Interaction between the *Adh* and *αGpdh* loci in *Drosophila melanogaster*: adult survival at high temperature

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The role of high temperature resistance in the world-wide cline of *Adh* and *αGpdh* allele frequencies of *Drosophila melanogaster* was investigated. Experimental strains were used with different combinations of *Adh* and *αGpdh* alleles but with similar genetic background. The survival time of adult males, reared at different pre-adult temperatures, was measured at 35°C. To investigate the relationship between survival and protein content, triglycerides content and ADH and *α*GPDH enzyme activity, the latter characters were measured before and after 12 h exposure to 35°C. Three-way ANOVA of survival at 35°C showed significant effects of rearing temperature and *Adh* genotype, interaction between *Adh* and *αGpdh*, interaction between *Adh* and rearing temperature and interaction between *Adh*, *αGpdh* and rearing temperature, *α*GPDH enzyme activity did not change significantly. Although protein content, triglycerides content and ADH enzyme activity decreased significantly at 35°C, no correlation was observed between survival and these traits. It is concluded that the world-wide cline of *Adh* and *αGpdh* allele frequencies cannot simply be explained by the genotypic differences in resistance to high temperature of adult *Drosophila melanogaster*.

Keywords: alcohol dehydrogenase, allozyme polymorphism, cline, *α*-glycerophosphate dehydrogenase, selection, temperature stress.

Introduction

Temperature stress is often suggested as one of the causes of the world-wide clines in allele frequencies of the alcohol dehydrogenase (*Adh*) and *α*-glycerophosphate dehydrogenase (*αGpdh*) loci of *Drosophila melanogaster* (see reviews by Van Delden, 1982 and Zera *et al.*, 1985).

The influence of high temperature on enzyme activity has been extensively investigated especially for the *Adh* locus. At high temperature the *in vitro* ADH enzyme activity of *Adh*^{SS} is generally found to be more stable than that of *Adh*^{FF} (Gibson, 1970; Day *et al.*, 1974; Van Delden & Kamping, 1980; Alahiotis, 1982). However, Oakeshott (1976) reported *Adh*^{FF} to be more stable than *Adh*^{SS}. Sampbell & Sims (1982) observed a decrease in ADH enzyme activity of *Adh*^{FF} *in vitro* after exposure to high temperature and no decrease in *Adh*^{SS}. Van Delden & Kamping (1980), however, found no decrease in ADH enzyme activity in adults for any *Adh* genotype after 24 h at 35°C. The *α*GPDH enzyme of adult flies is stable *in vitro* for at

least 5 min at temperatures up to 50°C, the larval enzyme is unstable at 50°C. No differences in temperature stability have been reported between *αGpdh* alleles (Bewley *et al.*, 1974; Miller *et al.*, 1975; Bewley, 1978; Alatossava & Lakovaara, 1981; Bewley *et al.*, 1984).

Quite a number of experiments have been published concerning the survival of *Adh* and *αGpdh* genotypes at high temperature. The data, however, are contradictory. Van Delden & Kamping (1980) reported that *Adh*^{FS} survived the longest at high temperature, and, in agreement with Sampsell & Sims (1982) and McElfresh & McDonald (1986) they found that *Adh*^{FF} survived longer than *Adh*^{SS}, but Johnson & Powell (1974) and Stephanou Alahiotis (1986) found *Adh*^{SS} to be the better survivor, and Oakeshott *et al.* (1985) found no differences at all in heat resistance between *Adh* genotypes. For *αGpdh* Oakeshott *et al.* (1985) found better survival at high temperature of *αGpdh*^{FF} when compared to *αGpdh*^{SS}, while *αGpdh*^{FS} was intermediate, but Schenfield & McKechnie (1979) found no differences between *αGpdh* genotypes.

A possible explanation of these contradictions may be that the strains tested had differences in their genetic background, and in particular the interaction between *Adh* and *αGpdh* might play a role. Resistance to high temperature may depend on (the rate of) metabolic processes, analogous to stress resistance in general (Hoffman & Parsons, 1989a,b). Metabolic interaction between *Adh* and *αGpdh* is plausible in two pathways: both enzymes play a role in lipid synthesis (Geer *et al.*, 1983; Lissimore *et al.*, 1990) and both enzymes can influence the NAD⁺/NADH ratio (Cavener, 1983); more details are given in Oudman *et al.* (1991). Because ADH enzyme stability depends on NAD⁺ concentration (Van Delden & Kamping, 1980), *αGPDH* theoretically may influence ADH enzyme stability via NAD⁺ concentration. Furthermore, catabolism and metabolism generally are less efficient under conditions of stress (Koehn & Bayne, 1989). The lipid content of flies can be used as an indicator for the net energy flux because lipids are the most important energy resource (e.g. Da Lage *et al.*, 1989). In addition, the lipid flux is especially interesting because Oudman *et al.* (1991) described differences in lipid content between *Adh* and *αGpdh* genotypes.

We investigated the interaction between the effects of the *Adh* and *αGpdh* loci on heat resistance by measuring adult survival at 35°C of all possible *Adh αGpdh* genotypes with similar genetic background. We investigated different pre-adult temperatures because survival might be influenced by rearing temperature (Maynard Smith, 1957; Levins, 1969). To investigate the balance between supply and utilization of resources we measured the initial content and change in content of protein and triglycerides during 12 h exposure at 35°C. *In vivo* enzyme stability was investigated by measuring ADH and *αGPDH* enzyme activity before and after a 12 h exposure at 35°C.

Materials and methods

Experimental lines

Flies were obtained from sublines derived from the polymorphic wild type laboratory strain Groningen 83. The sublines, described by Oudman *et al.* (1991), consisted of two sets (A and B) with all four possible homozygous combinations of *Adh*^F or *Adh*^S and *αGpdh*^F or *αGpdh*^S, and with similar genetic background within each set. Experimental lines were obtained from crosses between sublines from the sets A and B, resulting in *Adh αGpdh* genotypes with similar genetic background (A × B). Nine crosses (and their reciprocals) were performed to obtain all possible *Adh αGpdh* genotypes, see Table 1. Only one double hetero-

Table 1 Origin of the experimental lines from the sets A and B. The first and second letter refer to the *Adh* genotype, the third and fourth letter to the *αGpdh* genotype

	Set B			
	<i>Adh</i> ^{FF} <i>αGpdh</i> ^{FF}	<i>Adh</i> ^{FF} <i>αGpdh</i> ^{SS}	<i>Adh</i> ^{SS} <i>αGpdh</i> ^{FF}	<i>Adh</i> ^{SS} <i>αGpdh</i> ^{SS}
Set A <i>Adh</i> ^{FF} <i>αGpdh</i> ^{FF}	FF FF	FF FS		FS FS
<i>Adh</i> ^{FF} <i>αGpdh</i> ^{SS}		FF SS		FS SS
<i>Adh</i> ^{SS} <i>αGpdh</i> ^{FF}	FS FF		SS FF	
<i>Adh</i> ^{SS} <i>αGpdh</i> ^{SS}			SS FS	SS SS

zygous genotype (the coupling) was used for statistical convenience.

Rearing conditions

Standard rearing conditions were 25°C, ~ 50 per cent RH and 24 h light, and medium as described by Oudman *et al.* (1991). Flies from sets A and B were allowed to lay eggs for 12 h on dishes with fresh medium. The eggs were transferred to half-pint bottles with 30 ml fresh medium, 200 eggs per bottle. After eclosion virgin females and males were collected and stored separately for 5 days; the flies were then crossed according to the scheme given in Table 1; reciprocal crosses were kept apart. Eggs were collected from these crosses and incubated at 20, 25 or 29°C. In order to synchronize the age and time of testing of flies reared at different temperatures, eggs were collected on different days. On these days the flies were allowed to lay eggs for 12 h on dishes with fresh medium and a drop of live yeast. The eggs were transferred to half-pint bottles with 30 ml fresh medium, 200 eggs per bottle, and incubated at the appropriate temperature.

Experiments

Males were collected after eclosion, etherized and stored in groups of 20 in plastic vials of 80 × 23 mm with 5 ml medium at 25°C. At the age of about 6 days three vials of both reciprocals of each genotype were put at 35°C and the number of dead flies recorded every 6 h (survival test group). The survival time of individual flies was taken as the midpoint of this interval. The mean survival per vial was calculated as the average of the survival times of the individual flies. Data from reciprocal crosses were combined because

the design of the experiment did not allow us to discriminate between environmental and maternal effects. ANOVA was performed on survival data to determine the effects of *Adh* genotype, α Gpdh genotype and rearing temperature. Tukey's test for multiple comparisons was performed on groups of three temperatures or genotypes with a level of significance of 5 per cent; significant differences are indicated in Fig. 3.

Biochemical data were collected from flies reared at 25°C only. Of each genotype four replicate samples of 10 males, 6 days old, were collected before (control group) and after 12 h exposure at 35°C (treated group) and stored at -80°C. Care was taken not to collect dead flies. Biochemical assays were performed on the control groups and the treated groups. Protein content was measured using Bradford's (1979) method; triglycerides were assayed enzymatically; ADH enzyme activity was measured with isopropanol as substrate, α GPDH enzyme activity with DL- α -glycerophosphate as a substrate. Detailed descriptions of the biochemical methods are given in Oudman *et al.* (1991). Data from reciprocal crosses were combined and ANOVA was performed to determine the effects of treatment and *Adh* and α Gpdh genotype.

Results

The survival time at 35°C of genotypes reared at 25°C is shown in Fig. 1. Two periods of mortality could be distinguished: an initial mortality within 24 h (at least for some genotypes) and a second period later on. ANOVA (not shown) indicated that genotypic differences

in mortality during the first 24 h were independent of the pre-adult rearing temperature. Experimental analysis has shown that the first period of mortality was due to the occurrence of the heat-sensitive lethal *l(2)hs* (Oudman, 1991). This recessive conditional lethal allele occurred in the strain Groningen 83 with a frequency of about 50 per cent, estimated on the basis of the percentage early mortality in this strain. The frequency of lethal homozygotes in the experimental lines was between 0 and ~50 per cent (Fig. 1). To correct for this early mortality, the flies that survived the first 24 h were reset on 100 per cent (Fig. 2). This procedure was justified because there was no significant correlation between mortality during the first 24 h and survival time after 24 h ($r=0.17$ over genotype means). We will not deal with the phenomenon of the early mortality, which was independent from *Adh* and α Gpdh genotype effects, in the present paper (for further details see Oudman, 1991), and for the rest of the paper survival data are given only for flies which survived the first 24 h.

Mean survival times are shown in Fig. 3. ANOVA performed on these data (Table 2) shows that there were significant effects of rearing temperature, *Adh* genotype and all interactions except the interaction between α Gpdh genotype and rearing temperature. Flies reared at 25°C showed the longest survival (Fig. 3a). *Adh*^{FS} survived significantly longer than *Adh*^{SS} (Fig. 3b). α Gpdh genotype had no significant effect. Figure 3c shows the interaction between the effect of *Adh* genotype and rearing temperature. For flies reared at 20 and 25°C there were no significant differences between the *Adh*

Fig. 1 Survival curves at 35°C of adult males of different *Adh* α Gpdh genotypes reared at 25°C.

Fig. 2 Survival curves at 35°C, corrected for early mortality (see text), of adult males of different *Adh* α *Gpdh* genotypes reared at 25°C (symbols see Fig. 1).

genotypes, for flies reared at 29°C *Adh*^{FF} survived significantly longer than *Adh*^{SS}. The strong interactions between the effects of *Adh* and α *Gpdh* genotype are shown in Fig. 3d. Within α *Gpdh*^{FF} genotypes, *Adh*^{SS} survived significantly shorter than both the other *Adh* genotypes, within α *Gpdh*^{FS} genotypes *Adh*^{SS} survived shorter than *Adh*^{FS}, and within α *Gpdh*^{SS} no significant differences between *Adh* genotypes were found. Within *Adh*^{FF} genotypes α *Gpdh*^{FF} survived significantly longer than both the other α *Gpdh* genotypes, within *Adh*^{FS} no significant differences between α *Gpdh* genotypes were found, and within *Adh*^{SS} genotypes α *Gpdh*^{SS} survived longer than both the other α *Gpdh* genotypes. Figure 3e shows the interactions between the effects of *Adh* and α *Gpdh* genotype and rearing temperature. The two trends exhibited were that *Adh*^{FF} α *Gpdh*^{SS} survived relatively longer when reared at 29°C than at 20 and 25°C compared to other combinations containing *Adh*^{FF} or α *Gpdh*^{SS}, and *Adh*^{FS} α *Gpdh*^{FS} survived relatively shorter when reared at 29°C than at 20 and 25°C compared to other combinations containing *Adh*^{FS} or α *Gpdh*^{FS} genotypes. In general, flies reared at 29°C showed less significant differences in survival between genotypes than flies reared at 20 and 25°C.

Biochemical measurements were restricted to flies reared at a pre-adult temperature of 25°C. A summary of the ANOVA performed on the biochemical data is shown in Table 3. Protein content, triglycerides content and ADH enzyme activity showed genotypic effects [of *Adh* and α *Gpdh* (except for protein content) and interactions between them] and an effect of treatment. No

interactions were found between *Adh* or α *Gpdh* genotype and treatment, thus all genotypes reacted in a similar way. No significant differences between the genotypes were observed for α GPDH enzyme activity. Table 4 shows the mean values of the biochemical traits before and after 12 h exposure to 35°C. On the average protein decreased 2.8 per cent during 12 h exposure at 35°C, triglycerides decreased 4.3 per cent and ADH enzyme activity decreased 4.5 per cent. α GPDH enzyme activity increased 2.1 per cent during 12 h exposure to 35°C, but ANOVA indicated that this increase was not significant.

No significant correlations were found between survival time and biochemical characters measured before or after 12 h exposure at 35°C, nor between survival time and the change in the values of the biochemical traits during 12 h exposure at 35°C.

Discussion

The higher heat resistance of flies raised at a pre-adult temperature of 25°C compared to 20°C is in agreement with the literature on acclimation. Levins (1969) found survival at 38°C of *Drosophila melanogaster* to be positively related to rearing temperature (acclimation). Maynard Smith (1957) distinguished between pre-adult and adult acclimation of *Drosophila subobscura* and found both to improve adult heat resistance, although pre-adult temperature had less influence than adult temperature. Thus one would expect flies reared at 29°C to have the highest heat resistance. This, however, was not the fact, so pre-adult acclimation

Fig. 3 Survival time at 35°C (h) of males reared at different temperatures (20, 25 and 29°C). Vertical bars indicate 95 per cent confidence intervals. Arrows indicate significant differences with Tukey's test ($P < 5\%$).

might not occur over the whole range of rearing temperatures studied.

Although the effects of the *Adh* and *αGpdh* loci on survival at high temperature are small compared to the effects of other loci (compare Oudman, 1991) several interesting patterns were discovered. The longer survival we observed of *Adh*^{FS} compared to *Adh*^{SS} is in agreement with the data of Van Delden & Kamping (1980), who found a better survival of *Adh*^{FS} compared to *Adh*^{SS} at 35°C, with *Adh*^{FF} being intermediate. Sampsell & Sims (1982) and McElfresh & McDonald (1986) also observed better survival of *Adh*^{FF} compared to *Adh*^{SS} at high temperature on medium with little or no ethanol. However, Johnson & Powell (1974) and Stephanou & Alahiotis (1986) reported *Adh*^{SS} to be the best survivor at high temperatures, and Oakeshott *et al.* (1985) observed no differences between *Adh* genotypes. We found no main effect of

αGpdh genotype on heat resistance. This is in agreement with the results of Schenfield & McKechnie (1979), but in contradiction with Oakeshott *et al.* (1985), who observed differences in heat resistance between *αGpdh* genotypes. A possible explanation for the differences between survival experiments involving *Adh* or *αGpdh* genotypes in the literature may be differences in the genetic background of the tested strains. As shown in Fig. 3d we found a strong interaction between the effects of *Adh* and *αGpdh*. Survival of *Adh* genotypes shows opposing results for different *αGpdh* genotypes and vice versa. In view of this *Adh/αGpdh* interaction, heat resistance experiments performed for one allozyme polymorphism may provide quite different outcomes depending on the genetic composition of the other locus. Differences in the literature may be ascribed to this effect. We do, however, not know the background genotypes of the strains tested by other authors.

αGPDH enzyme activity was the only biochemical character studied that did not decrease at 35°C. Vossen *et al.* (1983) observed for *Drosophila hydei* an increase in *αGPDH* specific activity after activation of the heat shock genes; this increase was not due to *de novo* synthesis of the enzyme but to a decrease in the *K_m^{αGp}* of *αGPDH*. We found a significant decrease in ADH enzyme activity during 12 h at 35°C. Van Delden & Kamping (1980) found no measurable decrease in ADH enzyme activity after 24 h at 35°C, but a more detailed study of their data showed that after 6 and 8 h at 35°C ADH enzyme activity was about 10 per cent lower for all *Adh* genotypes. Thus ADH enzyme activity may decrease during exposure to 35°C in the first instance, but it may increase again later. A more detailed study on this subject will be necessary, especially in relation to the *in vitro* instability of ADH at

Table 2 ANOVA for survival time at 35°C of males of different *Adh* and *αGpdh* genotypes, reared at different temperatures

Source	SS	d.f.	MS	F	P <
<i>Adh</i> genotype (<i>a</i>)	212	2	106	4.7	0.05
<i>αGpdh</i> genotype (<i>g</i>)	60	2	30	1.3	ns
Rearing temperature (<i>t</i>)	318	2	159	7.0	0.001
<i>a</i> × <i>g</i>	733	4	183	8.1	0.001
<i>a</i> × <i>t</i>	249	4	62	2.7	0.05
<i>g</i> × <i>t</i>	195	4	49	2.1	ns
<i>a</i> × <i>g</i> × <i>t</i>	509	8	64	2.8	0.01
Explained	2275	26	87	3.8	0.001
Residual	3074	135	23		
Total	5349	161	33		

Table 3 Summary of ANOVA for protein content (μg/ind.), triglycerides content (μg/ind.) and ADH and *αGPDH* enzyme activity (nmol NADH/min/ind.) of flies of different *Adh αGpdh* genotypes, measured before and after 12 h exposure at 35°C

	Protein	Triglycerides	ADH	<i>αGPDH</i>
<i>Adh</i> genotype (<i>a</i>)	***	*	***	ns
<i>αGpdh</i> genotype (<i>g</i>)	ns	***	***	ns
Treatment (<i>t</i>)	***	*	*	ns
<i>a</i> × <i>g</i>	ns	***	***	ns
<i>a</i> × <i>t</i>	ns	ns	ns	ns
<i>g</i> × <i>t</i>	ns	ns	ns	ns
<i>a</i> × <i>g</i> × <i>t</i>	ns	ns	ns	ns

P* < 0.05. *P* < 0.01. ****P* < 0.001.

Table 4 Protein content ($\mu\text{g}/\text{ind.}$), triglycerides content ($\mu\text{g}/\text{ind.}$) and ADH and α GPDH enzyme activity (nmol NADH/min/ind.) of adult flies, measured before (control) and after 12 h exposure at 35°C (treated). Mean and standard deviation (s.d) of eight replicates of nine genotypes

	Control		Treated	
	Mean	s.d	Mean	s.d
Protein	67.4	3.5	65.5	3.4
Triglycerides	89.2	11.3	85.4	11.0
ADH	37.5	19.0	35.8	17.7
α GPDH	53.0	4.1	54.1	4.3

high temperature. Although the decrease in protein and triglycerides content during 12 h at 35°C was significant, this decrease was so small that mortality obviously was not caused by the depletion of these resources.

We found no correlation between the biochemical characters studied and survival at 35°C. In addition, Oudman *et al.* (1988) found no effect of body weight (manipulated by larval density) on survival at 37°C. Parsons (1973) found a positive correlation between desiccation tolerance and bodyweight in *Drosophila melanogaster*, but Hoffmann & Parson (1989a,b) found no long-term effect of selection for desiccation tolerance on body weight or lipid content. In addition Hoffman & Parsons (1989a,b) found that lines selected for increased desiccation resistance in adults had decreased metabolic rate and increased resistance to heatshock, starvation and toxic concentrations of ethanol. They concluded that there is a general component in stress resistance. The same might be concluded from the congruence of our data with the data of McKechnie & Geer (1988). They found epistatic interaction between *Adh* and α Gpdh for larval resistance to ethanol: *Adh*^{FF} α Gpdh^{FF} and *Adh*^{SS} α Gpdh^{SS} survived better than *Adh*^{FF} α Gpdh^{SS} and *Adh*^{SS} α Gpdh^{FF}. We found the same genotypic differences for adult survival at high temperature when only taking into account the four homozygous genotypes. Thus survival on alcohol medium may also be influenced by general stress resistance as well as a better specific detoxification capacity for ethanol, as argued by several authors (e.g. Geer *et al.*, 1990). Cavener & Clegg (1981), however, found in a long-term study that the *Adh*^{SS} α Gpdh^{SS} genotype was at a disadvantage in ethanol environments. Thus long-term effects may be different from direct effects. Clearly in long-term experiments more (complex) adaptations can occur (e.g. Kerver & Van Delden, 1985; Bijlsma-Meeles & Bijlsma, 1988).

Hoffman & Parsons (1989a,b) suggest that metabolic rate plays an important role in stress resistance. In general, increased stress resistance should be related to decreased metabolic rate. Possibly, interactions between *Adh* and α Gpdh for stress resistance occur via their influence on the NAD⁺/NADH ratio (Cavener, 1983) and thereby on metabolic rate. We plan further investigations on the relation of stress resistance and developmental rate [for which *Adh* and α Gpdh genotypic effects are reported by Oudman *et al.* (1991)] with metabolic rate.

In nature extreme high temperatures are expected to occur most frequently in equatorial regions. The most common genotype of *Drosophila melanogaster* in that area is *Adh*^{SS} α Gpdh^{FF} (Oakeshott *et al.*, 1982). We found, however, that this genotype was among the poorest survivors at 35°C (Fig. 3d). We thus see no causal relationship between the world-wide cline of *Adh* and α Gpdh allele frequencies and the resistance to high temperature. We have to emphasize that our experiments were restricted to adult males and one testing temperature. In addition, the situation is complicated by the occurrence of the inversion *In(2L)t*, which is nearly always associated with *Adh*^S and α Gpdh^F alleles. This inversion did not occur in our lines (Oudman *et al.*, 1991). Van Delden & Kamping (1989) found, besides an effect of temperature on α Gpdh allele frequencies in the absence of *In(2L)t*, that the inversion had a selective advantage at high temperatures compared to the same *Adh* α Gpdh genotype in the standard chromosome arrangement. Thus the occurrence of *In(2L)t*, favoured by high temperature, may be at least one of the causes of the world-wide *Adh* and α Gpdh allozyme clines, although it is not yet clear to what extent (for discussion see Van Delden & Kamping, 1989).

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References

- ALAHOTIS, S. N. 1982. Adaptation of *Drosophila* enzymes to temperature. IV. Natural selection at the alcohol dehydrogenase locus. *Genetica*, **59**, 81–87.
- ALATOSSAVA, T. AND LAKOVAARA, S. 1981. Thermal stability of α -glycerophosphate dehydrogenase in *Drosophila*. *Biochem. Genet.*, **19**, 311–320.

- BEWLEY, G. C. 1978. Heat stability studies at the α -glycerophosphate dehydrogenase locus in population of *Drosophila melanogaster*. *Biochem. Genet.*, **16**, 769-775.
- BEWLEY, G. C., NIESEL, D. W. AND WILKINS, J. R. 1984. Purification and characterization of the naturally occurring allelic variants of *sn*-glycerol-3-phosphate dehydrogenase in *Drosophila melanogaster*. *Comp. Biochem. Physiol.*, **79B**, 23-32.
- BEWLEY, G. C., RAWLS, J. M. AND LUCCHESI, J. C. 1974. α -Glycerophosphate dehydrogenase in *Drosophila melanogaster*: kinetic differences and developmental differentiation of the larval and adult isozymes. *J. Insect Physiol.*, **20**, 153-165.
- BIJLSMA-MEELES, E. AND BIJLSMA, R. 1988. The alcohol dehydrogenase polymorphism in *Drosophila melanogaster*: fitness measurements and predictions under conditions with no alcohol stress. *Genetics*, **120**, 743-753.
- BRADFORD, M. M. 1979. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.*, **72**, 248-254.
- CAVENER, D. R. 1983. The response of enzyme polymorphisms to developmental rate selection in *Drosophila melanogaster*. *Genetics*, **105**, 105-113.
- CAVENER, D. R. AND CLEGG, M. T. 1981. Multigenic response to ethanol in *Drosophila melanogaster*. *Evolution*, **35**, 1-10.
- DA LAGE, J. L., CAPY, P. AND DAVID, J. R. 1989. Starvation and desiccation tolerance in *Drosophila melanogaster* adults: effects of environmental temperature. *J. Insect Physiol.*, **35**, 453-457.
- DAY, T. H., HILLER, P. C. AND CLARKE, B. 1974. Properties of genetically polymorphic isozymes of alcohol dehydrogenase in *Drosophila melanogaster*. *Biochem. Genet.*, **11**, 141-153.
- GEER, B. W., HEINSTRAS, P. W. H., KAPOUN, A. M. AND VAN DER ZEL, A. 1990. Alcohol dehydrogenase and alcohol tolerance in *Drosophila melanogaster*. In: Barker, J. S. F. et al. (eds), *Ecological and Evolutionary Genetics of Drosophila*, Plenum Press, New York, pp. 231-252.
- GEER, B. W., MCKECHNIE, S. W. AND LANGEVIN, M. L. 1983. Regulation of *sn*-glycerol-3-phosphate dehydrogenase in *Drosophila melanogaster* larvae by dietary ethanol and sucrose. *J. Nutr.*, **113**, 1632-1642.
- GIBSON, J. 1970. Enzyme flexibility in *Drosophila melanogaster*. *Nature*, **227**, 959-960.
- HOFFMANN, A. A. AND PARSONS, P. A. 1989a. An integrated approach to environmental stress tolerance and life-history variation: desiccation tolerance in *Drosophila*. *Biol. J. Linn. Soc.*, **37**, 117-136.
- HOFFMANN, A. A. AND PARSONS, P. A. 1986b. Selection for increased desiccation resistance in *Drosophila melanogaster*: additive genetic control and correlated responses for other stresses. *Genetics*, **122**, 837-845.
- JOHNSON, F. M. AND POWELL, A. 1974. The alcohol dehydrogenases of *Drosophila melanogaster*: frequency changes associated with heat and cold shock. *Proc. Nat. Acad. Sci., U.S.A.*, **71**, 1783-1784.
- KERVER, J. W. M. AND VAN DELDEN, W. 1985. Development of tolerance to ethanol in relation to the alcohol dehydrogenase locus in *Drosophila melanogaster*. 1. Adult and egg-to-adult survival in relation to ADH activity. *Heredity*, **55**, 355-367.
- KOEHN, R. K. AND BAYNE, B. L. 1989. Towards a physiological and genetical understanding of the energetics of the stress response. *Biol. J. Linn. Soc.*, **37**, 157-171.
- LEVINS, R. 1969. Thermal acclimation and heat resistance in *Drosophila* species. *Am. Nat.*, **103**, 483-499.
- LISSEMORE, J. L., BAUMGARDNER, C. A., GEER, B. W. AND SULLIVAN, D. T. 1990. Effect of dietary carbohydrates and ethanol on expression of genes encoding *sn*-glycerol-3-phosphate dehydrogenase, aldolase, and phosphoglycerate kinase in *Drosophila* larvae. *Biochem. Genet.*, **28**, 615-630.
- MAYNARD SMITH, J. 1957. Temperature tolerance and acclimatization in *Drosophila subobscura*. *J. Exp. Biol.*, **34**, 85-96.
- MCELFRESH, K. C. AND MCDONALD, J. F. 1986. The effect of temperature on biochemical and molecular properties of *Drosophila* alcohol dehydrogenase. *Biochem. Genet.*, **24**, 873-889.
- MCKECHNIE, S. W. AND GEER, B. W. 1988. The epistasis of *Adh* and *Gpdh* allozymes and variation in the ethanol tolerance of *Drosophila melanogaster* larvae. *Genet. Res., Camb.*, **52**, 179-184.
- MILLER, S., PEARCY, R. W. AND BERGER, E. 1975. Polymorphism at the α -glycerophosphate dehydrogenase locus in *Drosophila melanogaster*. I. Properties of adult allozymes. *Biochem. Genet.*, **13**, 175-188.
- OAKESHOTT, J. G. 1976. Biochemical differences between alcohol dehydrogenases of *Drosophila melanogaster*. *Aust. J. Biol. Sci.*, **29**, 365-373.
- OAKESHOTT, J. G., GIBSON, J. B., ANDERSON, P. R., KNIBB, W. R., ANDERSON, D. G. AND CHAMBERS, G. K. 1982. Alcohol dehydrogenase and glycerol-3-phosphate dehydrogenase clines in *Drosophila melanogaster* on different continents. *Evolution*, **36**, 86-96.
- OAKESHOTT, J. G., WILSON, S. R. AND PARNELL, P. 1985. Selective effects of temperature on some enzyme polymorphisms in laboratory populations of *Drosophila melanogaster*. *Heredity*, **55**, 69-82.
- OUDMAN, L. 1991. A locus in *Drosophila melanogaster* affecting heat resistance. *Heredity*, **114**, 285-287.
- OUDMAN, L., KAMPING, A. AND VAN DELDEN, W. 1988. Body size and survival at high temperature in *Drosophila melanogaster*. *Dros. Inf. Serv.*, **67**, 63-64.
- OUDMAN, L., VAN DELDEN, W., KAMPING, A. AND BIJLSMA, R. 1991. Polymorphism at the *Adh* and *aGpdh* loci in *Drosophila melanogaster*: effects of rearing temperature on developmental rate, body weight, and some biochemical parameters. *Heredity*, **67**, 103-115.
- PARSONS, P. A. 1973. Genetics of resistance to environmental stresses in *Drosophila* populations. *Ann. Rev. Genet.*, **7**, 239-265.
- SAMPSELL, B. AND SIMS, S. 1982. Effect of *Adh* genotype and heat stress on alcohol tolerance in *Drosophila melanogaster*. *Nature*, **296**, 853-855.
- SCHENFIELD, E. AND MCKECHNIE, S. W. 1979. The α -glycerophosphate dehydrogenase (*aGpdh*) polymorphism in *Drosophila melanogaster*: adult survival under temperature stress. *Theor. Appl. Genet.*, **54**, 235-237.

- STEPHANOU, G. AND ALAHOTIS, S. N. 1986. Adaptive significance of the action of the *Drosophila melanogaster* alcohol dehydrogenase locus through the heat shock protein system. *Genetica*, **69**, 59–68.
- VAN DELDEN, W. 1982. The alcohol dehydrogenase polymorphism in *Drosophila melanogaster*. Selection at an enzyme locus. *Evol. Biol.*, **15**, 187–222.
- VAN DELDEN, W. AND KAMPING, A. 1980. The alcohol dehydrogenase polymorphism in populations of *Drosophila melanogaster*. IV. Survival at high temperature. *Genetica*, **51**, 179–185.
- VAN DELDEN, W. AND KAMPING, A. 1989. The association between the polymorphisms at the *Adh* and *αGpdh* loci and the *In(2L)t* inversion in *Drosophila melanogaster* in relation to temperature. *Evolution*, **43**, 775–793.
- VOSSEN, J. G. H. M., LEENDERS, H. J. AND KNOPPIEN, W. G. 1983. A change in the affinity of larval *α*-glycerophosphate dehydrogenase in relation to the activity of the heat shock genes of *Drosophila hydei*. *Insect Biochem.*, **13**, 349–359.
- ZERA, A. J., KOEHN, R. K. AND HALL, J. G. 1985. Allozymes and biological adaptation. In: Kerkut, G. A. and Gilbert, L. I. (eds), *Comprehensive Insect Physiology*, Vol. 10, Pergamon Press, New York, pp. 633–674.