DNA HYBRIDIZATION IN THE GENUS DROSOPHILA[1]

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EVOLUTIONARY trees have classically been based upon species characteristics which are phenotypic in nature. It is likely that direct measurement of the number or proportion of genes shared by different species will be of basic importance in understanding evolutionary relationships. A quite direct comparison of a large number of genes of different organisms can be made through the nucleic acid hybridization technique (HOYER and ROBERTS 1968). When species relationships are discussed, phenetic relationships must be distinguished from phylogenetic relationships. Phenetic relationships are based on indices of overall similarity between species. Phylogenetic relationships purport to trace the genealogies of species, and are based on specific characteristics selected to produce a genealogy which is most compatible with all the phenotypic characteristics of the species. The purpose of the present experiments is to determine if the species relationships which are derived from DNA hybridization data are phenetic or phylogenetic in nature.

A large amount of information about DNA similarity relationships among bacteria (MCCARTHY and BOLTON 1963) has been obtained, but in the absence of detailed morphological and paleontological data these studies have contributed little to our understanding of species relationships. On the other hand, only limited information is available from vertebrate DNA comparisons (HOYER *et al.* 1965), but that which exists is in general agreement with phylogenies derived from anatomical, embryological, and paleontological data. Since the phylogenetic and phenetic relationships of species in the genus Drosophila have been comparatively well established (STURTEVANT and NOVITSKI 1941; PATTERSON and STONE 1952; THROCKMORTON 1962a, 1962b; HUBBY and THROCKMORTON 1965), DNA hybridization between a number of Drosophila species was studied in order to compare DNA similarity data with cytogenetic, biochemical, and morphological data. LAIRD and MCCARTHY (1968) have compared the DNA of three Drosophila species using the hybridization technique and found relatively large differences among these species, showing that DNA hybridization can be used to distinguish between species in the same genus.

Not only have hybridization studies given insights into relationships between the DNAs of different organisms, but they have revealed some surprising properties of DNA itself. For example, intraspecific DNA reassociation studies have shown that eukaryotic DNA contains a fraction which, following denaturation,

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TABLE 1

List of species and strains used

Drosophila species	Subgenus	Collection No.	Collection locality
1. *victoria*	*Pholadoris*	1865.3	Nevo, Utah
2. *lebanonensis*	*Pholadoris*	1733.1	Beirut, Lebanon
3. *melanogaster*	*Sophophora*	Oregon-R	
4. *simulans*	*Sophophora*	H231.2	Raratonga, British Guinea
5. *willistoni*	*Sophophora*	2267.9	
6. *paulistorum*	*Sophophora*	1975.21	Belem, Brazil
7. *virilis*	*Drosophila*	1801.1	Texmelucan, Mexico
8. *virilis*	*Drosophila*	1736.7	Hang Chow, China
9. *borealis*	*Drosophila*	2077.4L	Itasca Park, Minnesota
10. *micromelanica*	*Drosophila*	2160.12	Madera Canyon, Arizona
11. *melanica*	*Drosophila*	1720.3	Cliff, New Mexico
12. *paramelanica**	*Drosophila*	28–12	St. Louis, Missouri
13. *euronotus**	*Drosophila*	45b	St. Louis, Missouri
14. *nigromelanica*	*Drosophila*	2551.3	Cold Spring Harbor, N.Y.
15. *pengi*	*Drosophila*	....	Japan
16. *hydeii*	*Drosophila*	2360.30	Cave Creek, Arizona
17. *fulvamacula*	*Drosophila*	H435.7	Latitia, Columbia
18. *funebris*	*Drosophila*	2082.1	Minneapolis, Minnesota
19. *pallidipennis*	*Drosophila*	H191.48	Bucaramanga, Columbia

* Obtained from H. STALKER.
Species classification is according to PATTERSON and STONE (1952) and THROCKMORTON (1962).

reanneals rapidly compared with the rest of the DNA (WALKER and MCCLAREN 1965; BRITTEN and KOHNE 1968). This DNA fraction might be localized in certain chromosome structures. Since *D. melanogaster* stocks with varying amounts of heterochromatin can be obtained, experiments were done to determine if the fast annealing DNA is localized only in the heterochromatic portions of the genome.

MATERIALS AND METHODS

Stocks: The 19 species and strains used are listed in Table 1 along with the collection numbers and localities. Flies were raised at 23°C on either standard cornmeal-agar medium, CARPENTER's (1950) medium, or banana-agar medium. Young adults were collected and stored at —23°C. *D. melanogaster* stocks of strains carrying varying amounts of *X*-centromeric heterochromatin were obtained from W. K. BAKER's laboratory at the University of Chicago. $In(1)sc^{4L}sc^{8R},yw/Y/ywf$ males lack the nucleolus organizer (NO) heterochromatin on the *X* but carry it on the *Y* chromosome, $sc^{8}\ B\ Inw^{a}\ sc^{4};sc^{19L}/cy\ cn^{2}$ females carry two NO heterochromatic regions on each *X* chromosome. *X/0* males collected from the cross of $Y^{s}\ wy{\cdot}Y^{1}\ y^{55f10}/0$ males and Oregon-R virgin females carry one dose of NO heterochromatin on the *X* chromosome but have no *Y* chromosome.

Isotopic labeling: To label Drosophila DNA, ^{3}H-methyl-thymidine (New England Nuclear, 17 c/mM) was added to either cornmeal or CARPENTER's medium to a final concentration of 50 μc/ml. ^{3}H-thymidine is a suitable label for yeast containing media since there is no incorporation of the label into the yeast DNA (LAIRD and MCCARTHY 1968). Adult females were allowed to lay eggs on this medium for 24 hr. Ten to eighteen days later emerging adults were collected and stored for extraction of DNA. Purified DNA was assayed for specific activity on nitrocellulose

filters (Schleicher and Schuell B-6) in a Nuclear-Chicago scintillation counter. Various preparations yielded DNAs containing from 680 to 2,675 cpm/μg.

Isolation of DNA: Drosophila DNA was extracted according to the procedures of RITOSSA and SPIEGELMAN (1965) and LAIRD and MCCARTHY (1968). Nucleic acid, protein, and polysaccharide concentrations were estimated from optical density measurements scanned between 230 and 360 mμ. The DNA concentration was determined by the DISCHE (1944) reaction and preparations containing more than 20% RNA were redigested with RNAase. The yield of purified DNA varied from 0.5–1.0 mg/g flies. *E. coli* DNA was extracted using the procedure of MARMUR (1961). All DNA solutions were stored at 4°C over chloroform.

Hybridization: To prepare filterbound DNA, about 0.5 mg of purified DNA (0.10 mg/ml) was denatured in 0.1 × SSC (1 × SSC is 0.15M sodium chloride, 0.015M trisodium citrate) by heating to 95°C for 10 min. The denatured DNA was plunged into ice and diluted to 0.05 mg/ml with 3× SSC and deposited on a membrane filter (Schleicher and Schuell B-6) which had been soaked in 3× SSC for 3 min. The filter was washed with 100 ml of 3× SSC, dried at room temperature overnight, and preincubated for 6 hr at 65°C in the medium specified by DENHARDT (1966). The filter was then dried in a vacuum oven at 80°C for two hours. The amount of DNA per filter was determined from OD_{260} measurements before and after filtration. Labeled and unlabeled DNAs used for duplex reactions were denatured as above and sheared by passing them through a Tomac 1 ml syringe (GILLESPIE and SPIEGELMAN 1965). These DNAs were incubated with the filterbound DNA in scintillation vials at 65°C for 16 hr at a concentration of 4–7 μg in 0.15 ml of 3× SSC. The filters were then washed with 40 ml of 3× SSC, dried overnight, and counted in a liquid scintillation counter. The fraction of DNA which reanneals under these conditions is called the *fast annealing* fraction.

The remaining DNA fraction was dialyzed, concentrated by lyophilization, dissolved in distilled water at 2–5 mg/ml and stored frozen. To initiate further hybridization this DNA fraction was adjusted to 3× SSC, redenatured, sheared, placed in 1 ml 3× SSC with 5 to 7 filters containing immobilized DNA, and incubated with mild shaking at 65°C in silicone-treated vials for a specified number of days. This DNA fraction is called the *slow annealing* fraction.

RESULTS

1. *Factors affecting hybridization:*

The proportion of fragmented DNA which annealed to homologous filterbound DNA reached a maximum of about 20% (Figure 1) at approximately 12 hr and remained constant for at least 72 hr. The lack of complete annealing did not appear to be due to the saturation of filterbound DNA since removal of the saturated filter and the insertion of new filterbound DNA led to only 3.8% additional annealing of the DNA fragments. Nor was the cessation of annealing due to significant reassociation of the DNA fragments themselves, since reheating the incubation mixture reinitiated only 4.8% further annealing. DNA which annealed under the above conditions is the fast annealing DNA. By varying the experimental conditions reassociation of a portion of the remaining DNA (slow fraction) can be obtained (Figure 2).

2. *Nucleotide sequence similarity among different Drosophila species:*

a. Similarity of the fast annealing DNA fraction. All the following experiments were done with total (nonfractionated) DNA under the conditions which allow annealing of the fast DNA fraction. A competition method was used to determine the percentage of DNA sequences held in common by different pairs of species: radioactive DNA fragments were reacted with homologous filterbound DNA in the presence of increasing and excessive amounts of nonradioactive frag-

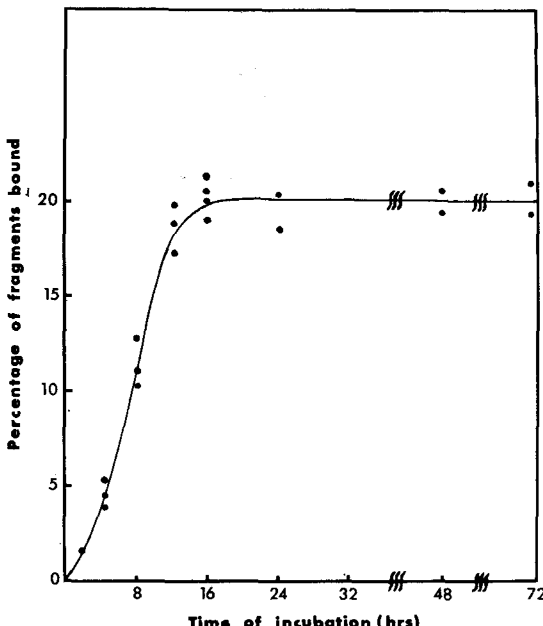

FIGURE 1.—The effect of different times of incubation on the proportion of DNA fragments bound. Fifty micrograms filterbound *melanogaster* DNA were placed in 1.15 ml 3× SSC containing 5 μg ^{3}H-*melanogaster* DNA (1576 cpm/μg) and were incubated at 65°C for specified time periods.

TABLE 2

*The relatedness of several Drosophila species as measured by DNA competition interactions**

	^{3}H-DNA fragments					
Competitor DNA	*D. melanogaster*	*D. willistoni*	*D. lebanonensis*	*D. funebris*	*D. virilis* (1801.1)	*D. hydeii*
D. melanogaster†	100	69.9	...	...	38.1	30.0
D. simulans	81.5	...	...	...	...	30.3
D. willistoni	. .	100	60.6	...	...	35.8
D. paulistorum	...	88.7	...	38.7	51.9	23.5
D. victoria	69.3	65.0	92.7	55.2	59.9	...
D. lebanonensis	...	...	100	...	55.6	...
D. funebris	34.2	38.2	47.3	100	52.5	35.1
D. virilis (1801.1)	40.7	52.8	...	...	100	53.2
D. virilis (1736.7)	42.6	...	...	...	100	...
D. borealis	39.0	...	...	...	73.3	56.1
D. hydeii	...	35.0	46.6	...	...	100
D. fulvamacula	28.0	29.8	53.9	...	...	75.5
D. micromelanica	29.7	...	...	...	...	...
D. pallidipennis	...	...	...	59.2	...	...

* Conditions of incubation of these competition experiments are as in legend to Figure 3. The specific activity of the DNAs varied from 976–2,675 cpm/μg DNA. The mean standard error was 2.4%; the range of the standard errors 1.2–3.8%. Means were computed from three or four samples.

† *melanogaster–simulans*, *willistoni–paulistorum*, and *victoria–lebanonensis* are sibling species of *Drosophila*.

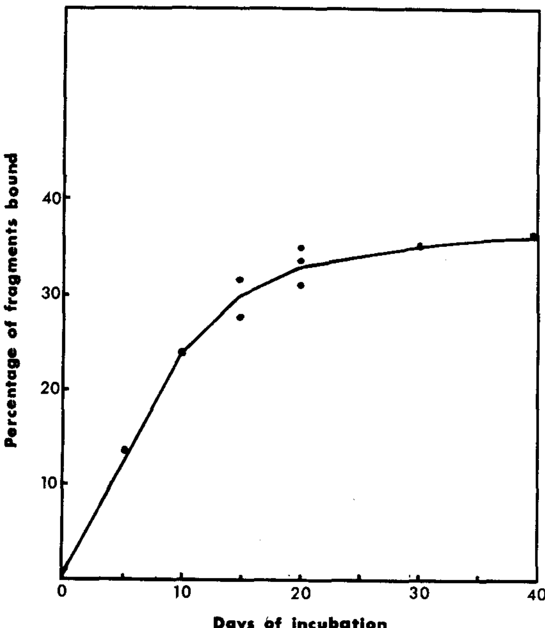

FIGURE 2.—The rate of reassociation of the slow annealing DNA fraction. One mg of ^{3}H-*melanogaster* DNA (688 cpm/μg; slow fraction, prepared as outlined in MATERIALS AND METHODS) was placed in 1 ml of 3× *SSC* with 5–7 filters containing from 2.8–3.7 mg of homologous, nonfractionated filterbound DNA, and was incubated with mild shaking in silicone-treated vials at 65°C for the specified number of days.

ments from the comparison species. It was predicted that *E. coli* DNA would not compete with the annealing of Drosophila DNA. Figure 3 shows the competition interactions of several DNAs with that of *D. melanogaster*. Table 2 presents the DNA comparisons of the species selected to represent the sections and subgenera of Drosophila. Six species were used as reference species, that is, they were used as the source of radioactive DNA in different competition experiments. These six species were selected to represent the respective species groups. This generated a large number of species comparisons and provided an opportunity to check the internal consistency of the results (see DISCUSSION). Table 3 presents DNA comparisons of a group of closely related flies, six species in the *melanica* subgroup.

b. Similarity of the slow annealing DNA fraction. The question naturally arises as to how the fast annealing DNA relationships compare with those based on the slow annealing DNA fraction. The nucleotide similarity relationships using the slow annealing DNA of five species were obtained and compared with the fast annealing DNA relationships. The species relationships based on the slow annealing fractions do not differ significantly from those based on the fast annealing fractions (Table 4). Since large quantities of DNA were needed, the direct annealing method rather than the competition method was used. *E. coli* DNA was again used as a nonannealing control.

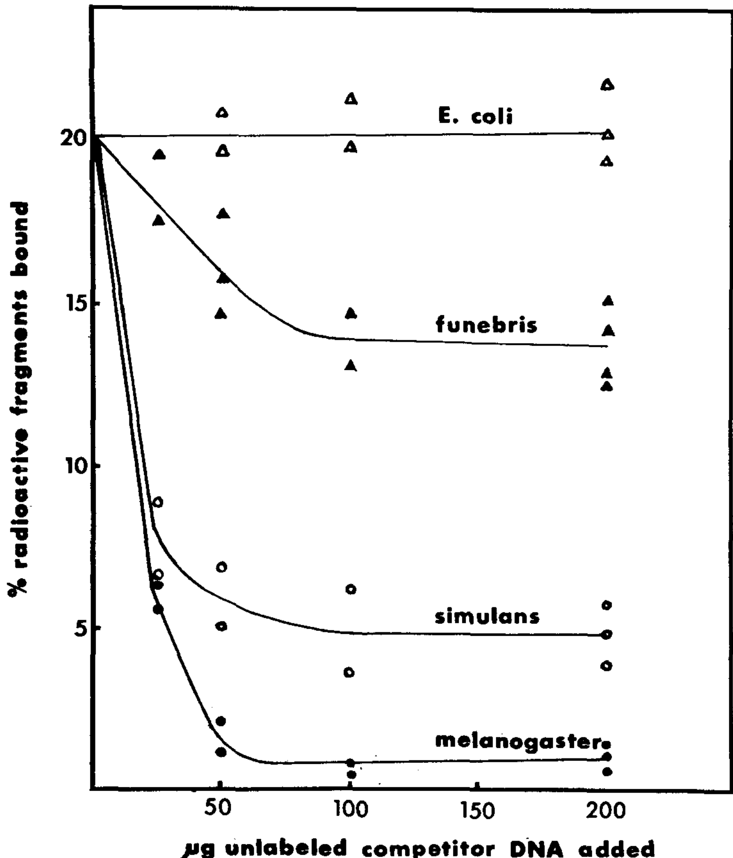

FIGURE 3.—Competition of DNA from different species in the reaction of ^{3}H-*melanogaster* DNA fragments and filterbound *melanogaster* DNA. Increasing amounts of unlabeled DNA from various species were incubated with 5 μg ^{3}H-*melanogaster* DNA (1576 cpm/μg) and 50 μg filterbound homologous DNA in 1.15 ml of 3× SSC at 65°C for 16 hours.

TABLE 3

The relatedness of the DNAs of several species in the Melanica species group of the genus Drosophila, as measured by competition interactions

Source of competitor DNA	Source of radioactive fragments *D. micromelanica* (1,112 cpm/μg)	*D. paramelanica* (1,816 cpm/μg)
D. pengi	67.7	66.4
D. micromelanica	100.0	72.8
D. nigromelanica	77.2	71.3
D. euronotus	73.3	88.2
D. paramelanica	71.2	100.0
D. melanica	70.2	92.1

Conditions of incubation as in legend to Figure 3. The percentage of radioactive fragments bound in the competition interactions with homologous DNA is used as a reference of 100% similarity. Values are averages of four samples with a mean standard error of 3.0% (range of standard error 2.0–4.9%).

TABLE 4

Comparison of species relationships based on hybridization studies with fast- and slow-annealing DNAs

Species compared with *D. melanogaster*	Nucleotide sequence similarity with the Slow DNA fraction*	Nucleotide sequence similarity with the Fast DNA fraction†
D. simulans	78.2, 80.1	81.5
D. victoria	59.3, 56.9	69.3
D. virilis	42.2, 37.8	42.6
D. funebris	27.5, 20.2	34.2

* Measured by the direct annealing method. One mg ^{3}H-*melanogaster* DNA fragments (slow fraction, prepared as in METHODS AND MATERIALS; 688 cpm/μg) was added to 1 ml 3× SSC with 5–7 filters containing 2.4–3.9 mg of nonfractionated DNA from various species. This mixture was incubated in silicone-treated vials for 20 days at 65°C.

† Measured by the direct competition method (see Figure 3). Values are taken from Table 4.

3. *Hybridization studies with DNAs containing varying amounts of heterochromatin:*

The exact nature and function of the fast annealing DNA is unknown. If the fast annealing DNA sequences originated solely from the heterochromatic portions of the genome, the amount of fast annealing DNA should be proportional to the amount of heterochromatin present. To test this hypothesis, DNA was extracted from *D. melanogaster* stocks containing varying amounts of *X* and *Y* heterochromatin, and their amount of fast annealing DNA was determined

Chromosomes	Description	% DNA
	$sc^8Binsw^asc^4$; $sc^{19L}/cycn^2$ females	21.04 ± 2.15
	Ins(1)$sc^{4L}sc^{8R}$ yw/Y/ywf: males	20.6 ± 2.65
	XXO males	21.5 ± 2.5

FIGURE 4.—The nature of *D. melanogaster* stocks (with varying amounts of heterochromatin) used and their repetitive DNA content. Fifty μg of filterbound DNA from the above stocks was placed in 1.15 ml of 3× SSC containing 5 μg of homologous ^{3}H-DNA fragments (730–1160 cpm/μg) and incubated at 65°C for 16 hours. The values are means of five samples ± one standard error.

(Figure 4). The results do not support the idea that the fast annealing sequences originate solely from all the heterochromatic portions of the Drosophila genome.

DISCUSSION

Certain properties of DNA reassociation must be considered before the relationships found among the Drosophila species can be evaluated. First, the degree of resolution of the technique can be varied with the experimental conditions used (Walker and McClaren 1965; Martin and Hoyer 1966). Thus the present data do not give absolute measures of nucleotide sequence similarity. But as long as incubation conditions are held constant, the technique gives reliable indications of relative nucleotide sequence similarity.

Second, in all higher organisms studied to date, a class of rapidly annealing DNA has been found in addition to slow annealing DNA sequences. The evidence that the rapidly annealing DNA consists of frequently repeated nucleotide sequences has been reviewed in detail by Britten and Kohne (1968). The function of this fast annealing DNA is unknown and the manner in which it influences the processes of evolution and speciation can only be speculated upon. Although the present results (Figure 4) indicate that the amount of nucleolar organizer heterochromatin present bears no direct relationship to the amount of rapidly annealing DNA, the possibility remains that the fast annealing DNA is related to a specific chromosome structure.

The primary purpose of this study was to evaluate the type of information obtained by comparing a large number of genes through the DNA hybridization technique. Before discussing the major results, inferences made from some previous hybridization studies should be pointed out. From vertebrate species, it appears that similarities in polynucleotide sequences are related to the time at which the lines of the vertebrate organisms examined diverged from one another (Hoyer *et al.* 1965). Similarly, Salthe and Kaplan (1966) using the microcomplement fixation technique, conclude that changes in the immunological properties of muscle-type lactate dehydrogenase in different amphibians show a linear relationship with time of phylogenetic divergence, as does the number of amino acid substitutions of the hemoglobin chains of mammals (Zuckerkandl and Pauling 1965) and of the cytochrome proteins of different organisms (Margoliash and Smith 1965). The term "phyletic distance" is often used to describe the species relationships inferred from the above types of data. As will be evident from the discussion below, one must be very cautious in assuming that "phyletic distance" is anything more than an inference from the data.

From the Drosophila data in Table 2 a number of inconsistencies become apparent. For example, there is a 29% difference in fast annealing nucleotide sequence similarity between *D. victoria* and *D. virilis* when *D. melanogaster* is used as the reference species, but only an 11% difference when *willistoni* is used as the reference species. Numerous analogous discrepancies are cited in Table 5. The phylogenetic position of the reference species "viewing" the remaining species is very important in determining the relationships observed among the remaining

TABLE 5

*Percentage differences between pairs of species depending upon the reference species used**

Species pair	Reference species *D.* *melanogaster*	*virilis*	*hydeii*	*willistoni*
D. simulans / *D. borealis*	42.5	...	25.8	...
D. paulistorum / *D. victoria*	23.7	8.0	...	...
D. paulistorum / *D. fulvamacula*	...	...	52.0	58.9
D. victoria / *D. borealis*	30.3	13.4	...	...
D. victoria / *D. fulvamacula*	41.3	...	...	35.2
D. borealis / *D. fulvamacula*	11.0	...	19.1	...

* The percentages are calculated from the data given in Table 2.

species. The discrepancies which arise when a simple relationship between nucleotide sequence similarity and time of divergence is assumed are not surprising. It is unlikely that the evolutionary divergence found between various species is due to changes in the same DNA segment in the same way and to the same extent in the various lineages. As a result different relationships would appear among the species, depending on which species was used as the reference species. DNA comparisons do indicate the degree of similarity, but they are imprecise indicators of phylogeny, i.e., they are phenetic rather than phylogenetic comparisons. The observation that DNA comparisons (and comparisons of homologous proteins) are phenetic comparisons has been made previously by THROCKMORTON (1968b).

The above statement can be tested by comparing the data on DNA comparisons with other types of data obtained from the same Drosophila species. THROCKMORTON (1968a) has coded phenetically 60 anatomical characters from a number of different Drosophila species (a number of different states can exist for each character). The character states are coded descriptively and weighted equally. No hypothesis as to the evolutionary sequence of the character states is implied. The percentage of morhopological character states that two species shared was calculated and compared with the percentage of repetitive nucleotide sequences that these same species shared. Figure 5 shows the regression of the percentage of character states shared by two species on the percentage of repetitive nucleotide sequences shared. The high correlation coefficient ($r = 0.79$) between nucleo-

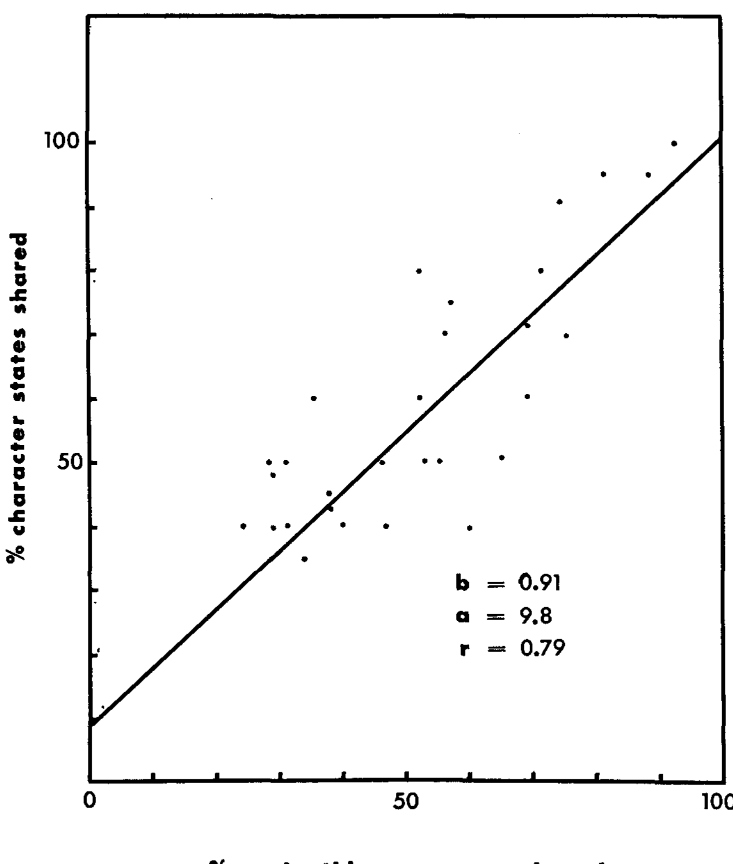

FIGURE 5.—Regression of the percentage of morphological character states shared by two species (from THROCKMORTON) on the relative percentage of nucleotides these two species share (from Table 2).

tide sequences and morphological character states supports the view that DNA comparisons are phenetic comparisons. Thus for the genus Drosophila generally, it appears that DNA comparisons between species yield overall similarity values analogous to similarity values which can be obtained by comparing a large number of equally weighted morphological character states of species.

From Figure 5 one infers that the greater the morphological similarity between two species the greater the repetitive nucleotide sequence similarity. Thus one would predict that a greater degree of nucleotide sequence similarity would exist between sibling species than between morphologically distinct species. The nucleotide sequence similarities between the sibling species used in this study are summarized in Table 6. It can be seen that a high percentage (an average of 89%) of nucleotide sequence similarity exists between sibling species. HUBBY and THROCKMORTON (1968) using electrophoretic techniques studied an average of 18 proteins per species of nine triads of species in the genus Drosophila. Each

TABLE 6

Comparisons of the genetic similarity between sibling species on the basis of protein analysis and DNA hybridization studies

Sibling species	Percentage of proteins in common (maximum estimate)*	Percentage of repetitive nucleotide sequences in common
D. melanica *D. paramelanica*	31.6	92.1
D. melanogaster *D. simulans*	52.9	81.5
D. willistoni *D. paulistorum*	22.5	88.7
D. victoria *D. lebanonensis*	85.7	92.7

* HUBBY and THROCKMORTON (1968).

triad consisted of a pair of sibling species and a related but morphologically distinct species. Their results show that, on the average, sibling species shared proteins with the same electrophoretic mobilities 18% of the time. Their results also indicate that a whole range of genetic similarity (from 85.7 to 22.5%) may exist between sibling species. Table 6 compares the percentage similarity between four sibling species pairs based on the protein data of HUBBY and THROCKMORTON (1968) and the nucleotide sequence data. More comparisons are necessary, but it appears that an analysis of the electrophoretic mobility of proteins is a more sensitive technique for determining genetic divergence than an analysis of DNA sequence similarity using the hybridization technique. DNA hybridization studies cannot detect single base changes, whereas these might be detected in an analysis of proteins (providing the base change caused an electrophoretic change). In the present experiments the hybridization technique could not distinguish between two strains of *D. virilis* (1801.1 and 1736.7). Melting curves of the reassociated duplexes of these strains might possibly distinguish the two strains. One would predict that an analysis of proteins would reveal divergence, since genetic polymorphism has been found in different populations of Drosophila species (HUBBY and LEWONTIN 1966; LEWONTIN and HUBBY 1966).

It can also be noted from Table 2 that the reciprocal comparisons which were made (*melanogaster* and *virilis; hydeii* and *willistoni; paramelanica* and *melanica*) all agreed. This would be the case only if these species have the same total complement of DNA. Whether this is true for all the Drosophila species needs to be determined from more reciprocal comparisons. The fact that all of the species tested have approximately 20% repetitive DNA is very interesting, although the implications of this are not clear.

All of the above comparisons were made with the fast annealing DNA frac-

tions. Although the present method of separating fast and slow annealing DNA is very crude when compared to the hydroxyapatite fractionation procedures of BRITTEN and KOHNE (1968), the fast annealing fraction is apparently a representative sample of the genome, since the species relationships based on the fast annealing fractions do not differ significantly from those based on the slow annealing fractions (Table 4). In contrast, different species of mammals are more divergent when the slow annealing DNA is compared instead of the fast annealing DNA. For example, there appear to be few, if any, common slow annealing sequences between the mouse and rat; although these organisms share over 50% of their fast annealing sequences (BRITTEN and KOHNE 1966). One interpretation of these data is that the slow annealing sequences change at a much faster rate than the fast annealing sequences. Another interpretation is inherent in the DNA hybridization technique. In order for two unique sequences to reassociate, base complementarity must be very precise; but when a number of similar sequences are present, a certain amount of base mispairing may exist without precluding the reassociation of these sequences. Thermal stability studies of the reassociated duplexes should be helpful in distinguishing between the above possibilities, and they might provide information as to why the slow and fast fractions reveal the same species relationships in the case of Drosophila.

One interesting feature of these results is the magnitude of the differences found between the individual species. A bacterial genus such as Bacillus (DUBNAU *et al.* 1965) can contain species even more diverse than the Drosophila species, but the taxon of higher plants showing this degree of diversity seems to be the family (BOLTON *et al.* 1965). In mammals, the taxon showing this degree of diversity seems to be the Order (BRITTEN and KOHNE 1966). This difference in magnitude of divergence between higher plants and animals and the genus Drosophila is probably due to differences in rates of divergence, as seen by the appearance of new genera and orders and by an increase in the number of species. Hopefully, a correlation of various patterns of speciation and degrees of nucleotide substitutions will eventually permit a determination of the rates of divergence within various groups of organisms.

I would like to express my gratitude to Dr. J. L. HUBBY for many helpful discussions, and to Dr. L. H. THROCKMORTON for criticizing the manuscript and for his contribution to Figure 5.

SUMMARY

Some experimental parameters of intraspecific and interspecific DNA/DNA duplex formation in the genus Drosophila were investigated. The genomes of eighteen Drosophila species were found to consist of approximately 20% fast annealing DNA, in addition to the slow annealing DNA. Investigations into the possible nature of *D. melanogaster* fast annealing DNA revealed that it does not originate solely from the heterochromatic portions of the genome. The relative percentage of fast annealing DNA sequences shared among these eighteen species and the percentage of slow annealing sequences shared among five of them were determined. The species relationships based on the respective DNA fractions did

not differ significantly. A simple relationship between the percentage of nucleotide sequences shared between two species and the time of their divergence was not found, and the importance of the phylogenetic position of the "reference" species in determining the relationships that will be observed among the remaining species was illustrated. It was found that DNA comparisons between species yield overall similarity values which are analogous to similarity values that can be obtained by comparing a large number of equally weighted morphological characters. Thus DNA comparisons are phenetic comparisons. The applications of the DNA hybridization technique and the relevance of DNA hybridization results in providing a better understanding of species relationships and evolutionary processes were discussed.

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