ELECTROPHORETIC HETEROGENEITY OF α-GLYCEROPHOSPHATE DEHYDROGENASE AMONG MANY SPECIES OF *DROSOPHILA*

JERRY A. COYNE, WALTER F. EANES, JOHN A. M. RAMSHAW, AND RICHARD K. KOEHN

Abstract

Coyne, J. A., W. F. Eanes, J. A. M. Ramshaw (Museum of Comparative Zoology, Harvard University, Cambridge, Massachusetts 02138) and R. K. Koehn (Department of Ecology and Evolution, State University of New York, Stony Brook, New York 11794). 1979. Electrophoretic heterogeneity of α-glycerophosphate dehydrogenase among many species of Drosophila. *Syst. Zool. 28:164–175.*—Electromorphic variation of α-glycerophosphate dehydrogenase in 71 species of *Drosophila* has been studied using sequential polyacrylamide gel electrophoresis, sequential starch gel electrophoresis, and isoelectric focusing. Although this locus was previously reported as nearly invariant among many species of the genus, this study shows a substantial increase in variation between species which is concordant with the known taxonomy of the genus. However, these methods revealed no additional variation within the species themselves. Each of the three methods found variation not detectable by the other two; thus, all are necessary for maximizing the observed variation between species. In comparison to previous electrophoretic studies of this enzyme in *Drosophila,* these new data show a much increased concordance with reported immunological and kinetic differences between this enzyme in different species. The implications of these results for electrophoretic studies of speciation and taxonomy are discussed. [*Drosophila* spp.; α-glycerophosphate dehydrogenase; electrophoresis; heterogeneity.]

In recent years electrophoresis has been extensively used to investigate questions of general interest in evolutionary biology and systematics (Ayala et al., 1974; King and Wilson, 1975; Mickevich and Johnson, 1976; and many others). Electrophoretic data is fairly simple to gather, and it has a number of advantages over more traditionally used taxonomic characters (Avise, 1974). Because it is assumed that electromorphs are only a step removed from single genes, differences between them are readily translated into measures of "genetic distance." Inherent in these investigations are assumptions about the nature of electrophoretic variation. It is often recognized that some genetic differences may be mistakenly described as genetic identities due to convergence of different variants to similar mobilities; but this problem is usually ignored, being considered insignificant given the weight of a total data set. Of course the generalities drawn from such data can seriously depend on the safety of these assumptions.

Evidence is now accumulating that electrophoretic phenotypes are not necessarily homogeneous molecular entities, but are often heterogeneous classes which will divide when subjected to a series of electrophoretic conditions (e.g., Weitkamp et al., 1973; Bernstein et al., 1973; Singh et al., 1976; Coyne and Felton, 1977). When used to investigate variation within species, this approach has had a significant effect on the interpretation of particular problems, such as descriptions of geographic variation of gene frequencies. So far these analyses have not been extended to comparisons between many species. However, the implications of undetected variation at this level may be even more profound than within species. In this report we examine the apparent electrophoretic identity of a single enzyme α-glycerophosphate dehydrogenase (α-GPDH, L-glycerol-3-phosphate oxidoreductase, E.C. 1.1.1.8.) in many species of the genus *Drosophila.* Our study clearly demonstrates that judgements of genetic

identity depend considerably on the discriminating ability of particular electrophoretic conditions.

Previous electrophoretic surveys of α-GPDH have shown it to be remarkably invariant not only within species of *Drosophila,* but also between them. Using starch gel electrophoresis in a single buffer system, Lakovaara et al. (1977a) reported only eleven electromorphs of the enzyme among 159 species of *Drosophila.* Six of these variants were found in three or fewer species each. Of the 87 non-Hawaiian species investigated by Lakovaara et al. (1977a), 16 were monomorphic for the *100* (slow) variant of α-GPDH, 52 for the *104* variant, and 11 for the *108* (fast) variant. Only the two species *D. melanogaster* and *D. subarctica* were polymorphic for the enzyme. In a later study (Lakovaara et al., 1977b), these workers found five additional thermostability variants in the genus, three of which were within the species *D. subarctica.*

Collier (1977) studied α-GPDH in 105 species of *Drosophila* using cellulose acetate electrophoresis. His results were consistent with those of Lakovaara et al. (1977a); only eight electromorphs were found, four of which were unique to single species. Four species contained electromorph *1* (corresponding to *100* of Lakovaara et al. [1977a]), 74 contained electromorph *4* (*104*), and 23 contained electromorph 7 (*108*). There were a few differences between this study and that of Lakovaara et al. (1977a) in the assignment of variants to species, but in general their findings were nearly identical. The invariance of this protein among fly species led Lakovaara et al. (1977a) to conclude that it was evolving slowly and was canalized to exist in only a few forms.

In contrast to these results, however, are several other pieces of biochemical evidence indicating a more extensive heterogeneity of the enzyme among *Drosophila* species. Collier and MacIntyre (1977) used microcomplement fixation to examine immunological differences between α-GPDH proteins of 31 species of *Drosophila.* Their results demonstrate apparent immunological differences between many of the electromorphs judged identical in the electrophoretic studies mentioned above. In another survey, Alahiotis et al. (1977) found some significant differences in thermostability and Michaelis constants among α-GPDH from six species of *Drosophila,* all of which share the same electromorph according to Lakovaara et al. (1977a, 1977b) and Collier (1977).

In an attempt to resolve the discrepancies between the results of electrophoretic studies of α-GPDH and those of studies using other biochemical characterizations, we investigated the variability of this enzyme within and among 71 species of *Drosophila* with a combination of electrophoretic methods. We have used sequential polyacrylamide gel electrophoresis (Singh et al., 1976), sequential starch gel electrophoresis, and isoelectric focusing; we can thus compare these various methods for their ability to detect previously unknown variation. The results show an enormous increase in the number of known variants of α-GPDH among *Drosophila* species and demonstrate previously unrecorded electrophoretic differences which are consistent with the known taxonomy of the genus. We also find a substantial increase in the concordance between electrophoretic and other non-electrophoretic characterizations of the enzyme in these many species.

MATERIALS AND METHODS

Seventy-one species of *Drosophila* were obtained from several sources; for 25 of these species we were able to obtain at least one duplicate culture. Most of the cultures were derived from isofemale lines, while others were mass cultures of a species from a single locality. In addition, we examined 29 lines of *D. pseudoobscura* isogenic for the fourth chromosome and 16 lines of *D. melanogaster* isogenic for the second chromosome (these chromosomes are known to carry the α-GPDH locus in these species).

All flies were maintained at 17°C on standard cornmeal food, but flies with more rigid food requirements did not produce progeny under these conditions. A full list of the sources of the experimental material may be obtained from the authors.

Polyacrylamide Gel Electrophoresis

Electrophoresis in polyacrylamide slab gels was performed as described by Coyne and Felton (1977) with the following modifications. Recrystallized acrylamide and bisacrylamide (Biorad) rather than technical grade acrylamide (Cyanogum; E-C Apparatus Corp.) were used to obtain the best resolution for this enzyme. The acrylamide used to prepare each gel consisted of 6 percent bisacrylamide and 94 percent acrylamide. Fresh buffer was used for each electrophoretic run.

Enzyme variation among species was studied using sequential electrophoresis as described in Singh et al. (1976) and Coyne and Felton (1977). The following sequence of four conditions was used: condition 1, 4.5 percent gels at pH 8.9 in 0.1 M tris-borate-EDTA run for 4 hours; condition 2, 7 percent gels at pH 8.9 in 0.1 M tris-borate-EDTA run for 6 hours; condition 3, 4.5 percent gels at pH 7.1 in 0.1 M tris-borate-EDTA run for 7 hours; condition 4, 11 percent gels at pH 8.9 in 0.1 M tris-borate-EDTA run for 12 hours. Gels at conditions 1, 2, and 4 were run at 300 volts; gels at condition 3 were run at 200 volts. In addition, all species were further examined in 4.5 percent gels at pH 10.5 in 0.1 M tris-HCl, but no additional variation was found. The results of the sequential analysis are presented as a hierarchical classification of electromorphs (Coyne and Felton, 1977).

Isoelectric Focusing

Isoelectric focusing was performed essentially as described by Ramshaw and Eanes (1978) with the following changes. Gels contained 1.0 percent w/v pH 4–6 and 1.0 percent w/v pH 5–7 ampholytes and 0.2 percent w/v Triton X-100. During electrophoresis the voltage was increased to 500 volts over one hour at which time the sample papers were removed from the gel and the voltage increased to 1,000 volts for a further 1–2 hours.

Starch Gel Electrophoresis

Horizontal starch gel electrophoresis was performed as described by Koehn and Rasmussen (1967) using a concentration of 14 percent Electrostarch. Sequential electrophoresis using two conditions was carried out, first in a 0.1 M tris-borate-EDTA buffer of pH 8.9, and then in 0.15 M tris-citrate buffer of pH 7.0 (Shaw and Prasad, 1970). Voltages were about 10 volts/cm and gels of each buffer were run for 8 and 12 hours respectively.

Staining and Scoring Procedure

All gels were stained according to the method of Hubby and Lewontin (1966) except that KCN was omitted from the stain in polyacrylamide and starch gels.

Differences between variants were scored visually, and then were confirmed by running the putative variants together in alternate pairs of pockets on single gels (e.g., Fig. 2). We prefer this qualitative method to methods which distinguish variants by comparing measured relative mobilities from gel to gel since the former method eliminates sources of error which could lead to spurious results. This scoring technique is especially useful in distinguishing variants with only slight differences in mobility.

RESULTS

The three electrophoretic methods enabled us to resolve 19 distinct variants of α-GPDH in the 71 fly species. Each distinguishable electromorph is designated in Table 1 by a separate letter of the alphabet. Also shown in Table 1 are the electromorph classes distinguishable in each of the three independent studies using sequential polyacrylamide gel, sequential starch gel, and isoelectric focusing electrophoresis.

A total of 10 electrophoretic classes of α-GPDH were found by starch gel elec-

TABLE 1. CLASSIFICATION OF 19 ELECTROPHORETIC VARIANTS OF α-GLYCEROPHOSPHATE DEHYDROGENASE BY COMBINING THE RESULTS OF 3 DIFFERENT ELECTROPHORETIC TECHNIQUES. THE 19 VARIANTS ARE EACH INDICATED BY A SEPARATE LETTER AND THE RESULTS OF THE 3 ELECTROPHORETIC STUDIES ARE SHOWN SEPARATELY. THE NUMBER OF DIFFERENT LINES EXAMINED BY POLYACRYLAMIDE GEL ELECTROPHORESIS IS GIVEN IN PARENTHESES. FOR EACH ELECTROPHORETIC TECHNIQUE VARIANTS ARE NUMBERED SEQUENTIALLY WITH THE SLOWEST MOVING OR MOST BASIC VARIANT BEING GIVEN THE LOWEST NUMBER.

	Electromorph Class			Acrylamide results	Starch results	Isoelectric focusing results
Subgenus *Drosophila*						
repleta group						
mulleri subgroup						
aldrichi		k		4100 (1)	7	2c
arizonensis		k		4100 (3)	7	2c
buzzatti		k		4100 (1)	*	2c
meridiana meridiana	a^+	k		1000^+ (1) 4100 (1)	* 7	* 2c
mohavensis		k		4100 (3)	7	2c
mulleri		k		4100 (2)	7	2c
navajoa		k		4100 (1)	*	2c
mercatorum subgroup						
mercatorum		k		4100 (1)	7	2c
paranaensis		k		4100 (1)	7	2c
melanopalpa subgroup						
fulvimacula		k		4100 (1)	*	2c
melanopalpa		k		4100 (1)	7	2c
repleta		k		4100 (1)	7	2c
hydei subgroup						
bifurca		k		4100 (1)	7	2
eohydei		k		4100 (1)	7	2c
hydei		k		4100 (2)	7	2c
neohydei		k		4100 (1)	7	2c
robusta group						
robusta	d^+		p	2001^+ (1) 5000 (1)	* 7	* 2c
melanica group						
micromelanica		k		4100 (1)	7	2c
cardini group						
cardini		k		4100 (1)	*	2
polymorpha		k		4100 (1)	7	2
immigrans group						
immigrans			n	4120 (1)	7	2
virilis group						
americana americana		l		4100 (3)	7	2d
americana texana		l		4100 (2)	7	2d
ezoana		l		4100 (2)	7	2d
littoralis		l		4100 (1)	7	2d
lummei		l		4100 (1)	7	2d
novamexicana		l		4100 (2)	7	2d
virilis		l		4100 (3)	7	2d
borealis		i		3100 (1)	5	2c
flavomontana		i		3100 (2)	5	2c
lacicola		i		3100 (2)	5	*
montana montana		i		3100 (2)	5	2c
montana ovivororum		i		3100 (2)	5	*

TABLE 1. CONTINUED.

	Electromorph Class			Acrylamide results	Starch results	Isoelectric focusing results
nannoptera group						
nannoptera		p		5000 (1)	9	2c
pachea		p		5000 (1)	9	2c
quinaria group						
kunzei	b			1100 (1)	*	*
testacea group						
testacea	j			4000 (1)	7	2b
funebris group						
funebris	d			2001 (1)	3	1c
Subgenus *Phalodoris*						
lebanonensis	c			2000 (1)	2	1b
Subgenus *Dorsilopha*						
busckii		o		4130 (1)	7	2b
Subgenus *Sophophora*						
willistoni group						
capricorni	f			3000 (1)	5	2a
equinoxialis	g			3000 (1)	5	2b
fumipennis	g			3000 (1)	*	2
nebulosa	g			3000 (1)	5	2b
paulistorum	g			3000 (1)	5	2b
pavlovskiana	h		r^+	3000 (1) 6010+ (1)	6 8	2 3a
tropicalis	g			3000 (1)	5	2b
willistoni	g			3000 (1)	5	2b
melanogaster group						
montium subgroup						
birchii	a^+	m		1000+ (1) 4110 (1)	* 7	1a 2
serrata		m		4110 (1)	7	2c
melanogaster subgroup						
erecta	a^+	m		1000+ (1) 4110 (1)	* 7	* 2c
mauritiana			s	7000 (1)	10	3b
melanogaster		m	s	4110 (7) 7000 (3 + 9§)	7 10	2c 3b
orena		m		4110 (1)	7	2c
simulans			s	7000 (3)	10	3b
teissieri			s	7000 (1)	10	3b
yakuba			s	7000 (1)	10	3b
ananassae subgroup						
ananassae			s	7000 (1)	10	*
bipectinata			s	7000 (1)	10	3b
obscura group						
obscura subgroup						
ambigua	a			1000 (1)	1	1a
guanche	a			1000 (1)	1	1a
miranda	a			1000 (2)	1	1a
persimilis	a			1000 (5)	1	1a
pseudoobscura	a			1000 (12 + 29§)	1	1a
subobscura	a		q	1000 (4) 6000 (1)	1 8	1a *

TABLE 1. CONTINUED.

	Electromorph Class	Acrylamide results	Starch results	Isoelectric focusing results
affinis subgroup				
affinis	e	3000 (3)	4	2a
algonquin	e	3000 (1)	4	2a
athabasca	e	3000 (3 ‖)	4	2a
azteca	a	1000 (2)	1	1a
narragansett	a	1000 (2)	*	*
tolteca	a	1000 (2)	1	1a

* Not determined.
+ Assumed rare electromorph (see text).
§ Isochromosomal lines.
‖ 3 different semispecies were examined.

trophoresis and are listed in Table 1. All of these mobility classes were observed using the first criterion, a tris-borate-EDTA buffer of pH 8.9. No additional variability was found using the pH 7.0 tris-citrate system with which the data of Lakovaara et al. (1977a) were collected. The variant 8 observed in *D. pavlovskiana* was unique to the starch system, and was not detected by the other methods.

The extensive variability observed at pH 8.9 in starch gels (see Fig. 1) generally collapsed under the pH 7.0 system into the electrophoretic classes described by Lakovaara et al. (1977a) and Collier (1977). It is noteworthy that the mobility differences observed on polyacrylamide gels using the tris-borate-EDTA pH 7.1 system (see below) were generally not seen on starch gels using the tris-citrate pH 7.0 system. Since tris-borate-EDTA of pH 7.1 is not compatible with starch gel electrophoresis, a suitable comparison with polyacrylamide gels in the neutral pH range could not be carried out.

The results of the sequential polyacrylamide gel electrophoresis are also given in Table 1, along with the number of lines investigated in each species using this system (most, but not all replicate lines of every species were studied in starch or isoelectric focusing gels). As in previous studies (Singh et al. 1976; Coyne and Felton, 1977), electromorph classes are designated by a string of integers giving the mobility of the enzyme under each condition relative to a standard mobility within each condition. The relative mobilities corresponding to these integers are given in Table 2.

The four different conditions of polyacrylamide electrophoresis distinguished 15 electromorphs among the 71 species examined. Seven of these classes were resolved with condition 1, 3 with condition 2, and 4 with condition 3. A single difference, that between *D. lebanonensis* (variant *2000*) and *D. funebris* (variant

TABLE 2. MEASURED RELATIVE ELECTROPHORETIC MOBILITIES IN SEQUENTIAL POLYACRYLAMIDE GEL ELECTROPHORESIS (SEE COYNE AND FELTON [1977] FOR EXPLANATION OF THE NOMENCLATURE).

Electromorph	Mobility
1000	1.00/1.00/1.00/1.00
1100	1.00/1.02/1.00/1.00
2000	1.05/1.00/1.00/1.00
2001	1.05/1.00/1.00/1.08
3000	1.11/1.00/1.00/1.00
3100	1.11/1.03/1.00/1.00
4000	1.14/1.00/1.00/1.00
4100	1.14/1.02/1.00/1.00
4110	1.14/1.02/1.01/1.00
4120	1.14/1.02/1.03/1.00
4130	1.14/1.02/1.07/1.00
5000	1.15/1.00/1.00/1.00
6000	1.21/1.00/1.00/1.00
6010	1.21/1.00/1.02/1.00
7000	1.25/1.00/1.00/1.00

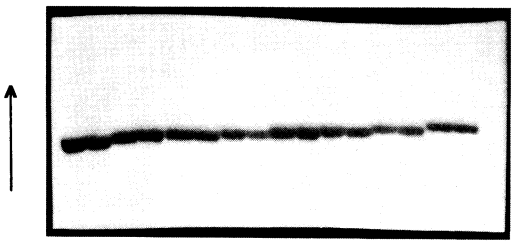

FIG. 1.—Separation of 8 variants of α-GPDH on starch gels of pH 8.9 in tris-borate-EDTA. Bands are numbered from left to right. 1, 2: *D. pseudoobscura*; 3, 4: *D. lebanonensis*; 5, 6: *D. funebris*; 7, 8: *D. affinis*; 9, 10: *D. borealis*; 11, 12: *D. virilis*; 13, 14: *D. nannoptera*; 15, 16: *D. melanogaster* (fast variant). Several of these variants were not resolved from one another in previous electrophoretic studies.

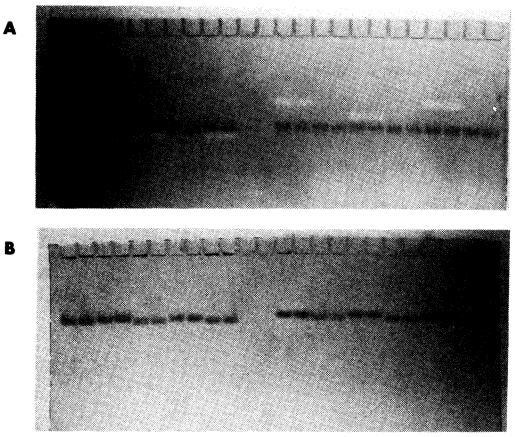

FIG. 2.—Polyacrylamide gels which show variation not detected by the other electrophoretic methods used in this study. Pockets are numbered from left to right. *Gel A.* 4.5 percent gel, pH 8.9. 1, 2, 5, 6, 9, 10: *D. immigrans*; 3, 4, 7, 8: *D. virilis*; 13, 14, 21, 22: *D. affinis*; 15, 16, 19, 20, 23, 24: *D. borealis*; 17, 18: *D. willistoni. Gel B.* 4.5 percent gel, pH 7.1. Order of species same as in Gel A.

2001), was revealed by condition 4. This latter system was the poorest at resolving differences since it produced broader bands and hence small changes in mobility were less detectable.

Several of the differences found in polyacrylamide gels were not found with starch gel electrophoresis (e.g., see Fig. 2). Notable among these are the difference between the electromorphs of the *virilis* group (*4100*) and the slow *melanogaster* group (*4110*), and the difference between the *willistoni* group variant *3000* and that of the slow *virilis* group (*montana* subphylad of the *montana* phylad) variant *3100*.

In contrast to the increased variability *between* species, polyacrylamide gel electrophoresis shows that the individual species themselves remain essentially monomorphic. No differences were seen in any of the replicate lines of 25 species, including the 41 lines of *D. pseudoobscura*. We also investigated several lines of each of the two common variants in the polymorphic species *D. melanogaster* (see Table 1), but no further heterogeneity was seen within either of these electromorph classes. Milkman (1976) and Bewley (1978) also found no additional variation in this species using thermostability criteria. Six species (*D. meridiana, D. robusta, D. pavlovskiana, D. erecta, D. subobscura,* and *D. birchii*) showed more than a single allele segregating in some of the lines we examined. In these cases one variant always possessed the same electromorph as closely related species, and from the assumption of continuing near-monomorphism in these species we designated the other variant "rare" in Table 1. Three of these "rare" variants (in *D. meridiana, D. birchii,* and *D. erecta*) appear identical in mobility, falling into the *1000* class.

Isoelectric focusing distinguished many of the classes of α-GPDH variants. The use of this technique separates the species into three general pI groups (Fig. 3). In species with more than a single variant (e.g., *D. pavlovskiana, D. melanogaster*), segregating variants are always found in adjacent groups. This suggests that these separate groups represent classes of variants which are a single unit charge apart (Ramshaw and Eanes, 1978). There is also smaller charge variation within each group. This variation may represent enzymes with the same absolute number of acidic and basic residues, but possessing slightly different net charges depending

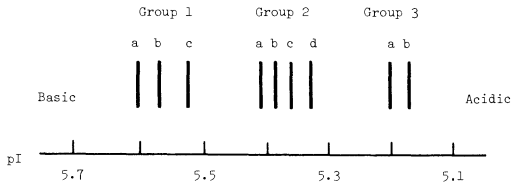

FIG. 3.—Relative positions of the charge groups observed by isoelectric focusing. The nomenclature of the various subgroups which were observed is indicated.

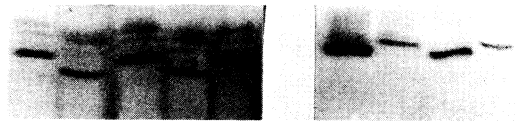

FIG. 4.—Sections from isoelectric focusing gels which show variation not detected by the other electrophoretic methods used in this study. Gel section 1 (L–R): *D. hydei, D. texana, D. hydei, D. virilis, D. hydei.* Gel section 2 (L–R): *D. equinoxialis, D. affinis, D. tropicalis, D. capricorni.*

on the *in situ* pK's of the individual amino acids.

A total of nine classes were clearly distinguished by isoelectric focusing (Table 1). For most species it was possible to unambiguously assign membership to one of these classes. In certain cases, however, assignment to a class was difficult, although assignment to one of the three charge groups was never a problem. The principal difficulties encountered were poorly focused bands and bands with low activity, presumably caused by insolubility or instability of the enzyme near its isoelectric point. For some of the species we occasionally observed two bands of enzyme activity. The spacing between the two bands was, however, very small, being less than the smallest differences between classes. This rules out modification by a major charge change, such as deamidation, as the cause of this phenomenon. As it was not reproducible and only intermittently observed, its nature remains unknown. The assignments given in Table 1 are based only on *reproducible single bands.*

When compared with the resolution obtained with starch and polyacrylamide gels, isoelectric focusing distinguished fewer classes of variants. Nevertheless, several unique differences were detected by this method (Fig. 4). *D. capricorni* (variant 2*a*) is separated from the other members of the *willistoni* group (variant 2*b*), and a clear difference is found between the main allele of the *virilis* group (variant 2*d*) and that of the *repleta* group (variant 2*c*). In addition, focusing has separated three members of the *affinis* subgroup (2*a*) from the main variant in the *willistoni* group (2*b*), a distinction which was also observed by Collier (1977) using cellulose acetate electrophoresis.

DISCUSSION

The three methods of analysis used in this study have revealed a total of 19 variants of α-GPDH in 71 species of *Drosophila* (Table 1). This represents a substantial increase in the number of known variants at this locus when our work is compared with previous studies of the same group of species. Fifty-six of our 71 species were investigated by Lakovaara et al. (1977a), and they report seven electromorphs in this group. Three more variants were added to this total by their later (1977b) thermostability studies. We find fourteen electromorphs in this same group of species. Thirty-nine of our seventy-one species were also studied by Collier (1977). He resolved six variants in this group, while we have found 14. It is obvious that electrophoresis using a variety of methods is capable of revealing considerably more differentiation at this locus than studies which use only one electrophoretic condition.

In contrast to this differentiation *between* species is the apparent lack of additional variation *within* species. This is true for the 41 lines of *D. pseudoobscura* and the replicate cultures of 24 other species. The monomorphism of this locus within almost every species is obviously a real phenomenon, since the newly discovered differences *between* species show that our methods are not insensitive

to finding variation. This continuing monomorphism of a locus previously judged monomorphic reinforces the pattern of a lack of hidden variation within loci possessing few alleles and low heterozygosity (Coyne et al., 1978). It also supports the finding of Koehn and Eanes (1977) that small subunit enzymes such as α-GPDH (subunit m.w. about 32,000 according to Bewley et al., 1974) are in general the least variable.

Of particular interest is the absence of extra variation of α-GPDH in *D. melanogaster*, a species known to be polymorphic for two alleles. If there is additional intraspecific variation for this enzyme, it is not likely to be as extensive as that seen for enzyme loci such as xanthine dehydrogenase and esterase-5, at which the high heterozygosity is the consequence of many variants (Singh et al., 1976; Coyne et al., 1978).

Six of our species showed an additional allele segregating within a line, and by comparison with other members of their taxonomic group and the previous demonstration of near-monomorphism in these species it is possible to infer which of these alleles is most likely to be rare within natural populations (Table 1). It is notable that three of the species (*D. birchii*, *D. erecta*, and *D. meridiana*) appear to share the same rare electromorph although they are not closely related. This may represent independent mutations to the same allele or, more probably, different mutations which *converge* in appearance to the same electromorph class.

The interspecific distribution of the new variants revealed in this study is consistent with the known taxonomy of the genus *Drosophila* (Throckmorton, 1974). Notable among these is the split between the common alleles within the subgenus *Drosophila* (variants *k* and *l* in Table 1) and those of the subgenus *Sophophora* (variants *e*, *g*, and *m*). Within the subgenus *Drosophila*, the *repleta* and *virilis* groups have different electromorphs (*k* in the former and *i* and *l* in the latter); and within the *virilis* group itself we find a previously undetected difference between the *montana* subphylad of the *montana* phylad (variant *i*) and the *virilis* phylad together with the *littoralis* subphylad of the *montana* phylad (variant *l*). In the subgenus *Sophophora*, the most notable differences are the distinction between the variants of the *willistoni* group (*f*, *g*, and *h*), the *affinis* subgroup (*e*), and the slow *melanogaster* group variant (*m*). In addition, species which are seemingly well separated from the main radiations of the genus (e.g., *D. lebanonensis*, *D. immigrans*, *D. funebris*, *D. busckii*, and *D. testacea*) are now seen to have unique electromorphs.

Of the three methods used to distinguish variants, sequential polyacrylamide gel electrophoresis was the most successful; but under the single condition pH 8.9, starch gels proved to have the most resolving power. It should be emphasized, however, that each of the three methods used showed variation not revealed by the other two. The difference between the *virilis/repleta* variants *k* and *l* and the *melanogaster* group variant *m* was seen only in polyacrylamide gels, the unique *D. pavlovskiana* variant *h* was seen only on starch gels, and the difference between the *repleta* group variant *k* and the *virilis* group variant *l* was seen only with isoelectric focusing. Thus, *a combination of all three methods is necessary to detect the maximum amount of variation.* In another comparison of polymorphism in the human enzyme phosphoglucomutase-1, Kühnl et al. (1977) found that isoelectric focusing was most powerful in detecting variation. It seems likely that the optimal combination of electrophoretic methods for maximum resolution must be determined separately for each protein.

The pattern of new electromorphs revealed in our study leads to a much greater taxonomic concordance with the results of other, non-electrophoretic studies. Alahiotis et al. (1977) found differences in Michaelis constants and thermostabilities of α-GPDH enzymes among the

species *D. willistoni, D. equinoxialis, D. arizonensis, D. virilis, D. americana,* and the *D. melanogaster* slow allele, although previous studies had indicated that all 6 species shared the same electromorph. In reality, however, we find that these six species have at least four electrophoretically distinct variants of α-GPDH (Table 1). Similarly, Collier and MacIntyre (1977) demonstrated immunological differences among α-GPDH enzymes of different species which at the time appeared to share the same electromorph. It is now apparent that many of these α-GPDH enzymes do indeed belong to different electromorph classes (Table 1). This increase in concordance with the detected immunological differences can be examined further. There are 17 species examined in common between the present study and those of MacIntyre and Collier (1977), Lakovaara et al. (1977a, 1977b), and Collier (1977). Therefore, 136 distinct comparisons can be made between pairs of species to judge whether their α-GPDH proteins are different. MacIntyre and Collier (1977) suggested that an immunological distance of five or more units from the *D. melanogaster* A/A (variant *s*) antiserum results from one or more amino acid substitutions. Thus, if the proteins of two species differ in their distance from the *D. melanogaster* standard by five or more units, we judged them as having "different" α-GPDH proteins. (Of course, this comparison is imperfect since two proteins with the same immunological distance from a given standard may still differ from one another.) The pairwise *immunological* comparisons were then inspected for concordance with pairwise *electromorph* comparisons from the other four studies.

A comparison of the estimate of immunological differences with the electrophoretic and thermostability differences found by Lakovaara et al. (1977a, 1977b) shows 29 cases in which immunological differences of α-GPDH between species were not detected electrophoretically. However, 27 of these 29 discrepancies were resolved in our study as we confirmed the immunological differences electrophoretically. Similarly, the comparison of the immunological data with the electrophoretic data of Collier (1977) shows 20 cases of immunological differences which are not electrophoretically distinguishable. Of these 20 cases, 17 are now resolved by us as electrophoretically different. In neither of these two sets of comparisons is there any case of an interspecific difference found by both immunology and a previous electrophoretic study which is also not found by us as a difference.

Several points must be considered when interpreting our data. Since we were unable to cross species possessing different variants, we are unable to say with certainty that our different electromorphs represent changes coded by the structural locus for α-GPDH. For example, it appears that the larval isozyme of α-GPDH in *D. melanogaster* may be produced by the same structural locus as the adult isozyme but that it differs from it by an epigenetic modification (Bewley and Lucchesi, 1977). However, the taxonomic consistency of our results suggests that even if the variation is not due to differences at the structural locus, it is differentially fixed between species and can be used as a taxonomic marker. The possibility of epigenetic modification of enzymes represents a problem for all studies showing electrophoretic differences between non-crossable species, and it can only be resolved by determination of the amino acid sequences of the proteins.

It is now clear that there is significant molecular heterogeneity which remains undetected by any single electrophoretic condition. The work to date suggests that the amount of undetected genetic variation is proportional to that observed under any one condition (Coyne et al., 1978) and that the average heterozygosity of an individual may not change substantially with a more complete analysis of the level of genic variation. Average heterozygosity is, however, only one statistic of

population genetic interest; and many others may be significantly affected by the failure to detect much variation. These include the profile of allele frequencies within populations, geographic differences in allele frequencies (Singh et al., 1976; Coyne and Felton, 1977), and measures of gene frequency difference between species (Coyne, 1976; Cobbs and Prakash, 1977).

The latter measures are, as the present study shows, particularly sensitive to a failure to discriminate differences in electromorphs among species. If one fails to detect alternate fixations at a locus between two species, for example, a complete genetic non-identity may be mistakenly described as complete genetic identity. This would have drastic effects on measures of interspecific distances. It is obvious, then, that one must exercise great caution when using electrophoretic data to make inferences about taxonomic relationships and rates of genetic change during speciation. The "evolution" of electromorph variation may be confined to a limited set of phenotypic classes under any *single* electrophoretic condition, and apparent convergence of different alleles to similar electromorphs could be a likely phenomenon. This appears to have happened repeatedly in the case of α-GPDH in *Drosophila*.

ACKNOWLEDGMENTS

We would like to thank the following people for sending us stocks of flies and for other assistance during this study: Andy Leigh-Brown, Hampton Carson, Paula Chandoha, Alphonso Coleman, Jean David, Alex Felton, William Heed, Donal Hickey, P. T. Ives, Costas Krimbas, S. Lakovaara, Charles Langley, Doreen Lewis, Richard Lewontin, Jenifer Lichtenfels, Michael Loukas, Dwight Miller, Rodney Norman, Jeffrey Powell, Satya Prakash, A. Prevosti, R. H. Richardson, Marta Rico, Rollin Richmond, B. Shorrocks, Susan Sluss, D. Sperlich, Lynn Throckmorton, and El. Zouros. We are especially grateful to John Ellison and Marietta Reveley for repeated mailings of flies from the University of Texas *Drosophila* stock center. This work was supported by grant GM-21179 to R. C. Lewontin from the Institute of General Medical Sciences of the National Institutes of Health.

REFERENCES

ALAHIOTIS, S., S. MILLER, AND E. BERGER. 1977. Natural selection at the α-GPDH locus in *Drosophila*. Nature 269:144–145.

AVISE, J. C. 1974. Systematic value of electrophoretic data. Syst. Zool. 23:465–481.

AYALA, F. J., M. L. TRACEY, L. G. BARR, J. F. MCDONALD, AND S. PEREZ-SALAS. 1974. Genetic variation in natural populations of five *Drosophila* species and the hypothesis of the selective neutrality of protein polymorphisms. Genetics 77:343–384.

BERNSTEIN, S. C., L. H. THROCKMORTON, AND J. L. HUBBY. 1973. Still more genetic variability in natural populations. Proc. Nat. Acad. Sci. U.S.A. 70:3928–3931.

BEWLEY, G. C. 1978. Heat stability studies at the α-glycerophosphate dehydrogenase locus in populations of *Drosophila melanogaster*. Biochem. Genet. 16:769–775.

BEWLEY, G. C., AND J. C. LUCCHESI. 1977. Origin of α-glycerophosphate dehydrogenase isozymes in *Drosophila* and their functional relationship in the α-glycerophosphate cycle. Biochem. Genet. 15:235–251.

BEWLEY, G. C., J. M. RAWLS, JR., AND J. C. LUCCHESI. 1974. Alpha-glycerophosphate dehydrogenase in *Drosophila melanogaster:* kinetic differences and developmental differentiation of larval and adult isozymes. J. Insect Physiol. 20:153–165.

COBBS, G., AND S. PRAKASH. 1977. A comparative study of the esterase-5 locus in *Drosophila pseudoobscura, D. persimilis* and *D. miranda*. Genetics 85:697–711.

COLLIER, G. E. 1977. Inter- and intraspecific variation of α-glycerophosphate dehydrogenase among *Drosophila*. Isozyme Bull. 10:58–60.

COLLIER, G. E., AND R. J. MACINTYRE. 1977. Microcomplement fixation studies on the evolution of α-glycerophosphate dehydrogenase within the genus *Drosophila*. Proc. Nat. Acad. Sci. U.S.A. 74:684–688.

COYNE, J. A. 1976. Lack of genic similarity between two sibling species of *Drosophila* as revealed by varied techniques. Genetics 84:593–607.

COYNE, J. A., AND A. A. FELTON. 1977. Genic heterogeneity at two alcohol dehydrogenase loci in *Drosophila pseudoobscura* and *Drosophila persimilis*. Genetics 87:285–304.

COYNE, J. A., A. A. FELTON, AND R. C. LEWONTIN. 1978. Extent of genetic variation at a highly polymorphic esterase locus in *Drosophila pseudoobscura*. Proc. Nat. Acad. Sci. U.S.A. 75:5090–5093.

HUBBY, J., AND R. C. LEWONTIN. 1966. A molecular approach to the study of genic heterozygosity in natural populations. I. The number of alleles at different loci in *Drosophila pseudoobscura*. Genetics 54:577–594.

KING, M.-C., AND A. C. WILSON. 1975. Evolution at two levels in humans and chimpanzees. Science 188:107–116.

KOEHN, R. K., AND W. F. EANES. 1977. Subunit size and genetic variation of enzymes in natural populations of *Drosophila*. Theoret. Pop. Biol. 11:330–341.

KOEHN, R. K., AND D. J. RASMUSSEN. 1967. Polymorphic and monomorphic serum esterase heterogeneity in catastomid fish populations. Biochem. Genet. 1:131–144.

KÜHNL, P., U. SCHMIDTMANN, AND W. SPIELMANN. 1977. Evidence for two additional common alleles at the PGM_1 locus (phosphoglucomutase—E.C.:2.7.5.1.). Human Genet. 35:219–223.

LAKOVAARA, S., A. SAURA, AND P. LANKINEN. 1977a. Evolution at the α-GPDH locus in Drosophilidae. Evolution 31:319–330.

LAKOVAARA, S., A. SAURA, AND P. LANKINEN. 1977b. Natural selection and the α-GPDH locus in Drosophilidae. *In* Christiansen, F. B., and T. M. Fenchel (eds.), Measuring selection in natural populations, Springer-Verlag, Berlin, pp. 245–264.

MICKEVICH, M. F., AND M. S. JOHNSON. 1976. Convergence between morphological and allozyme data in evolutionary inference and character evolution. Syst. Zool. 25:260–270.

MILKMAN, R. 1976. Further evidence of thermostability variation within electrophoretic mobility classes of enzymes. Biochem. Genet. 14:383–387.

RAMSHAW, J. A. M., AND W. F. EANES. 1978. Study of the charge-state model for electrophoretic variation using isoelectric focusing of esterase-5 from *Drosophila pseudoobscura*. Nature 275:68–70.

SHAW, C. R., AND R. PRASAD. 1970. Starch gel electrophoresis of enzymes—a compilation of recipes. Biochem. Genet. 4:297–320.

SINGH, R. S., R. C. LEWONTIN, AND A. A. FELTON. 1976. Genetic heterogeneity within electrophoretic "alleles" of xanthine dehydrogenase in *Drosophila pseudoobscura*. Genetics 84:609–629.

THROCKMORTON, L. H. 1974. The phylogeny, ecology and geography of *Drosophila*. *In* King, R. C. (ed.), Handbook of genetics. Vol. 3. Plenum Publishing Corp., New York, pp. 421–469.

WEITKAMP, L. R., F. M. SALZANO, J. V. NEEL, F. PORTA, R. A. GEERDINK, AND A. C. TARNOKY. 1973. Human serum albumin: twenty-three genetic variants and their population distribution. Ann. Human Genet. London 36:381–392.

Manuscript received October 1978
Revised January 1979