Milkman, R. University of Iowa, Iowa City, Iowa. Thermostability variation in D. m. allozymes.

Electrophoretic mobility and thermostability were examined in sets of individual flies from wild (Cedar Rapids, IA; Berkeley, CA) and laboratory strains. Mobility studies utilized electrophoresis for 30 min at 400V with 1/4-strength Gelman HR buffer, or 50 min at 210V with 1/2-strength Gelman HR buffer (or occasionally 100 min when the loaded cellulose acetate strips are coated with oil to reduce evaporation). Thermostability tests involved 10 min electrophoresis at 200 or 400V, after which the strips were enclosed in a cellophane bag and immersed in a water bath for 20-120 sec, then removed and stained. Mobility class frequencies are listed in Table 1, with those of Band (1975) below for comparison. (Extensive ADH and α-GPDH data are reported by Sampsell 1977.) 88-110 strains were tested for each enzyme.

Table 1

Enzyme	Slow	Commonest	Fast	Faster	Test Temp.
ACPH	0.02	0.98			47°C
	0.05	0.95			
AK		1.00			54
		-			
AO		0.90	0.09	0.01	70
		0.88	0.12		
ME		1.00			57
		0.99	0.01		
IDH		0.97	0.03		53
	0.03	0.94	0.01	0.02	
PGM	0.17	0.78	0.05		65
	0.03	0.95	0.01		
XDH		1.00			72
	0.02	0.98			
Est-6		0.74	0.26*		59
		0.57	0.41 [sic]		
MDH		0.95**	0.05		60
		1.00			

*Thermostability classes: 0.18, 0.03, 0.04.
**0.94, 0.01. Order of increasing sensitivity.
No thermostability variation was detected in the other enzymes.

The Est-6 classes likely correspond to Cochrane's (1976) 1.10 classes. Trippa et al. (1976) clearly demonstrated variants in PGM. In MDH, one sensitive slow strain was found. The thermostability difference was mapped to the Mdh locus (or very close) as follows. Progeny of an S^S (slow sensitive) X F^+ cross were propagated for 10 generations, after which 60 heterozygotes were examined. In all cases, the slow band was sensitive and the fast band was not (heterodimer appeared intermediate). In a similar test of S^+ X F^+ 10th generation heterozygote progeny, both homodimer bands invariably evinced equal thermostability. These tests were made after 60 min at 200V.

The observed stability differences were in the range of four-fold differences in the treatment durations required for criterion response. These differences corresponded to differences in $\Delta F^{\ddagger}$ on the order of 800 cal/mol, which is in the range of one van der Waals bond energy. While somewhat subtler differences could have been detected, none were. Since bond substitutions, as well as conformational changes, could alter bond energies by far smaller values, thermostability analysis is not a sensitive detector of amino acid substitutions. In any event, the increased genetic variability indicated by the present tests is negligible. On the other hand, the asymmetry evident so far in thermostability variation (most variants are more sensitive than the commonest class) should be noted as potential evidence for the Ohta and Kimura (1975) model of very slightly deleterious mutations. Supported by NSF Grant DEB 76-01903.

References: Band, H.T. 1975, Genetics 80:761-771; Cochrane, B. 1976, Nature 263:131-132; Ohta, T. and M. Kimura 1975, Am. Nat. 109:137-145; Sampsell, B. 1977, Biochem. Genet. 14: 971-988; Trippa, G., A. Loverre and A. Catamo 1976, Nature 260:42-44.