AN ASSESSMENT OF "HIDDEN" HETEROGENEITY WITHIN ELECTROMORPHS AT THREE ENZYME LOCI IN DEER MICE

CHARLES F. AQUADRO[1] AND JOHN C. AVISE

Department of Molecular and Population Genetics, University of Georgia, Athens, Georgia 30602

Manuscript received October 9, 1981
Revised copy accepted July 3, 1982

ABSTRACT

Allelic heterogeneity within protein electromorphs at three loci was examined in populations of deer mice (*Peromyscus maniculatus*) collected from five localities across North America. We used a variety of electrophoretic techniques (including several starch and acrylamide conditions, gel-sieving, and isoelectric focusing) plus heat denaturation. Of particular interest was the supernatant glutamate oxalate transaminase system (GOT-1; aspartate aminotransferase-1 of some authors), which under standard electrophoretic conditions had been shown to exhibit basically a two-allele polymorphism throughout the range of maniculatus. The use of all of the above techniques failed to uncover any additional variation for GOT-1 in these populations. Similarly, no new scorable variation was resolved at the essentially monomorphic malate dehydrogenase-1 locus by additional conditions of electrophoresis. In marked contrast to the results for the above two enzymes, the use of multiple conditions of electrophoresis resolved the 8 standard-condition electromorphs of esterase-1 into a total of 23 variants showing strong geographic differentiation in frequency. These 23 electromorphs were further divided into a total of 35 variants by thermal stability studies. However, the allelic nature of all of the thermal stability esterase variants remains to be documented. The results of this study, taken together with the remarkable geographic heterogeneity for this species in ecology, morphology, karyotype and mitochondrial DNA sequence, suggest that some form of balancing selection may be acting to maintain the GOT-1 polymorphism.

DISTRIBUTIONS of frequencies of electrophoretic "alleles" (electromorphs) within and between populations have commonly been used to infer the role of natural selection in the maintenance of variation in nature. For example, the geographic uniformity of electromorph frequencies at several loci in Drosophila, coupled with geographic heterogeneity at other loci (and often in chromosomal inversion frequencies), has been advanced as evidence for an important role of natural selection in maintaining protein polymorphism (e.g., AYALA, POWELL and TRACY 1972; AYALA *et al.* 1972; AYALA and TRACY 1974; AYALA *et al.* 1974). Selection may be acting on the heterogeneous loci (and inversions) and/or the geographically uniform proteins, but it must be acting on at least one of them.

Geographic homogeneity of allele frequencies could be maintained in the face of isolation by selective constraints on amino acid sequence. Alternatively, the geographic uniformity may have resulted from a sufficient level of gene flow between populations to "homogenize" populations in electromorph frequency

[1] Present address: Laboratory of Genetics, National Institute of Environmental Health Sciences, P. O. Box 12233, Research Triangle Park, NC 27709.

(KIMURA and MARUYAMA 1971; KIMURA and OHTA 1971; MARUYAMA and KIMURA 1974). However, according to neutrality predictions, a level of gene flow sufficient to homogenize allele frequencies would also increase the effective size of the population, which should result in a total number of alleles substantially greater than has commonly been observed. As suggested by KING (1974) and others, the data would not be inconsistent with neutrality predictions if the electromorphs observed to display geographic uniformity in frequency were actually heterogeneous classes of structural alleles.

KING (1974) went on to predict "... when time and techniques are available, it will be found that the commoner electrophoretic 'alleles' in almost every polymorphic system in large populations will be shown to consist of a number of sequentially distinct alleles." Through the use of varied electrophoretic conditions, including different separation pHs, gel types, gel concentration and buffer ions, as well as nonelectrophoretic criteria, such as resistance to denaturation by heat and other agents, it has become clear that an electromorph can often camouflage a remarkable number of "hidden" genetic variants (e.g., BERNSTEIN, THROCKMORTON and HUBBY 1973; COCHRANE 1976; COYNE 1976; JOHNSON 1976; MCDOWELL and PRAKASH 1976; SINGH, LEWONTIN and FELTON 1976; COYNE and FELTON 1977; COYNE *et al.* 1979; GOSLING 1979).

Although a great deal of attention has been focused on hidden variation in Drosophila and several other invertebrates, little is known of the amount of cryptic variation in vertebrates. BONHOMME and SELANDER (1978) surveyed variation in both electrophoretic charge and thermostability at 14 structural loci in inbred strains of the house mouse (Mus) and estimated that conventional "one-pass" electrophoresis could detect an average of only one-half of the alleles present at structural loci. Similarly, it is clear that for human hemoglobins there exists a great wealth of variation not detected by a single condition of electrophoresis (e.g., BASSET *et al.* 1978; RAMSHAW, COYNE and LEWONTIN 1979; FUERST and FERRELL 1980). However, the amount of cryptic variation in natural vertebrate populations remains largely unknown.

In this paper, we report an examination of hidden electrophoretic variation in the deer mouse (*Peromyscus maniculatus*), a small mammal native to North America. There exists considerable conventional electrophoretic data for this species (LOUDENSLAGER 1978; AVISE, SMITH and SELANDER 1979; AQUADRO and KILPATRICK 1981; MASSEY and JOULE 1981). At some polymorphic loci, standard electrophoretic techniques show remarkably uniform electromorph frequencies across North America (AVISE, SMITH and SELANDER 1979). For example, at the glutamate oxalate transaminase-1 locus (*Got-1*; aspartate aminotransferase-1 of some authors), the same two electromorphs appear in similar frequencies in populations ranging from southern Mexico to northern Canada and from California to Pennsylvania (AVISE, SMITH and SELANDER 1979). Although there exists significant interpopulation heterogeneity in electromorph frequencies at other loci (e.g., 6-phosphogluconate dehydrogenase-1, *6-Pgd*), the overall geographic pattern is one of high electrophoretic similarity throughout the immense range of the species (mean genetic similarity of 0.93; AVISE, SMITH and SELANDER 1979). This contrasts with the striking geographic heterogeneity shown by this species in morphology, ecology, karyotype and mitochondrial DNA sequence (OSGOOD 1909; BLAIR 1950; BAKER 1968; BOWERS, BAKER and SMITH 1973; R. A. LANSMAN *et al.*, unpublished). We are, thus, especially interested in reassessing the previously reported biochemical similarity among *P. maniculatus* populations. In particular, do the GOT-1 electromorphs really represent the same two alleles across the continent, or do they camouflage heterogeneous classes of alleles at two favored charge states?

MATERIALS AND METHODS

Deer mice were live-trapped from natural populations at the following localities: North Carolina, Macon County (N = 16); Vermont, Rutland County (N = 16); Kansas, Jefferson County (N = 17); Colorado, Gunnison County (N = 19); and California, Amador County (N = 20). Mice were maintained in the lab on an *ad libitum* diet of Purina Laboratory Mouse Chow and water.

Three enzymes have been surveyed for variation: glutamate oxalate transaminase (GOT-1, EC 2.6.1.1); malate dehydrogenase (MDH-1, EC 1.1.1.37) and esterase-1 (EST-1, EC 3.1.1.2). Blood was obtained from the suborbital canthol sinus with a 0.8–1.10-mm × 100-mm capillary tube. Hemolysate samples were prepared by gently shaking a volume of two capillary tubes of blood in a test tube containing 0.5 ml of 4% sodium citrate solution. After centrifugation at 1000 × g to remove plasma, the red blood cells were washed three times in buffered saline solution (1.21 g dibasic sodium phosphate, 0.62 g monobasic potassium phosphate, 14.45 g sodium chloride per 1700 ml deionized water; JENSEN 1970) and lysed by the addition of two volumes of distilled water. Concentrated hemolysate samples, necessary to confidently assay GOT-1 from the blood, were prepared by lysing the red blood cells with two volumes of toluene. This treatment did not affect the pattern observed on gels. Muscle extracts were prepared according to methods described by SELANDER *et al.* (1971).

Conventional starch gel electrophoresis was carried out using gels containing 12.5% electrostarch generally as described by SELANDER *et al.* (1971), although additional buffers were employed and gels were often run longer than originally suggested. The electrophoretic conditions used for each enzyme are listed in Table 1. For Peromyscus, the following electrophoretic systems described by SELANDER *et al.* (1971) are generally considered the "standard conditions" for the three enzymes examined in the study: Tris-citrate, pH 8.0, run at 100 V for 1.5 hr (GOT-1); Tris-citrate, pH 6.7/6.3, run at 170 V for 3 hr (MDH-1); and Tris-HCl pH 8.5/borate, pH 8.2, run at 250 V for 1.5 hr (EST-1). Additionally, GOT-1 and MDH-1 were assayed on both 5% and 10% acrylamide slab gels. The amount of bisacrylamide relative to total acrylamide was kept constant at 5%. Electrophoresis was carried out at 20 mA for 20 min after which the gels were run at 50 mA until the bromphenol blue marker reached the bottom of the gel (approximately 7 hr). Acrylamide gel electrophoresis of EST-1 variants proved impractical because of scoring difficulties associated with poor staining. The use of more concentrated hemolysate samples to increase EST-1 activity often resulted in distorted bands because of the large amounts of hemoglobin present.

Enzyme nomenclature and histochemical stains are those used by SELANDER *et al.* (1971) and AVISE, SMITH and SELANDER (1979). EST-1 was assayed using the substrate Napthol AS-D Acetate (Sigma). This substrate has been found particularly specific for EST-1 in *P. maniculatus* (VAN DEUSEN and KAUFMAN 1978), readily allowing EST-1 electromorphs to be distinguished from those of other esterases. Electromorphs were denoted by percent migration relative to a common electromorph arbitrarily designated "100". Electromorphs of similar mobility were grouped together and run using a different buffer type or pH. This sequential process was repeated until the conditions that had been chosen empirically to give sharp resolution for each enzyme were exhausted.

Since we were particularly interested in the homogeneity of the GOT-1 electromorphs, we further examined these variants from the North Carolina population by isoelectric focusing and by the method of gel sieving described by JOHNSON (1976). Flatbed isoelectric focusing was carried out in 5% acrylamide gels as modified from BASSET *et al.* (1978) and RAMSHAW and EANES (1978). Gels contained 1% pH 4–6 and 1% pH 5–7 ampholytes. Anodic and cathodic solutions were 1 M H_3PO_4 and 1 M NaOH, respectively. Gels were prefocused at 300 V for 20 min, after which samples were applied to the gel surface on 5- × 5-mm 3 MM Whatman paper wicks. Focusing was performed by gradually increasing the voltage to 500 V over 1 hr. The sample wicks were then removed and the voltage increased to 1000 V for an additional 2 hr.

JOHNSON'S (1976) method of gel sieving involves the determination of band mobility over a range of gel pore sizes (4, 5, 6, 7, 8 and 9% acrylamide). Variation in both protein shape (K_r) and charge (M_o) can thus be independently characterized through the following relationship:

$$R_f = (M_o/\mu_f)e^{K_r T}$$

where R_f is the mobility of the protein relative to the buffer front, T is the percent acrylamide and μ_f is the constant of the buffer system (RODBARD and CHRAMBACH 1971). This equation can also be expressed as follows:

$$\log R_f = \log\left(\frac{M_o}{\mu_f}\right) + K_r \times T$$

TABLE 1

Electrophoretic conditions used in this study[a]

No.	Gel type	Gel buffer	Electrode buffer	Running conditions	Reference[b]	Enzyme[c]
1	Starch	Amine-citrate, pH 6.0	Amine-citrate, pH 6.1	75 mA, 5 hr	1	G, M, E
2	Starch	Tris-citrate, pH 6.3	Tris-citrate, pH 6.7	75 mA, 5 hr	2	G, M, E
3	Starch	Tris-citrate, pH 8.0	Tris-citrate, pH 8.0	100 V, 4 hr	2	G, M
4	Starch	Lithium hydroxide, pH 8.2	Lithium hydroxide, pH 8.1	350 V, 12-cm boundary	2	G, E
5	Starch	Tris-HCl, pH 8.5	0.3 M borate, pH 8.2	250 V, 1.5 hr	2	E
6	Starch	Tris-HCl, pH 8.5	0.3 M borate, pH 8.2	250 V, 4 hr	2	G, M, E
7	Starch	Tris-borate-EDTA, pH 9.0	Tris-borate-EDTA, pH 9.0	50 mA, 7 hr, buffer mixing	3	G, M, E
8	Acrylamide	378 mM Tris-HCl, pH 8.9	5 mM Tris-glycine, pH 8.3	5% acrylamide, see text	4	G, M
9	Acrylamide	378 mM Tris-HCl, pH 8.9	5 mM Tris-glycine, pH 8.3	10% acrylamide, see text	4	G, M
10	Acrylamide	Tris-HCl, pH 6.7/ 8.9	Tris-glycine, pH 8.3	4–9% acrylamide, see text	5	G

[a] All enzymes were studied using heat denaturation (see text).

[b] References: 1) ALLENDORF *et al.* (1977); 2) SELANDER *et al.* (1971); 3) modified from AYALA *et al.* (1972), gel and electrode: 174 mM Tris, 17.5 mM boric acid, 2.4 mM EDTA, pH 9.0; 4) modified from small pore gel and discontinuous buffer system described by DAVIS (1964); 5) DAVIS (1964) and JOHNSON (1977).

[c] G = GOT-1, M = MDH-1, E = EST-1.

By regressing log R_f on T, one obtains a linear plot with an intercept whose antilog is M_o divided by μ_f and whose slope is K_r. As suggested by JOHNSON (1977), we have used *Mid-Y*, rather than M_o, as an indicator of charge variation. *Mid-Y* is calculated as the antilog of the R_f corresponding to 6.5% T. Confidence ellipses for the variants were calculated empirically from the run-to-run variation observed for a canine hemoglobin standard (Sigma) run in the same gels (JOHNSON 1977).

Heat denaturation studies were carried out using methods similar to those described by BONHOMME and SELANDER (1978). Aliquots (30 μl) of sample in 6- × 50-mm culture tubes sealed with Parafilm were heated for 20 min in a constant temperature circulating water bath at a series of four temperatures empirically determined for each enzyme. A fifth aliquot was kept on ice as a control. After heat treatment, the tubes were immediately placed on ice. Fifteen microliters of distilled water were added to each aliquot and the tube was agitated. This additional water was necessary to liquify the high temperature aliquots that had become semisolid. Samples were then loaded in sequence, lowest to highest temperature, on the standard condition gel appropriate for the enzyme. After electrophoresis, the gels were sliced, stained and scored visually for activity differences. EST-1 and MDH-1 were examined using hemolysate. Because of low GOT-1 activity in red blood cells, it was necessary to carry out thermal denaturation for this enzyme using muscle homogenates.

RESULTS

Glutamate oxalate transaminase-1: The standard electrophoretic condition (Tris-citrate, pH 8.0) revealed two GOT-1 electromorphs ("130" and "100") common to all populations and a third electromorph ("68") seen only in a single heterozygote from North Carolina (Table 2). It is clear that "130" and "100" are the same variants observed as a two-allele polymorphism throughout North America by AVISE, SMITH and SELANDER (1979). These workers observed a few rare variants (frequencies < 0.05) in four populations. It is unclear whether these included the "68" variant of the present study.

The use of five additional starch gel electrophoretic conditions ranging in pH

TABLE 2

Glutamate oxalate transaminase-1 (GOT-1) and malate dehydrogenase-1 (MDH-1) variants observed in five populations of deer mice

Locality	No. genes sampled	GOT-1 electromorph			MDH-1 electromorph		
		130	100	68	100	75	50
NC	32	0.094	0.875	0.031	0.969	—	0.031
VT	32	0.813	0.188	—	1.000	—	—
KS	30	0.500	0.500	—	0.967	0.033	—
CO	32[a]	0.313	0.688	—	1.000	—	—
CA	40	0.625	0.375	—	1.000	—	—

[a] 38 genes sampled for MDH-1.

from 6.0–9.0 (Table 1) failed to reveal any additional mobility variation among the 166 genes sampled in this survey. Similarly, electrophoresis in both 5% and 10% acrylamide slab gels resolved only the three standard-condition electromorphs. However, a single heterozygous individual from Tulare County, California exhibited a "133" electromorph on the amine-citrate, pH 6.0/6.1, buffer. This electromorph subsequently proved to be distinguishable under all electrophoretic conditions except the standard (Tris-citrate, pH 8.0). This variant was found when surveying populations not included in this study, but is mentioned here to indicate that our techniques are sensitive to small variation in gel migration.

As an additional check of the homogeneity of the two common electromorphs, mice from three populations were also examined using isoelectric focusing and the disc acrylamide gel-sieving method described by JOHNSON (1976). Both of these techniques have been reported capable of resolving variants not detected by other approaches (RAMSHAW and EANES 1978; COYNE *et al.* 1979; JOHNSON 1976, 1977). No new variants were detected by either technique.

The results of the gel-sieving analysis for the North Carolina population, for five Vermont mice (genotype "130/130"), and for the California mouse heterozygous for the hidden variant ("133/100") are presented in Table 3. The values of Mid-Y are plotted *vs.* K_r in Figure 1. There is no clear evidence of heterogeneity within the electromorph classes. The single hidden variant ("133") was clearly distinct. Variation between the electromorphs appears to be caused almost entirely by differences in molecular net charge rather than conformation.

Heat denaturation studies were carried out on GOT-1 electromorphs. The results were not particularly satisfactory, in part because thermal stability was influenced by the concentration of the sample. However, no repeatable differences were noted either between or within electromorph classes. Only a single thermal stability profile (Table 4) was consistently observed.

Results of limited breeding experiments (Table 5) are consistent with an interpretation that the variants of GOT-1 are inherited in a Mendelian fashion as codominant alleles at a single autosomal locus. In addition, examination of the wild progeny of the female mouse from California (genotype "133/100") suggests a similar mode of inheritance for this hidden variant.

Malate dehydrogenase-1: MDH-1 has been reported to be monomorphic in most deer mouse populations (MASCARELLO and SHAW 1973; LOUDENSLAGER 1978; AVISE, SMITH and SELANDER 1979; AQUADRO and KILPATRICK 1981). We

TABLE 3

Results of gel sieving (JOHNSON 1977) analysis of GOT-1 electromorphs

Locality	Individual	GOT-1 electromorphs	GOT-1		Canine HB	
			K_r	Mid-Y	K_r	Mid-Y
NC	1	130	−0.072	0.323	−0.054	0.440
		100	−0.062	0.272		
	2	100	−0.073	0.267	−0.062	0.433
	3	100	−0.071	0.266	−0.059	0.422
	4	100	−0.064	0.271	−0.055	0.426
	5	130	−0.069	0.332	−0.057	0.441
		100	−0.071	0.268		
	6	100	−0.073	0.260	−0.060	0.429
	7	100	−0.067	0.262	−0.053	0.430
	8	100	−0.073	0.262	−0.059	0.430
	9	100	−0.071	0.262	−0.057	0.430
	10	100	−0.074	0.261	−0.061	0.432
		68	−0.071	0.208		
	11	100	−0.074	0.258	−0.061	0.425
	12	100	−0.068	0.271	−0.054	0.434
	13	130	−0.072	0.314	−0.059	0.424
		100	−0.071	0.256		
	14	100	−0.068	0.275	−0.056	0.444
	15	100	−0.071	0.257	−0.059	0.421
	16	100	−0.070	0.258	−0.057	0.424
VT	17	130	−0.068	0.320	—	—
	18	130	−0.064	0.313	—	—
	19	130	−0.066	0.317	—	—
	20	130	−0.072	0.317	—	—
	21	130	−0.070	0.313	—	—
CA	22	133	−0.070	0.354	−0.055	0.430
		100	−0.069	0.260	—	—

observed a total of three electromorphs for MDH-1 in our samples using the standard electrophoretic condition, but one electromorph greatly predominated or was fixed in all populations (Table 2). The use of three additional starch gel electrophoretic conditions (ranging from pH 6.0–9.0, Table 1) resolved no new variants. Neither could we detect additional mobility variation for MDH-1 on 5% and 10% acrylamide slab gels.

The use of heat denaturation criteria revealed only a single thermal stability profile for MDH-1 electromorphs from Colorado and California (profile 3.1, Table 4). The same stability profile was observed to predominate in the other three populations, but these populations also contained MDH-1 electromorphs with thermal stabilities grading, nearly continuously, from the 2.1 to the 3.1 pattern. We were not, however, able to score distinct stability variants. This was due in part to the continuous gradation of stabilities, and to the fact that the stability profiles for the possible variants were poorly replicable. The two rare electrophoretic variants at *Mdh-1* also had thermal stability profiles of 2.1. However, they were generally faintly stained on conventional gels, suggesting an effect of enzyme concentration on heat stability. It is clear that the establishment of homozygous lines for these variants and careful breeding studies

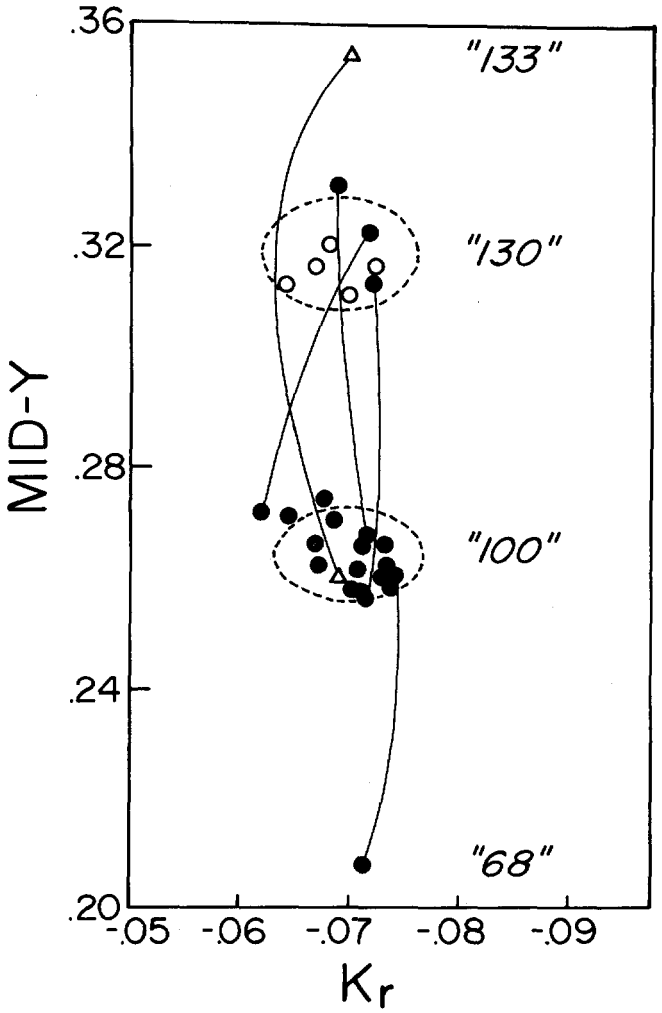

FIGURE 1.—Gel-sieving analysis of GOT-1 variation in *P. maniculatus*. Each shape represents an individual or an "allele"; individuals heterozygous are represented by two shapes and a connecting line. The 95% confidence envelopes were calculated from mobility variation of a hemoglobin standard run in the same gels. The symbols represent mice from North Carolina (●), Vermont (○) and California (△).

will be necessary to document the reality of hidden thermal stability variation at *Mdh-1*. The inheritance of the MDH-1 electromorphs does appear to be simple and Mendelian (Table 5).

Esterase-1: Electromorph frequencies for EST-1, found to be quite variable in New England (AQUADRO and KILPATRICK 1981), have not previously been reported from across the species range. Eight variants were observed in this study under the standard condition (Tris-HCl, pH 8.5, run for 1.5 hr), with each population exhibiting three to six electromorphs (Table 6).

From slight but unscorable migrational differences observed within several EST-1 electromorphs on the standard condition gels, it was apparent that additional "hidden" variation might be present. By running the standard condition gels for longer times (4 hr instead of the normal 1.5 hr), three new variants

TABLE 4

Designations of thermal stability profiles for GOT-1, MDH-1 and EST-1[a]

Enzyme	Temperature (20 min)					Profile designation
	0°	69°	72°	75°	78°	
GOT-1[b]	++++	++++	+–––	––––	––––	2.1
	0°	50°	53°	56°	59°	
MDH-1[b]	++++	++++	+–––	––––	––––	2.1
	++++	++++	+++–	+–––	––––	3.1
EST-1	++++	++++	––––	––––	––––	2.0
	++++	++++	++++	––––	––––	3.0
	++++	++++	++++	++––	––––	3.5
	++++	++++	++++	++++	––––	4.0
	++++	++++	++++	++++	++++	5.0

[a] ++++, full enzymatic activity; +++–, slight reduction in activity; ++––, one-half full activity; +–––, low activity; ––––, no activity.
[b] Homodimers.

TABLE 5

Results of breeding experiments for electromorphs at Got-1 *and* Mdh-1[a]

Parental genotype			No. of progeny	Offspring genotypes			
Got-1	Male	Female		133/100	130/130	130/100	100/100
	130/100	× 100/100	18	—	—	11 (9)	7 (9)
	130/130	× 130/130	6	—	6 (6)	—	—
	100/100	× 130/130	6	—	—	6 (6)	—
	(Wild male)	× 133/100	6	4 (3)	—	—	2 (3)
Mdh-1	Male	Female		100/100	100/75	75/75	
	100/100	× 100/100	12	12 (12)	—	—	
	100/100	× 100/75	18	11 (9)	7 (9)	—	

[a] Expected number of progeny are given in parentheses.

TABLE 6

Esterase-1 variants observed in five populations of deer mice using the standard electrophoretic system (12.5% starch, Tris-HCl pH 8.9 gel buffer, run 1.5 hr)

Locality	No. of genes sampled	EST-1 electromorphs (standard condition)								No. of variants
		112	110	108	106	100	92	83	70	
NC	32	—	—	—	0.063	0.500	0.375	0.063	—	4
VT	32	—	—	—	—	0.438	0.438	0.125	—	3
KS	34	—	0.029	—	0.147	0.353	0.441	—	0.029	5
CO	38	0.053	—	—	0.395	0.289	0.237	—	0.026	5
CA	40	—	—	0.050	0.275	0.325	0.250	0.075	0.025	6

were resolved (Table 7). The distribution of these variants did not substantially change the geographic pattern observed. However, the use of additional electrophoretic conditions resulted in a dramatic increase in both within- and between-population variation (Table 7). The amine-citrate, pH 6.0/6.1, buffer system allowed discrimination of an additional 10 electromorphs. Gels run with Tris-citrate, pH 6.7/6.3, buffer further split the *510-* electromorph into three variants.

TABLE 7

Variants detected for EST-1 by using varied conditions of electrophoresis and heat denaturation

Electrophoretic condition				Locality					
5 (STD)	6	1	Thermal stability[a]	NC (32)[b]	VT (32)	KS (34)	CO (38)	CA (40)	Composite variant
112	112	169	3.2				0.053		1000
110	110	150	3			0.029			2000
108	108	141	3					0.050	3000
107	107	121	5				0.053	0.125	4000
107	107	121	4			0.059			4001
107	107	118	5				0.053		4010
107	107	118	4				0.053		4011
107	107	118	2				0.026		4012
107	106	144	4				0.053		4100
107	106	132	5				0.026		4110
107	106	132	4	0.063					4111
107	106	115	5				0.053	0.150	4120
107	106	115	4			0.088	0.078		4122
100	100	100	5				0.105	0.275	5000
100	100	100	4			0.147	0.053		5001
100	100	96	5				0.053		5010
100	100	96	4	0.375	0.219	0.206			5011
100	100	96	3	0.125			0.026		5012
100	100	96	2				0.026		5013
100	100	94	5					0.025	5020
100	99	93	5[c]					0.025	5100
100	99	93	4[c]				0.026		5101
100	99	93	3[c]		0.219				5102
92	92	76	4			0.353	0.026	0.050	6000
92	92	75	4		0.031	0.059	0.211	0.075	6010
92	92	75	3					0.050	6011
92	92	74	4	0.188	0.125			0.075	6020
92	92	74	3	0.188	0.094				6021
92	92	74	2		0.188				6022
92	91	56	3			0.029			6100
83	83	56	3		0.125				7000
83	83	53	4					0.075	7010
83	83	52	2	0.063					7020
70	71	36	3			0.029			8000
70	71	42	4				0.026	0.025	8010
(Number of variants per locality)				(6)	(7)	(9)	(18)	(12)	Total = 35

Composite variant designations are assigned as described by COYNE (1976).

[a] See Table 4 for interpretation of thermal stability designation.

[b] Numbers in parentheses are number of genes sampled.

[c] These three variants distinguished by thermal stability were also distinct on starch gels run using Tris-citrate, pH 6.7/6.3.

No additional variation was uncovered using starch gels run with lithium hydroxide, pH 8.2/8.1, or the Tris-borate-EDTA, pH 9.0, buffer systems.

Unlike the situation for GOT-1 and MDH-1, EST-1 variants showed clear and distinct differences in the temperature at which they lost enzymatic activity. Different thermal stabilities were noted between numerous electromorphs, including several that had been identical in mobility under the standard elec-

trophoretic condition. For example, composite electromorph *510-* was resolved into three mobility variants on Tris-citrate, pH 6.7/6.3, (*5100*, *5101*, and *5102*). These three variants also differ in thermal stability (Table 7). In addition, heat denaturation revealed a total of 12 additional variants previously not resolved by any electrophoretic condition.

Thus, a total of 35 EST-1 variants were observed for the 88 deer mice (176 genes) examined. It is of course crucial to know whether these variants have a genetic basis, and whether that genetic determination lies at the structural locus. To date we only have a preliminary indication that at least some of the variants represent genetic variation and are probably allelic.

Mobility variants of EST-1 (for single conditions) have been shown to act as codominant alleles and segregate in a Mendelian fashion for *P. maniculatus* (RANDERSON 1965), *P. leucopus* (VAN DEUSEN and KAUFMAN 1980) and *P. truei* (ZIMMERMAN and KILPATRICK 1975). Our preliminary breeding results for EST-1 variants are presented in Table 8. Crosses 1–4 are consistent with a codominant allele, Mendelian mode of inheritance for mobility variants. Cross 3 suggests that thermally stable variants could be, however, dominant to less stable mobility variants of *identical mobility*, at least as scored visually on our gels. In contrast, the results of cross 5 are not consistent with a simple genetic model concerning thermal stability. Although the parents possessed electromorphs with stability profiles of 4.0 and 5.0, an electromorph carried by one offspring had a profile of 2.0. Repeated heat denaturation tests using the same and fresh samples produced identical results for that animal.

The reproducibility of the profiles and mobilities from samples prepared from blood drawn several months apart suggest they are not caused by some short-term physiological variation. EST-1 thermal profiles for concentrated and dilute samples from the same mouse were identical. Thus, the heat stability differences do not seem to be caused simply by variations in the level of enzyme present. Evidence for an allele-specific genetic basis for the thermal stabilities comes from the observation of dissimilar stability profiles for different mobility variants observed together in heterozygous individuals (for example, individuals of genotype *5001/8000* and *5001/6010* in crosses 2 and 3, Table 8). Because of the high level of polymorphism for mobility variation, the two EST-1 "alleles" of a given individual nearly always differed in mobility. Thus, the thermal stability of individual variants was readily assayed without the confounding problems of dominance noted in cross 3.

Clearly, additional breeding studies are needed, particularly concerning the thermal stability variation. Without the documentation of the allelic nature of the EST-1 variants observed in this study, we must at the present time conclude only that there does appear to be significant heterogeneity within electromorphs resolved under only the single standard condition.

DISCUSSION

Results from the application of varied electrophoretic conditions and heat denaturation to several enzymes in Drosophila prompted COYNE and his colleagues (COYNE and FELTON 1977; COYNE, FELTON and LEWONTIN 1978) to suggest that the increase in the number of variants resolved by additional techniques will be proportional to the number resolved by the standard condition. To a

TABLE 8

Results of breeding experiments for EST-1 electromorphs

Parental genotypes	Offspring genotypes (No. observed)		Mendelian?
1) *5001/5001* × *5001/6000*	*5001/5001* (2)	*5001/6000* (4)	Yes
2) *5001/5001* × *5001/8000*	*5001/5001* (10)	*5001/8000* (8)	Yes
3) *5000/5001* × *6010/6001*	*5000/6010* (3)	*5001/6101* (3)	Yes
4) *5101/6010* × *5001/7001*[a]	*5101/5001* (2)	*5101/7001* (1)	Yes
	6010/5001 (2)	*6010/7001* (1)	
5) *5000/5101* × *5101/8010*	*5000/5101* (1)	*5000/8010* (1)	No
	5101/5101 (3)	*5101/8010* (0)	
	5103[b]*/8010* (1)		

[a] An electromorph observed in a New Mexico deer mouse, not included in the survey. It had a mobility like that of variant *7000* but had a thermal stability profile of 5.

[b] This variant had a mobility like that of *5101* but had a thermal stability profile of 2. This variant was not seen in either parent for the cross.

general approximation, the results of our study of hidden electrophoretic variation in the deer mouse, *P. maniculatus*, support that view. For example, we have been able to distinguish a remarkable amount of hidden variation at the highly polymorphic *Est-1* locus. The use of five additional conditions of starch gel electrophoresis resolved the 8 original electromorphs into a total of 23 mobility variants across the 5 populations examined. The use of heat denaturation further resolved these 23 electromorphs into a total of 35 variants, more than four times the number of variants revealed under standard conditions. This pattern of hidden variation is similar to that seen for the highly polymorphic xanthine dehydrogenase and esterase-5 loci in *Drosophila pseudoobscura* (COYNE 1976; SINGH, LEWONTIN and FELTON 1976; MCDOWELL and PRAKASH 1976; COBBS and PRAKASH 1977).

In contrast, we could find no scorable hidden variation in deer mice at *MDH-1*, either through use of varied electrophoretic conditions or heat treatments. This situation is reminiscent of the pattern observed for other monomorphic loci, such as malic dehydrogenase and several hexokinase loci in *D. pseudoobscura* (BECHENBACH and PRAKASH 1977; COYNE and FELTON 1977). These loci have also remained relatively monomorphic under closer scrutiny. Finally, at the *Got-1* locus in deer mice, we have observed an intermediate situation apparently analogous to that previously reported for alcohol dehydrogenase (*Adh*) in *D. melanogaster* (KREITMAN 1980); for both loci, standard surveys detect two common electromorphs throughout the respective species' ranges, and refined assays fail to reveal any additional hidden variation.

The effects of hidden variation on the interpretation of geographic differentiation have been varied. For example, even with the large increase in the number of new variants at *Xdh* in *D. pseudoobscura* there is little increase in geographic differentiation among North and Central American populations (SINGH, LEWONTIN and FELTON 1976). The hidden variants at Est-5, however, show more marked geographic differentiation in the same populations (SINGH 1979). For deer mice, there is not only a large increase in the number of EST-1 variants, but also a marked decrease in similarity between different populations. Electromorphs considered under standard conditions to be shared by two or more populations from across the country often became split into several

variants for which there was little or no overlap in frequency between samples. For example, the populations from North Carolina and California originally appeared to share both the "100" and "92" electromorphs (frequencies of 0.500 *vs.* 0.325 for "100" and 0.375 *vs.* 0.250 for "92" in NC and CA, respectively; Table 6). Additional conditions revealed that the "100" electromorph was encoded by different alleles in the two populations. Similarly, a total of five alleles were resolved within the "92" electromorph class (Table 7). However, it is not yet certain that all of the EST-1 variants represent products of different alleles at the structural gene locus. The non-Mendelian behavior of thermal stability variation in one cross and the remarkably large total number of variants observed for this enzyme raise the possibility of the influence of modifier loci such as have been reported for several protein loci in Drosophila (COCHRANE and RICHMOND 1979; FINNERTY and JOHNSON 1979; TSAKAS and DIAMANTOPOULOU-PANOPOULOU 1980).

Whether the remarkable diversity of EST-1 reflects the action of selection for a wide variety of molecular forms or is simply the result of the accumulation of selectively equivalent (*i.e.*, neutral) variants is not known. Our inability to distinguish between these alternative hypotheses stems largely from our lack of knowledge of the physiological role of EST-1. Similarly, we do not know whether the variants differ in any functionally significant way from each other. A high level of polymorphism is, however, typical of esterases in both vertebrates and invertebrates (SELANDER 1976; SINGH 1976). GILLESPIE and KOJIMA (1968) proposed a relationship between level of polymorphism and metabolic function: enzymes such as esterases which are not involved in glycolysis and the citric acid cycle are commonly more variable than enzymes involved in energy metabolism. High levels of allozyme diversity for esterases may also be related to greater variability of substrates, many of which are of external origin (KOJIMA, GILLESPIE and TOBARI 1970; GILLESPIE and LANGLEY 1974; JOHNSON 1974). Esterases may also be less constrained physiologically because of the presence of several loci with products of similar function (SINGH 1976).

The similarities between estimates of hidden variation in *P. maniculatus* and *D. melanogaster* are particularly relevant to the main question motivating this investigation: does the pattern of geographic distribution of protein variants document the action of some form of selection? As discussed by CAVALLI-SFORZA (1966) and LEWONTIN and KRAKAUER (1973), the breeding structure of populations, even if unknown, must nonetheless be the same for all alleles and loci. Different magnitudes of heterogeneity in allele frequencies across loci would suggest the action of natural selection, either in causing geographic differentiation in the face of gene flow, or in maintaining geographic homogeneity in the face of isolation. With results of the present study, the magnitude of heterogeneity across loci in interlocality allele frequency variances in *P. maniculatus* is even more striking than before. Frequencies of the two alleles at *Got-1* remain relatively uniform across the continent, whereas the number of alleles and level of differentiation at *Est-1* is very high.

The geographic homogeneity of allele frequencies at *Got-1* contrasts strongly not only with the allele frequency heterogeneity at *Est-1*, but also with the remarkable geographic diversity in morphology, ecology, karyotype and mitochondrial DNA sequence of *P. maniculatus*. For example, although the diploid number of chromosomes remains constant at 48, the number of acrocentric

chromosomes ranges from 4 to 20 across populations (BOWERS, BAKER and SMITH 1973). Similarly, although mitochondrial DNA sequence shows regions of geographic uniformity, the continent-wide pattern is one of marked regional differentiation (R. A. LANSMAN *et al.*, unpublished). It thus seems very unlikely that gene flow alone can account for the geographic uniformity in frequencies of *Got-1* alleles (AVISE, SMITH and SELANDER 1979).

It has been pointed out that variation in mutation rates across loci can also produce different levels of geographic heterogeneity (NEI and MARUYAMA 1975). For instance, one could argue that the mutation rate at *Got-1* was significantly lower than for other loci, particularly *Est-1*. However, two common *Got-1* alleles are present in uniformly high frequencies throughout the species range, whereas additional alleles are present only in total frequency of about 0.01. There is increasing molecular evidence (independent of geographic considerations) that the *Adh* polymorphism in *D. melanogaster* is maintained by some form of selection (MCDONALD, ANDERSON and SANTOS 1980; CAVENER and CLEGG 1981). In view of the remarkable diversity of the deer mouse in morphology, ecology and a variety of genetic markers, and the strong analogies between the observed patterns of variation at *Adh* and *Got-1* in *D. melanogaster* and *P. maniculatus*, respectively, it now seems likely that some form of balancing selection is acting to maintain the *Got-1* polymorphism as well. A recent selection component analysis of *Got-1* electromorph frequencies in Colorado populations of *P. maniculatus*, in fact, found significant changes attributable to selection in different life history stages (BACCUS, JOULE and KIMBERLING 1980; however, see NADEAU and BACCUS 1981).

We thank WENDY AQUADRO for assistance in the field, KENT TANKERSLEY for competent technical assistance, WYATT ANDERSON and ALAN PRZYBYLA for the use of their laboratory equipment, and GEORGE JOHNSON for teaching us his gel-sieving technique. WYATT ANDERSON, MICHAEL CLEGG and ROBERT LANSMAN read and commented on a draft of this manuscript. This work was supported by a Public Health Service Predoctoral Genetics Traineeship to C.F.A., by the Theodore Roosevelt Memorial Fund of the American Museum of Natural History, by a Grant-in-Aid from the American Society of Mammalogists, and by National Science Foundation Grant DEB7814195.

LITERATURE CITED

ALLENDORF, F. W., N. MITCHELL, N. RYMAN and G. STAHL, 1977 Isozyme loci in brown trout (*Salmo trutta* L.): detection and interpretation from population data. Hereditas **86:** 179–190.

AQUADRO, C. F. and C. W. KILPATRICK, 1981 Morphological and biochemical variation and differentiation in insular and mainland deer mice (*Peromyscus maniculatus*). pp. 214–230. In: *Mammalian Population Genetics*, Edited by M. H. SMITH and J. JOULE. University of Georgia Press, Athens.

AVISE, J. C., M. H. SMITH and R. K. SELANDER, 1979 Biochemical polymorphism and systematics in the genus *Peromyscus*. VII. Geographic differentiation in members of the *truei* and *maniculatus* species groups. J. Mammal. **60:** 177–192.

AYALA, F. J., J. R. POWELL and M. L. TRACEY, 1972 Enzyme variability in the *Drosophila willistoni* group. V. Genic variation in natural populations of *Drosophila equinoxialis*. Genet. Res., Camb. **20:** 19–42.

AYALA, F. J., J. R. POWELL, M. L. TRACEY, C. A. MOURÃO and S. PÉREZ-SALAS, 1972 Enzyme variability in the *Drosophila willistoni* group. IV. Genic variation in natural populations of *Drosophila willistoni*. Genetics **70:** 113–139.

AYALA, F. J. and M. L. TRACEY, 1974 Genetic differentiation within and between species of the *Drosophila willistoni* group. Proc. Natl. Acad. Sci. USA **71:** 999–1003.

AYALA, F. J., M. L. TRACEY, L. G. BARR, J. F. MCDONALD and S. PÉREZ-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.

BACCUS, R., J. JOULE and W. J. KIMBERLING, 1980 Linkage and selection analysis of biochemical variants in *Peromyscus maniculatus*. J. Mammal. **61:** 423–435.

BAKER, R. H., 1968 Habitats and distribution. pp. 98–126. In: *Biology of* Peromyscus (*Rodentia*), Edited by J. A. KING. Spec. Publ. Amer. Soc. Mammal. No. 2.

BASSET, P., Y. BEUZARD, M. C. GAREL and J. ROSA, 1978 Isoelectric focusing of human hemoglobin: its application to screening, to the characterization of 70 variants, and to the study of modified fractions of normal hemoglobin. Blood **5:** 971–982.

BECKENBACH, A. T. and S. PRAKASH, 1977 Examination of allelic variation at the hexokinase loci of *Drosophila pseudoobscura* and *D. persimilis* by different methods. Genetics **87:** 743–761.

BERNSTEIN, S. C., L. H. THROCKMORTON and J. L. HUBBY, 1973 Still more genetic variability in natural populations. Proc. Natl. Acad. Sci. USA **70:** 3928–3931.

BLAIR, W. F., 1950 Ecological factors in speciation of *Peromyscus*. Evolution **4:** 253–275.

BONHOMME, F. and R. K. SELANDER, 1978 Estimating total genic diversity in the house mouse. Biochem. Genet. **16:** 287–297.

BOWERS, J. H., R. J. BAKER and M. H. SMITH, 1973 Chromosomal, electrophoretic, and breeding studies of selected populations of deer mice (*Peromyscus maniculatus*) and black-eared mice (*P. melanotis*). Evolution **27:** 378–386.

CAVALLI-SFORZA, L., 1966 Population structure and human evolution. Proc. R. Soc. Lond. (Biol.) **164:** 362–379.

CAVENER, D. R. and M. T. CLEGG, 1981 Multigenic response to ethanol in *Drosophila melanogaster*. Evolution **35:** 1–10.

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.

COCHRANE, B. J., 1976 Heat stability variants of esterase-6 in *Drosophila melanogaster*. Nature **263:** 131–132.

COCHRANE, B. J. and R. C. RICHMOND, 1979 Studies of esterase 6 in *Drosophila melanogaster*. I. The genetics of a posttranslational modification. Biochem. Genet. **17:** 167–183.

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., W. F. EANES, J. A. M. RAMSHAW and R. K. KOEHN, 1979 Electrophoretic heterogeneity of α-glycerophosphate dehydrogenase among many species of *Drosophila*. Syst. Zool. **28:** 164–175.

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. Natl. Acad. Sci. USA **75:** 5090–5093.

DAVIS, B. J., 1964 Disc electrophoresis. II. Method and application to human serum proteins. Ann. New York Acad. Sci. **121:** 404–427.

FINNERTY, V. and G. JOHNSON, 1979 Post-translational modification as a potential explanation of high levels of enzyme polymorphism: xanthine dehydrogenase and aldehyde oxidase in *Drosophila melanogaster*. Genetics **91:** 695–722.

FUERST, P. A. and R. E. FERRELL, 1980 The stepwise mutation model: an experimental evaluation utilizing hemoglobin variants. Genetics **94:** 185–201.

GILLESPIE, J. H. and K. KOJIMA, 1968 The degree of polymorphisms in enzymes involved in energy production compared to that in nonspecific enzymes in two *Drosophila ananassae* populations. Proc. Natl. Acad. Sci. USA **61:** 582–585.

GILLESPIE, J. H. and C. H. LANGLEY, 1974 A general model to account for enzyme variation in natural populations. Genetics **76:** 837–848.

GOSLING, E., 1979 Hidden genetic variability in two populations of a marine mussel. Nature **279:** 713–715.

JENSEN, J. N., 1970 Blood protein polymorphism in natural populations of the brush mouse. Ph.D. dissertation, Arizona State Univ., Tempe.

JOHNSON, G. B., 1974 Enzyme polymorphism and metabolism. Science **184:** 28–37.

JOHNSON, G. B., 1976 Hidden alleles at the α-glycerophosphate dehydrogenase locus in Colias butterflies. Genetics **83:** 149–167.

JOHNSON, G. B., 1977 Characterization of electrophoretically cryptic variation in the alpine butterfly *Colias meadii*. Biochem. Genet. **15:** 665–693.

KIMURA, M. and T. MARUYAMA, 1971 Pattern of neutral polymorphism in a geographically structured population. Genet. Res., Camb. **18:** 125–131.

KIMURA, M. and T. OHTA, 1971 Protein polymorphism as a phase of molecular evolution. Nature **229:** 467–469.

KING, J. L., 1974 Isoallele frequencies in very large populations. Genetics **76:** 607–613.

KOJIMA, K., J. H. GILLESPIE and Y. N. TOBARI, 1970 A profile of *Drosophila* species enzymes assayed by electrophoresis. I. Number of alleles, heterozygosities and linkage disequilibrium in glucose-metabolizing systems and some other enzymes. Biochem. Genet. **4:** 627–637.

KREITMAN, M., 1980 Assessment of variability within electromorphs of alcohol dehydrogenase in *Drosophila melanogaster*. Genetics **95:** 467–475.

LEWONTIN, R. C. and J. KRAKAUER, 1973 Distribution of gene frequency as a test of the theory of the selective neutrality of polymorphisms. Genetics **74:** 175–195.

LOUDENSLAGER, E. J., 1978 Variation in the genetic structure of *Peromyscus* populations. I. Genetic heterozygosity—its relationship to adaptive divergence. Biochem. Genet. **16:** 1165–1179.

MARUYAMA, T. and M. KIMURA, 1974 Geographical uniformity of selectively neutral polymorphisms. Nature **249:** 30–32.

MASCARELLO, J. T. and C. R. SHAW, 1973 Analysis of multiple zymograms in certain species of *Peromyscus*, Rodentia: Cricetidae. Texas Rep. Biol. Med. **31:** 507–518.

MASSEY, D. R. and J. JOULE, 1981 Spatial-temporal changes in genetic composition of deer mouse populations. pp. 180–201. In: *Mammalian Population Genetics*, Edited by M. H. SMITH and J. JOULE. University of Georgia Press, Athens.

MCDONALD, J. F., S. M. ANDERSON and M. SANTOS, 1980 Biochemical difference between products of the *Adh* locus in *Drosophila*. Genetics **95:** 1013–1022.

MCDOWELL, R. E. and S. PRAKASH, 1976 Allelic heterogeneity within allozymes separated by electrophoresis in *Drosophila pseudoobscura*. Proc. Natl. Acad. Sci. USA **73:** 4150–4153.

NADEAU, J. H. and R. BACCUS, 1981 Selection components of four allozymes in natural populations of *Peromyscus maniculatus*. Evolution **35:** 11–20.

NEI, M. and T. MARUYAMA, 1975 Lewontin-Krakauer test for neutral genes. Genetics **80:** 395.

OSGOOD, W. H., 1909 Revision of the mice of the American genus *Peromyscus*. N. Amer. Fauna **28:** 1–285.

RAMSHAW, J. A. M., J. A. COYNE and R. C. LEWONTIN, 1979 The sensitivity of gel electrophoresis as a detector of genetic variation. Genetics **93:** 1019–1037.

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.

RANDERSON, S., 1965 Erythrocyte esterase forms controlled by multiple alleles in the deer mouse. Genetics **52:** 999–1005.

RODBARD, D. and A. CHRAMBACH, 1971 Estimation of molecular radius, free mobility, and valence using polyacrylamide gel electrophoresis. Anal. Biochem. **40:** 95–134.

SELANDER, R. K., 1976 Genic variation in natural populations. pp. 21–45. In: *Molecular Evolution*. Edited by F. J. AYALA. Sinauer, Sunderland, Massachusetts.

SELANDER, R. K., M. H. SMITH, S. Y. YANG, W. E. JOHNSON and J. B. GENTRY, 1971 Biochemical polymorphism and systematics in the genus *Peromyscus*. I. Variation in the old-field mouse (*Peromyscus polionotus*). pp 49–90. In: *Studies in Genetics VI*, Univ. Texas Publ. **7103.**

SINGH, R. S., 1976 Substrate-specific enzyme variation in natural populations of *Drosophila pseudoobscura*. Genetics **82:** 507–526.

SINGH, R. S., 1979 Genic heterogeneity within electrophoretic "alleles" and the pattern of variation among loci in *Drosophila pseudoobscura*. Genetics **93:** 997–1018.

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.

TSAKAS, S. C. and E. DIAMANTOPOULOU-PANOPOULOU, 1980 Does the "hidden heat-sensitive polymorphism" in electrophoresis of crude extracts involve loci other than the structural locus examined? Experiments with *D. subobscura*. Biochem. Genet. **18:** 1159–1174.

VAN DEUSEN, M. and D. W. KAUFMAN, 1978 Esterase-1 as a genetic marker for *Peromyscus*. J. Mammal. **59:** 185–186.

VAN DEUSEN, M. and D. W. KAUFMAN, 1980 Inheritance and expression of Esterase-1 allozymes in *Peromyscus leucopus*. Experientia **36:** 1162–1163.

ZIMMERMAN, E. G. and C-W. KILPATRICK, 1975 Genetic control of erythrocyte esterase (Es-1) in the pinon mouse, *Peromyscus truei* (Shufeldt). Experientia **31:** 420–421.

Corresponding editor: W. W. ANDERSON