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THE DNA SEQUENCES OF SIX ENDEMIC HAWAIIAN DROSOPHILA SPECIES

By

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Abstract : *DNA was extracted from the following six Hawaiian Drosophila species: D. crucigera, D. pilimana, D. engyochracea, D. silvarentis, D. picticornis and D. mimica. Examinations of the DNAs in neutral CsCl gradients reveal distinct satellite bands, except in D. crucigera. The proportion of the satellite DNA relative to the main DNA varies among the different species. The buoyant densities of the main band as well as of the satellites were calculated. The G+C composition of each species was inferred from thermal denaturation of native DNA and analytical density gradient centrifugation in neutral CsCl.*

Three classes of nucleotide sequences can be recognized in the genome of the studied species: (a) a very rapidly renaturing fraction comprising from 9% (D. crucigera) to 24% (D. pilimana) of the genome, (b) a moderately reassociating fraction representing from 12% (D. pilimana) to 25% (D. mimica) of the genome and having repetition frequency from 49 (D. picticornis) to 484 (D. engyochracea), and (c) a slowly reassociating fraction comprising from 60% (D. silvarentis and D. mimica) to 76% (D. crucigera) of the genome. From the results it is obvious that the percentage of the very highly reassociating fraction is correlated directly to the amount of the satellite DNA of each species.

Kinetic fractions II (intermediate) and III (non-repetitive) were prepared from total cell DNA and reassociated separately. The corrected rate constant and the kinetic complexity for these fractions were estimated. Based on the reassociation kinetics of the non-repeated DNA sequences, the genome size of the Hawaiian Drosophila species were calculated. The genome size of these species varies from 6.6×10^{10} *daltons (D. engyochracea) to* 10.7×10^{10} *daltons (D. mimica).*

INTRODUCTION

The family Drosophilidae is most remarkably developed in the Hawaiian islands, and represents one of the most unusual faunas in the world. About one-fourth of the described species of Drosophila

are endemic to the Hawaiian archipelago (Wheeler and Hamilton, 1972). A remarkable combination of habital, behavioral, electrophoretical, cytogenetical and other studies is being applied to understanding the evolutionary processes that have produced the extraordinary diversity of the Hawaiian Drosophilidae (see Carson et al., 1970). In order to understand more fully the evolutionary processes of the Hawaiian Drosophila species it is necessary to analyze the molecular composition of their nuclear DNAs. In this paper we report our results on the reassociation kinetics, the multiplicity and the density of the DNAs of six Hawaiian Drosophila species.

MATERIALS AND METHODS

Material

Six endemic Hawaiian Drosophila species (Carson et al., 1970) were used in this investigation. They were obtained from the National Drosophila Species Resource, University of Texas at Austin. The species used, their code numbers, taxonomy, and geographic origin are shown in Table I. All species were cultured as previously described by Wheeler and Clayton (1965).

TABLE I

Species examined in this study

Species	Subgroup	Group*	Stock. No.	Collection Locality
D. crucigera	grimshawi	P.W.	C63.4	Pupukea, Oahu
D. pilimana	pilimana	P.W.	C53.3	Tantalus, Oahu
D. engyochracea	orphnopeza	P.W.	J39C2	Upper Olaa, For. Hawaii
D. silvarentis	hawaiiensis	P.W.	K18M3	Humuula, Hawaii
D. picticornis	planitibia	P.W.	M.11J3	Kokee, Kauai
D. mimica	mimica	M.M.	Q.73M	Kapuka Ki, Hawaii

*P.W. = Picture-Winged
M.M. = Modified Mouthparts

Methods

Preparation of DNAs: Pupae were collected and stored at - 70°C until sufficient quantities were obtained for DNA extraction and purification (Lagowski et al., 1973). The DNA in 0.12M sodium phosphate

buffer (PB) was sheared with an Aminco pressure cell (14,000 psi, 1ml/min) into fragments averaging a length of 500 base pairs. The sheared DNA was then loaded on a hydroxyapatite column (HAP, Clarkson Chemical Co.) at 60°C, washed with five bed volumes of 0.15M PB, pH 6.8, and eluted with 0.35M PB. The double-stranded DNA was heat-denatured in 0.12M PB (15 min at 100°C), and then incubated at 60°C to Cot_{10}. Repeated sequences (double strands) were then separated from single copy sequences (single strand, SS) by HAP chromatography (Britten and Kohne, 1968). The repeated sequence fractions were concentrated and dialyzed against 0.12M PB, after which they were again heat-denatured, and reassociated in 0.12M PB at 60°C to a $Cot_{0.05}$ (Wu et al., 1972; Manning et al., 1975) for the separation of the highly repeated (double strands, HR) and intermediate repeated (single strands, IR) sequences by HAP chromatography.

The purity and concentration of the DNA solutions were determined by their absorption spectra (47μg native DNA/ml = 1 A). All total DNAs exhibited 260nm/230nm and 260nm/280nm ratios of 2.4 and 2.0, or greater, respectively, and sharply melted with a hyperchromicity of about 35 percent (Gilford 250 Spectrophotometer).

The yields of DNA varied within different Drosophila species. The yields from D. crucigera averaged about seven mg of purified DNA per 100gr net weight of frozen pupae, while the yields from the other Drosophila species were about two times greater.

DNA from Bacteria: Escherichia coli DNA (strain BB Thy⁻, labeled with thymidine H^3) and Micrococcus luteus DNA (courtesy of Dr C.S. Lee and his associates, University of Texas at Austin) were extracted by the procedure of Marmur (1961). The E. coli DNA was sheared to an average fragment length of 500 nucleotides, and had a specific activity of 38,600 cpm/μg DNA. Labeled material was counted with a Beckman LS-100C scintillation counter.

Analytical ultracentrifugation: Buoyant densities were determined by equilibrium centrifugations in CsCl using a Spinso model E analytical ultracentrifuge. Approximately 2 to 3 μg of Drosophila DNA and 1 μg of Micrococcus luteus DNA, as reference (p=1.731 gr/cm³), were mixed and centrifuged in neutral CsCl solutions at 44,770 rpm for 22

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hours at 25° C using an ANF rotor. Buoyant densities were calculated according to Szybalski (1968), while the G+C content was calculated according to Schildkraut et al. (1962). The satellite DNA profiles were examined also in crude extracts from brains, using the procedure of Cordeiro et al. (1975).

Determination of molecular weights: Molecular weights of sheared DNA samples were determined by boundary velocity sedimentation in a Spinco model E analytical ultracentrifuge. The bulk medium contained 0.9 M NaCl, 0.1 M NaOH, and DNA at concentration of 20 to 30 μg/ml. The velocity sedimentation was done at 44,770 rpm and 20° C. The equation of Studier (1965) was used to convert sedimentation coefficients to molecular weights.

DNA reassociation rate measurements: DNA/DNA renaturation was carried out in solution. In some experiments, sheared ^{3}H-labeled E. coli DNA was used as an internal standard. DNA samples were heat-denatured in an ethylene glycol bath for fifteen nimutes at 100° C. The samples were immediately transfered to a water bath at 60° C and were allowed to renature to the desired Cot-value. Separation of single-stranded molecules from molecules bearing duplex regions was performed by hydroxyapatite chromatography using stepwise elutions with 0.12 M PB and 0.5 M PB at 60° C in a water-jacketed chromatography column. The general procedures used were those described hy Britten and Kohne (1968). The data were then plotted as equivalent Cot (E Cot) versus percent DNA bound to HAP. Renaturation proceeded usually in 0.12 M PB. When renaturation took place in higher molarity buffers, the equivalent Cot was calculated according to Britten et al. (1974). The kinetic parameters of the reassociation were calculated as described by Britten et al. (1974) on the CDC 6600/6400 computer at the University of Texas at Austin. The rate constants (K) obtained for each DNA fraction were then corrected for the number of nucleotides per DNA fragment (Wetmur and Davidson, 1968); they were not corrected for any possible effects of differing G+C content, since contradictory reports of such effects have been published (Wetmur and Davidson, 1968; Gillis et al., 1970).

Melting temperature measurements: Thermal denaturation of DNA samples was carried out using the hydroxyapatite column chromatography method of Lagowski et al. (1973). Then, the G+C content was calculated according to the formula of Marmur and Dotty (1962) from the Tm value of the total DNA.

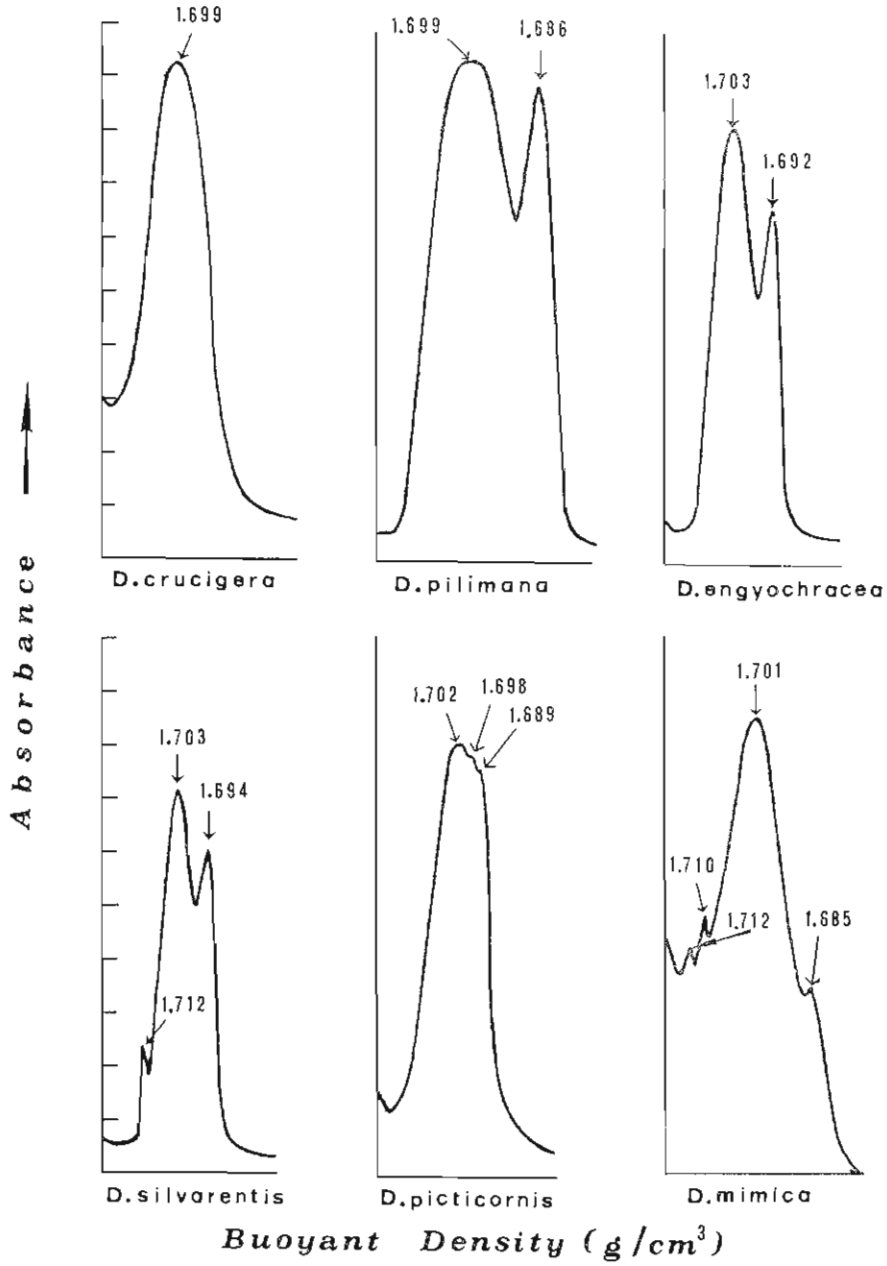

Fig. 1. Analytical CsCl gradient equilibrium ultraccntrifugation of unsheared total DNA of Hamaiian Drosophila species. Band densities were calculated relative to M. luteus DNA (p = 1.731), aeeording to Szybalski (1968).

RESULTS

Buoyant density

The purified unsheared total DNA of the six species studied was analyzed in neutral CsCl sedimentation gradients. Figure 1 shows the microdensitometric tracing profile after analytical centrifugation, while Table II shows the buoyant density of the various bands found in these species. The density distributions revealed one major band for D. crucigera and, in addition to that, one or more satellite DNA components for each of the other Hawaiian species. Using brains and imaginal discs of D. crucigera, we still did not observe any satellite DNA. Therefore, satellites were not lost in this species during purification.

TABLE II

Melting Point, Density and Base Composition

DNA Source	Total DNA		Main Band		Satellite DNA	
Species	Tm*	Percent G+C	Buoyant Deusity	Percent G+C	Buoyant Density	Percent G+C
D. crucigera	88.0	45	1.699	40	-	-
D. pilimana	88.3	45	1.699	40	1.686	27
D. engyochracea	89.9	49	1.703	44	1.692	33
D. silvarentis	90.0	49	1.703	44	1.694	35
					1.712	53
D. picticornis	90.5	50	1.702	43	1.689	30
					1.698	39
D. mimica	89.0	47	1.701	42	1.685	26
					1.710	51
					1.712	53

* Tm is the temperature at which 50% of the DNA is eluted (HAP column).
Note: Figures were rounded after final calculations.

The relative amounts of the satellite DNA have been determined from several gradient profiles obtained by analytical ultracentrifugation. The total satellite DNA fractions represent approximately 13% to 17% of the total DNA of diploid tissues of D. pilimana, 12% to 16% of D. engyochracea and D. silvarentis, 6% to 8% of D. picticornis, and 2% to 5% of D. mimica.

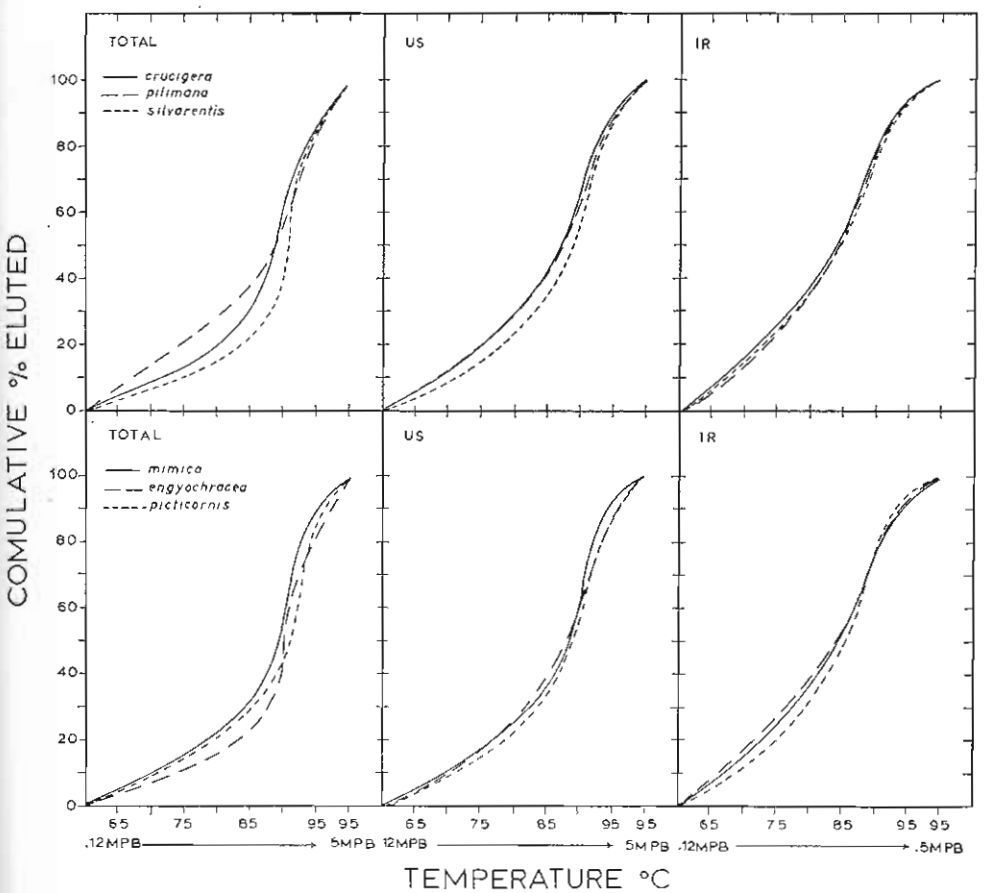

Fig. 2. HAP thermal denaturation profiles of sheared total DNA and reassociated non-repetitive (US) and intermediately (IR) DNA sequences in six Hawaiian Drosophila species.

Thermal denaturation profiles

Melting profiles were obtained for sheared total (native) DNA, and renatured fraction III (unique) and II (intermediate) DNA. The melting behavior of the previous DNAs is portrayed in Fig. 2, while in Table II, Tm values are presented only for total DNAs. Examinations of the melting curves reveal that the thermal stability (Tm value) of homoduplexes of unique DNAs is higher than that of the homoduplexes from the intermediate DNAs. Similarly, the Tm values of

the unfractionated native DNAs are higher than those of the unique DNAs. Differences of Tm values of native and renatured fractions II and III (Δ Tm) reveal about 1.8° C decrease in thermal stability of the homoduplexes of the unique fraction and about 5.1°C for the intermediate fraction. In other words, a comparison between the ΔTm (total-unique) and ΔTm (total-intermediate) sequences indicates that the ΔTm for the reassociated intermediate DNAs is about three or four times greater than that of the reassociated unique DNAs. This decrease in melting temperatures of the reassociated intermediate and unique DNAs probably results from base mismatching (Britten and Kohne, 1968; Renjekar et al., 1974).

DNA base compositions

The percent G+C content of each species was inferred from buoyant density determinations by analytical CsCl centrifugation and from the Tm value of unsheared native DNA. The DNA base compositions of the six species are shown in Table II. In general, there is a relatively good agreement between the G+C content estimated from the Tm value of the native DNA and from the buoyant densities of the main band (about 5% higher estimates are obtained for G+C base composition from the estimation based on Tm).

Reassociation kinetics

The total DNA was denatured, incubated to Cot_{10} and the repetitive (rapidly reassociating) and non-repetitive (slowly reassociating) fraction were recovered on hydroxyapatite as double and single stranded components respectively (see Materials and Methods for the details). The repetitive fraction was further divided into highly repetitive and moderately repetitive fractions by denaturing the DNA solution to $Cot_{0.05}$ as described in the Methods. The non-repetitive and the moderately repetitive fractions (but not the highly repetitive) were collected, concentrated and used for further reassociation kinetic experiments (Fig. 3a and 3b).

The relative amounts and Cots of the DNA fractions described above for the genomes of the studied species are given in Table III. The proportion of the different fractions shows some variation between the species, particularly for repetitive sequence fractions. Drosophila crucigera is especially unusual in having small amounts of re-

TABLE III

Fractions of Total DNA, Redundancy and Genome Complexity Determined by Reassociation Kinetics

Species	Fraction I Cot<0.05	Fraction II 0.05<Cot<10		Fraction III Cot > 10		Minimum Genome Size	
	%	%	Redundancy*	%	Redundancy	Relative to E. coli Haploid Genome	Minimum Size of Haploid Genome (dalton) x 10^{10}
D. crucigera	9	15	64	76	1	27	7.3
D. pilimana	24	12	**	64	1	31	10.0
D. engyochracea	20	17	484	63	1	24	6.6
D. silvarentis	22	18	266	60	1	36	9.7
D. picticornis	20	19	49	61	1	32	8.5
D. mimica	15	25	159	60	1	40	10.7

* Calculated according to Britten et al. (1974).

** There were no calculations.

Note: Figures were rounded after the final calculations.

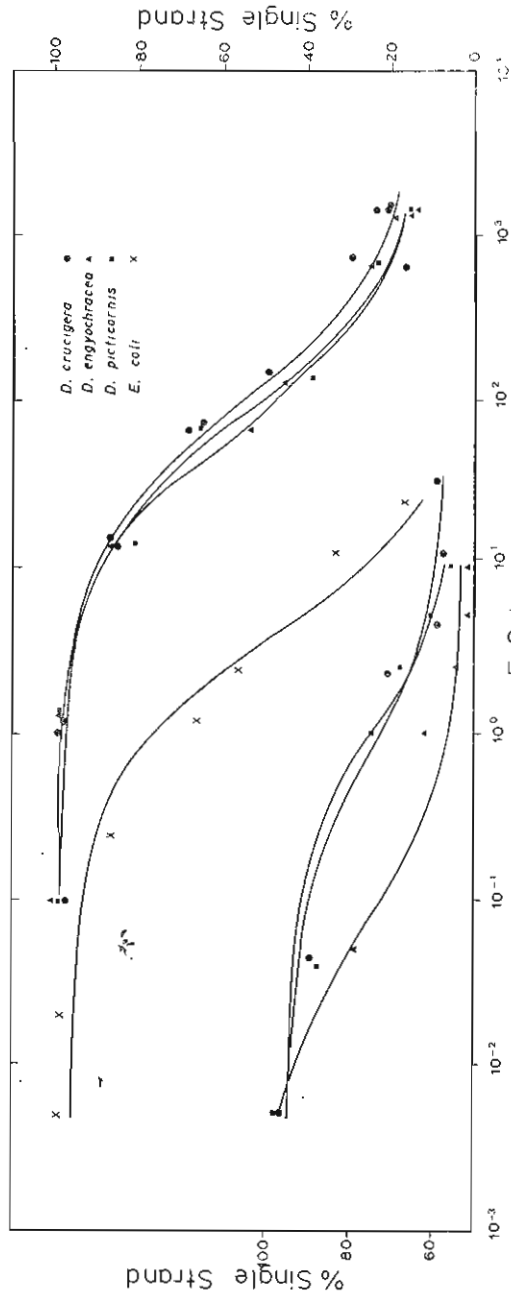

Fig. 3a. Computer derived reassociation profiles of the nonrepetitive DNA fraction (upper) and $0.05 < Cot < 10$ DNA fraction (below) from different Drosophila species compared with E. coli DNA. DNA concentrations (μg/ml) during the reaction were as follows: D. crucigera (U.S. 1280; I.R. 20), D. engyochracea (U.S. 775 and I. R. 16), D. picticornis (U.S. 784 and I. R. 15), E. coli (170). Note: US = non-repetitive fraction and I.R. = $0.05 < Cot < 10$ DNA fraction.

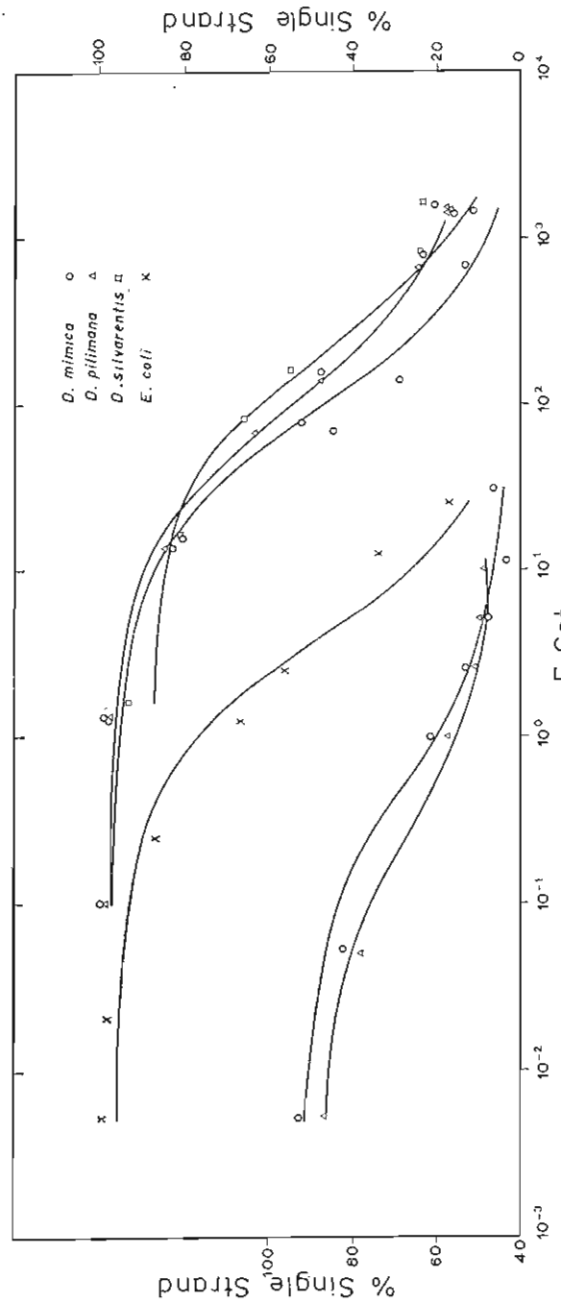

Fig. 3b. DNA concentrations during the reaction were: D. mimica (U.S. 890 and I.R. 26), D. pilimana (U.S. 840) and D. silvarentis (U.S. 860 and I.R. 25).

petitious sequences. Furthermore, it is worth-while to mention that species having large amounts of satellite DNA., have higher percentage of highly repetitive DNA.

(a) Non-repetitive fraction

Figs 3a and 3b show Cot curves for the slowly reassociating fraction of the species studied. These Cot curves were fitted to the data by the least square procedure of Britten et al. (1974) modified for a CDC 6600/6400 computer. The kinetic curves are of second order as expected from single copy DNA sequences. ^3H- labeled E. coli DNA was added to the kinetically derived single copy DNA during reassociation to provide an internal kinetic standard.

Reassociation of E. coli DNA extends over a narrow range of Cot units. Approxinately 96% of the E. coli DNA was bound to hydroxyapatite at 60° C in 0.12 M PB at a Cot about 24. From the other hand the reassociation curves of Drosophila unique DNAs are much broader and extend over a very wide range of Cot values. Approximately 10% of these DNA sequences remained dissociated even at a Cot of 1500. From Figs 3a and 3b it is obvious that the reassociation profiles of the studied species is quite similar.

Table IV gives the data obtained from the reassociation kinetics (Fig. 3) of E. coli DNA and the unique DNA sequences of each Hawaiian Drosophila species. The reassociation kinetics of E. coli DNA, that we included as an internal control with the unique DNA from D. crucigera, had an observed rate constant equal to 0.247 $M^{-1}.s^{-1}$. Since Cot $1/2=K^{-1}$, E. coli has a Cot 1/2, under our conditions, which is equal to 4. The corrected reassociation rate constant for E. coli is 0.258 $M^{-1}.s^{-1}$ a value similar to that obtained by Chattopadhyay et al. (1972) and Dusenbery (1975). The kinetic complexity of E. coli DNA was estimated to be $4.3x10^6$ nucleotide pairs. This value is similar to other values reported (Cairns, 1963; Laird and McCarthy, 1969). However, we will use kinetic complexity value $4.5x10^6$ nucleotide pairs or 2.7 $x10^9$ daltons (Cairns 1963, Laird and McCarthy, 1969), because the kinetic complexities for most Drosophila species in the literature are based on this figure.

An estimate of the kinetic complexity of the non-repetitive DNA fraction of the six Hawaiian Drosophila species was calculated according to Jordan and Brosemer (1974) (but see the section Material and Methods). The estimates are given in Table IV. From these results

TABLE IV

Estimates of Reassociation Parameters from Hawaiian Drosophila Species

Species	Slowly Reassociating Component					
	Proportion of «US» Fraction of DNA Assayed	$Cot_{1/2}$ (observed) (M.s)	K (observed) $(M.s)^{-1}$	K* (pure) $(M.s)^{-1}$	Relative to E. coli Kinetic Complexity	Kinetic Complexity (daltons) $x10^{10}$
E. coli	.96	4	.247	.258	1	.27*
D. crucigera	.84	94	.011	.013	20	5.47
D. pilimana	.88	125	.008	.010	23	6.40
D. engyochracea	.86	69	.015	.017	15	4.13
D. silvarentis	.85	98	.010	.012	21	5.75
D. picticornis	.87	85	.012	.013	19	5.17
D. mimica	.97	95	.011	.011	24	6.40

* Refer to Laird and McCarthy (1969) and Cairns (1963).
Note: Figures were rounded after the fiual calculations.

it is obvious that the kinetic complexity of the non-repetitive DNA of the Hawaiian Drosophila species is 15 to 23 times greater than that of E. coli.

The total haploid genome size of the Hawaiian Drosophila DNAs is estimated by the relation:

$$G_i = \frac{g_i \text{ unique Drosophila}}{f}$$

where G_i is the genomic kinetic complexity of a Drosophila species; g_i unique Drosophila is the kinetic complexity of the non-repetitive fraction for each Drosophila species (Table IV), and f is the fraction of the total genome which comprises the unique DNA. The results obtained are shown in Table III. Therefore, the minimum amount of DNA in the haploid genome of Hawaiian Drosophila species is about 24 to 40 times that of E. coli or from 6.6×10^{10} daltons to 10.7×10^{10} daltons. These values are in agreenent with the values reported for other Drosophila species (see Laird, 1973).

(b) Intermediate repetitive fraction

In order to determine the kinetic complexity and the degree of repetition of the isolated $0.05 < Cot < 10$ DNA fraction of the studied Drosophila species, reassociation kinetic studies were undertaken. Figures 3a and 3b (below) show the computer-derived reassociation profiles for that compoment. The kinetics of reassociation of that fraction reveals that it contains primarily moderate repetitious DNA sequences: in addition it contains some slowly reassociating and perhaps some highly repetitive DNA sequences. Further, the kinetic curves show that a large portion of that component fails to reassociate or to form duplex-containing structures precociously, at the lowest Cots studied.

From Table V it is seen that the isolated $0.05 < Cot < 10$ DNA fraction has corrected K values ranging between 2.10 and 29.62 $M^{-1}.s^{-1}$, which correspond to kinetic complexities of 0.24×10^8 daltons to 3.31×10^8 daltons. These complexities range from about 1% (D. picticornis) to 12% (D. engyochracea) of the size of the E. coli genome. Table III gives the repetition frequency for each species for that fraction. The average multiplicity of the isolated $0.05 < Cot < 10$ DNA fractions varies among the different Hawaiian Drosophila species, from D. picticornis (49) to D. silvarentis (484). The average repetition frequency is about 204 copies.

TABLE V

Estimates of Reassociation Parameters from Hawaiian Drosophila Species

Species	Intermediate Reassociating Component					
	Proportion of Intermediate Fraction of DNA Assayed	Cot 1/2 (observed) (M.s)	K (observed) $(M.s)^{-1}$	K* (pure) $(M.s)^{-1}$	Relative to E. coli Kinetic Complexity $x10^{-2}$	Kinetic Complexity (daltons) $(g_1)x10^8$
D. crucigera	.37	.68	1.472	3.98	6.5	1.75
D. engyochracea	.45	.07	13.392	29.62	.9	.24
D. silvarentis	.38	.25	3.956	10.42	2.5	.67
D. picticornis	.41	1.16	.859	2.10	12.0	3.31
D. mimica	.47	.52	1.926	4.11	6.3	1.69

DISCUSSION

In this study we have examined the buoyant density of DNA from six species of the Hawaiian Drosophila group. Each of these species, except D. crucigera, has one or more satellite components. It seems probable that a correlation exists between the amount of satellite DNA in a given species and the amount of highly repetitive DNA (Table III) as separated by HAP chromatography. Furthermore, the satellite DNAs in the six Hawaiian Drosophila species differ quite grossly in buoyant density, as in the genus Apodemus (Henning and Walker, 1970) and in other Drosophila species (Walker, 1971).

The G+C content of the main band DNA ranges between 40 to 44% from buoyant density analysis, and from 45 to 50% from the thermal melting profile of sheared native DNA (Table II). That means that G+C content values based on Tm are about 5% higher than expected from buoyant densities. This difference is probably due to the hydroxyapatite that we used during this investigation (Clarkson Chemical Company). According to Chilton and McCarthy (1973), this kind of hydroxyapatite has a good column flow rate, and thus Tm values in melting curves are several degrees higher than other brands of HAP. At any rate, the G+C content of the Hawaiian Drosophila species is characteristic of Metazoan DNAs (Laird, 1973).

An examination of Table III reveals that the genome of each of the Drosophila species studied is composed of three classes of DNA sequences on the bases of their renaturation kinetics: a highly reassociating fraction, an intermediately reassociating fraction, and a slowly reassociating fraction. Approximately 9% to 24% of the genome consists of highly repetitive DNA sequences which, at least in the case of D. silvarentis, are predominantly located in the heterochromatic regions (Triantaphyllidis, 1978). The intermediately reassociating DNA represents about 12% to 25% of the genomes, and it appears that these sequences are both euchromatic and heterochromatic in location (Triantaphyllidis, 1978). The slowly reassociating DNA sequences account for about 60% to 76% of the genomes. This is probably a slight underestimate since some of these sequences are included in the intermediately reassociating DNA because of the selection of our Cot 10 for their separation (see Manning et al., 1975). Thus, the non-repetitive DNA sequences represent a larger proportion of the DNA in Hawaiian Drosophila species, under our renaturation conditions. These

sequences are predominantly localized in euchromatic regions (Triantaphyllidis, 1978).

Complete renaturation kinetics have been done with the isolated non-repetitive DNA fraction (unique sequences) and the DNA fraction II ($0.05 < \text{Cot} < 10$) for D. crucigera, D. engyochracea, D. silvarentis, D. picticornis and D. mimica, and only for the non-repetitive fraction of D. pilimana. Our data on the reassociation kinetics of the non-repetitive DNA clearly show second order kinetic curves, as expected from unique sequences. On the other hand, an anomalous feature was observed in the kinetic behavior of the moderately renaturing material: Following fractionation of DNA by Cot 0.05 to 10, a portion of this DNA is apparently unable to reassociate even when annealed to Cot_{30} (Fig. 3). Furthermore, the reassociation curves are heterogeneous. The same kinetic profiles have been observed in Nassaria obsoleta DNA by Davidson et al. (1971), Phycomyces blakesleeanus DNA by Dusenbery (1975) and in Spinacia oleracea DNA by Ojhia (1978). The previous authors suggest various explanations for the above mention observation. Further studies may provide information as to whether this is simply a contaminant phenomenon (see Manning et al., 1975) or a true kinetic aberration. According to Murray et al. (1978) new approaches, such as the use of molecular clones representing individual repetitive sequences, will be required to resolve the problem.

From Table III it is evident that there are variations in the proportion of the intermediate fraction, and that the repetition frequency of this fraction varies markedly among the species. Similar results have been reported for closely related species of rodents (Henning and Walker, 1970). Also, the repetition number of this fraction for D. crucigera and D. picticornis is similar to that reported for D. melanogaster (Laird and McCarthy, 1969). From the other hand, the other four Hawaiian Drosophila species have higher repetitious number than D. melanogaster.

The D. silvarentis genome contains about 1.465×10^8 base pairs, while D. crucigera 1.1×10^8 base pairs (Table III). Some 18% of the DNA of D. silvarentis, and 15% of the DNA of D. crucigera or 2.7×10^7 base pairs and 1.7×10^7 base pairs respectively, are in the middle repetitive class. If this middle repetitive DNA is interspersed in stretches of average length 5,600 nucleotides (Manning et al., 1975), there are 4,795 and 3,015 such stretches in the genome of the two species reprectively. The total number of middle repetitive stretches is thus of the same order (or less) as the number of chromomeres in Hawai-

ian Drosophila species (Dr Yoon, personal communication).

The size of the haploid genome of the studied Hawaiian Drosophila species varies from 6.6×10^{10} daltons to 10.7×10^{10} daltons (Table III), but is in reasonable agreement with the haploid genome size of other Drosophila species (Laird, 1973). An interesting point seems to be the type of genome organization changes which must have occured during subsequent divergence of Hawaiian Drosophila species. The big differences in haploid genome size (Table III) between the six species studied seems to be entirely due to the repetititive DNA sequences (Table III and V), while the variations of the amount (complexities) of unique DNAs are small, as described for other phylogenetic groups (i.e. Balbari and Amaldi, 1976). Britten and Davidson (1971) and Schmidtke et al. (1979) speculate about a possible functional meaning of variation of the amount of the highly and moderately repetitive DNA sequences.

In view of the results presented above it is interesting to ask whether the variation in haploid genome size (Table III) is related to the variation in either chromosome size or the amount of heterochromatin. Clayton (1969 and 1971) presented the karyotypes of more than one hundred Hawaiian Drosophila species. From her results it is clear that the five Picture-Winged species studied in this investigation (Table I), all have five pairs of rods and one pair of dots in their metaphase chromosomes, while D. mimica has six pairs of rods. According to Clayton, the latter species has six pairs of rods because of bigger euchromatic regions and the additions of heterochromatin to the dot chromosome. Yoon et al. (1972) confirmed the results of Clayton concerning the addition of heterochromatin in the rod and the sex chromosome. Furthermore, Yoon and Wheeler (1973) reported a triplication of 10 or more bands in a D. mimica polytene chromosome. Our results (Table III) indicate that D. mimica has the larger haploid genome size of all species studied. Then, our results give a clear impression that there may be a positive relatioship between the minimum haploid genome size, as determined by renaturation kinetics, and the relative amount of euchromatin and heterochromatin found in the karyotypes of the species.

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ΠΕΡΙΛΗΨΗ

ΜΕΛΕΤΗ ΤΟΥ DNA ΣΕ ΕΞΙ ΕΙΔΗ DROSOPHILA ΠΟΥ ΕΙΝΑΙ ΕΝΔΗΜΙΚΑ ΣΤΑ ΝΗΣΙΑ ΤΗΣ ΧΑΒΑΗΣ

Ὑπὸ

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Στὴν ἐργασία αὐτὴ ἀπομονώσαμε DNA ἀπὸ τὰ 6 ἀκόλουθα εἴδη Drosophila: D. crucigera, D. pilimana, D. engyohracea, D. silvarentis, D. picticornis καὶ D. mimica. Ἡ μελέτη τοῦ DNA τῶν προηγούμενων εἰδῶν σὲ διαβάθμιση πυκνότητας CsCl ἔδειξε χαρακτηριστικὲς ζῶνες δορυφορικοῦ DNA σὲ ὅλα τὰ εἴδη ἐκτὸς ἀπὸ τὸ εἶδος D. crucigera. Τὸ ποσοστὸ τοῦ δορυφορικοῦ DNA σὲ σχέση μὲ τὴν κύρια ζώνη ποικίλλει ἀνάμεσα στὰ πέντε εἴδη. Γιὰ κάθε εἶδος ὑπολογίσαμε τὴν ἑκατοστιαία σύσταση G+C, τόσο ἀπὸ θερμικὴ ἀποδιάταξη φυσικοῦ DNA ὅσο καὶ ἀπὸ ὑπερφυγοκέντρηση φυσικοῦ DNA σὲ διαβάθμιση πυκνότητας CsCl. Στὸ γονιδίωμα (γένωμα) καὶ τῶν 6 εἰδῶν ὑπάρχουν τρεῖς κλάσεις νουκλεοτιδικῶν ἀκολουθιῶν: α) ἕνα ταχυσυνδεόμενο κλάσμα ποὺ ἀποτελεῖ ἀπὸ τὸ 9% (D. crucigera) μέχρι τὸ 24% (D. pilimana) τοῦ γονιδιώματος, β) ἕνα μεσοσυνδεόμενο κλάσμα ποὺ ἀποτελεῖ ἀπὸ τὸ 12% (D. pilimana) μέχρι τὸ 25% (D. mimica) τοῦ γονιδιώματος, καὶ γ) ἕνα ἀργοσυνδεόμενο κλάσμα ποὺ ἀποτελεῖ τουλάχιστο τὸ 60% τοῦ γονιδιώματος κάθε εἴδους. Τὰ ἀποτελέσματά μας δείχνουν ὅτι τὸ ποσοστὸ τοῦ δορυφορικοῦ DNA εἶναι ἀνάλογο μὲ τὸ ποσοστὸ τοῦ ταχυσυνδεόμενου κλάσματος στὸ γονιδίωμα κάθε εἴδους ποὺ μελετήσαμε. Μὲ βάση τὴν κινητικὴ τοῦ ἐπανασχηματισμοῦ τοῦ ἀργοσυνδεόμενου DNA ὑπολογίσαμε γιὰ κάθε εἶδος τὸ μέγεθος τοῦ γονιδιώματος· αὐτὸ κυμαίνεται ἀπὸ 6.6×10^{10} daltons (D. engyochracea) μέχρι 10.7×10^{10} daltons (D. mimica).