*THE DEGREE OF POLYMORPHISMS IN ENZYMES INVOLVED IN ENERGY PRODUCTION COMPARED TO THAT IN NONSPECIFIC ENZYMES IN TWO DROSOPHILA ANANASSAE POPULATIONS**

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In the past two years, several estimates of genetic variability in *Drosophila* populations in which gel electrophoresis was used to detect enzyme and protein variants have been published.[1-3] The results have indicated that 30–50 per cent of the loci examined are polymorphic and that an average individual will be heterozygous at 10–25 per cent of these loci. Whether these figures reflect the total variation of the genome depends, in part, on the choice of enzymes. As a high proportion of the proteins used in these studies are of unknown physiological function and most enzymes used have broad substrate specificities, it would be interesting to compare the degree of genetic variability in these systems (group II) with enzymes known to be active in energy metabolism (group I). Group I enzymes examined in this study include two from glycolysis, hexokinase (HK) and aldolase (ALD); one from the glucose-6-phosphate oxidation system, glucose-6-phosphate dehydrogenase (G-6-PD); one from the citric acid cycle, fumarase (FUM); one from the α-glycerophosphate cycle, α-glycerophosphate dehydrogenase (α-GPD); and two involved in the oxidation of malate, cytoplasmic malic dehydrogenase (MDH) and malic enzyme (ME). The results of this survey will be compared to those of Stone *et al.*,[3] who surveyed the following group II enzymes: acid phosphatase, esterase C, alcohol dehydrogenase, and octanol dehydrogenase. At the time of analysis, Stone *et al.* chose these four enzymes strictly on the basis of the availability of assay techniques.

Materials and Methods.—The dark *Drosophila ananassae* used in this survey were collected at the same time as those used by Stone *et al.*[3] Lines were initiated from females inseminated in the wild in July and August, 1967, and were maintained in mass culture on standard cornmeal medium until the present investigation began in May, 1968. The adult flies from each of 42 lines from the Upolu population in Western Samoa and from each of 45 lines from the Viti Levu population in Fiji were examined.

Flies were homogenized individually in about 0.01 ml distilled water for α-GPD, ALD, FUM, MDH, and ME; and in about 0.01 ml of 0.1 *M* Tris buffer, pH 8.5, with 0.25

Table 1. *Constituents of enzyme stains.*

Enzyme	Substrate(s)	Coenzymes	Coupling enzymes	Inorganics
α-GPD	α-Glycerophosphate	NAD	—	—
MDH	Malic acid	NAD	—	—
ME	Malic acid	NADP	—	$MgCl_2$
G-6-PD	Glucose-6-phosphate	NADP	—	$MgCl_2$
HK	Glucose, ATP	NADP	G-6-PD	$MgCl_2$
FUM	Fumaric acid	NAD	MDH	—
ALD	Fructose-1,6-diphosphate	NAD	G-3-PD*	0.005 *M* arsenate

See *Materials and Methods* for concentrations.
* Glyceraldehyde-3-phosphate dehydrogenase.

TABLE 2. *Allele frequencies observed for seven enzymes involved in energy production (Group I) and four nonspecific enzymes (Group II).*

Enzyme	Group I				
	f(1)	*f*(2)	*f*(3)	*f*(4)	No.
MDH					
Samoa	0.042	0.923	0.024	0.012	84
Fiji	0.039	0.944	0.000	0.017	90
α-GPD					
Samoa	1.000				84
Fiji	1.000				90
ME					
Samoa	1.000				84
Fiji	1.000				90
FUM					
Samoa	0.000	1.000			84
Fiji	0.011	0.989			90
HK					
Samoa	0.000	1.000			84
Fiji	0.011	0.989			90
G-6-PD					
Samoa	1.000	0.000			84
Fiji	0.989	0.011			90
ALD					
Samoa	1.000				84
Fiji	1.000				90

Enzyme	Group II					
	f(1)	*f*(2)	*f*(3)	*f*(4)	Others	No.
Esterase C						
Samoa	0.907	0.041	0.039	0.013		344
Fiji	0.519	0.338	0.102	0.026	0.015	133*
Acid phosphatase						
Samoa	0.102	0.835	0.007	0.047	0.009	349†
Fiji	0.285	0.715				
Alcohol dehydrogenase						
Samoa	0.998	0.001			0.001	345*
Fiji	0.992	0.004			0.004	133*
Octanol dehydrogenase						
Samoa	0.993	0.003	0.004			349
Fiji	0.966	0.009	0.025			117

Data for group II were taken from Stone *et al.*[3] Allele numbers were changed for convenience.

* One more allele.

† Three more alleles.

mg/ml NADP for HK and G-6-PD. Starch-gel electrophoresis was carried out horizontally in Poulik's discontinuous buffer system[4] for FUM and ME; in a Tris-citrate buffer, pH 7.0,[5] for α-GPD, MDH, and ALD; and in a Tris-boric acid-EDTA buffer with 0.013 mg/ml NADP[6] for G-6-PD and HK. All staining solutions were made up in 0.1 *M* Tris buffer, pH 8.5, with 0.25 mg/ml nitro blue tetrazolium, about 0.05 mg/ml phenazine methasulfate, and the following concentrations of reagents, as appropriate: 0.25 mg/ml NAD or NADP, 0.2–0.25 mg/ml substrate, 1 EU/ml coupling enzymes, and 5 mM $MgCl_2$. The requirements for each stain are given in Table 1.

Results and Discussion.—Distinct differences in electrophoretic mobility were interpreted as evidence of different genetically determined enzyme molecules. Single-banded patterns were scored as homozygotes, and double- or triple-banded patterns as heterozygotes. A complete analysis of the genetics of these enzymes is under way. Alleles are numbered according to increasing electrophoretic mobility.

Allele frequency data are summarized in Table 2. It is immediately apparent that group I enzymes exhibit much less variation than the group II systems reported in Stone *et al.*[3] The frequency of the most common allele in the group I enzymes is never less than 0.92. It would seem unlikely, therefore, that any of the variation in nature is maintained by heterozygote superiority, in view of Robertson's[7] theoretical work on heterosis in finite populations. The genetic variants in group I enzymes are probably maintained by mutation-selection balance.

The mean proportion of heterozygote loci per individual for group I enzymes is compared to that for group II enzymes in Table 3. Group I enzymes are only 9–24 per cent as variable as group II enzymes by this criterion. Since the group I enzymes are all components of pathways in which most of the intermediates are required in other processes, there will be rigid standards for the kinetic parameters of these enzymes in order that the intermediate pool sizes remain optimal. The group II enzymes, being peripheral to the major energy-producing and anabolic pathways, may not have these rigid kinetic standards and may thus be able to vary over a wider range of kinetic activities. This would, under mutation pressure, create a more diverse spectrum of molecules acceptable from a kinetic point of view and available for selection on the basis of other parameters such as molecular stability. This idea of "critical enzymes" is developed more fully by Kacser.[8] However, there seem to be no *a priori* reasons why a heterozygote group I enzyme should or should not be kinetically superior to both homozygote enzymes.

The fact that grouping enzymes by functional characteristics can result in different degrees of variation suggests that the problem of estimating the total variation in the genome becomes the more physiological question of discovering the proportion of loci that are in some way restricted in the amount of variation they can tolerate.

TABLE 3. *Mean proportion of heterozygous loci per individual in the Samoan and Fijian populations of D. ananassae.*

Population	Group I	Group II	Group I/Group II (%)
Samoa	0.0278	0.1155	24.07
Fiji	0.0246	0.2643	9.31

The abbreviations used are: Tris, tris(hydroxymethyl)aminomethane; NADP, nicotinamide-adenine dinucleotide phosphate; EDTA, ethylenediaminetetraacetate; ATP, adenosine 5′-triphosphate.

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[1] Johnson, F. M., C. G. Kanapi, R. H. Richardson, M. R. Wheeler, and W. S. Stone, these PROCEEDINGS, **56**, 119–125 (1966).

[2] Lewontin, R. C., and J. L. Hubby, *Genetics*, **54**, 595 (1966).

[3] Stone, W. S., M. R. Wheeler, F. M. Johnson, and K. Kojima, these PROCEEDINGS, **59**, 102–109 (1968).

[4] Poulik, M. D., *Nature*, **180**, 1477 (1957).

[5] Gel buffer: 8.12 mM Tris and 3 mM citric acid; electrode buffer: 0.233 *M* Tris and 0.086 *M* citric acid.

[6] Young, W. J., J. E. Porter, and B. Childs, *Science*, **143**, 140–141 (1964).

[7] Robertson, A., *Genetics*, **47**, 1291–1300 (1962).

[8] Kacser, H., in *Biological Organization at the Cellular and Supercellular Level*, ed. R. J. C. Harris (New York: Academic Press, 1963), pp. 25–41.