PROTEIN POLYMORPHISM AND GENIC HETEROZYGOSITY IN A WILD POPULATION OF THE HOUSE MOUSE (*MUS MUSCULUS*)[1]

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PROTEIN polymorphisms demonstrable by zone electrophoresis are finding increasing use in studies of the population genetics, systematics, and ecology of rodents. For wild populations of the house mouse (*Mus musculus*), studies to date have dealt largely with esterases and hemoglobin (PETRAS 1967; SELANDER and YANG 1969; SELANDER, YANG and HUNT 1969), although genetically determined variation in a variety of other proteins has been described in laboratory strains and may be expected to occur in wild mice from which the domestic forms were derived. In an effort to increase the number of protein types available for studies of wild populations, and to assess the extent of genic polymorphism in wild populations of this species for comparison with estimates available for Drosophila and other organisms, we have examined electrophoretic variation in 35 enzymes and nonenzymatic proteins in samples of mice taken in several barns at a farm in southern California.

MATERIALS AND METHODS

Adult mice were trapped in 10 large chicken barns at Hallowell Farm, near Ramona, California, on 4 December 1968 and maintained in the laboratory for 10 to 20 days on a diet of Purina laboratory chow and water. Variation in hemoglobin, in plasma and erythrocyte esterases, and in albumin, transferrin, and other nonenzymatic plasma proteins was studied in several hundred mice in samples from all 10 barns, but our examination of most other proteins was limited to a total of 56 mice taken in Barns 1, 2, 3, and 16. Alkaline phosphatase phenotypes were determined for 67 mice from Barns 2 through 6, and variation in isocitrate dehydrogenase was studied in 90 mice from seven barns.

Preparation of tissue extracts: Samples of blood, kidney, and liver tissues were processed for electrophoresis as follows: 1.0 ml heparinized blood from each mouse was centrifuged at 2,500 × *g* for 10 min and the plasma removed. To prepare hemolysates, erythrocytes were resuspended and washed twice in 10 ml saline, lysed in 1.0 ml deionized water and 0.5 ml toluene, and centrifuged at 49,500 × *g* for 30 min. Plasma and hemolysate samples, as well as extracts of other tissues, were stored at 4°C for from 12 to 48 hr before electrophoresis.

In the preparation of extracts of kidney and liver, tissue samples were homogenized in two volumes of deionized water and centrifuged at 49,500 × *g* for 30 min, following which the supernatant was shaken with a half-volume of toluene and recentrifuged.

Electrophoretic and staining techniques: Electrophoresis was performed on a horizontal apparatus with paper inserts, as described by SELANDER and YANG (1969). Buffers employed in electrophoresis and stains used for the demonstration of proteins were as follows:

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Hemoglobin and erythrocyte esterases: Gel buffer 0.01 M Tris, pH 8.5 (adjusted with hydrochloric acid). Electrode buffer: 0.30 M boric acid and 0.06 M sodium hydroxide, pH 8.2. Stains: Amido black for hemoglobin; α-naphthyl propionate and Fast Garnet GBC salt for esterases.

Non-hemoglobin erythrocytic protein: Gel buffer: 1:4 dilution of stock solution of 0.125 M sodium acetate and 0.0085 M disodium salt of EDTA, pH 5.4. Electrode buffer: 1:1 dilution of stock solution. Stain: Amido black (BIDDLE and PETRAS 1967).

Lactate dehydrogenase regulator in erythrocytes: Gel buffer: 1:19 dilution of stock solution of 0.9 M Tris, 0.02 M tetrasodium salt of EDTA, and 0.5 M boric acid, pH 8.6. Electrode buffer: 1:4 dilution of stock solution for negative electrode tray and 1:6 dilution for positive electrode tray. Stain: See SHOWS and RUDDLE (1968a).

6-Phosphogluconate dehydrogenase in erythrocytes: Gel buffer: 1:9 dilution of electrode buffer. Electrode buffer: 0.1 M Tris, 0.1 M maleic acid, 0.01 M magnesium chloride, and 0.01 M disodium salt of EDTA, pH 7.4 (adjusted with 1.0 M sodium hydroxide). Stain: See SHAW and KOEN (1968a p. 351).

Phosphoglucose isomerase and phosphoglucomutases in erythrocytes: Gel buffer: 1:19 dilution of electrode buffer. Electrode buffer: 0.14 M monobasic potassium phosphate and 0.06 M sodium hydroxide, pH 6.7. Stains: See CARTER and PARR (1967) for phosphoglucose isomerase, and SPENCER, HOPKINSON and HARRIS (1964) for phosphoglucomutases.

Esterases and other proteins in plasma: Gel buffer: Stock solution A: 0.03 M monohydrate lithium hydroxide and 0.19 M boric acid, pH 8.1. Stock solution B: 0.008 M monohydrate citric acid and 0.05 M Tris, pH 8.4. Gel buffer: 1:9 mixture of stock solutions A and B. Electrode buffer: Stock solution A. Stains: α-Naphthyl butyrate and Fast Blue RR salt for esterases; amido black for other proteins.

NADP-Isocitrate dehydrogenases and alcohol dehydrogenase in liver: Gel buffer: 6.07 mM dibasic potassium phosphate and 1.21 mM monohydrate citric acid, pH 7.0. Electrode buffer: 0.214 M dibasic potassium phosphate and 0.027 M monohydrate citric acid, pH 7.0. Stains: See HENDERSON (1965) for isocitrate dehydrogenases, and SHAW and KOEN (1968a) for alcohol dehydrogenase.

Hexose-6-phosphate dehydrogenase, glucose-6-phosphate dehydrogenase, and fumarase in kidney; aldolase in liver: Gel buffer: 1:9 dilution of electrode buffer (7 mg NADP added to gel after cooking). Electrode buffer: 0.50 M Tris, 0.02 M disodium salt of EDTA, and 0.65 M boric acid, pH 8.0. Stains: Hexose- and glucose-6-phosphate dehydrogenases as described by RUDDLE, SHOWS and RODERICK (1967), except dipotassium salt of glucose-6-phosphate substituted for disodium salt; aldolase and fumarase as described by GILLESPIE and KOJIMA (1968).

NAD-Malate and NADP-malate dehydrogenases in kidney or liver: Gel buffer: 0.008 M Tris and 0.003 M monohydrate citric acid, pH 6.7 (adjusted with 1.0 M sodium hydroxide). Electrode buffer: 0.223 M Tris and 0.086 M monohydrate citric acid, pH 6.3. Stains: See SHOWS and RUDDLE (1968b).

Lactate dehydrogenase in kidney and alkaline phosphatase, xanthine dehydrogenase, and indophenol oxidase in liver: Gel buffer: 0.08 M Tris and 0.005 M monohydrate citric acid, pH 8.7. Electrode buffer: 0.30 M boric acid and 0.06 M sodium hydroxide, pH 8.2. Stains: Lactate dehydrogenase as described by MARKERT and MASSARO (1966); alkaline phosphatase and xanthine dehydrogenase as described by SHAW and KOEN (1968a).

Statistics: Significant interbarn heterogeneity in allele frequencies is apparent for many of the loci studied in the Hallowell Farm mice, indicating interbarn subdivision of the total population. This situation, analyzed in detail by SELANDER and YANG (1969) and by SELANDER, YANG and HUNT (1969), is typical for wild house mouse populations. Allele frequencies, derived by direct count or, where a "silent" (null) allele was involved, from maximum likelihood estimates, were tested for homogeneity by chi-square analysis, as described by SNEDECOR and COCHRAN (1967 pp. 240–241). For loci at which interbarn heterogeneity was not demonstrated, data from several barns are pooled (Table 1), but, for other loci, data are presented for each barn, and the overall frequencies of alleles are represented by unweighted barn means rather than by pooling samples from different barns.

Allele frequencies and expected numbers of individuals of various phenotypes were calculated

using the unbiased formulae of HALDANE (1956) and LEVENE (1949) for small samples. For loci at which silent alleles occur, allele frequencies for certain samples were calculated by a maximum likelihood method utilizing information on the frequency of homozygotes for the silent allele and the degree of deficiency of heterozygotes not involving non-silent alleles. When homozygotes for the silent allele are not represented in a sample, application of the maximum likelihood method is arbitrary, since heterozygote deficiency, even when marked, may be due wholly or in part to intrabarn subdivision rather than to the presence of a silent allele (SELANDER and YANG 1969).

RESULTS

In the following account, the proteins examined electrophoretically for variation are arranged according to function.

Hydrolases: Alkaline phosphatase (liver): In liver extracts stained for alkaline phosphatase, three phenotypes are represented in proportions suggesting the segregation of two codominant alleles at a single locus (Table 1). The allele presumed to produce the rapidly migrating band, which is present in low frequency, is provisionally designated *Ap-1*^{a}; that producing the slowly migrating band, *Ap-1*^{b}. This apparent polymorphism has not previously been described for the house mouse, and remains to be confirmed by breeding tests.

Esterase 1 (plasma): Three alleles, *Es-1*^{a}, *Es-1*^{b}, and *Es-1*^{c}, are represented in

TABLE 1

Variation at several loci

Number of						
Barns	Mice	Genotypes and phenotypes: observed (expected)			Allele frequency	
		Alkaline phosphatase				
		Ap-1^a/*Ap-1*^{a}	*Ap-1*^{b}/*Ap-1*^{b}	*Ap-1*^{a}/*Ap-1*^{b}	*Ap-1*^{a}	*Ap-1*^{b}
		Fast	Slow	FS		
5	67	1 (0.2)	61 (60.2)	5 (6.7)	0.052	0.948
		6-Phosphogluconate dehydrogenase				
		Pgd-1^a/*Pgd-1*^{a}	*Pgd-1*^{b}/*Pgd-1*^{b}	*Pgd-1*^{a}/*Pgd-1*^{b}	*Pgd-1*^{a}	*Pgd-1*^{b}
		Fast	Slow	FS		
4	56	2 (3.7)	29 (30.7)	25 (21.7)	0.259	0.741
		Phosphoglucomutase 1				
		Pgm-1^a/*Pgm-1*^{a}	*Pgm-1*^{b}/*Pgm-1*^{b}	*Pgm-1*^{a}/*Pgm-1*^{b}	*Pgm-1*^{a}	*Pgm-1*^{b}
		Fast	Slow	FS		
4	56	48 (48.2)	(0.3)	8 (7.5)	0.929	0.071
		Esterase 5				
		Es-5^a/*Es-5*^{a}	*Es-5*^{b}/—		*Es-5*^{a}	*Es-5*^{b}
		Band absent	Band present			
10	224	198	26		0.940	0.060
		Lactate dehydrogenase regulator				
		Ldr^a/*Ldr*^{a}	*Ldr*^{b}/—		*Ldr*^{a}	*Ldr*^{b}
		B subunit absent	B subunit present			
4	56	7	49		0.359	0.641

TABLE 2

Esterase 1

Sample	Number of mice	Genotypes and phenotypes: observed (expected)				Allele frequency		
		Es-1^a/– Fast	*Es-1^b*/– Slow	*Es-1^a*/*Es-1^b* FS	*Es-1^c*/*Es-1^c* "Silent"	*Es-1^a*	*Es-1^b*	*Es-1^c*
Barn 1	31	2(1.6)	24(23.7)	4(4.5)	1(1.2)	0.10	0.70	0.20
Barn 2	44	(0.4)	40(40.3)	2(1.6)	2(1.8)	0.02	0.78	0.20
Barn 3	26	....	25(25.0)	1(1.0)	....	0.02	0.98	...
Barn 4	21	....	20(20.0)	....	1(1.0)	...	0.76	0.24
Barn 5	9	....	8(8.0)	1(1.0)	....	0.06	0.94	...
Barn 6	6	....	6(6.0)	....	....	...	1.00	...
Barn 9	29	1(0.3)	27(26.5)	1(1.8)	(0.4)	0.04	0.85	0.11
Barn 12	17	2(1.1)	13(12.3)	1(2.0)	1(1.5)	0.09	0.60	0.30
Barn 15	17	1(0.2)	16(15.5)	(0.9)	(0.4)	0.03	0.81	0.16
Barn 16	24	....	24(24.0)	....	....	...	1.00	...
Mean	224					0.036	0.842	0.121

$\chi^2_{18} = 54.55^{**}$.

the Hallowell Farm samples (Table 2). Mice homozygous for the silent allele *Es-1^c* were taken in Barns 1, 2, 4, and 12, and the presence of this allele in Barn 9 and Barn 15 is suggested by a marked deficiency of *Es-1^a*/*Es-1^b* heterozygotes.

Esterase 1 shows an unusual pattern of geographic variation in North America. With the exception of populations in southern California and southern Arizona, all North American populations tested are homozygous for *Es-1^b*, as are those in Jamaica and Venezuela (SELANDER, YANG and HUNT 1969). Yet *Es-1^a* and *Es-1^b* are in approximately equal frequencies in the Hawaiian Islands (L. WHEELER, unpublished). The overall mean frequencies for the *Es-1^a* allele in southern California is 0.046. Both *Es-1^a* and *Es-1^b* occur in inbred strains, but *Es-1^c* has yet to be detected in inbred strains or in wild populations other than those at Hallowell Farm.

Esterase 2 (plasma): Three alleles, *Es-2^a*, *Es-2^b*, and *Es-2^d*, are present at the *Es-2* locus (Table 3). Direct evidence of the presence of the silent allele *Es-2^a* is provided by the occurrence of homozygotes in Barns 1 and 5. Marked deficiencies of heterozygotes (*Es-2^b*/ *Es-2^d*) in Barns 3, 6, 12, and 15 suggest the presence of *Es-2^a* in low frequency.

The alleles *Es-2^a* and *Es-2^b* are widespread in North America, the latter being the predominant allele in wild house mouse populations generally (SELANDER, YANG and HUNT 1969) as well as in inbred strains (PETRAS 1963). *Es-2^d* occurs in moderate frequency in southern California, southern Arizona, and southern Texas, but is unknown elsewhere.

Esterase 5 (plasma): This locus is polymorphic, with the *Es-5^a* allele common and *Es-5^b* present, in low frequency, in all barns except 3 and 6 (Table 1). As indicated in Table 1, the allele *Es-5^b*, which is responsible for the presence of a band, is dominant. Both alleles are widespread in wild house mouse populations in North America and elsewhere (PETRAS and BIDDLE 1967; SELANDER, YANG and HUNT 1969).

TABLE 3

Esterase 2

Sample	Number of mice	Genotype and phenotypes: observed (expected)				Allele frequency		
		Es-2^a/Es-2^a "Silent"	*Es-2^b/–* Fast	*Es-2^d/–* Slow	*Es-2^b/Es-2^d* FS	*Es-2^a*	*Es-2^b*	*Es-2^d*
Barn 1	31	1(0.9)	8(8.3)	11(11.2)	11(10.6)	0.17	0.37	0.46
Barn 2	44	....	12(11.3)	11(10.4)	21(22.2)	...	0.51	0.49
Barn 3	26	(0.2)	12(11.4)	5(4.3)	9(10.1)	0.08	0.58	0.33
Barn 4	21	....	9(9.2)	2(2.2)	10(9.6)	...	0.67	0.33
Barn 5	9	1(1.0)	8(8.0)	.....	.....	0.39	0.61	...
Barn 6	6	(0.4)	3(2.9)	1(0.9)	2(2.2)	0.08	0.61	0.30
Barn 9	29	....	19(19.0)	1(1.0)	9(9.1)	...	0.81	0.19
Barn 12	17	(0.1)	10(9.7)	2(1.6)	5(5.6)	0.08	0.68	0.24
Barn 15	17	(0.5)	8(7.0)	5(3.9)	4(5.7)	0.17	0.49	0.34
Barn 16	24	....	14(14.2)	1(1.2)	9(8.7)	...	0.77	0.23
Mean	224					0.097	0.610	0.291

$\chi^2_{18} = 89.85^{**}$.

Other plasma esterases: The number of esterase bands appearing on gels of mouse plasma varies with the electrophoretic system and stain employed, but is, in any event, large. PANTELOURIS and ARNASON (1966) resolved 19 esterase fractions in plasma of C3H mice, whereas PETRAS (1963) identified 11 bands in inbred and wild mice, and HUNTER and STRACHAN (1961), working with Pallid and Swiss albino strains, found only nine bands. On our gels, three prominent esterase fractions are consistently present, in addition to bands of the *Es-1*, *Es-2*, and *Es-5* systems. These three, designated A, B, and C for reference purposes, are monomorphic in the Hallowell Farm mice. For the purpose of estimating total

TABLE 4

Esterase 3

Sample	Number of mice	Genotypes and phenotypes: observed (expected)						Allele frequency		
		Es-3^b/Es-3^b Moderate	*Es-3^c/Es-3^c* Slow	*Es-3^d/Es-3^d* Fast	*Es-3^b/Es-3^c* MS	*Es-3^b/Es-3^d* MF	*Es-3^c/Es-3^d* SF	*Es-3^b*	*Es-3^c*	*Es-3^d*
Barn 1	31	25(25.2)	(0.1)	....	4(3.7)	2(1.8)	(0.1)	0.90	0.07	0.03
Barn 2	44	32(32.8)	....	(0.6)	1(0.9)	11(9.6)	(0.1)	0.86	0.01	0.13
Barn 3	26	18(16.9)	(0.3)	(0.1)	4(4.9)	2(3.3)	2(0.5)	0.81	0.11	0.08
Barn 4	21	12(12.1)	1(0.4)	(0.2)	4(4.7)	4(3.1)	(0.6)	0.76	0.14	0.10
Barn 5	10	6(6.3)	....	(0.2)	1(0.8)	3(2.5)	(0.2)	0.80	0.05	0.15
Barn 6	6	5(5.0)	....	....	1(1.0)	....	....	0.92	0.08	...
Barn 9	31	26(25.3)	1(0.1)	....	1(2.8)	3(2.8)	(0.1)	0.90	0.05	0.05
Barn 12	17	8(8.4)	(0.6)	(0.1)	6(5.1)	2(2.2)	1(0.6)	0.71	0.21	0.08
Barn 15	17	16(16.0)	....	....	1(1.0)	....	....	0.97	0.03	...
Barn 16	24	22(21.1)	1(0.02)	....	(1.9)	1(1.0)	....	0.94	0.04	0.02
Mean	227							0.857	0.079	0.064

$\chi^2_{18} = 35.66^{**}$.

genic variation, it is assumed that each of these esterases is controlled by a different locus.

Esterase 3 and esterase D (erythrocytes): Of the two prominent esterases appearing in hemolysates (HUNTER and STRACHAN 1961), one, *Es-3*, is polymorphic, and the other, designated esterase D, is monomorphic. At Hallowell Farm, $Es\text{-}3^b$ is the predominant allele at the *Es-3* locus, and both $Es\text{-}3^c$ and $Es\text{-}3^d$ are in low frequency (Table 4).

Two other *Es-3* alleles not represented in the Hallowell Farm material have been recorded in other populations. These are $Es\text{-}3^a$, an allele apparently limited to C57BL and certain other inbred strains (POPP 1966), and a silent allele, $Es\text{-}3^e$, discovered in samples from Fiji (L. WHEELER, unpublished) and occasionally found in Texas (SELANDER, YANG and HUNT 1969).

Aldolase (liver): Extracts of liver stained for aldolase uniformly show a 5-banded pattern, indicating homozygosity at both the *A* and *B* loci (RUTTER *et al.* 1968). Genetic variants have not been reported for the house mouse at either locus.

Dehydrogenases: Alcohol dehydrogenase (liver): This enzyme is monomorphic, appearing as a single cathodally migrating band in all individuals tested.

Lactate dehydrogenase (kidney): The Hallowell Farm samples are homozygous at both the *A* and *B* loci for lactate dehydrogenase, all individuals tested showing a uniform 5-banded pattern. Other wild populations examined, as well as the inbred strains, appear to be similarly invariant.

Lactate dehydrogenase regulator locus (erythrocytes): The Hallowell Farm mice are polymorphic at the *Ldr* locus (Table 1), originally described from inbred and wild mice by SHOWS and RUDDLE (1968a). For unknown reasons, the polymorphic types, involving the presence or absence of B subunits, are more difficult to score in wild mice than in inbred strains. A possible explanation is suggested by SHOWS and RUDDLE's (1968a p. 577) observation that the expression of a lactate dehydrogenase phenotype is influenced by genetic background. In any event, the frequencies recorded here must be regarded as only approximate, since we may have erred in scoring some individuals.

Malate dehydrogenases (kidney): All three malate dehydrogenases, the NAD-dependent and the supernatant and mitochondrial forms of NADP-dependent are monomorphic in the Hallowell Farm samples. Supernatant NADP-MDH is polymorphic (two alleles, $Mdh\text{-}1^a$ and $Mdh\text{-}1^b$) in inbred strains (HENDERSON 1966) and in many wild populations in North America (SHOWS and RUDDLE 1968b; SELANDER and YANG, unpublished). In our experience, the $Mdh\text{-}1^a$ allele, which is fixed in the Hallowell population, generally predominates in wild populations.

Xanthine dehydrogenase (liver): This enzyme is monomorphic in our samples, showing a single band.

Hexose-6-phosphate dehydrogenase and glucose-6-phosphate dehydrogenase (kidney): At the autosomal locus *Hpd-1* controlling the B or hexose-specific form of this enzyme (SHAW and KOEN 1968b), two alleles ($Hpd\text{-}1^a$ and $Hpd\text{-}1^b$) are present at moderate frequencies in all four barns (Table 5). These alleles, corresponding to those designated $Gpd\text{-}1^a$ and $Gpd\text{-}1^b$ by RUDDLE, SHOWS and ROD-

TABLE 5

Hexose-6-phosphate dehydrogenase

Sample	Number of mice	Genotypes and phenotypes: observed (expected)			Allele frequency	
		Hpd-1^a/Hpd-1^a Slow	*Hpd-1^b/Hpd-1^b* Fast	*Hpd-1^a/Hpd-1^b* SF	*Hpd-1^a*	*Hpd-1^b*
Barn 1	13	7(6.8)	1(0.8)	5(5.3)	0.73	0.27
Barn 2	15	5(4.7)	3(2.7)	7(7.6)	0.57	0.43
Barn 3	14	3(3.9)	2(2.9)	9(7.2)	0.54	0.46
Barn 16	14	2(1.0)	8(7.0)	4(5.9)	0.29	0.71
Mean	56				0.532	0.468

$\chi^2_3 = 11.07^*$.

ERICK (1967) on the basis of studies of inbred strains, are widespread in wild populations (SELANDER and YANG, unpublished). Glucose-6-phosphate dehydrogenase appears to be monomorphic.

6-Phosphogluconate dehydrogenase (erythrocytes): Two alleles, producing anodally migrating bands of differing mobility, are represented (Table 1), of which *Pgd-1^a* (faster band) is present in lower frequency than *Pgd-1^b* (slower band). The heterozygote has three bands, suggesting a dimer molecular configuration, as reported in the rat (PARR 1966) and a variety of other organisms (CARTER *et al.* 1968). This polymorphism has not previously been described in the house mouse but is widespread in wild populations (SELANDER and YANG, unpublished).

NADP-Isocitrate dehydrogenases (liver): At Hallowell Farm, the mitochondrial form of NADP-dependent isocitrate dehydrogenase (migrating cathodally under the electrophoretic conditions employed) is monomorphic, whereas the anodal, supernatant form is polymorphic for slower migrating and faster migrating bands, corresponding to the alleles *Idh-1^a* and *Idh-1^b*, respectively, described by HENDERSON (1965, 1968) from inbred strains. The two alleles are present in approximately equal frequencies (Table 6). Interbarn variation in allele fre-

TABLE 6

NADP-Isocitrate dehydrogenase (supernatant)

Sample	Number of mice	Genotypes and phenotypes: observed (expected)			Allele frequency	
		Idh-1^a/Idh-1^a Slow	*Idh-1^b/Idh-1^b* Fast	*Idh-1^a/Idh-1^b* SF	*Idh-1^a*	*Idh-1^b*
Barn 1	13	1(2.6)	2(3.6)	10(6.7)	0.46	0.54
Barn 2	15	5(6.5)	(1.6)	10(6.9)	0.67	0.33
Barn 3	14	1(1.3)	6(6.3)	7(6.3)	0.32	0.68
Barn 4	13	10(10.1)	(0.1)	3(2.8)	0.88	0.12
Barn 5	13	(0.6)	7(7.6)	6(4.8)	0.23	0.77
Barn 6	8	2(1.4)	3(2.4)	3(4.2)	0.44	0.56
Barn 16	14	9(9.4)	(0.4)	5(4.3)	0.82	0.18
Mean	90				0.546	0.454

$\chi^2_6 = 40.08^{**}$.

quencies is marked, with the frequency of *Idh-1*^{a} ranging from 0.23 in Barn 5 to 0.88 in Barn 4. These alleles are widespread in North American populations (SELANDER and YANG, unpublished). We were unable to demonstrate NAD-dependent IDH activity.

Oxidases: Indophenol oxidase (liver): This enzyme has been partially characterized by BREWER (1967), who reported a genetic variant in a human family. Comparable variants have been detected in kangaroo rats (Dipodomys) by W. E. JOHNSON (unpublished). Because the achromatic bands produced by activity of this enzyme, which are especially well shown in gels stained for xanthine dehydrogenase, are uniform in mobility in the Hallowell samples, the controlling locus is judged to be fixed for a single allele.

Isomerases and mutases: Phosphoglucomutases (erythrocytes): Two prominent phosphoglucomutase systems appear in mouse hemolysates. The more anodal of these is monomorphic in the Hallowell samples, but the other system exhibits polymorphism of a type explicable in terms of two codominant alleles producing rapidly and slowly migrating bands. These have been designated *Pgm-1*^{a} (fast) and *Pgm-1*^{b} (slow). Only *Pgm-1*^{a} was recorded in Barn 1, but *Pgm-1*^{b} was present in low to moderate frequency in the other three barns sampled (Table 1). Many North American populations are polymorphic for these alleles (SELANDER and YANG, unpublished).

Phosphoglucose isomerase (erythrocytes): The polymorphism recently described for this enzyme in wild mice and in inbred strains by CARTER and PARR (1967) is not apparent in the Hallowell samples, all of which have a single band and are, therefore, judged to be monomorphic.

Hydrases: Fumarase (kidney): We find no individual variation in mobility of bands in the fumarase phenotype in the Hallowell samples. The genetic control of this enzyme in the house mouse has yet to be determined, but, judging from the presence of five bands in the phenotype, we presume that it involves two loci.

Nonenzymatic proteins: Hemoglobin (erythrocytes): Three alleles at the *Hbb* locus (β-chain) affecting electrophoretic mobility patterns of hemoglobin are known in inbred strains: *Hbb*^{s}, *Hbb*^{d} and *Hbb*^{p}. *Hbb*^{p}, discovered in the inbred strain AU/Ss (MORTON 1962, 1966; RUSSELL, BLAKE and NORWOOD 1968), has yet to be demonstrated in wild populations, which, however, are segregating for *Hbb*^{s} and *Hbb*^{d}. Electrophoretic mobility appears to be unaffected by known allelic variation at the α-chain *Hba* locus (RUSSELL and BERNSTEIN 1966).

Subpopulations in all 10 Hallowell barns are polymorphic for *Hbb*^{d} and *Hbb*^{s}, with an average frequency of 0.574 for *Hbb*^{d} (Table 7). Over most of North America, *Hbb*^{s} has a higher frequency than *Hbb*^{d}, but *Hbb*^{d} predominates in southern California, with an average frequency of 0.565 (SELANDER, YANG and HUNT 1969).

Non-hemoglobin erythrocytic protein (erythrocytes): Polymorphism at the *Pro-1* locus has been described in inbred strains by BIDDLE and PETRAS (1967), who also reported that 197 mice from wild populations in the Windsor, Ontario, area were monomorphic for a slowly migrating band similar to that produced by

TABLE 7

Hemoglobin (Hbb)

Sample	Number of mice	Genotypes and phenotypes: observed (expected)			Allele frequency	
		Hbb^d/Hbb^d Strong diffuse	*Hbb^s/Hbb^s* Single	*Hbb^d/Hbb^s* Weak diffuse	*Hbb^d*	*Hbb^s*
Barn 1	31	16(14.1)	5(3.1)	10(13.8)	0.68	0.32
Barn 2	44	21(22.4)	2(3.4)	21(18.1)	0.72	0.28
Barn 3	26	7(7.4)	5(5.4)	14(13.2)	0.54	0.46
Barn 4	21	7(7.3)	3(3.3)	11(10.4)	0.60	0.40
Barn 5	10	3(2.9)	2(1.9)	5(5.2)	0.55	0.45
Barn 6	6	1(1.4)	1(1.4)	4(3.3)	0.50	0.50
Barn 9	31	10(8.7)	8(6.7)	13(15.7)	0.53	0.47
Barn 12	17	4(4.6)	3(3.6)	10(8.7)	0.53	0.47
Barn 15	17	3(2.4)	7(6.4)	7(8.3)	0.38	0.62
Barn 16	24	12(11.9)	2(1.9)	10(10.1)	0.71	0.29
Mean	227				0.574	0.426

$\chi^2_9 = 18.97^*$.

the *Pro-1^a* allele of C57BL/10 mice. Mice from Hallowell Farm are similarly monomorphic.

Prealbumins (plasma): Variation in the presence or absence of a prealbumin component, Pre, apparently corresponding to prealbumin 2 in the classification of REUTER *et al.* (1968), as described by SHREFFLER (1964), is shown in samples from Hallowell Farm and in those from most wild populations in North America, with the *Pre-a* allele predominant. As noted by SHREFFLER (1964 p. 630), the phenotypes are difficult to score consistently, particularly in females, so we have not calculated allele frequencies for our samples. However, the mean frequency of the less common allele, *Pre-b*, exceeds 20%, and, thus, the locus is, by conventional standards, polymorphic.

Prealbumin 3, a fraction migrating slightly slower than Pre, appears to be invariably present and constant in mobility in our samples. Prealbumin 1, a protein reportedly confined to adult males (REUTER *et al.* 1968), is not resolved on our gels.

Albumin (plasma): The Hallowell Farm samples are monomorphic for this protein. Genetic variation in electrophoretic mobility of albumin has not been described for the house mouse.

Transferrin (plasma): Interstrain polymorphism involving two alleles at the plasma transferrin locus (*Trf*) has been described (COHEN 1960; SHREFFLER 1960) for domestic mice, but comparable variation has not been reported in wild populations (PETRAS 1967; SELANDER, YANG and HUNT 1969). Like all other wild populations we have sampled, those from Hallowell Farm are monomorphic for the *Trf^b* allele.

Other plasma proteins: In addition to the prealbumins, albumin, and transferrin, three darkly staining protein bands, one of which may represent ceruloplasmin, are visible on our plasma gels. In Hallowell Farm mice, all three, designated A, B, and C for reference purposes, are monomorphic.

TABLE 8

Electrophoretic variation in proteins in house mice from Hallowell Farm

Protein	Number of controlling loci	Phenotypic variation	Number of alleles
Hydrolases			
Alkaline phosphatase	1	Polymorphic	2
Esterase 1	1	Polymorphic	3
Esterase 2	1	Polymorphic	3
Esterase 3	1	Polymorphic	3
Esterase 5	1	Polymorphic	2
Plasma esterase A	1 ?	Monomorphic	1
Plasma esterase B	1 ?	Monomorphic	1
Plasma esterase C	1 ?	Monomorphic	1
Erythrocyte esterase D	1 ?	Monomorphic	1
Aldolase	2	Monomorphic	1 (Locus *A*) 1 (Locus *B*)
Dehydrogenases			
Alcohol dehydrogenase	1 ?	Monomorphic	1
Lactate dehydrogenase	2	Monomorphic	1 (Locus *A*) 1 (Locus *B*)
Lactate dehydrogenase regulator	1	Polymorphic	2
Malate dehydrogenases			
NADP-MDH in mitochondria	1	Monomorphic	1
NADP-MDH in supernatant	1	Monomorphic	1
NAD-MDH in supernatant	1	Monomorphic	1
Xanthine dehydrogenase	1 ?	Monomorphic	1
Hexose-6-phosphate dehydrogenase	1	Polymorphic	2
Glucose-6-phosphate dehydrogenase	1	Monomorphic	1
6-Phosphogluconate dehydrogenase	1	Polymorphic	2
NADP-Isocitrate dehydrogenases			
Supernatant form	1	Polymorphic	2
Mitochondrial form	1	Monomorphic	1
Oxidases			
Indophenol oxidase	1 ?	Monomorphic	1
Isomerases and Mutases			
Phosphoglucomutases			
Form 1	1	Polymorphic	2
Form 2	1 ?	Monomorphic	1
Phosphoglucose isomerase	1 ?	Monomorphic	1
Hydrases			
Fumarase	2	Monomorphic	1 (Locus *A*) 1 (Locus *B*)
Nonenzymatic proteins			
Hemoglobin			
Hbb (β-chain)	1	Polymorphic	2
Hba (α-chain)	1	Monomorphic	1
Non-hemoglobin erythrocyte protein	1	Monomorphic	1
Prealbumin 2	1	Polymorphic	2
Prealbumin 3	1	Monomorphic	1
Albumin	1	Monomorphic	1
Transferrin	1	Monomorphic	1
Plasma protein A	1 ?	Monomorphic	1
Plasma protein B	1 ?	Monomorphic	1
Plasma protein C	1 ?	Monomorphic	1

DISCUSSION

The results of our survey of protein variation in the Hallowell Farm population of house mice are summarized in Table 8. A total of 35 protein species was examined, and, additionally, variation at the locus regulating B lactate dehydrogenase subunits in erythrocytes was recorded. The observed variation is believed to be controlled by 40 loci, none of which is sex-linked.

In the course of our survey, polymorphism was detected in two enzymes not previously reported to exhibit genetic polymorphism in the house mouse, namely alkaline phosphatase and 6-phosphogluconate dehydrogenase. Two other enzymes, phosphoglucose isomerase and the supernatant form of NADP-malate dehydrogenase, known to be polymorphic in other wild populations in North America and in inbred strains, proved to be monomorphic in Hallowell populations.

For the loci that are polymorphic in the Hallowell Farm population, the mean number of alleles per locus is 2.25. Three of the esterase loci are segregating for three alleles, but only two alleles are represented at other polymorphic loci. This situation is characteristic of mouse populations generally, for our investigations of variation at four esterase loci and the hemoglobin locus *Hbb* in some 8,000 mice from several hundred localities in North America (SELANDER, YANG and HUNT 1969) demonstrate that, with few exceptions, not more than three alleles are segregating at any locus in a single population. Over the whole range of the house mouse in North America, however, as many as seven alleles may be represented at a single locus.

From a sample of 18 proteins controlled by 18 loci in *Drosophila pseudoobscura*, LEWONTIN and HUBBY (1966) have estimated the proportion of the loci in the genome that are polymorphic. Inherent in this approach, which we have adopted in our study, are several potential sources of bias, as discussed in detail by LEWONTIN and HUBBY (1966 pp. 604–605) and SHAW (1969). Since only a minority of possible amino acid substitutions in peptides will affect the electrophoretic mobility of a protein by altering its net charge, estimates of total genic polymorphism based on electrophoretically demonstrable phenotypes are minimal. For the house mouse, our results are almost certainly biased in favor of polymorphic loci by the fact that methods for the detection of an enzyme are normally published only when a polymorphism has been detected, generally in the inbred strains. In an effort to minimize the effect of this bias, we purposely examined a number of protein types not reported in the literature on biochemical variation in the house mouse.

On the assumption that our sample of proteins, although not selected randomly, provides a basis for making at least a crude estimate of the total genic polymorphism in wild populations of the house mouse, we may proceed to compare our results with those reported for other organisms.

In a study of five natural populations of *Drosophila pseudoobscura*, LEWONTIN and HUBBY (1966) estimated that (1) 39% of the loci in the genome are polymorphic over the whole species, (2) the average population is polymorphic for 30% of all loci, and (3) 12% (range, 8% to 15%) of the loci in an average

individual are in the heterozygous state. For the house mouse, we have obtained remarkably similar estimates: (1) 14 of 40 loci, or 35%, are polymorphic over the whole species, as exemplified by the Hallowell Farm population and populations in Texas and elsewhere in North America, (2) 30% of the 40 loci examined are polymorphic in the Hallowell population, and (3) 11% of the loci in an average individual are heterozygous. (In making this estimate, which is based on mean barn frequencies, a frequency of 0.50 was arbitrarily assigned to each of the two *Pre* alleles.)

HARRIS, HOPKINSON and LUFFMAN (1968; see also HARRIS 1966), using an approach similar to that employed by LEWONTIN and HUBBY (1966) and by us, report that, for human populations, three of 12 (25%) arbitrarily chosen enzymes exhibit striking polymorphism. LEWONTIN's (1967a) analysis of the time sequence of the discovery of erythrocytic antigens in the English population yields estimates of 36% for the proportion of polymorphic loci in the genome and 16% for the proportion of loci heterozygous in an average individual.

Several additional estimates of levels of genic polymorphism in animal species are available, as follows: (1) 40% to 50% of loci polymorphic in insular populations of *Drosophila ananassae* and *D. nasuta* in the South Pacific region (JOHNSON *et al.* 1966; STONE *et al.* 1968); (2) 52% and 63%, respectively, of 27 loci polymorphic in the snails *Cepaea hortensis* and *C. nemoralis* in southwestern England (MANWELL and BAKER 1968); (3) 43% of 48 loci polymorphic in the pheasant *Phasianus colchicus* (BAKER *et al.* 1966); and (4) 54% and 58%, respectively, of 24 loci polymorphic in two populations of the quail *Coturnix coturnix* (BAKER and MANWELL 1967; MANWELL and BAKER 1968). Because these estimates were derived from studies that were not specifically designed to estimate proportions of polymorphic loci, they probably are more strongly biased toward polymorphic loci than those obtained for *Drosophila pseudoobscura*, the house mouse, and man.

Available information on the genetics of the house mouse does not contribute materially to a solution of the perplexing problem of how the unexpectedly large amount of genic polymorphism revealed by recent studies of natural populations of animals is maintained (LEWONTIN and HUBBY 1966; SVED, REED and BODMER 1967; MILKMAN 1967; LEWONTIN 1967b; CROW 1968). Although circumstantial evidence of heterosis at the *Es-3* and *Hbb* loci is provided by observations of an excess of heterozygotes in wild populations in Texas (SELANDER, YANG and HUNT 1969), for no biochemical locus do we have any real understanding of the basis for maintenance of observed polymorphisms.

GILLESPIE and KOJIMA (1968) have recently suggested that the frequency of polymorphism may vary among groups of enzymes with different functional characteristics. For *Drosophila ananassae* from Samoa and Fiji, polymorphism was found in only one of seven enzymes that have narrow substrate specificities and are involved in energy metabolism (Group I, "critical" enzymes) but in two of four enzymes that have relatively broad substrate specificities and are functionally peripheral to the major energy-producing and anabolic pathways (Group II, "peripheral" enzymes). A similar comparison based on the present data is presented in Table 9, in which the loci have been assigned to three groups.

TABLE 9

Protein variation in relation to functional classification

Protein type	Number of controlling loci	Polymorphic loci		Mean proportion of heterozygous loci per individual
		Number	Percent	
Group I: "Critical" enzymes	18	5	28	0.110
Group II: "Peripheral" enzymes	12	5	42	0.106
Group III: Nonenzymatic proteins	10	2	20	0.099
Pooled	40	12	30.0	0.1059

Group I consists of loci controlling aldolase, the phosphoglucomutases, phosphoglucose isomerase, fumarase, and all dehydrogenases except xanthine dehydrogenase. Group II includes loci controlling all other enzymes analyzed; and Group III includes loci controlling the nonenzymatic proteins.

Of the 18 loci in Group I, 28% are polymorphic, as compared with 42% of the 12 in Group II and 20% of the 10 in Group III. The intergroup variation is not statistically significant but, as in the case of *D. ananassae*, the percentage of polymorphic loci is higher in Group II than in Group I. In evaluating this variation in the house mouse, it should be noted that four of the five polymorphic loci in Group II control enzymes of a single category, the esterases. It is apparent that a wider array of "peripheral" enzymes must be examined before the pattern of variation in the house mouse can be properly evaluated in terms of the GILLESPIE-KOJIMA hypothesis.

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SUMMARY

An analysis of electrophoretically demonstrable polymorphic variation in 35 enzymes and nonenzymatic proteins in wild house mice collected in 10 barns at Hallowell Farm, California, provides a basis for estimating the proportion of polymorphic loci in the genome. Of 40 loci controlling the proteins examined, 30% are polymorphic; and, for the species as a whole, as represented by North American populations, 35% are polymorphic. It is estimated that 11% of the loci in an average individual of the Hallowell Farm population are in heterozygous state. These estimates for wild populations of the house mouse are remarkably similar to those previously reported for *Drosophila pseudoobscura* by LEWONTIN and HUBBY (1966).

LITERATURE CITED

BAKER, C. M. A. and C. MANWELL, 1967 Molecular genetics of avian proteins. VIII. Egg-white proteins of the migratory quail, *Coturnix coturnix*.—New concepts of "hybrid vigour." Comp. Biochem. Physiol. **23**: 21–42.

BAKER, C. M. A., C. MANWELL, R. F. LABISKY and J. A. HARPER, 1966 Molecular genetics of avian proteins. V. Egg, blood and tissue proteins of the ring-necked pheasant, *Phasianus colchicus* L. Comp. Biochem. Physiol. **17**: 467–499.

BIDDLE, F. G. and M. L. PETRAS, 1967 The inheritance of a non-hemoglobin erythrocytic protein in *Mus musculus*. Genetics **57**: 943–949.

BREWER, G. J., 1967 Achromatic regions of tetrazolium stained starch gels: inherited electrophoretic variation. Am. J. Human Genet. **19**: 674–680.

CARTER, N. D. and C. W. PARR, 1967 Isoenzymes of phosphoglucose isomerase in mice. Nature **216**: 511.

CARTER, N. D., R. A. FILDES, L. I. FITCH and C. W. PARR, 1968 Genetically determined electrophoretic variations of human phosphogluconate dehydrogenase. Acta Genet. **18**: 109–122.

COHEN, B. L., 1960 Genetics of plasma transferrins in the mouse. Genet. Res. **1**: 431–438.

CROW, J. F., 1968 The cost of evolution and genetic loads. pp. 165–178. In: *Haldane and Modern Biology*, Edited by K. R. DRONAMRAJU. Johns Hopkins Press, Baltimore.

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. U.S. **61**: 582–585.

HALDANE, J. B. S., 1956 The estimation and significance of the logarithm of a ratio of frequencies. Ann. Human Genet. **20**: 309–311.

HARRIS, H., 1966 Enzyme polymorphisms in man. Proc. Roy. Soc. London B **164**: 298–310.

HARRIS, H., D. A. HOPKINSON and J. LUFFMAN, 1968 Enzyme diversity in human populations. Ann. N. Y. Acad. Sci. **151**: 232–242.

HENDERSON, N. S., 1965 Isozymes of isocitrate dehydrogenase: subunit structure and intracellular location. J. Exptl. Zool. **158**: 263–274. —— 1968 Intracellular location and genetic control of isozyme of NADP-dependent isocitrate dehydrogenase and malate dehydrogenase. Ann. N. Y. Acad. Sci. **151**: 429–440.

HUNTER, R. L. and D. S. STRACHAN, 1961 The esterases of mouse blood. Ann. N. Y. Acad. Sci. **94**: 861–867.

JOHNSON, F. M., C. G. KANAPI, R. H. RICHARDSON, M. R. WHEELER and W. S. STONE, 1966 An analysis of polymorphisms among isozyme loci in dark and light *Drosophila ananassae* strains from American and Western Samoa. Proc. Natl. Acad. Sci. U.S. **56**: 119–125.

LEVENE, H., 1949 On a matching problem arising in genetics. Ann. Math. Statistics **20**: 91–94.

LEWONTIN, R. C., 1967a An estimate of average heterozygosity in man. Am. J. Human. Genet. **19**: 681–685. —— 1967b Population genetics. Ann. Rev. Genet. **1**: 37–70.

LEWONTIN, R. C. and J. L. HUBBY, 1966 A molecular approach to the study of genic heterozygosity in natural populations. II. Amount of variation and degree of heterozygosity in natural populations of *Drosophila pseudoobscura*. Genetics **54**: 595–609.

MANWELL, C. and C. M. A. BAKER, 1968 Genetic variation of isocitrate, malate and 6-phosphogluconate dehydrogenases in snails of the genus Cepaea—introgressive hybridization, polymorphism and pollution? Comp. Biochem. Physiol. **26**: 195–209.

MARKERT, C. L. and E. J. MASSARO, 1966 *In vitro* hybridization of lactate dehydrogenase in the presence of arsenate and nitrate ions. Arch. Biochem. Biophys. **115**: 417–426.

MILKMAN, R. D., 1967 Heterosis as a major cause of heterozygosity in nature. Genetics **55**: 493–495.

MORTON, J. R., 1962 Starch-gel electrophoresis of mouse haemoglobins. Nature **194**: 383–384. —— 1966 The multiple electrophoretic bands of mouse haemoglobins. Genet. Res. **7**: 76–85.

PANTELOURIS, E. M. and A. ARNASON, 1966 Ontogenesis of serum esterases in *Mus musculus*. J. Embryol. Exptl. Morphol. **16**: 55–64.

PARR, C. W., 1966 Erythrocyte phosphogluconate dehydrogenase polymorphism. Nature **210**: 487–489.

PETRAS, M. L., 1963 Genetic control of a serum esterase component in *Mus musculus*. Proc. Natl. Acad. Sci. U.S. **50**: 112–116. —— 1967 Studies of natural populations of Mus. I. Biochemical polymorphisms and their bearing on breeding structure. Evolution **21**: 259–274.

PETRAS, M. L. and F. G. BIDDLE, 1967 Serum esterases in the house mouse, *Mus musculus*. Can. J. Genet. Cytol. **9**: 704–710.

POPP, R. A., 1966 Inheritance of an erythrocyte and kidney esterase in the mouse. J. Heredity **57**: 197–201.

REUTER, A. M., F. KENNES, A. LEONARD and A. SASSEN, 1968 Variations of the prealbumin in serum and urine of mice, according to strain and sex. Comp. Biochem. Physiol. **25**: 921–928.

RUDDLE, F. H., T. B. SHOWS and T. H. RODERICK, 1967 Autosomal control of an electrophoretic variant of glucose-6-phosphate dehydrogenase in the mouse (*Mus musculus*). Genetics **58**: 599–606.

RUSSELL, E. S. and S. E. BERNSTEIN, 1966 Blood and blood formation. pp. 351–372. In: *Biology of the Laboratory Mouse*, 2nd edition, Edited by E. L. GREEN. McGraw-Hill, New York.

RUSSELL, E. S., S. L. BLAKE and M. S. NORWOOD, 1968 Search for mouse hemoglobin polymorphisms and for linkage relation of the alpha-chain locus. Genetics **60**: 218.

RUTTER, W. J., T. RAJKUMAR, E. PENHOET, M. KOCHMAN and R. VALENTINE, 1968 Aldolase variants: structure and physiological significance. Ann. N. Y. Acad. Sci. **151**: 102–117.

SELANDER, R. K. and S. Y. YANG, 1969 Biochemical genetics and behavior in wild house mouse populations. In: *Contributions to Behavior-genetic Analysis: The Mouse as a Prototype*, Edited by G. LINDZEY and D. D. THIESSEN. Appleton-Century-Crofts, New York (in press).

SELANDER, R. K., S. Y. YANG and W. G. HUNT, 1969 Polymorphism in esterases and hemoglobin in wild populations of the house mouse (*Mus musculus*). Studies in Genetics V. Univ. Texas Publ. **6918**: 271–338.

SHAW, C. R., 1969 Isozymes: classification, frequency, and significance. Intern. Rev. Cytol. **25**: 297–332.

SHAW, C. R. and A. L. KOEN, 1968a Starch gel zone electrophoresis of enzymes. pp. 325–364. In: *Chromatographic and Electrophoretic Techniques*, Vol. 2, Edited by I. SMITH. Wiley, New York. —— 1968b Glucose 6-phosphate dehydrogenase and hexose 6-phosphate dehydrogenase of mammalian tissues. Ann. N. Y. Acad. Sci. **151**: 149–158.

SHOWS, T. B. and F. H. RUDDLE, 1968a Function of the lactate dehydrogenase B gene in mouse erythrocytes: evidence for control by a regulatory gene. Proc. Natl. Acad. Sci. U.S. **61**: 574–581. —— 1968b Malate dehydrogenase: evidence for tetrameric structure in *Mus musculus*. Science **160**: 1356–1357.

SHREFFLER, D. C., 1960 Genetic control of serum transferrin type in mice. Proc. Natl. Acad. Sci. U.S. **46**: 1378–1384. —— 1964 Inheritance of a serum pre-albumin variant in the mouse. Genetics **49**: 629–634.

SNEDECOR, G. W. and W. G. COCHRAN, 1967 *Statistical methods*. 6th edition. Iowa State Univ. Press, Ames.

SPENCER, N., D. A. HOPKINSON and H. HARRIS, 1964 Phosphoglucomutase polymorphism in man. Nature **204**: 742–745.

STONE, W. S., M. R. WHEELER, F. M. JOHNSON and K. KOJIMA, 1968 Genetic variation in natural island populations of members of the *Drosophila nasuta* and *Drosophila ananassae* subgroups. Proc. Natl. Acad. Sci. U.S. **59**: 102–109.

SVED, J. A., T. E. REED and W. F. BODMER, 1967 The number of balanced polymorphisms that can be maintained in a natural population. Genetics **55**: 469–481.