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The α-Glycerophosphate Dehydrogenase (α-gpdh) Polymorphism in *Drosophila melanogaster:* Adult Survival Under Temperature Stress

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Summary. As a test of the hypothesis that adult temperature stress is an important component of natural selection maintaining the *α-gpdh* polymorphism, we have looked for differential survival among genotypes subjected to (i) heat shock and (ii) cold shock. Factorial ANOVAR, taking account of genotype, sex and temperature-stress indicated that genotype did not contribute to the variance of survival proportion per vial. We have not therefore found evidence to support our hypothesis. Incidental to the above was a significant sex-temperature interaction. Thus, adult females showed higher survival than males under heat stress, while under cold stress, there was no indication of a survival difference between the sexes.

Key words: *Drosophila* – α-Glycerophosphate dehydrogenase – Polymorphism – Temperature selection

Introduction

The soluble α-glycerophosphate dehydrogenase enzyme is polymorphic in populations of *D. melanogaster* throughout the world. Two electrophoretic alleles are reported and the locus maps to position 17.8 on chromosome II. Much work has been carried out on the development, physiology and genetics of *α-gpdh* (review by Dickinson and Sullivan 1975) including a detailed study on the biochemistry of the three genotypes (Miller et al. 1975). Hence, this enzyme now becomes very attractive for studies directed towards an understanding of the possible selective forces responsible for the maintenance of structural gene variability in nature.

The *α-gpdh* enzyme is likely to be involved in at least two separate metabolic roles, (i) energy metabolism, especially during adult flight, and (ii) larval lipid metabolism (see Rechsteiner 1970; Bewley et al. 1974). Environmental correlations and seasonal changes in *α-gpdh* gene frequency in natural populations (Johnson and Schaffer 1973; Berger 1971) indicate that environmental temperature variation may play a role in the maintenance of this polymorphism. Miller et al. (1975) have investigated temperature dependent biochemical parameters of the adult enzymes produced by all three genotypes. They found that at lower temperatures the heterozygous form of the enzyme has, at saturation but not inhibitory levels of NADH, the greatest affinity for dihydroxyacetone phosphate, the natural substrate of this enzyme. The S/S form of the enzyme lies between the other two forms in its affinity for substrate at lower temperatures. At higher temperatures, greater than about 20°C, the F/F enzyme is superior in this regard (i.e. it has a lower K_m). Also, as temperature increases, the specific activity of the F/F form increase to a value above that of the enzyme products of the other two genotypes. These observations are consistent with the hypothesis that in *D. melanogaster*, a poikilotherm, environmental temperature may select via fitnesses determined directly by the differential efficiencies of *α-gpdh* gene products.

It now needs to be shown the detailed way in which selection might operate on this locus. Can temperature dependent fitness differences among genotypes be directly related to the biochemical parameters of the enzyme? In this communication we have focused on the adult enzyme and its possible role in survival under stressful temperature environments. With this hypothesis, we are suggesting that intermittent extremes of temperature selection may play a role in the maintenance of *α-gpdh* variability in nature. Analogous data have been obtained for the alcohol dehydrogenase polymorphism of this species by Johnson and Powell (1974).

Method

Two lines homozygous for the alterate *α-gpdh* alleles were established and maintained at 25°C for several generations before use,

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from a laboratory population – Tahbilk. The Tahbilk population was initiated two years earlier from at least 200 field-collected adults and maintained at 25°C by mass transfer in two half-pint bottles. The homozygous lines were each obtained by pooling six individual homozygous lines derived entirely by outbreeding methods from the Tahbilk bottles. Heterozygous adults were obtained by mating virgin FF females with SS males and vice-versa. For this experiment both types of heterozygotes were pooled in equal numbers.

For genotype determination individual adults were ground in 20 µl of distilled water and electrophoresed at 4°C in horizontal gels of 10% Sigma starch using Poulik's buffer system (2hr; 200 V; 75 mA/gel of 30 flies). Staining was carried out using the method of Ayala et al. (1972).

Vials containing a yeast-treacle maize-meal medium and 30 adults (6 to 7 days from pupal eclosion) were placed, for the heat stress, in an incubator at 37°C for 13.5 hr and, for the cold stress, in a salt-ice bath at −1°C to −2°C for 7.5 hr. These temperatures were our best estimates of the extremes which might be encountered by adults in natural populations, especially in Victoria, and they are similar to the temperature stresses applied to this species by other workers (see Parsons 1974, 1977; Johnson and Powell 1974). The exposure times were predetermined to cause reasonable mortality rates. Fifteen flies of a single sex and genotype were placed together in one vial with 15 flies of the other sex and a different genotype. This arrangement of sexes and genotypes was used in an effort to spread the observed heterogeneity in survival within treatments as evenly as possible among treatments. We found this heterogeneity in survival proportion very difficult to control, notwithstanding (i) every care in the positioning of vials during the temperature stress, and (ii) efforts to create a uniform pretreatment environment during development. Survivors were scored between 12 and 24 hours after the vials had been removed from the temperature stress.

Results and Discussion

A minimum of 26 replicates were scored for proportion surviving for each combination of genotype, sex and stress. Results were analysed by a three way factorial analysis of variance (ANOVAR) on the angularly transformed proportions and are presented in Table 1.

No significant effect of genotype was detected either separately or in interaction with sex and/or temperature stress (Table 1). Within each of the four sex-temperature classes, three pairwise t tests were carried out between genotypes. Of the 12 tests no significant differences were detected. We have not therefore found evidence to support our initial hypothesis that the *α-gpdh* gene products play a role, via their differential temperature dependent biochemical activities, in the determination of fitness under adult temperature stress. In the histograms (Fig. 1), we have not distinguished genotype effects. Though we have pooled only six outbred lines to obtain our original homozygous lines, we consider that this should have been sufficient to avoid spurious differences in survival among the three tested lines (i.e. to avoid differences due to chance sampling of background genes or to avoid differ-

Table 1. Three way factorial analysis of variance (genotype, sex, temperature stress) of angularly transformed proportion surviving per vial

Source of variation	d.f.	Mean square	F ratio
Main effects			
Genotype	2	12.3	0.02
Sex	1	1951.2	3.83
Temperature stress	1	86047.1	168.76[b]
Two-way interactions			
Genotype-sex	2	140.0	0.27
Genotype-temperature stress	2	13.6	0.03
Sex-temperature stress	1	3515.0	6.89[a]
Three-way interaction			
Genotype-sex-temperature stress	2	651.0	1.28
Error	300	509.9	

[a] $p < 0.01$, [b] $p < 0.001$

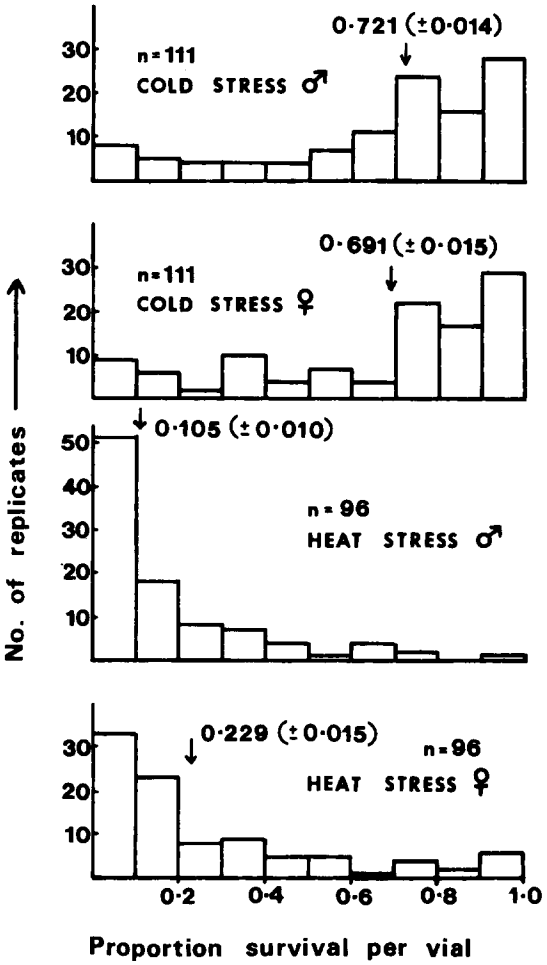

Fig. 1. A comparison between sexes of the distribution among replicates of adult survival proportions for both cold stress and heat stress conditions. Arrows indicate the mean proportion surviving per vial averaged over the n replicates (± standard error)

ences in response due to electrophoretically identical allelomorphs which may be present).

The highly significant difference in survival between treatments is of course due simply to the experimental design. This design would have been improved if mean survival could have been manipulated to be closer to 50% for both treatments.

Although, overall, no significant effect of sex on the proportion surviving has been detected by ANOVAR, there has been a significant ($p < 0.01$) sex-temperature interaction. A t test on the difference between the means of the sexes (genotypes pooled) for each of the temperature treatments, indicated (i) no significant difference under cold stress ($t = 0.59$, $df = 220$, $p \approx 0.6$), and (ii) females survived better under the heat stress ($t = 3.26$, $df = 190$, $p < 0.01$). The lack of a difference between the sexes under cold stress is consistent with results obtained by Parsons (1977) who subjected adults to $-1°C$ for 48 hrs. However, the survival difference between the sexes we have detected here under heat stress, was not found by Hosgood and Parsons (1968) when they exposed adults to 33.5°C for 24 hrs. This effect is, however, consistent with data indicating that female *D. melanogaster* are generally more resistant than males to environmental stresses, e.g. dessication, longevity at 29.5°C and resistance to anoxia (Parsons 1974). This result is also in line with data obtained by McKenzie (1978) where females outsurvive males when development occurs at extreme, though permissive, temperatures. If heat stresses of the type used in these studies occur in nature, we might expect the female/male ratio to increase with increasing spring to summer temperatures.

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Literature

Ayala, F.J.; Powell, J.R.; Tracey, M.L.; Mourao, C.A.; Perez-Salas, S.: Enzyme variability in the *Drosophila willistoni* group. IV Genetic variation in natural populations of *Drosophila willistoni*. Genetics **70**, 113-139 (1972)

Berger, E.M.: A temporal survey of allellic variation in natural and laboratory populations of *D. melanogaster*. Genetics **67**, 121-136 (1971)

Bewley, G.C.; Rawls, J.M.; Lucchesi, J.A.: α-Glycerophosphate dehydrogenase in *Drosophila melanogaster*: kinetic differences and developmental differentiation of the larval and adult isozymes. J. Insect Physiol. **20**, 153-165 (1974)

Dickinson, W.J.; Sullivan, D.T.: Gene-enzyme systems in *Drosophila*. Berlin-Heidelberg-New York: Springer 1975

Hosgood, S.M.W.; Parsons, P.A.: Polymorphism in natural populations of *Drosophila* for the ability to withstand temperature shocks. Experientia **24**, 727-728 (1968)

Johnson, F.M.; Powell, A.: The alcohol dehydrogenases of *D. melanogaster*: frequency changes associated with heat and cold shock. Proc. Nat. Acad. Sci. (USA) **71**, 1783-1784 (1974)

Johnson, F.M.; Schaffer, H.E.: Isozyme variability in species of the genus *Drosophila*. VII Genotype environment relationships in populations of *D. melanogaster* from the eastern United States. Biochem. Genet. **10**, 149-163 (1973)

McKenzie, J.A.: The effect of developmental temperature on population flexibility in *Drosophila melanogaster* and *D. simulans*. Austr. J. Zool. **26**, 105-112 (1978)

Miller, S.; Pearcy, R.W.; Berger, E.: Polymorphism at the α-glycerophosphate dehydrogenase locus in *D. melanogaster*. I Properties of adult allozymes. Biochem. Genet. **13**, 175-188 (1975)

Parsons, P.A.: Genetics of resistance to environmental stresses in *Drosophila* populations. Annu. Rev. Genet. **7**, 239-265 (1974)

Parsons, P.A.: Resistance to cold temperature stress in populations of *Drosophila melanogaster* and *D. simulans*. Austr. J. Zool. **25**, 693-698 (1977)

Poulik, N.D.: Starch gel electrophoresis in a discontinuous system of buffers. Nature. **180**, 1477 (1957)

Rechsteiner, M.C.: *Drosophila* lactate dehydrogenase and α-glycerophosphate dehydrogenase: distribution and change in activity during development. J. Insect Physiol. **16**, 1179-1192 (1970)

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