# GENETIC DIFFERENTIATION BETWEEN GEOGRAPHICALLY DISTANT POPULATIONS OF *DROSOPHILA MELANOGASTER*

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ABSTRACT

We have studied allozyme variation at 26 gene loci in nine populations of *Drosophila melanogaster* originating on five different continents. The distant populations show significant genetic differentiation. However, only half of the loci studied have contributed to this differentiation; the other half show identical patterns in all populations. The genetic differentiation in North American, European and African populations is correlated with the major climatic differences between north and south. These differences arise mainly from seven loci that show gene-frequency patterns suggestive of latitudinal clines in allele frequencies. The clinal variation is such that subtropical populations are more heterozygous than temperate populations. These results are discussed in relation to the selectionist and neutralist hypotheses of genetic variation in natural populations.

GENERALLY, there is a remarkable similarity in allozyme frequencies between populations of sexually-reproducing mobile organisms like Drosophila (*e.g.*, LEWONTIN 1974; AYALA *et al.* 1972; PRAKASH 1977). This similarity of allele frequency between populations has been presented as evidence that some sort of balancing selection must be involved; otherwise different populations should show differences in gene frequency due to random genetic drift. On the other hand, such similarity in allele frequencies is also expected under the neutral theory provided that some gene exchange takes place between populations (KIMURA and MARUYAMA 1971; KIMURA and OHTA 1971). Consequently, both balancing selection and migration can explain the results. Similarity in gene frequency between populations is potentially a function of three different factors —distance between the populations, the mobility of the organism, and the intensity of natural selection. These factors may be acting singly or in combination in any particular situation. Recently, in the well-studied Drosophila species *D.*

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*pseudoobscura*, which has striking population similarity, individual flies were found to possess an unexpectedly high dispersal potential (JONES *et al.* 1981). Migration would seem to be a likely explanation for population similarities in this case. However, the existence of even a single gene-locus which showed significant between-population differentiation in allele frequency would be conclusive evidence against the hypothesis of high effective migration rates. This is the kind of evidence that we are able to provide for populations of *D. melanogaster*.

We have chosen to study *Drosophila melanogaster* because its population structure provides an excellent opportunity for sorting out the effects of mobility, distance and natural selection on allele frequencies. This species is commensal with man and has world-wide distribution. It experiences a wide range of climatic and ecological habitats and, since individuals of this species are quite mobile, local founder effects are minimized. On the other hand, populations on different continents thousands of miles apart and separated by oceans can be expected to exchange alleles at a relatively low rate despite possible transportation by man. Genetic variation within populations of *D. melanogaster* has already been studied extensively (KOJIMA, GILLESPIE and TOBARI 1970; O'BRIEN and MACINTYRE 1969; BERGER 1970; GIRARD, PALABOST and PETIT 1977; LANGLEY, TOBARI and KOJIMA 1974; MUKAI and VOELKER 1977; VOELKER, MUKAI and JOHNSON 1977; JOHNSON and SCHAFFER 1973; SCHAFFER and JOHNSON 1974; VIGUE and JOHNSON 1973; METTLER, VOELKER and MUKAI 1977; SAMPSELL 1977; MILKMAN 1976; BAND 1975; TRIANTAPHYLLIDIS *et al.* 1980). Less attention has been paid to comparisons between populations because it has been assumed that little differentiation would be observed due to the commensalism with man. This assumption is supported by those studies that include a comparison of several populations from within a single geographical area. In this report, we show that the above assumption is not true for populations from widely separated locations. There is already some evidence available for the existence of gene-frequency differentiation between geographically distant populations of *D. melanogaster* (*e.g.*, VOELKER *et al.* 1978; HICKEY 1979b). We have simply extended the sampling range to cover most of the species range; we have also chosen to study a large number of allozyme loci. This enables us to present the results from a world-wide survey of allozyme polymorphism in *D. melanogaster*. We have shown that, contrary to findings in other Drosophila species, in *D. melanogaster* some loci show remarkable similarity in allele frequency between populations, while others show extensive variation. This provides compelling evidence that, despite a potentially high rate of migration, populations of *D. melanogaster* remain partially genetically separate. Moreover, a comparison of the specific patterns of variation at different loci suggests that this genetic differentiation is due to the action of natural selection, rather than being due entirely to random genetic drift.

## MATERIALS AND METHODS

The Drosophila stocks used in this survey were derived from wild-caught females and were maintained as separate iso-female lines at 18° in the laboratory. In some cases, each iso-female line was divided into two sublines to reduce the loss of segregating alleles. Most iso-female lines

had been maintained as separate cultures in the laboratory for a period of one to two years. The populations sampled were: Ottawa, Canada (20 lines); Hamilton, Ontario, Canada (40); Amherst, Massachusetts, U.S.A. (30); Brownsville, Texas, U.S.A. (30); Villeurbanne, France (2 × 20); Benin, West Africa (2 × 28); Taiwan (26); Ho-Chi-Mihn City, Vietnam (2 × 20); and Fairfield, Australia (26). Since we are interested in regional rather than local patterns, these populations will be referred to as Ottawa (OT), Hamilton (HA), Massachusetts (MA), Texas (TE), France (FR), West Africa (WA), Taiwan (TA), Vietnam (VI) and Australia (AU). The location of each of these populations' origins is shown in Figure 1.

Allozyme variability was assayed by vertical polyacrylamide slab-gel electrophoresis, using Aardvark gel boxes (PRAKASH, LEWONTIN and HUBBY 1969). For most enzymes the commercial grade acrylamide (Cyanogum) was adequate, but for others, *e.g.*, α-GPD and PGM, pure acrylamide and bis-acrylamide were used. All gels contained either 5% Cyanogum or 5% acrylamide-bisacrylamide (5% bis). A continuous buffer system was used, usually 0.1 M Tris-Borate or its variation as indicated in Table 1. The enzyme staining methods were adapted from HARRIS and HOPKINSON (1976) and from SHAW and PRASAD (1970).

There is some variation in the literature with respect to the nomenclature of isozyme loci and their alleles: isozyme loci have been named by numbers as well as by letters; alleles have been labelled by letters as well as by their relative mobilities. For uniformity, the isozyme loci in this report are numbered in order of their electrophoretic mobility, beginning with the slowest-migrating band. For instance, Esterase-9 migrates faster than Esterase-6 according to this nomenclature. In some cases, both the previous lettering system and the present numbering system are given; *e.g.*, LAP-D is the same as LAP-3. Allozyme variants at a given locus were labelled as follows: if two allozyme variants were found we retained the lettering system, Slow (S) and Fast (F). However, when more than two alleles were found at a given locus, they were numbered in the order of their electrophoretic mobilities in the same manner as for the isozymes. For example, there are four allelic variants (numbered 1 to 4) for Esterase-6, and the alleles 2 and 3 in our system are the same as the previously described Slow (S) and Fast (F) allozymes respectively. Amylase allozyme bands were numbered beginning with the fastest-migrating band; this is consistent with the existing numbering system. We will point


FIGURE 1.—Locations of various *Drosophila melanogaster* populations studied: OT = Ottawa, HA = Hamilton, MA = Massachusetts, TE = Texas, FR = France, WA = West Africa, TA = Taiwan, VI = Vietnam, AU = Australia.

TABLE 1

*Enzymes, their International Union of Biochemistry numbers, genetic map position, electrophoresis buffers and subunit structure*

| Enzymes or proteins | I.U.B. number | Genetic map position[1] | Electrophoresis buffer[2] | Subunit structure[3] |
|---|---|---|---|---|
| *Chromosome* I: | | | | |
| Glucose-6-phosphate dehydrogenase (G-6PD) | (1.1.1.49) | 1–63 (*Zw*) | C | 1 |
| Larval Protein-11 (Pt-11) | ..... | 1–39 (*LSP-1α*) | A | 1 |
| 6-Phosphogluconate Dehydrogenase (6-PGD) | (1.1.1.44) | 1–0.9 (*Pgd*) | A | 2 |
| *Chromosome* II: | | | | |
| Alcohol Dehydrogenase (ADH) | (1.1.1.1) | 2.50.1 (*Adh*) | A | 2 |
| α-Amylase (AMY) | (3.2.1.1) | 2.77.7 (*Amy*) | B | 1 |
| α-Glycerophosphate Dehydrogenase (α-GPDH) | (1.1.99.5) | 2–20.5 (*α-Gpdh*) | A | 2 |
| Malate Dehydrogenase (MDH) | (1.1.1.3) | 2–37.0 (*Mdh-1*) | A | 2 |
| Larval Protein-10 (Pt-10) | ..... | 2–1.9 ((*Lsp-1β*) | A | 1 |
| *Chromosome* III: | | | | |
| Acid Phosphatase (ACPH) | (3.1.3.2) | 3–101.3 (*Acph-1*) | C | 2 |
| Aldehydeoxidase (AO) | (1.2.3.1) | 3–56.7 (*Aldox*) | A | 2 |
| Alkaline phosphatase-larval (APH) | (3.1.3.1) | 3–46.3 (*Aph*) *Larval* | C | 2 |
| Esterase-C (EST-C) | (3.1.3.2) | 3–47.7 (*Est-C*) | B | 1 |
| Esterase-6-(EST-6) | (3.1.3.2) | 3–36.8 (*Est-6*) | B | 1 |
| Leucine aminopeptidase-A (LAP-A) | (3.4.1.1) | 3–98.3 (*Lap-A*) | C | 1 |
| Leucine aminopeptidase-D | (3.4.1.1) | 3.98.3 (*Lap-D*) | C | 1 |
| Malic enzyme (ME) | (1.1.1.40) | 3–51.7 (*Men*) | A | .. |
| Octanol Dehydrogenase (ODH) | ..... | 3.49.2 (*Odh*) | A | 2 |
| Phosphoglucomutase (PGM) | (2.7.5.1) | 3–43.4 (*Pgm*) | C | 1 |
| Larval Protein-9 (Pt-9) | ..... | 3–0.00 (*Lsp-1γ*) | A | 1 |
| Larval Protein-15 (Pt-15) | ..... | 3–37.0 (*Lsp-2*) | A | 1 |
| Xanthine Dehydrogenase(XDH) | (1.2.3.2) | 3–52.0 (*ry+*) | A | 2 |
| Tetrazolium oxidase-1 (TO-1) | (1.15.1.1) | 3–32 (*TO-1*) | A | 2 |

TABLE 1—Continued

| Enzymes or proteins | I.U.B. number | Genetic map position[1] | Electrophoresis buffer[2] | Subunit structure[3] |
|---|---|---|---|---|
| *Loci unmapped*: | | | | |
| Esterase-9 (EST-9) | (3.1.1.2) | ..... | B | 1 |
| Esterase-10 (EST-10) | (3.1.1.2) | ..... | A | 1 |
| Leucine aminopeptidase-6 (LAP-6) | (3.4.1.1) | ..... | C | 1 |
| Tetrazolium oxidase-2 (TO-2) | (1.15.1.1) | ..... | A | 2 |

[1] The genetic map positions are taken from DOANE and TREAT-CLEMONS (1981).

[2] *Buffers*: A = 0.1 M Tris-Borate EDTA, pH 8.9.
B = Same as A but no EDTA.
C = Same as B but 0.05 M $MgCl_2$ was added in the gel- and sample buffers.

[3] *Subunit Structure*: 1 = monomeric; 2 = dimeric.

out the equivalence between the previously described alleles and those reported in this study whenever necessary.

A total of 26 loci (21 enzymes and 5 larval proteins) were studied (see Table 1). Most of these loci are well mapped and are distributed on all the major chromosomes. Since we are interested in geographical patterns of polymorphisms, we generally chose loci that were known to be polymorphic. For the 24 polymorphic loci, the results are presented both in terms of individual allozyme frequencies and the expected heterozygosity for each locus, using the method of LEWONTIN and HUBBY (1966). For the amylase locus, because of the complication of gene duplication, allele frequencies are not available and hence the data are presented as

TABLE 2

*Frequency of alternative alleles of polymorphic loci in various populations of* Drosophila melanogaster

| Loci and alleles | Ottawa (Canada) 45.3°N | Hamilton (Canada) 43.3°N | Massa-chusetts (U.S.A.) 42.4°N | Texas (U.S.A.) 25.8°N | France 46.1°N | West Africa 6.3°N | Taiwan 25°N | Vietnam 11°N | Australia 33.4°S |
|---|---|---|---|---|---|---|---|---|---|
| | | | | *Chromosome* I | | | | | |
| Glucose-6 phosphate Dehydrogenase | | | | | | | | | |
| 1 | 0.050 | 0.280 | 0.905 | 1.00 | 0.160 | 0.416 | .... | 0.088 | 0.260 |
| 2 | 0.950 | 0.720 | 0.095 | .... | 0.840 | 0.584 | 0.929 | 0.795 | 0.740 |
| 3 | .... | .... | .... | .... | .... | .... | 0.071 | 0.117 | .... |
| HET. | 0.095 | 0.404 | 0.172 | 0 | 0.269 | 0.486 | 0.132 | 0.347 | 0.385 |
| Larval proteins-11 | | | | | | | | | |
| 1 | .... | .... | .... | .... | .... | 0.011 | .... | .... | .... |
| 2 | 0.205 | 0.196 | 0.013 | 0.032 | 0.210 | 0.043 | 0.029 | 0.026 | 0.040 |
| 3 | 0.975 | 0.655 | 0.777 | 0.871 | 0.633 | 0.707 | 0.472 | 0.472 | 0.940 |
| 4 | .... | 0.071 | 0.131 | 0.097 | 0.157 | 0.163 | 0.117 | 0.105 | 0.020 |
| 5 | .... | 0.078 | 0.079 | .... | .... | 0.065 | .... | 0.407 | .... |
| 6 | .... | .... | .... | .... | .... | .... | 0.382 | .... | .... |
| 7 | .... | .... | .... | .... | .... | 0.011 | .... | .... | .... |
| HET. | 0.049 | 0.522 | 0.373 | 0.231 | 0.531 | 0.468 | 0.617 | 0.600 | 0.114 |
| 6-Phosphogluconate Dehydrogenase | | | | | | | | | |
| 1 | 0.047 | .... | .... | .... | .... | .... | .... | .... | .... |
| 2 | 0.286 | 0.238 | 0.596 | 0.472 | .... | .... | .... | 0.105 | 0.159 |
| 3 | 0.667 | 0.762 | 0.404 | 0.528 | 1.00 | 1.00 | 1.00 | 0.895 | 0.841 |
| HET. | 0.472 | 0.363 | 0.482 | 0.499 | 0 | 0 | 0 | 0.188 | 0.268 |
| | | | | *Chromosome* II | | | | | |
| $\alpha$-Glycerophosphate Dehydrogenase | | | | | | | | | |
| S | 0.20 | 0.190 | 0.166 | 0.318 | 0.526 | .... | 0.125 | 0.235 | 0.214 |
| F | 0.80 | 0.810 | 0.834 | 0.682 | 0.474 | 1.00 | 0.875 | 0.765 | 0.786 |
| HET. | 0.320 | 0.308 | 0.277 | 0.434 | 0.499 | 0 | 0.219 | 0.360 | 0.333 |
| Alcohol Dehydrogenase | | | | | | | | | |
| S | 0.500 | 0.238 | 0.514 | 0.861 | .... | 0.971 | 0.050 | .... | 0.220 |
| F | 0.500 | 0.762 | 0.476 | 0.139 | 1.00 | 0.029 | 0.950 | 1.00 | 0.780 |
| HET. | 0.500 | 0.363 | 0.510 | 0.240 | 0 | 0.057 | 0.095 | 0 | 0.344 |
| Malate Dehydrogenase | | | | | | | | | |
| S | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 0.857 | 1.00 | 1.00 | 1.00 |

TABLE 2—Continued

| Loci and alleles | Ottawa (Canada) 45.3°N | Hamilton (Canada) 43.3°N | Massa-chusetts (U.S.A.) 42.4°N | Texas (U.S.A.) 25.8°N | France 46.1°N | West Africa 6.3°N | Taiwan 25°N | Vietnam 11°N | Australia 33.4°S |
|---|---|---|---|---|---|---|---|---|---|
| F | .... | .... | .... | .... | .... | 0.143 | .... | .... | .... |
| HET. | 0 | 0 | 0 | 0 | 0 | 0.246 | 0 | 0 | 0 |
| Larval Protein-10 | | | | | | | | | |
| 1 | .... | .... | .... | .... | .... | 0.011 | 0.058 | .... | .... |
| 2 | .... | .... | .... | .... | 0.039 | .... | .... | 0.050 | .... |
| 3 | 0.950 | 0.929 | 0.921 | 0.806 | 0.909 | 0.902 | 0.854 | 0.900 | 0.980 |
| 4 | 0.050 | 0.071 | 0.026 | 0.113 | 0.026 | .... | 0.088 | 0.050 | 0.020 |
| 5 | .... | .... | 0.053 | 0.081 | 0.026 | 0.087 | .... | .... | .... |
| 6 | .... | .... | .... | .... | .... | .... | .... | .... | .... |
| HET | 0.095 | 0.132 | 0.149 | 0.332 | 0.171 | 0.179 | 0.260 | 0.185 | 0.039 |
| | | | | *Chromosome* III | | | | | |
| Acid Phosphatase | | | | | | | | | |
| 1 | 0.050 | 0.017 | .... | 0.068 | .... | .... | 0.023 | .... | 0.019 |
| 2 | 0.950 | 0.983 | 1.000 | 0.932 | 1.00 | 0.988 | 0.977 | 1.00 | 0.981 |
| 3 | .... | .... | .... | .... | .... | 0.012 | .... | .... | .... |
| HET. | 0.095 | 0.034 | 0 | 0.127 | 0 | 0.024 | 0.045 | 0 | 0.038 |
| Aldehyde Oxidase | | | | | | | | | |
| 1 | 0.025 | 0.016 | .... | .... | 0.065 | .... | .... | .... | .... |
| 2 | .... | .... | 0.017 | 0.029 | .... | 0.018 | .... | 0.012 | .... |
| 3 | 0.700 | 0.888 | 0.566 | 0.501 | 0.818 | 0.129 | 0.838 | 0.763 | 0.929 |
| 4 | .... | 0.064 | 0.150 | 0.088 | .... | 0.092 | 0.023 | .... | .... |
| 5 | .... | .... | 0.067 | 0.191 | 0.078 | 0.558 | .... | 0.200 | .... |
| 6 | 0.255 | 0.032 | 0.133 | 0.029 | .... | 0.037 | 0.139 | .... | .... |
| 7 | .... | .... | 0.067 | 0.059 | 0.028 | 0.018 | .... | .... | .... |
| 8 | .... | .... | .... | .... | .... | .... | .... | .... | .... |
| 9 | 0.050 | .... | .... | 0.044 | 0.013 | 0.111 | .... | 0.025 | 0.071 |
| 10 | .... | .... | .... | 0.059 | .... | 0.037 | .... | .... | .... |
| HET. | 0.457 | 0.207 | 0.631 | 0.695 | 0.320 | 0.648 | 0.278 | 0.378 | 0.132 |
| Alkaline phosphatase (larval) | | | | | | | | | |
| 1 | .... | .... | .... | .... | 0.095 | .... | .... | .... | 0.057 |
| 2 | 0.975 | 1.00 | 1.00 | 0.983 | 0.905 | 0.978 | 0.857 | 0.938 | 0.943 |
| 3 | 0.205 | .... | .... | 0.017 | .... | 0.022 | 0.143 | 0.062 | .... |
| HET. | 0.049 | 0 | 0 | 0.034 | 0.172 | 0.044 | 0.246 | 0.117 | 0.108 |
| Esterase-C | | | | | | | | | |
| 1 | .... | .... | 0.069 | 0.022 | .... | 0.186 | .... | .... | .... |
| 2 | .... | 0.023 | 0.552 | 0.478 | 0.025 | 0.457 | 0.023 | 0.125 | .... |
| 3 | 1.00 | 0.977 | 0.345 | 0.391 | 0.975 | 0.357 | 0.977 | 0.875 | 1.00 |
| 4 | .... | .... | 0.017 | 0.065 | .... | 0.014 | .... | .... | .... |
| HET. | 0 | 0.045 | 0.573 | 0.614 | 0.049 | 0.629 | 0.045 | 0.219 | 0 |
| Esterase-6 | | | | | | | | | |
| 1 | .... | .... | .... | .... | .... | 0.009 | 0.186 | 0.017 | .... |
| 2 | 0.700 | 0.460 | 0.511 | 0.365 | 0.693 | 0.340 | 0.628 | 0.762 | 0.620 |
| 3 | 0.300 | 0.540 | 0.454 | 0.635 | 0.307 | 0.623 | 0.186 | 0.167 | 0.357 |
| 4 | .... | | 0.023 | .... | .... | 0.038 | .... | .... | 0.023 |
| HET. | 0.420 | 0.497 | 0.533 | 0.464 | 0.426 | 0.495 | 0.537 | 0.392 | 0.488 |
| Leucine amino-peptidase-3 (=D) | | | | | | | | | |
| 1 | .... | .... | .... | .... | 0.100 | 0.190 | .... | 0.047 | .... |

TABLE 2—Continued

| Loci and alleles | Ottawa (Canada) 45.3°N | Hamilton (Canada) 43.3°N | Massa-chusetts (U.S.A.) 42.4°N | Texas (U.S.A.) 25.8°N | France 46.1°N | West Africa 6.3°N | Taiwan 25°N | Vietnam 11°N | Australia 33.4°S |
|---|---|---|---|---|---|---|---|---|---|
| 2 | 0.025 | 0.125 | 0.136 | 0.225 | 0.350 | 0.668 | 0.147 | 0.214 | 0.340 |
| 3 | 0.975 | 0.875 | 0.864 | 0.775 | 0.550 | 0.142 | 0.853 | 0.739 | 0.660 |
| HET. | 0.049 | 0.219 | 0.236 | 0.349 | 0.565 | 0.498 | 0.251 | 0.406 | 0.449 |
| Leucine amino-peptidase-7 (=A) | | | | | | | | | |
| 1 | 0.075 | 0.100 | 0.208 | 0.300 | 0.318 | 0.619 | 0.214 | 0.500 | 0.080 |
| 2 | 0.925 | 0.725 | 0.709 | 0.550 | 0.387 | 0.381 | 0.786 | 0.476 | 0.880 |
| 3 | .... | 0.175 | 0.083 | 0.150 | 0.295 | .... | .... | 0.024 | 0.040 |
| HET. | 0.139 | 0.434 | 0.448 | 0.585 | 0.663 | 0.472 | 0.337 | 0.523 | 0.218 |
| Phosphoglucomutase | | | | | | | | | |
| 1 | 0.100 | 0.016 | 0.024 | 0.095 | 0.020 | 0.062 | .... | .... | 0.142 |
| 2 | 0.900 | 0.834 | 0.905 | 0.786 | 0.980 | 0.709 | 1.00 | 1.00 | 0.835 |
| 3 | .... | 0.150 | 0.071 | 0.119 | .... | 0.187 | .... | .... | 0.023 |
| 4 | .... | .... | .... | .... | .... | 0.042 | .... | .... | .... |
| HET. | 0.180 | 0.282 | 0.176 | 0.360 | 0.039 | 0.457 | 0 | 0 | 0.283 |
| Octanol Dehydrogenase | | | | | | | | | |
| S | 0.100 | 0.069 | 0.090 | 0.382 | .... | 0.135 | 0.047 | .... | .... |
| F | 0.900 | 0.931 | 0.910 | 0.618 | 1.00 | 0.865 | 0.953 | 1.00 | 1.00 |
| HET. | 0.180 | 0.129 | 0.164 | 0.473 | 0 | 0.234 | 0.090 | 0 | 0 |
| Xanthine Dehydrogenase | | | | | | | | | |
| 1 | .... | .... | .... | .... | .... | .... | .... | 0.025 | .... |
| 2 | .... | .... | .... | .... | 0.065 | .... | .... | .... | .... |
| 3 | .... | 0.016 | 0.100 | .... | .... | .... | .... | 0.037 | .... |
| 4 | 0.050 | 0.032 | 0.050 | 0.059 | .... | .... | .... | .... | .... |
| 5 | 0.175 | 0.161 | 0.099 | 0.206 | 0.607 | 0.277 | 0.524 | 0.663 | 0.200 |
| 6 | 0.525 | 0.727 | 0.660 | 0.677 | 0.276 | 0.612 | 0.452 | 0.250 | 0.660 |
| 7 | 0.250 | 0.064 | 0.066 | 0.058 | 0.052 | 0.111 | 0.024 | 0.025 | 0.140 |
| 8 | .... | .... | 0.016 | .... | .... | .... | .... | .... | .... |
| HET. | 0.629 | 0.441 | 0.538 | 0.493 | 0.549 | 0.537 | 0.521 | 0.496 | 0.505 |
| Larval Protein-9 | | | | | | | | | |
| 1 | 1.00 | 1.00 | 0.908 | 0.791 | 0.869 | 0.957 | 0.912 | 1.00 | 1.00 |
| 2 | .... | .... | 0.066 | 0.048 | 0.118 | .... | 0.088 | .... | .... |
| 3 | .... | .... | 0.026 | 0.129 | 0.013 | 0.043 | .... | .... | .... |
| 4 | .... | .... | .... | 0.032 | .... | .... | .... | .... | .... |
| HET. | 0 | 0 | 0.171 | 0.357 | 0.231 | 0.083 | 0.161 | 0 | 0 |
| Larval Protein-15 | | | | | | | | | |
| 1 | .... | 0.107 | 0.145 | .... | 0.026 | .... | 0.029 | .... | 0.020 |
| 2 | .... | .... | .... | .... | .... | 0.228 | .... | .... | .... |
| 3 | 1.00 | 0.893 | 0.855 | 1.00 | 0.974 | 0.772 | 0.942 | 1.00 | 0.980 |
| 4 | .... | .... | .... | .... | .... | .... | 0.029 | .... | .... |
| HET. | 0 | 0.192 | 0.248 | 0 | 0.051 | 0.353 | 0.111 | 0 | 0.039 |
| *Loci Unmapped* | | | | | | | | | |
| Tetrazolium Oxidase-1 | | | | | | | | | |
| S | .... | .... | 0.150 | 0.176 | 0.238 | .... | .... | .... | 0.104 |
| F | 1.00 | 1.00 | 0.850 | 0.824 | 0.762 | 1.00 | 1.00 | 1.00 | 0.896 |
| HET. | 0 | 0 | 0.255 | 0.291 | 0.363 | 0 | 0 | 0 | 0.187 |

TABLE 2—Continued

| Loci and alleles | Ottawa (Canada) 45.3°N | Hamilton (Canada) 43.3°N | Massa-chusetts (U.S.A.) 42.4°N Massa- | Texas (U.S.A.) 25.8°N | France 46.1°N | West Africa 6.3°N | Taiwan 25°N | Vietnam 11°N | Australia 33.4°S |
|---|---|---|---|---|---|---|---|---|---|
| Tetrazolium Oxidase-2 | | | | | | | | | |
| S | .... | .... | .... | 0.059 | .... | .... | .... | .... | .... |
| F | 1.00 | 1.00 | 1.00 | 0.941 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 |
| HET. | 0 | 0 | 0 | 0.112 | 0 | 0 | 0 | 0 | 0 |
| Leucineamino peptidase-6 | | | | | | | | | |
| 1 | .... | 0.166 | .... | 0.300 | .... | .... | .... | .... | .... |
| 2 | 1.00 | 0.691 | 0.875 | 0.600 | 0.421 | 1.00 | 0.176 | 0.750 | 0.204 |
| 3 | .... | 0.143 | 0.125 | 0.100 | 0.526 | .... | 0.824 | 0.250 | 0.796 |
| 4 | .... | .... | .... | .... | 0.053 | .... | .... | .... | .... |
| HET. | 0 | 0.475 | 0.219 | 0.540 | 0.544 | 0 | 0.291 | 0.375 | 0.325 |
| Esterase-9 | | | | | | | | | |
| 1 | .... | .... | .... | .... | .... | 0.119 | 0.023 | .... | .... |
| 2 | 0.400 | 0.381 | 0.625 | 0.524 | 0.421 | 0.334 | 0.716 | 0.825 | 0.460 |
| 3 | 0.600 | 0.619 | 0.375 | 0.452 | 0.553 | 0.476 | 0.238 | 0.175 | 0.540 |
| 4 | .... | .... | .... | 0.024 | 0.026 | 0.047 | 0.023 | .... | .... |
| 5 | .... | .... | .... | .... | .... | 0.024 | .... | .... | .... |
| HET. | 0.480 | 0.412 | 0.469 | 0.521 | 0.517 | 0.645 | 0.430 | 0.289 | 0.497 |
| Esterase-10 | | | | | | | | | |
| 1 | .... | 0.050 | .... | .... | .... | .... | .... | .... | .... |
| 2 | .... | 0.050 | .... | .... | .... | .... | .... | 0.053 | .... |
| 3 | 1.00 | 0.900 | 0.976 | 1.000 | 1.00 | 0.955 | 1.00 | 0.947 | 1.00 |
| 4 | .... | .... | 0.024 | .... | .... | .... | .... | .... | .... |
| 5 | .... | 0.050 | .... | .... | .... | 0.045 | .... | .... | .... |
| HET. | 0 | 0.185 | 0.047 | 0 | 0 | 0.086 | 0 | 0.101 | 0 |

frequencies of the various amylase phenotypes that were seen on the gels. An index of heterozygosity in this case was calculated from these phenotypic frequencies. To facilitate the North-South comparison of gene frequencies, the populations are listed (in Tables 2 and 7) from North to South; first those from North America, followed by those from Europe and Africa, followed by those from Taiwan, Vietnam and Australia. The loci are listed in the order of their occurrence on chromosomes *I*, *II* and *III*, followed by those loci that are unmapped.

## RESULTS

Of the 26 loci studied, only two, Malic enzyme and Larval Protein-16, were found to be uniformly monomorphic in all populations. We have defined a locus to be polymorphic when the frequency of the most common variant is 99% or less. The results for the 24 polymorphic loci are presented in Tables 2 and 3 and are summarized in Table 4. Rather than describe each locus separately, we will identify the general trends, which emerge from the data in Tables 2 and 3.

All populations studied here were highly polymorphic, but temperate populations (Ottawa, Hamilton, France) are less polymorphic than the subtropical or tropical populations (Texas and West Africa). This is apparent in all three statistics calculated—proportion of polymorphic loci, average number of alleles per

TABLE 3

*Frequency of different* Amylase *phenotypes in various populations of* D. melanogaster

| Amylase phenotypes | Ottawa (Canada) | Massa-chusetts (U.S.A.) | Texas (U.S.A.) | France | West Africa | Taiwan | Vietnam | Australia |
|---|---|---|---|---|---|---|---|---|
| 1 | 0.450 | 0.738 | 0.800 | 1.00 | 0.064 | 0.158 | 0.476 | 0.84 |
| 1, 2 | .... | .... | .... | .... | .... | .... | 0.190 | 0.040 |
| 1, 3 | 0.050 | 0.071 | 0.143 | .... | 0.011 | 0.316 | 0.048 | 0.080 |
| 1, 4 | .... | .... | .... | .... | .... | 0.053 | 0.238 | .... |
| 1, 5 | .... | .... | .... | .... | 0.117 | .... | .... | .... |
| 1, 6 | .... | 0.095 | 0.043 | .... | 0.085 | 0.263 | .... | .... |
| 1, 2, 3 | 0.400 | 0.036 | .... | .... | .... | .... | .... | .... |
| 1, 2, 6 | .... | .... | .... | .... | .... | .... | 0.048 | .... |
| 1, 3, 5 | .... | .... | .... | .... | 0.043 | .... | .... | .... |
| 1, 3, 6 | .... | 0.024 | 0.014 | .... | 0.021 | 0.105 | .... | .... |
| 1, 5, 6 | .... | .... | .... | .... | 0.170 | .... | .... | .... |
| 2 | .... | .... | .... | .... | 0.043 | .... | .... | .... |
| 2, 3 | 0.050 | 0.048 | .... | .... | .... | .... | .... | .... |
| 2, 3, 5 | .... | .... | .... | .... | 0.011 | .... | .... | 0.040 |
| 2, 3, 5. 6 | .... | .... | .... | .... | 0.011 | .... | .... | .... |
| 3, 4 | .... | .... | .... | .... | 0.021 | .... | .... | .... |
| 3, 5 | .... | .... | .... | .... | 0.021 | .... | .... | .... |
| 3, 4, 5 | .... | .... | .... | .... | 0.011 | .... | .... | .... |
| 3, 5, 6 | .... | .... | .... | .... | 0.032 | .... | .... | .... |
| 4, 5 | .... | .... | .... | .... | 0.074 | .... | .... | .... |
| 4, 6 | .... | .... | .... | .... | 0.064 | .... | .... | .... |
| 4, 5, 6 | .... | .... | .... | .... | 0.117 | .... | .... | .... |
| 4, 5, 7 | .... | .... | .... | .... | 0.011 | .... | .... | .... |
| 5 | .... | .... | .... | .... | 0.074 | .... | .... | .... |
| 5, 6 | .... | .... | .... | .... | 0.085 | .... | .... | .... |
| 5, 7 | .... | .... | .... | .... | 0.021 | .... | .... | .... |
| 5, 6, 7 | .... | .... | .... | .... | 0.011 | .... | .... | .... |
| 6 | .... | .... | .... | .... | 0.011 | .... | .... | .... |
| 3, 6 | .... | .... | .... | .... | .... | 0.105 | .... | .... |

locus, and average heterozygosity (Table 4). This North-South trend is present in populations from North America and is repeated in populations from France and West Africa, but is not seen in the populations from Taiwan, Vietnam and Australia; these latter three populations are relatively similar to each other in terms of levels of polymorphism (Table 4). The North-South trend in average polymorphism is, of course, a reflection of what is also true of individual loci. Many loci show a particular pattern of allele frequency change, suggestive of latitudinal clines, such that heterozygosity increases from north to south. For example, at the *G-6PD* locus allele 1 increases from 5% in Ottawa to 28% in Hamilton, 90% in Massachusetts and 100% in Texas; similarly it goes from 16% in France to 41% in West Africa (Table 2). On other hand, some loci such as *XDH* do not show clinal patterns. The loci that show parallel North-South patterns in allozyme frequencies among populations from North America,

TABLE 4

*Summary of allozyme polymorphisms in various populations of* Drosophila melanogaster

| Statistics | Ottawa (Canada) 45.3°N | Hamilton (Canada) 43.3°N | Massa-chusetts (U.S.A.) 42.4°N | Texas (U.S.A.) 25.8°N | France 46.1°N | West Africa 6.3°N | Taiwan 25°N | Vietnam 11°N | Australia 33.4°S |
|---|---|---|---|---|---|---|---|---|---|
| Proportion of loci polymorphic | 0.615 | 0.769 | 0.807 | 0.807 | 0.654 | 0.769 | 0.731 | 0.615 | 0.731 |
| Average No. Alleles[1] | 1.80 | 2.24 | 2.48 | 2.52 | 2.20 | 2.64 | 2.08 | 2.08 | 1.92 |
| Average Heterozygosity[1] | 0.168 | 0.225 | 0.267 | 0.310 | 0.238 | 0.266 | 0.187 | 0.199 | 0.190 |

[1] Excluding the Amylase locus.

and between France and West Africa are *G-6PD*, ADH, AO, EST-C, EST-6, LAP-D and ODH (Table 2).

The remarkable observation is not that we have some loci which show variation between temperate and tropical populations, but that these patterns are repeated on two separate continents. Moreover this set includes those loci that have shown similar clinal patterns in previous studies, although those studies were usually on a smaller geographical scale (JOHNSON and SCHAFFER 1973; VOELKER, MUKAI and JOHNSON 1977; VIGUE and JOHNSON 1973). However, the fact that latitudinal clines cannot be the sole determinant of the patterns of variation is clear from an examination of the Taiwan and Vietnam populations. These populations are similar latitudinally to Texas and West Africa but the allele frequencies, for those loci that show evidence of latitudinal clines, are more similar to those from the temperate populations of North America and Europe. This effect is seen most dramatically at the ADH locus. Generally, the $ADH^F$ allozyme is found at high frequency in temperate populations and at a much lower frequency in tropical and subtropical populations. However both the Taiwan and Vietnam populations show very high frequencies of $ADH^F$ (Table 2). In general, clinal patterns which are seen in North America and in Europe and Africa, seem not to be repeated in the East Asian populations.

For the amylase locus all populations except France are polymorphic, although some are much more polymorphic (*e.g.*, West Africa) than others (*e.g.*, Australia and Texas). The amylase variation in West Africa is remarkably high with a total of 23 amylase phenotypes and the most common phenotypes accounting for only 17% of the total frequency (Table 3). This peculiar pattern of variation at the amylase locus has been reported previously (HICKEY 1979b). It is interesting to note that the Vietnam population, which is similar to the temperate populations at many loci (*e.g.*, ADH), has a high combined frequency (over 40%) of two amylase phenotypes, $Amy^{1,2}$ and $Amy^{1,4}$, which are absent from the temperate populations (Table 3).

The loci have been classified according to their function and subunit size, and the polymorphism statistics for each of these groups are presented in Table 5. As has been shown before (GILLESPIE and KOJIMA 1968; KOJIMA, GILLESPIE and

TOBARI 1970; GILLESPIE and LANGLEY 1974) Group 1 loci are less polymorphic than Group II loci; Group III, comprised of the larval proteins, is very polymorphic in terms of average number of alleles per locus, but is less so in terms of heterozygosity. As has also been shown before (ZOUROS 1976; HARRIS, HOPKINSON and EDWARDS 1977; WARD 1977) monomeric loci, including the larval proteins, are generally more polymorphic than the dimeric ones. Since known polymorphic loci were chosen for this study, and since most of the monomorphic loci not included here are in Group I (GILLESPIE and LANGLEY 1974), the differences between Group I and Group II loci which are shown in Table 5 are an underestimate of the real differences between these two groups.

Heterozygosity was partitioned into its within-population and between-population components. The proportion of heterozygosity attributable to among-population differentiation is shown in terms of Wright's fixation index (Table 6). These data allow us to summarize the results on a per locus basis. The main features to be noted in Table 6 are, first, that some loci such as ADH and G-6PD are highly differentiated between populations, while others which are equally polymorphic, such as XDH and EST-6 are not. Secondly, when we plot the fixation index for each locus against the average within-population heterozygosity (Figure 2) we see that this differentiation between populations is not always correlated with the level of heterozygosity. While the average heterozygosity and fixation index are positively correlated for weakly polymorphic loci as would be expected, this is not the case for some of the highly polymorphic loci, for example, ADH and XDH. Generally, the correlation between fixation index ($F_{st}$) and average within population heterozygosity was not significant (Figure 2). The loci which display the highest level of allelic differentiation between populations are those that are di- or tri-allelic and which have intermediate levels of heterozygosity (see Figure 2 and Table 2). Naturally, many of the loci which show evidence for latitudinal clines in allele frequency also contribute significantly to the between-population differentiation as measured by the fixation index (Table 6). On the average, about 20% of the total heterozygosity is attributable to between-population differentiation. However, there is much heterogeneity between loci; *i.e.*, while some loci are remarkably even in their allele-frequency distribution, others are very different. This is, indeed, the principal finding of this study.

TABLE 5

*Average alleles and average heterozygosity of allozyme loci grouped according to variou criteria*

| Allozyme[1] groups | No. loci | Av. alleles | Av. het. |
|---|---|---|---|
| Group I | 5 | 2.80 | 0.281 |
| Group II | 15 | 4.00 | 0.329 |
| Group III | 4 | 5.25 | 0.194 |
| Monomeric | 13 | 4.30 | 0.340 |
| Dimeric | 11 | 3.54 | 0.254 |

[1] Enzyme loci in Group I and Group II are classified according to GILLESPIE and LANGLEY (1974), Group III includes all larval protein loci. For the subunit structure of loci see Table 1.

TABLE 6

*Total heterozygosity, average heterozygosity among populations and fixation index for various allozyme loci in* D. melanogaster. *Loci are arranged in order of decreasing fixation index*

| Loci | Total heterozygosity ($H_T$) | Av. heterozygosity per population ($H_S$) | Fixation index ($F_{ST}$) $(H_T-H_S)/H_T$ |
|---|---|---|---|
| ADH | 0.4673 | 0.2343 | 0.4986 |
| G-6PDH | 0.4812 | 0.2544 | 0.4713 |
| LAP-6 | 0.4996 | 0.3076 | 0.3843 |
| Est-C | 0.3767 | 0.2415 | 0.3589 |
| 6-PGD | 0.3356 | 0.2524 | 0.2479 |
| LAP-D | 0.4262 | 0.3357 | 0.2123 |
| AO | 0.5127 | 0.4162 | 0.1882 |
| LAP-A | 0.5029 | 0.4243 | 0.1562 |
| ODH | 0.1661 | 0.1411 | 0.1505 |
| Lpt-11 | 0.4577 | 0.3849 | 0.1492 |
| MDH | 0.0312 | 0.0273 | 0.1250 |
| XDH | 0.5979 | 0.5232 | 0.1249 |
| AMY[1] | 0.6612 | 0.5856 | 0.1143 |
| To-1 | 0.1374 | 0.1217 | 0.1142 |
| αGPD | 0.3425 | 0.3055 | 0.1080 |
| Lpt-15 | 0.1235 | 0.1104 | 0.1060 |
| Est-9 | 0.5283 | 0.4733 | 0.1041 |
| Est-6 | 0.5236 | 0.4724 | 0.0977 |
| PGM | 0.2137 | 0.1974 | 0.0762 |
| Lpt-9 | 0.1195 | 0.1114 | 0.0677 |
| APH | 0.0899 | 0.0855 | 0.0489 |
| To-2 | 0.0130 | 0.0124 | 0.0461 |
| Est-10 | 0.0486 | 0.0465 | 0.0432 |
| Lpt-10 | 0.1766 | 0.1713 | 0.0300 |
| ACPH | 0.0410 | 0.0403 | 0.0170 |
| Mean (excluding AMY) | 0.3005 | 0.2371 | 0.1636 |

[1] See text for explanation of figures for the Amylase locus.

The same trends are evident when we look at population differentiation in terms of NEI's (1975) genetic identity and genetic distance (Table 7). Here the emphasis is on a per-population analysis, rather than per locus, as was the case for the fixation indices. The temperate populations, Ottawa, Hamilton and France, along with the Asian and Australian populations, Taiwan, Vietnam and Australia, are all very similar to one another. Within this group, the coefficient of genetic identity is close to 95%—a figure not very different from other studies on Drosophila populations (AYALA *et al.* 1974). In contrast, populations from Texas and West Africa are very different from most other populations. The Massachusetts population is intermediate between these two groups. It is quite clear from the results (Figure 3) that the differentiation between populations is not simply a function of the distance between them. The fact that temperate populations, regardless of the continent are similar to each other, as are (to a lesser extent) the tropical populations, shows that lack of migration (distance) alone

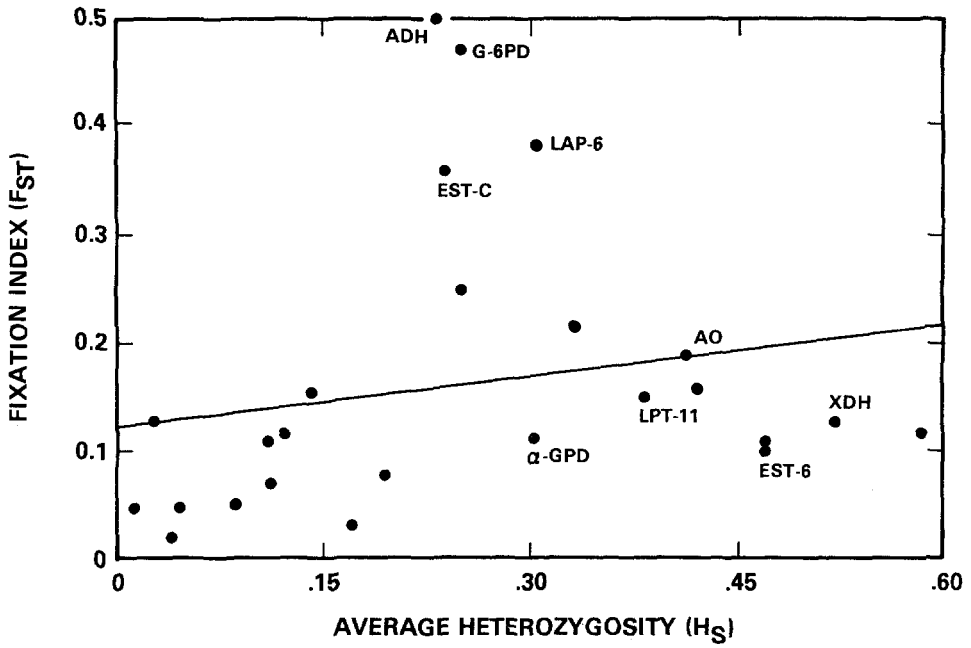


FIGURE 2.—Regression of Fixation Index ($F_{st}$) on average heterozygosity ($H_s$) at allozyme loci in several geographical populations of *Drosophila melanogaster*. $Y = 0.12 + 0.16\ X$. Correlation coefficient ($r^2$) $= 0.20$ ($p > 0.05$).

cannot explain these patterns. The average genetic identity coefficient for all populations is 90% with a range of 80 to 98%. Compared to the identity coefficient of 97% between populations of *D. willistoni* (AYALA *et al.* 1974), this figure is relatively low. Moreover, the average genetic identity between the West African population and all other populations is only 85%. However, the inclusion of loci that are identically monomorphic in all populations would increase these measures of genetic identity.

TABLE 7

*Average genetic identity (upper diagonal) and genetic distance (lower diagonal) between geographical populations of* Drosophila melanogaster

| | OT | HA | FR | MA | TE | WA | TA | VI | AU |
|---|---|---|---|---|---|---|---|---|---|
| OT | | 0.975 | 0.911 | 0.918 | 0.884 | 0.866 | 0.913 | 0.928 | 0.943 |
| HA | 0.025 | | 0.948 | 0.933 | 0.900 | 0.873 | 0.943 | 0.947 | 0.969 |
| FR | 0.093 | 0.053 | | 0.868 | 0.843 | 0.812 | 0.934 | 0.955 | 0.961 |
| MA | 0.086 | 0.069 | 0.142 | | 0.971 | 0.878 | 0.849 | 0.887 | 0.905 |
| TE | 0.123 | 0.101 | 0.171 | 0.030 | | 0.887 | 0.806 | 0.839 | 0.880 |
| WA | 0.144 | 0.136 | 0.209 | 0.130 | 0.120 | | 0.802 | 0.844 | 0.831 |
| TA | 0.091 | 0.059 | 0.067 | 0.163 | 0.215 | 0.220 | | 0.952 | 0.952 |
| VI | 0.075 | 0.055 | 0.046 | 0.119 | 0.175 | 0.169 | 0.049 | | 0.933 |
| AU | 0.059 | 0.031 | 0.040 | 0.099 | 0.128 | 0.186 | 0.049 | 0.069 | |

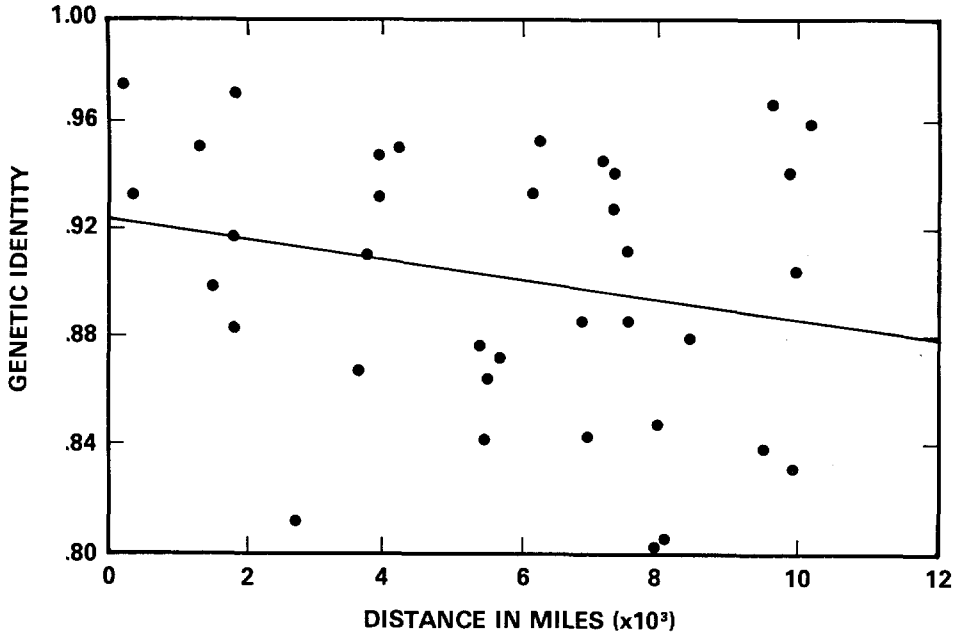


FIGURE 3.—Regression of average genetic identity on the minimum physical distance between populations of *Drosophila melanogaster*. $Y = 0.924 - 0.004X$. Correlation coefficient $(r^2) = -0.23$ $(p > 0.05)$.

Since various loci contribute very different amounts to between-population differentiation (Table 6), it is useful to ask what the average genetic identity between populations would be when calculations are based on each individual locus. It quickly becomes apparent (Table 8) that the genetic identity, averaged over all loci, can be very misleading because, as was clear from Table 6, there are two more or less clearcut groups of loci. One group, which includes several highly polymorphic loci, yields a genetic identity coefficient of about 98%. The other group of loci, ranging from moderately to highly polymorphic, are highly differentiated, giving a mean identity coefficient of 78% with a range of 60% to 90%. Thus, it is obvious that the average genetic identity of 90% for all loci is not very meaningful in this case, because it is an average based on a very heterogeneous set of loci. It is remarkable that, for some loci, the average differentiation between populations (Table 8) is as great as has been shown to exist between sibling species of Drosophila (*e.g.*, AYALA *et al.* 1974; PRAKASH 1977; TRIANTAPHYLLIDIS *et al.* 1980.

## DISCUSSION

Recent doubts about the adaptive significance of enzyme polymorphisms stem from several sources. The first, and most important, is the general lack of clearcut cases of allozyme loci where natural selection can be shown to occur. Alcohol dehydrogenase and amylase polymorphisms in *Drosophila melanogaster* readily

TABLE 8

*Mean and range of genetic identity for allozyme loci in various population of* Drosophila melanogaster. *(Loci arranged in order of increasing genetic identity)*

| Locus | Genetic identity | |
|---|---|---|
| | Mean | Range |
| AMY | 0.599 | 0.155–0.993 |
| G-6PD | 0.653 | 0–0.995 |
| ADH | 0.656 | 0.030–1.000 |
| LAP-6 | 0.684 | 0.209–1.000 |
| EST-C | 0.787 | 0.527–1.000 |
| AO | 0.796 | 0.229–0.990 |
| XDH | 0.816 | 0.488–0.991 |
| LAP-D | 0.834 | 0.224–1.000 |
| LAP-A | 0.855 | 0.591–0.999 |
| 6-PGD | 0.868 | 0.561–1.000 |
| LPt-11 | 0.875 | 0.602–1.000 |
| EST-6 | 0.887 | 0.655–1.000 |
| EST-9 | 0.907 | 0.714–0.998 |
| α-GPD | 0.939 | 0.669–1.000 |
| ODH | 0.966 | 0.851–1.000 |
| TO-1 | 0.986 | 0.955–1.000 |
| LPt-15 | 0.986 | 0.946–1.000 |
| PGM | 0.987 | 0.962–1.000 |
| LPt-9 | 0.994 | 0.984–1.000 |
| LPt-10 | 0.995 | 0.988–0.999 |
| APH | 0.995 | 0.981–1.000 |
| MDH | 0.996 | 0.986–1.000 |
| ACPH | 0.999 | 0.997–1.000 |
| TO-2 | 0.999 | 0.998–1.000 |
| EST-10 | 0.999 | 0.997–1.000 |
| Mean | 0.882 | |
| | 0.893 (Excluding AMY) | |

come to mind, but such loci are rare among the hundreds studied in various organisms. Secondly, very little of the allozyme variation seems to be involved in the process of speciation (HUBBY and THROCKMORTON 1968; LEWONTIN 1974; CARSON 1975). Thirdly, the concept of a molecular clock in protein evolution (ZUCKERKANDL and PAULING 1962; MAXSON, SARICH and WILSON 1975) and the claim that protein polymorphism is a phase of molecular evolution (KIMURA and OHTA 1971) have also helped to reduce the perceived significance of protein variation in adaptive evolution. Fourthly, the role of protein variation in evolution has also been questioned because of the proposition that adaptive evolution is propelled, not by variation of the structural genes, but by variation in the regulatory genes, mainly by gene-rearrangements (WILSON, MAXSON and SARICH 1974; WILSON, SARICH and MAXSON 1974). Finally, the current controversy about the decoupling of micro- and macro-evolution and the role of macrogenetic changes in evolution have also given some support, although an indirect one, to the idea that allozyme variations, or point mutations in general, do not play an important role in evolution (ELDREDGE and GOULD 1972; WILSON 1976;

VALENTINE and CAMPBELL 1975). Thus, the lack of authenticated examples of selection acting on protein polymorphisms has been only partly responsible for the present negative state of our attitude about the adaptive role of allozyme variation in evolution.

Before discussing our results in detail, it is important to determine if our sample populations are really representative of the gene-pools in the sampled regions. This view is supported by what we know about the population structure of this species. First, populations of *D. melanogaster* sampled over several consecutive years show a stable allele frequency distribution (MUKAI and VOELKER 1977). Secondly, when populations have been sampled over seasons in a single year, a stable frequency distribution is observed for many but not all, loci (BERGER 1970; GIRARD, PALABOST and PETIT, 1977). The Hamilton population was sampled over a period of several months and no significant differences between samples have been observed (R. A. MORTON, personal communication). Thirdly, local populations from a given region have similar allelic frequencies, suggesting that migration in this species is probably too large to allow much local differentiation (BERGER 1970; GIRARD, PALABOST and PETIT 1977; COCHRANE and RICHMOND 1979; DAVID 1981). Fourthly, in our study, several loci have shown parallel patterns in two different continents, strongly suggesting that our samples are indeed representative of those regions. Finally, a comparison of our results with some published data on the same regions indicates that our samples are again representative of the regions from which they come. For example, our results for the alcohol dehydrogenase and amylase loci are similar to those described in other studies (VIGUE and JOHNSON 1973; DAVID 1981; HICKEY 1979b). Also, the Amherst and Brownsville populations have been studied before and those results are generally in agreement with ours (LANGLEY, TOBARI and KOJIMA 1974; CHARLESWORTH and CHARLESWORTH 1973; BAND 1975).

Another obvious question is whether allozyme clines are merely a reflection of inversion clines. Since we do not have inversion data, we cannot answer this question directly. However, there are several facts which seem to indicate that inversions alone cannot explain the allozyme clines observed in this species. While latitudinal clines for inversions (METTLER, VOELKER and MUKAI 1977) have been reported, it was found that these were not always correlated with allozyme clines (SCHAFFER and JOHNSON 1974; VOELKER *et al.* 1978). VOELKER *et al.* (1978) concluded that the clines for ADH and $\alpha$-GPD alleles could not be explained on the basis of associated inversion clines alone. In our study, not all loci showing North-South differentiation are associated with inversions. EST-C, ODH, LAP-A and LAP-D are all outside commonly occurring inversions; EST-6 and $\alpha$-GPD lie inside inversion regions; while AMY and ADH are both close to inversion regions but on different arms of chromosome *II*. In our study, ADH shows latitudinal differentiation but AMY does not; conversely, AMY shows extremely high levels of polymorphism in the West African population while ADH does not. These results would suggest that inversion clines alone cannot explain allozyme clines, but more data are required to prove this point on a global scale.

Latitudinal patterns in allele frequency are repeated, in trend if not in magnitude, in both North America and in European and African populations. More-

over, these trends are consistent with the clinal patterns of variation that have previously been described for the North American continent (JOHNSON and SCHAFFER 1973). However, there is no evidence for a similar latitudinal cline in the populations from Taiwan, Vietnam and Australia. For example, the $ADH^F$ allele, which is typical of temperate American and European populations, is fixed in Vietnam and almost fixed in Taiwan. A possible explanation is that the $ADH^F$ allozyme in the Asian populations is not identical to the $ADH^F$ of Europe and America; however, the Vietnam and Taiwan populations seem to have a high frequency of the "wrong" alleles at many other loci too. Some factors, other than simply latitude, must be operating to generate such a seemingly "wrong" pattern. The high frequencies of otherwise rare alleles in Vietnam and Taiwan for Larval Protein-II would indicate the role of a bottleneck effect, but the similarity of allele frequency distribution to that in other populations at some highly polymorphic loci (*e.g.*, XDH) argues against this hypothesis. It has been suggested, based on differences in biometrical traits, that the Far Eastern populations of *D. melanogaster* may constitute a distinct genetic race (DAVID *et al.* 1976).

Now we turn to the question: why should geographical populations of *D. melanogaster* show large-scale genetic differentiation whereas no such pattern is seen in populations of *D. pseudoobscura* (LEWONTIN 1974) and *D. willistoni* (AYALA *et al.* 1972)? One answer is that balancing selection is responsible for the geographic similarity observed in these previous studies, although this explanation is now less probable in the case of *D. pseudoobscura*, since this species has been shown to possess a remarkable dispersal potential (JONES *et al.* 1981). This is not to say that migration alone must be responsible for the geographical similarity of allele frequencies in *D. pseudoobscura*, but merely that migration is now a plausible alternative to balancing selection in this case.

Our general argument is that the striking differences observed in the patterns of variation shown by different groups of loci must be the result of natural selection, though we cannot, at this point, characterize the exact nature of the inferred selective forces. As pointed out by JONES *et al.* (1981), if populations are connected by extensive migration, large differences in gene frequencies could then plausibly be attributed to locally differentiated selective forces. The ecology of *D. melanogaster* leads one to suppose that migration, even over long distances, is considerable. Moreover, the North-South differentiation in allelic frequencies which occurs in both the New and Old World is very suggestive of the action of regionally-differentiated selective forces.

A major problem in determining the significance of protein polymorphisms has been that the allozyme frequency data that are presented as evidence in favor of the selection hypothesis can also be explained by the neutral hypothesis with a suitable choice of parameters (LEWONTIN 1974). Because of this problem, a general consensus seems to have been reached that the selective significance of protein polymorphisms can only be determined in laboratory experiments involving detailed biochemical and physiological techniques. The studies with alcohol dehydrogenase (KAMPING and VAN DELDEN 1977) and amylase (SCHARLOO *et al.* 1977; HICKEY 1979a) are the paradigms for such investigations. However, we think that laboratory experiments can only be a complement to natural field

studies. Laboratory experiments can, at best, only show what to measure, and when to measure, and may tell us the biochemical and physiological mechanisms whereby natural selection works at a given locus. However, in order to evaluate the evolutionary significance of allozyme polymorphisms, we must not only demonstrate that natural selection can occur, but also show that it does occur in nature. Laboratory experiments only show the potential for natural selection and the probable magnitude of the selection coefficients. On the other hand, surveys of natural populations, such as the present study, can only tell us whether or not selection may be involved, but not how and when; this information is obtainable only through laboratory experiments. Therefore, we do not subscribe to the view that showing selection at, say, half-a-dozen allozyme loci, using laboratory experiments, would be sufficient evidence in favor of the selectionist hypothesis for the maintenance of protein polymorphisms. An approach that combines both laboratory investigations and surveys in nature is not only desirable but essential. The potential usefulness of this combined approach becomes apparent when we consider the fact that the loci showing clines or between-population differentiation in the present study (and in some previous studies) are the very loci that are showing promising results in laboratory selection experiments (McDONALD and AVISE 1974; MORGAN 1976; COCHRANE and RICHMOND 1979; HICKEY 1979a; SCHARLOO *et al.* 1977; GIBSON 1970; VAN DELDEN *et al.* 1978; DAY, HILLIER and CLARKE 1974a, 1974b; KOJIMA and YARBROUGH 1967; KOJIMA and TOBARI 1969; KAMPING and VAN DELDEN 1977; ALAHIOTIS, MILLER and BERGER 1977; COLLIER and MACINTYRE 1977). Another excellent example of where such a combined approach has paid off is in the case of leucine aminopeptidase variants in Mytilis (KOEHN *et al.* 1980).

The results presented here do not provide a complete description of the genetic composition of *D. melanogaster*. They do serve, however, to illustrate that extensive genetic differentiation has taken place over the species range. An obvious expansion of this work would be to do more extensive sampling from other areas as well (*e.g.*, South America, Central Asia, South Africa) in order to get more precise quantitative measures of the trends noted here. Parallel studies of karyotypic, morphometric and physiological variability would be especially interesting. Some morphometric analyses have already shown significant differences between populations (DAVID and BOCQUET 1975; DAVID *et al.* 1976). Because of its world-wide distribution, its abundance, and the wealth of background genetic information, *D. melanogaster* provides an excellent opportunity for studying the significance and interrelationships of the various phenotypic expressions (allozymic, karyotypic, morphological and physiological) of naturally-occurring genetic polymorphism; and it may also shed some light on the functional relationships between these various manifestations of genetic variation.

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