilization this percentage decreases and by the pre-larval embryo stage is only 3.8% of the total DNA. Also, our measurements of the dAT in several inbred strains of wild-type Drosophila embryos range from 3.8% of the total DNA in Oregon R-S pre-larval embryos to 6.0% of the total DNA in pre-larval embryos of Canton S and Swedish C strains (Table 1). These data fall within the range of the dAT values found by BLUMENFELD and FORREST (1971) for the variation they attribute to the *Y* chromosome composition of the embryonic genome. On the basis of our results, it would appear that the dAT in the Drosophila embryo is not uniquely associated with the *Y* chromosome.

DISCUSSION

We have used a quantitative method to recover the total DNA in *D. melanogaster* and have shown that the DNA consists of three types: a bulk DNA, M-DNA, $\rho = 1.699$ g cm^{-3}, and two satellite DNA's, Class II, $\rho = 1.685$ g cm^{-3}, and Class I, $\rho = 1.669$ g cm^{-3}. The Class II and Class I DNA's appear to be in constant ratio to each other throughout development. In the fertilized egg, 80% of the total DNA is satellite DNA; this decreases to 15–18% during the first three hours after fertilization and then remains constant through embryogenesis. There is a concomitant increase of Class I and Class II DNA's with M-DNA after blastoderm formation.

Several investigators (LAIRD and MCCARTHY 1968; GALL, COHEN and POLAN 1971; BOTCHAN *et al.* 1971) have extracted DNA from crude nuclear pellets of

D. melanogaster tissue using the phenol extraction method of RITOSSA and SPIEGELMAN (1966) and have shown the presence of the M-DNA and Class II DNA. LAIRD (1968) and BOTCHAN (1971) have shown that the amount of Class II DNA extracted varied with the purity of the nuclear pellet and concluded that the Class II DNA was probably of cytoplasmic mitochondrial origin. RAE (1970) and GALL *et al.* (1971), by means of *in situ* hybridization, have shown at least some of the Class II DNA to be of nuclear origin, although GALL *et al.* (1971) do not exclude the possibility that there may be some cytoplasmic DNA of the same density. The M-DNA is considered by these investigators to be the major portion of the nuclear DNA. Class I DNA, which is primarily dAT, is probably soluble in phenol as is the synthetic poly dAT, and the crab dAT (MORGAN and WELLS 1968; SKINNER and TRIPLETT 1967; SKINNER *et al.* 1970) and would not have been detected using the phenol method of extraction. GALL *et al.* (1971) have detected trace amounts of the Class I DNA in CsCl extracts of larval tissue.

The Class I DNA is a remarkable polymer; FANSLER *et al.* (1970) have shown it to be composed of 83% alternating and 15% apposed adenine-thymine sequences. Similar types of deoxyribotide polymers have been reported in a variety of crab species (SUEOKA and CHENG 1962b; SMITH 1963, 1964; SWARTZ *et al.* 1962; SKINNER *et al.* 1967, 1970; ASTELL *et al.* 1969), and in the petite mutants of yeast (BERNARDI *et al.* 1968). dAT's in the crab are believed to be of nuclear origin, those of yeast to be of mitochondrial origin. To date, no one has found a function for this type of polymer *in vivo*. In the petite mutants of yeast, the presence of dAT seems to be involved with a serious dysfunction of the mitochondria; as the petite mutation becomes more extreme, more of the normal mitochondrial DNA appears to be replaced by a dAT polymer. This is not the case in *D. melanogaster*, where several strains of the wild-type fly appear to have a constant amount of the Class I DNA and have no mitochondrial dysfunction. Study of the DNA's in other species of Drosophila may indicate that the Class I DNA may be some evolutionary end-product in the evolution of the fly.

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