SATELLITE DNAs IN THE EMBRYOS OF VARIOUS SPECIES OF THE GENUS DROSOPHILA[1]

E. C. TRAVAGLINI, J. PETROVIC[2] AND J. SCHULTZ[3]

The Institute for Cancer Research, Fox Chase, Philadelphia, Pennsylvania 19111

Manuscript received May 23, 1972
Revised copy July 12, 1972

ABSTRACT

A tentative evolutionary pattern has been found for two classes of the multiple satellite DNA's found in the genus Drosophila. The satellite DNA's from five Drosophila species (*D. melanogaster, D. simulans, D. nasuta, D. virilis* and *D. hydei*) were analyzed and found to fall into three arbitrary CsCl buoyant density classes: Class I, $\rho = 1.661$–1.669 g cm^{-3}, DNA molecules composed of primarily dA and dT moieties; Class II, $\rho = 1.685$ and $\rho = 1.692$, DNA molecules of low GC content; and Class III, $\rho = 1.711$, a DNA of high GC composition. The dAT satellite DNA's appear in all the species studied except *D. hydei*, the species of most recent evolutionary divergence, whereas the heavy satellite appears only in the two species of most recent divergence, *D. virilis* and *D. hydei*.

THE DNA in *D. melanogaster* can be fractionated into three classes of molecules by buoyant density equilibrium centrifugation in CsCl (TRAVAGLINI, PETROVIC and SCHULTZ 1972): a main band DNA, M-DNA, $\rho = 1.699$ g cm^{-3}, and two satellite DNA's, Class I DNA, $\rho = 1.669$ g cm^{-3} which has been shown to be composed primarily of deoxyadenylate, dA, and thymidylate, dT, residues (FANSLER *et al.* 1970), and Class II DNA, $\rho = 1.685$ g cm^{-3} which contains all four deoxyribonucleotides but has a lower density than M-DNA. Since the phylogeny of the genus Drosophila has been established in considerable detail (THROCKMORTON 1962; STURTEVANT 1942), we felt that it would be of interest to study the evolutionary pattern of these satellites.

Using the phylogeny of Drosophila as depicted by THROCKMORTON, we chose five species from the genus to study. Their phylogenic relationship is depicted in Figure 1. *D. melanogaster* and its sibling, *D. simulans*, belong to the sub-genus Sophophora which branched from the main phylogenetic stem at an earlier time in evolution than the sub-genus Drosophila in which the other three species we studied arose. One of these species is *D. nasuta* which belongs to the immigrans group, closest in time to the sub-genus Sophophora but deriving directly from the main stem of the sub-genus Drosophila. Another is *D. virilis* which belongs to the virilis group and also derives from the main stem of the sub-genus but at a later

[1] Work supported by USPHS grants CA-01613, CA-06927 and RR-05539 from the National Institutes of Health, and by an appropriation from the Commonwealth of Pennsylvania.
[2] Present address: Institute for Biological Research, Beograd, Yugoslavia.
[3] Deceased April 29, 1971.

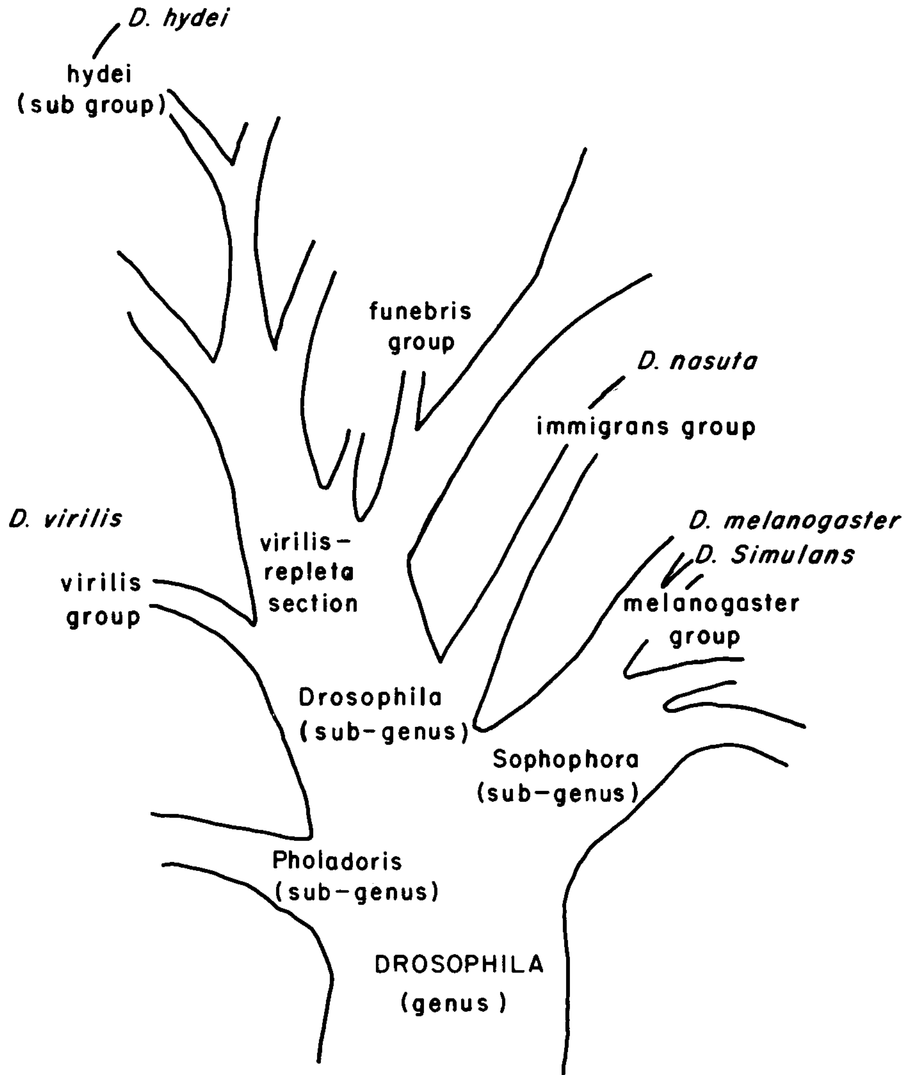

FIGURE 1.—A simplified version of the phylogeny of Drosophila as depicted by THROCKMORTON (1962) showing the relationships of the genera to the various phyletic lines within the genus.

time than immigrans. The most recently evolved species in this study is *D. hydei* which belongs to the virilis-repleta section of the sub-genus.

When fractionated in a CsCl equilibrium gradient, the DNA from each of these species of Drosophila appears to consist of a heterogeneous main band DNA, M-DNA, and one or more satellite DNA's. The buoyant density of the M-DNA is the same for all of the species studied, $\rho = 1.699$ g cm^{-3}. The densities of the satellite DNA's vary with the species; because of the multiplicity of these satellites, we have arbitrarily placed them into three classes: DNA I, a class of DNA's

whose low densities indicate that they consist mainly of dA and dT (WELLS and BLAIR 1967); DNA II, molecules which contain dG and dC but have the same or lower densities than M-DNA; and DNA III, a class of molecules with a higher dG,dC content, as reflected by their buoyant density, than M-DNA. DNA III is only present in the two species of most recent divergence, *D. hydei* and *D. virilis*.

MATERIALS AND METHODS

Biological material: The flies were bred and embryos collected as described for *D. melanogaster* in the preceding paper of this series (TRAVAGLINI, PETROVIC and SCHULTZ 1972) with the following changes: for collections of *D. nasuta* eggs, the molasses agar trays were coated with a heavy layer of yeast to facilitate egg collections. Fewer flies were mated when *D. virilis* and *D. hydei* were bred; six females and eight males were used per bottle. Their offspring took three weeks to hatch at 25°C; during the second and third weeks, the stock bottles had to be supplemented with fresh yeast (approximately 1 g/bottle/week).

DNA analyses: The extraction and analyses of the DNA from each species were the same as that described for *D. melanogaster* in the preceding paper (TRAVAGLINI, PETROVIC and SCHULTZ 1972).

RESULTS

When the DNA from the 18-hr embryos of the various species is centrifuged to equilibrium in neutral CsCl, results such as those shown in Figure 2 are obtained; the density gradient profiles of the DNA's are surprisingly different from each other, although M-DNA has the same buoyant density for all the species studied.

D. simulans, the sibling species of *D. melanogaster*, has a poly dAT of the same density as that of *D. melanogaster*; however, it does not contain the satellite DNA, $\rho = 1.685$ g cm^{-3}, which is found in *D. melanogaster*. Yet *D. simulans* does contain a satellite DNA of approximately the same density in CsCl as the more heterogeneous M-DNA. Such a cryptic satellite can be detected in the presence of M-DNA by denaturing the DNA at 100°C for 10′, rapidly cooling it to 0°C and determining its density in CsCl. The strands of a denatured DNA such as satellite DNA will reassociate more completely than those of a heterogeneous DNA and thus the satellite DNA will have a different density in CsCl than the M-DNA. When *D. simulans* DNA is treated in this manner, a satellite DNA, $\rho = 1.692$ g cm^{-3}, is made apparent on a CsCl gradient (Figure 3).

The density gradient profile completely at variance with all the species studied is that of *D. nasuta*; this species is closest to *D. melanogaster* in terms of evolutionary time although it belongs to a different sub-genus. There appear to be two types of poly dAT molecules, one of the same density as the low density satellite in *D. melanogaster*, and another whose density is 1.661 g cm^{-3}. Melting and reannealing the main band DNA present in *D. nasuta* does not disclose the presence of another satellite DNA.

D. virilis has four kinds of DNA; two have the same densities as those of *D. melanogaster*. However, the poly dAT has a slightly lower density (1.664 g cm^{-3}) than the *D. melanogaster* dAT, which is 1.669 g cm^{-3}, and there is also a DNA present whose density (1.711 g cm^{-3}) is indicative of a high dG, dC content. *D. hydei* also has this heavy dG,dC satellite but unlike the other species, it has no

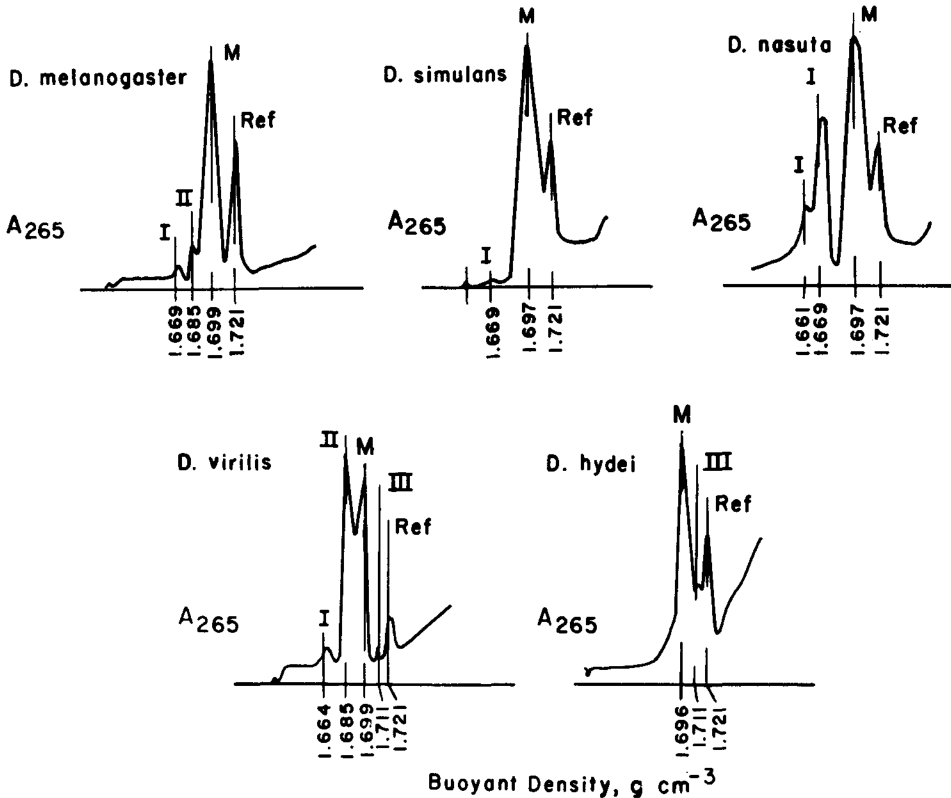

FIGURE 2.—Density gradient profiles of the DNA in five species of Drosophila. Approximately 20 μgs of DNA from the 18-hr embryos of each species of Drosophila DNA and 3 μg of a reference DNA (*P. fluorens* DNA $\rho = 1.721$ g cm^{-3}) were dissolved in 0.7 ml of CsCl $\rho = 1.700$ g cm^{-3} and centrifuged to equilibrium for 20 hr at 44,770 rpm in a Spinco Model E analytical centrifuge. The gradient was scanned on a recording densitometer and the buoyant densities of the DNA's calculated as described by VINOGRAD and HEARST (1962). The Roman numerals refer to the density classes of DNA. See text.

detectable poly dAT. Melting and reannealing its main band DNA reveals a satellite DNA whose density (1.691 g cm^{-3}) is similar to that of *D. simulans* (Figure 3).

The occurrence of a high dG,dC satellite in *D. virilis* and *D. hydei* made us question the possibility that it might have been a nucleic acid other than DNA, i.e., an RNA-DNA hybrid; however, MAK column chromatography of the DNA from *D. virilis* (Figure 4) shows that this heavy DNA behaves characteristically like a high dG,dC DNA insofar as it comes off the column at a low salt concentration (SUEOKA and CHENG 1962).

Table 1 shows how these DNA's are distributed in terms of the percentage of total DNA present in the 18-hr embryo of each species. It is apparent that the satellite DNA's in these Drosophila species fall into three arbitrary classes: DNA I, a type of DNA which is composed of over 95% adenine and thymine residues;

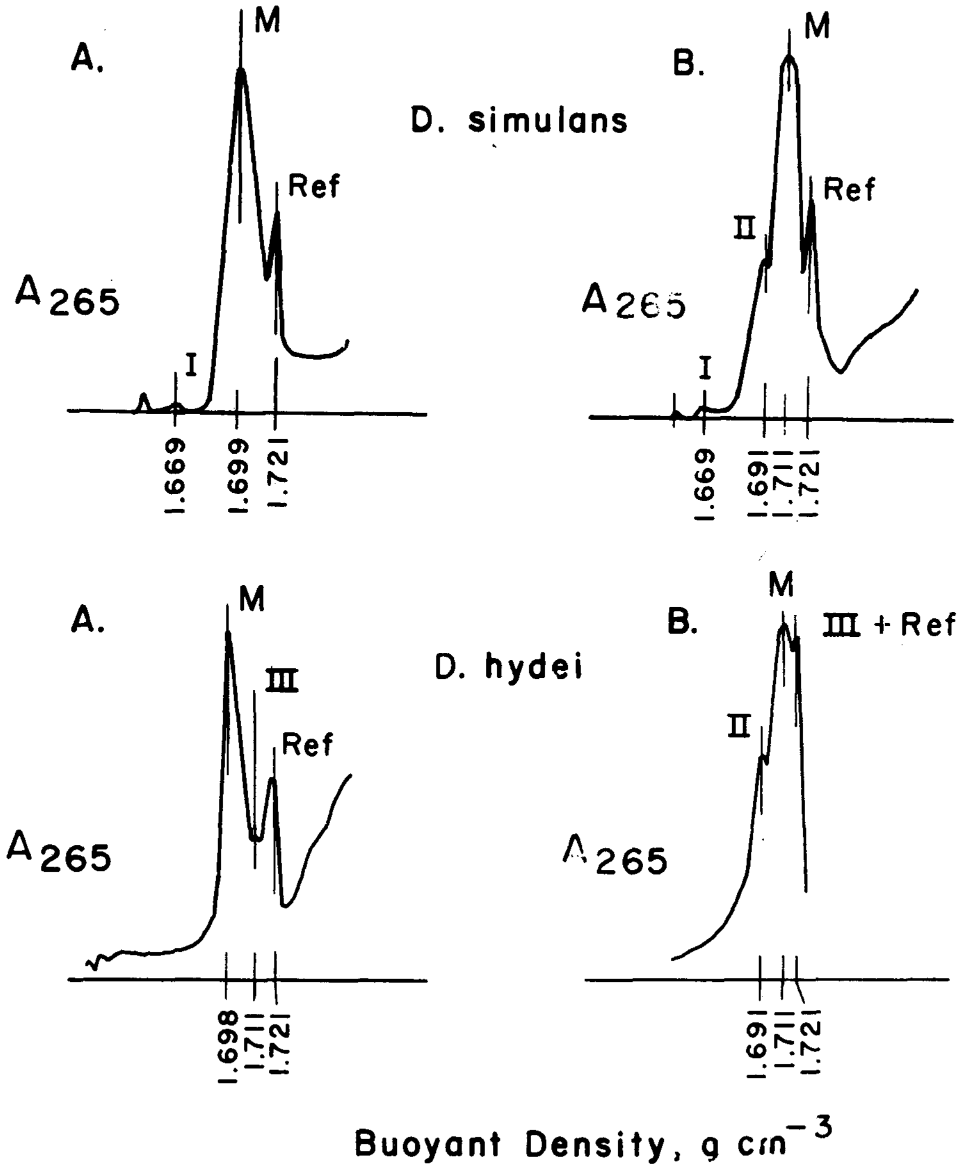

FIGURE 3.—Density gradient profiles of the DNA in two species of Drosophila DNA before and after thermal denaturation and reannealing. The density gradient profiles were obtained as described in Figure 2. Figure A for each species is the profile for the native DNAs. Figure B is the profile for the DNAs which have been heated at 100°C for 10 min in 1 × SSC (0.15 M NaCl, 0.015 M Na_3 citrate) and then quickly immersed in an ice bath prior to the addition of CsCl. The Roman numerals refer to the DNA density classes.

DNA II, a DNA with a low moiety of guanine, G, and cytosine, C, bases, which can be either of two densities, 1.685 g cm^{-3} or 1.692 g cm^{-3}; and DNA III, a DNA of high density which is indicative of a high GC base composition. In Table 1,

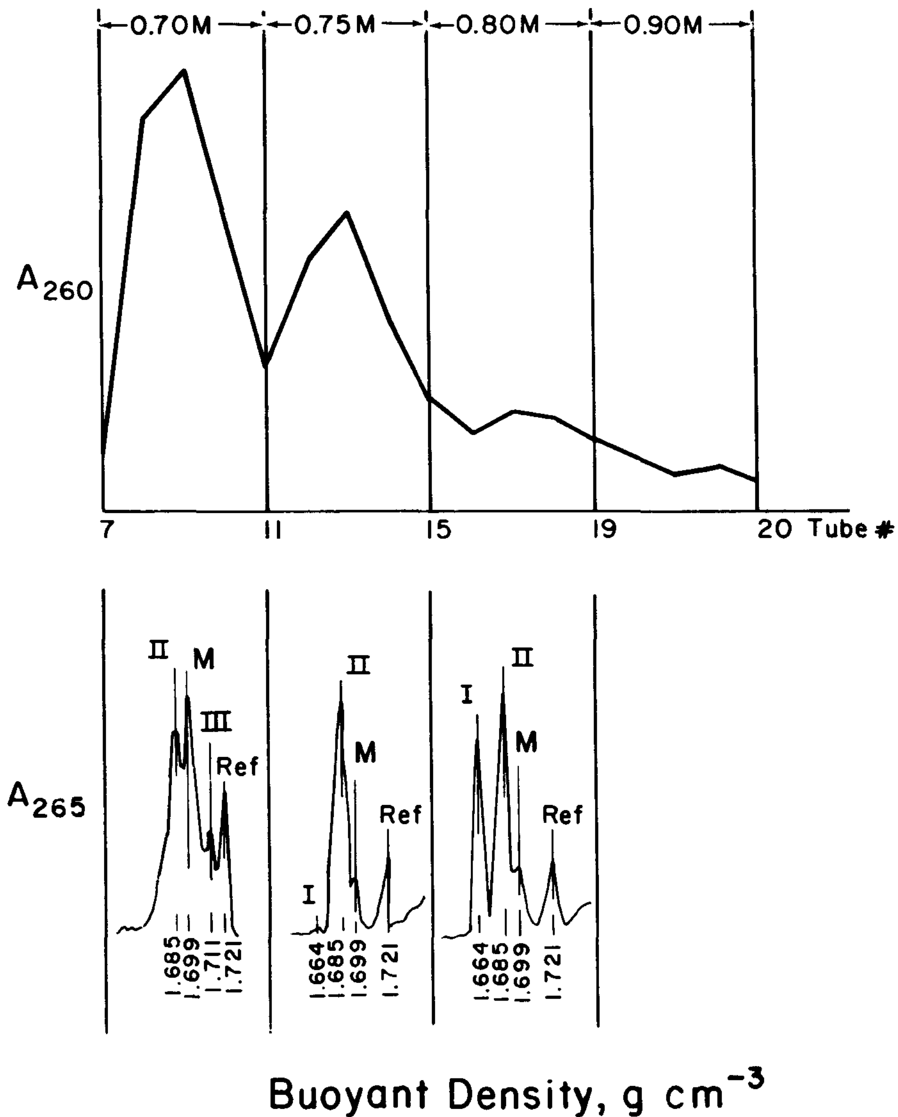

FIGURE 4.—Fractionation of the DNA from *Drosophila virilis*. Above: a MAK column chromatogram of the DNA from 18-hr embryos of *D. virilis*. 250 μgs of DNA were applied to a MAK column and eluted step-wise by increasing NaCl concentrations. Below: CsCl density equilibrium patterns for the nucleic acids obtained from the eluates at each salt concentration. The density profiles were obtained and the DNAs denoted as described in Figure 2.

where the species are listed from the most primitive to the most recent, it can be seen that poly dAT is present in all but the most recently evolved species to be studied, *D. hydei*. On the other hand, DNA III, rich in GC, is only present in the two species of most recent divergence.

DISCUSSION

The 18-hr embryos which we have chosen as our source of DNA for these studies do not represent the same stage of embryogenesis for all the species analyzed. *D. hydei* and *D. virilis* develop at approximately two-thirds the rate of the other three species. However, our results with *D. melanogaster* show that the ratios of the various DNA's to each other are relatively constant from fertilization to the pre-larval embryo (TRAVAGLINI, PETROVIC and SCHULTZ 1972), so we felt reasonably confident that, although the actual amounts of a particular DNA would change as development proceeded, the relative amounts would not.

The M-DNA has been extracted from purified nuclear pellets and salivary gland nuclei from various species of Drosophila by several investigators (TRAVAGLINI, PETROVIC and SCHULTZ 1968; LAIRD and MCCARTHY 1968, 1969; HENNIG, HENNIG and STEIN 1970; GALL, COHEN and POLAN 1971; DICKSON, BOYD and LAIRD 1971) and considered to be of chromosomal origin. It will not be discussed in detail here. Three types of Class I DNA, which is primarily of dA and dT, can be distinguished by density gradient centrifugation. The three most primitive species studied have a dAT similar to the *D. melanogaster* dAT, which consists of 83% d(A·T), 15% (dA·dT) and 2% (dG·dC) (FANSLER *et al.* 1970). Besides this dAT, *D. nasuta* has another type dAT whose density is 1.661 g cm^{-3}, a density which by analogy might indicate that approximately half of the molecule consists of (dA·dT) sequences. The poly dAT found in *D. virilis* has an intermediate density which would likewise indicate that it has more apposed sequences than are present in *D. melanogaster* dAT. In three of these species, DNA I is approximately 4% of the total DNA in the embryo; in *D. nasuta* this class of molecules makes up 30% of the total DNA and in *D. hydei* there is no detectable dAT.

Since only two bases are involved in this class of molecules, the buoyant density in CsCl could reflect differences in the base sequences. WELLS and BLAIR (1967) have shown that the densities of the synthetic polymers poly d(A·T) and poly (dA·dT) are very different; the alternating dAT has a density of 1.672 g cm^{-3}, whereas the apposed dAT has one of 1.638 g cm^{-3}. The results of FANSLER *et al.* (1970) show that the CsCl density of *D. melanogaster* dAT, ρ 1.669 g cm^{-3}, a natural dAT polymer, reflects its base sequence as determined by nearest neighbor analysis. In Drosophila these dAT molecules are composed of both alternating and apposed sequences; it would be possible for them to furnish messages which would code for proteins containing six possible amino acids: UAU, tyrosine; UUA, leucine; AUU, or AUA, isoleucine; AAU, asparagine; UUU, phenylalanine; and AAA, lysine. Insects incorporate poly-aromatic amino acids into their structure (BRUNET 1965), particularly in the structural proteins of their cuticle and hinge joints. It may be that the poly dAT's are actively involved in the formation of poly-aromatic amino acids such as poly-tyrosine or poly-phenylalanine which contribute to the formation of the Drosophila's cutaneous skeleton.

The Class II DNA's, although they appear to fall into distinct density types, do not seem to follow any evolutionary trend in terms of species divergence. They all appear to be localized in the cytoplasm and will be discussed in greater detail in a subsequent paper of this series.

TABLE 1

Distribution of DNA's in five species of Drosophila

Sub-genus	Group	Species	Number of samples	Percent DNA in each class: I			II		M	III
				1.661 ± .001	1.664 ± .001	1.669 ± .001	1.685 ± .001	1.692* ± .001	1.698 ± .002	1.711 ± .000
Sophophora	*melanogaster*	*melanogaster*	5	—	—	4	10	—	86	—
		simulans	4	—	—	3	—	19	78	—
Drosophila	*immigrans*	*nasuta*	3	7	—	23	trace	—	70	—
	virilis	*virilis*	5	—	4	—	47	—	45	4
Drosophila (*virilis–repleta* section)	*hydei*	*hydei*	4	—	—	—	2	27	58	13

* Density of these DNA's taken after native DNA was melted and reannealed.

The high GC DNA, DNA III, which is found in *D. virilis* and *D. hydei*, appears to be of nuclear origin. We have made no attempt to localize it in the cell. HENNIG, HENNIG and STEIN (1970) have shown that this class of DNA from *D. neohydei* is localized at the kinetochore and at various sites throughout the genome of *D. neohydei*. They have done similar hybridization experiments for *D. pseudoneohydei* and found hybridization only at the kinetochore. It is interesting to note that this class of DNA is found in our study only in the two species of most recent occurrence in the genus Drosophila.

The suggestions and discussion of Drs. T. F. ANDERSON, J. GEARHART, L. LOEB and R. PERRY have proven invaluable during the preparation of this manuscript.

LITERATURE CITED

BRUNET, P. C. J., 1965 The metabolism of aromatic compounds. pp. 49–77. In: *Biochem. Soc. Symposia No. 25*. Academic Press, New York.

DICKSON, E., J. B. BOYD and C. LAIRD, 1971 Sequence diversity of polytene chromosome DNA from *Drosophila hydei*. J. Mol. Biol. **61**: 615–627.

FANSLER, B. S., E. C. TRAVAGLINI, L. A. LOEB and J. SCHULTZ, 1970 Structure of *Drosophila melanogaster* dAT replicated in an *in vitro* system. Biochem. Biophys. Res. Commun. **40**: 1266–1272.

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

HENNIG, W., I. HENNIG and H. STEIN, 1970 Repeated sequences in the DNA of Drosophila and their localization in giant chromosomes. Chromosoma **32**: 31–63.

LAIRD, C. D. and B. J. MCCARTHY, 1968 Magnitude of interspecific nucleotide sequence variability in Drosophila. Genetics **60**: 303–322. ——, 1969 Molecular characterization of the Drosophila genome. Genetics **63**: 865–882.

STURTEVANT, A. H., 1942 The classification of the genus Drosophila with descriptions of nine new species. University of Texas Publication No. **4213**: 5–51.

SUEOKA, N. and T. Y. CHENG, 1962 Fractionation of nucleic acids with the methylated albumin column. J. Mol. Biol. **4**: 161–172.

THROCKMORTON, L. H., 1962 The problem of phylogeny in the genus Drosophila. University of Texas Publication No. **6205**: 207–343.

TRAVAGLINI, E. C., J. PETROVIC and J. SCHULTZ, 1968 Two satellite cytoplasmic DNA's in Drosophila. J. Cell Biol. **39**: 136a. ——, 1972 Characterization of the DNA in *Drosophila melanogaster*. Genetics **72**: 419–430.

VINOGRAD, J. and J. E. HEARST, 1962 Equilibrium sedimentation of macromolecules and viruses in a density gradient. Fort. der Chemie organischer Naturstoffe **20**: 372–422.

WELLS, R. D. and J. E. BLAIR, 1967 Sedimentation and buoyant density studies of some DNA-like polymers with repeating nucleotide sequences. J. Mol. Biol. **27**: 273–288.