4-1998

Experimental Manipulation of the Cost of Thermal Acclimation in Drosophila melanogaster

Robert A. Krebs
Cleveland State University, r.krebs@csuohio.edu

Martin E. Feder
University of Chicago

Publisher's Statement

This is the accepted version of the following article: Krebs RA, Feder ME. Experimental manipulation of the cost of thermal acclimation in drosophila melanogaster. Biol J Linn Soc 1998;63(4):593-601., which has been published in final form at http://onlinelibrary.wiley.com/doi/10.1111/j.1095-8312.1998.tb00331.x/abstract

Experimental manipulation of the cost of thermal acclimation in *Drosophila melanogaster*

ROBERT A. KREBS[1*] AND MARTIN E. FEDER[1,2]

[1]*Department of Organismal Biology and Anatomy,* [2]*The Committee on Evolutionary Biology, and the College, The University of Chicago, 1027 East 57th Street, Chicago, IL 60637, U.S.A.*

Acclimation to environmental change can impose costs on organisms. One potential cost is the energy and nutrients consumed by a physiological response, e.g. the resources required for expression of heat-shock proteins (Hsps). We examined the significance of this cost by genetic manipulation. We isolated four isofemale lines from a *Drosophila melanogaster* population previously transformed with a *hsp70-lacZ* fusion. Lines were similar in Hsp70 expression but differed in β-galactosidase expression upon heat shock, and replicates of each line were reared on a high quantity and low quantity medium. Multiple heat shock reduced survival in all lines, but did not increase developmental time. Variation in expression of β-galactosidase among lines, which differed more than 4-fold in response to heat treatment, was unrelated to the decreased survival. Thus the predicted effects of β-galactosidase expression on components of fitness were not evident. The superimposition of costs upon those normal for acclimation had no effect on mortality or developmental time, even when resources were especially limiting.

ADDITIONAL KEY WORDS:—genetics – heat shock – metabolism – stress – trade-offs.

CONTENTS

* Correspondence to: R. A. Krebs: Department of Biological, Geological and Environmental Sciences, Cleveland State University, 2399 Euclid Ave., Cleveland OH 44115-2403, U.S.A.
Email: rkrebs@biology.csuohio.edu

INTRODUCTION

Individuals of many species can change their phenotype when they encounter a novel thermal environment. Such change, termed thermal acclimation, is sometimes adaptive in that it can improve organismal performance in the novel thermal environment. A growing number of studies, however, report that thermal acclimation is neutral or deleterious with respect to fitness in some circumstances, i.e. acclimated individuals perform no better or even worse than non-acclimated individuals (Bennett & Lenski, 1997). One of several possible mechanistic explanations for such non-adaptive outcomes of thermal acclimation is that the expression of a new thermal phenotype (Feder, 1996) can consume too much energy or nutrients, monopolize the expression apparatus and related cellular pathways, and thereby jeopardize other cellular functions. For example, in expression of heat-shock proteins (Hsps), a near-universal response to high temperatures, Hsps can be virtually absent from the cell before heat stress and suddenly undergo 10 000-fold increases in expression (Lindquist, 1993). These proteins may accumulate to account for 10–15% of soluble protein in the cell (Loomis & Wheeler, 1982; Palter *et al.*, 1986), displacing routine protein synthesis. The negative consequences of this intense protein expression for performance and fitness can be profound: *Drosophila* cells that express Hsps at benign temperatures grow more slowly than normal cells (Feder *et al.*, 1992), a yeast strain that cannot express Hsp104 grows faster than its wild-type counterpart on some media (Sanchez *et al.*, 1992), fecundity declines in adult *Drosophila melanogaster* (Meigen) treated to induce the heat-shock response (Krebs & Loeschcke, 1994), and overexpression of Hsp70 reduces larva-to-adult survival of *D. melanogaster* (Krebs & Feder, 1997a). Heckathorn *et al.* (1996a) found that when nitrogen availability is limiting, other proteins are catabolized to provide amino acids for stress protein production. Consequently, loci such as the *hsp* genes face antagonistic selection, as costs of stress tolerance potentially cancel out benefits of acclimation (Calow, 1991; Coleman, Heckathorn & Hallberg, 1995; Hoffmann, 1995; Krebs & Loeschcke, 1996; Parsons, 1996).

These negative outcomes of thermal acclimation, while consistent with an excessive energetic cost of Hsp expression, are also consistent with other candidate mechanisms such as toxicity of Hsps at high concentration (Krebs & Feder, 1997a). Therefore, to examine the energetic cost explanation explicitly, we exploited *Drosophila* that had been genetically engineered with a *hsp70-lacZ* fusion. Even without this transgene, *Drosophila* larvae express Hsps massively in response to a mild heat shock, such as those that may occur in nature (Junge-Berberovic, 1996; Feder, 1997; Feder, Blair & Figueras, 1997). With the transgene, larvae also express β-galactosidase in response to mild heat shock. β-galactosidase expression typically does not benefit *Drosophila*, but its production consumes energy and amino acids. From a mass population of this strain, we founded four isofemale lines that express variable amounts of this innocuous protein as a response to heat. Thus these four lines are expected to differ primarily in their cost of thermal acclimation. Replicates of each line were reared with and without repeated induction of β-galactosidase expression, and at high and low resource levels (manipulated by diluting available food at equal volumes). If the resource cost of β-galactosidase expression is non-negligible to fitness, replicates reared with repeated induction of β-galactosidase expression should perform less well than lines without β-galactosidase expression, and this difference should be

more pronounced in replicates in the low resource treatment than in the high resource treatment.

Changes in performance were assayed by effects on survival and developmental time. Survival, we predicted, would decline where costs are extreme (Gebhardt & Stearns, 1988), while developmental time, which varies in response to minor changes in nutrient resources (Robertson, 1960; Bakker, 1961), would be a more sensitive assay of energetic costs.

MATERIAL AND METHODS

Origin of hsp70-lacZ *lines*

Simon & Lis (1987) constructed *Drosophila* lines that express bacterial β-galactosidase as a response to heat. In summary, the transformation vector, inserted within a P-element, contained wild-type *hsp70* sequence from −194 to the *lacZ* fusion point and has the *hsp70* poly(A)+ signal region downstream of *lacZ*. The fusion protein produced has only the first 7 amino acids of Hsp70 and a functional β-galactosidase with improved expression relative to original trials (Simon *et al.*, 1985).

Although Simon & Lis (1987) originally screened lines to eliminate those with atypical expression, new variation developed over the 10+ years of maintenance. After subculturing singly mated females from the original line, preliminary assays identified several isofemale lines with relatively high and low β-galactosidase expression, of which we chose four for further study.

Chemical and fitness assays

From the same group of parental flies from each line, we collected larvae to assay β-galactosidase activity, Hsp70 expression, survival to adult and developmental time. Flies oviposited on petri dishes containing a yeast-cornmeal-molasses-agar medium, from which larvae could easily be collected from the surface. The assays of β-galactosidase activity and Hsp70 expression each required 10–15 2nd-instar larvae per replicate. These larvae were transferred to microtubes with 10 µl phosphate buffered saline and treated either for 1 h at 36°C and 2 h at 25°C before assaying β-galactosidase activity, or for 1 h at 36°C and 1 h at 25°C for Hsp70 concentration. These treatments induce near-maximal levels of expression of each protein (Krebs & Feder, 1997b, unpublished data). Larvae were frozen in liquid nitrogen and stored at −80°C for subsequent biochemical analysis. To assay survival and developmental time, we transferred groups of 40 larvae to a series of vials containing either a high quantity resource (8 ml of undiluted yeast-cornmeal-molasses-agar medium) or a low quantity resource (8 ml of the same batch of medium diluted to 25% with 1% agar). Larvae in half the vials within each resource treatment group developed in a constant 25°C rearing environment (controls), and half received three acclimation induction treatments, 1 h at 36°C, in a thermostatted water bath, at 2, 24 and 48 h after collection. These treatment times correspond to the larval developmental stages, 1st-instar, early 2nd-instar and early 3rd-instar. When not undergoing heat treatment, all larvae developed within a large covered container at 25°C with moistened toweling to ensure high humidity.

To assay β-galactosidase activity, we first sonicated each sample of 10–15 larvae in reaction buffer (Simon & Lis, 1987) on ice and then centrifuged the lysate at 4°C for 30 min at 13 000 rpm. β-galactosidase activity of the supernatant was determined according to Simon & Lis (1987); reactions contained 6.125 µl supernatant and 1 mM chlorophenol red-β-D-galactopyranosidase in 200 µl volume reaction buffer, and were run in triplicate at 37°C. Absorbance of the coloured reaction product was measured at 405 nm with a microplate reader. The protein content of the supernatant was determined with the BCA assay (Pierce Biochemical), and results expressed as $mOD_{562} \cdot min^{-1} \cdot \mu g$ soluble $protein^{-1}$. Hsp70 expression was determined for 6 or 7 groups of 10–15 larvae per line by enzyme linked immunosorbent assay (ELISA), which has been described elsewhere (Welte *et al.*, 1993; Feder *et al.*, 1996; Krebs & Feder, 1997c). Results were expressed as a percent of a standard, expression in S2 *Drosophila* cells treated at 36.5°C for 1 h and 1 h at 25°C.

For each line, we determined the proportion of larvae that emerged as adults from each vial; data underwent arcsine square-root transformation before statistical analysis. We estimated the mean developmental time per vial by averaging that for males and females, each determined as $\log^{-1}$ of the log day-of-emergence per individual.

RESULTS

Lines varied in β-galactosidase activity after treatment at 36°C (Fig. 1A, $F_{3,16} = 22.4$, $P<0.001$), and each line differed from all others (Tukey's multiple comparisons test, $P<0.05$). By contrast, Hsp70 expression varied little (Fig. 1B, $F_{3,22} = 2.0$, NS). These lines also varied in larva-to-adult survival (Fig. 2A, $F_{3,112} = 3.2$, $P<0.05$) and in developmental time (Fig. 2B, $F_{3,104} = 3.2$, $P<0.05$). The low quantity medium reduced survival ($F_{1,112} = 63.2$, $P<0.001$) and lengthened larval development ($F_{1,104} = 235$, $P<0.001$).

The predicted effects of β-galactosidase expression on components of fitness were not evident. Repeated exposure to heat shock, with consequent induction of both Hsps and β-galactosidase, did not affect developmental time in a combined analysis of all lines ($F_{1,104} = 1.0$, NS). Only in line 2 did repeated induction of β-galactosidase expression affect development time. This effect was opposite in direction (decrease) to that expected if resources were limiting, occurred in both high and low quantity medium, and was in a line with intermediate β-galactosidase expression. Although repeated heat shock reduced larva-to-adult survival ($F_{1,112} = 13.8$, $P<0.001$), other findings were inconsistent with predictions: First, effects of repeated heat shock were greater in the high quantity medium than in the low quantity medium ($F_{1,112} = 6.5$, $P<0.05$), in which resource limitation should have been more problematic. Second, while lines varied in response to medium quantity ($F_{3,112} = 4.3$, $P<0.01$), the depression in larva-to-adult survivorship when grown on the low-quantity medium bore no consistent relationship to the β-galactosidase expression in the four lines. In fact, the line with by far the greatest β-galactosidase expression was intermediate to the other lines in this respect. Thus, despite producing very different levels of β-galactosidase, all lines responded to heat similarly in both traits (line × heat-treatment interactions; survival, $F_{3,112} = 0.1$, NS; developmental time, $F_{3,104} = 1.2$, NS) in the high and low quantity environments (line × heat treatment × medium quantity, survival, $F_{3,112} = 0.7$, NS; developmental time, $F_{3,104} = 0.4$, NS).

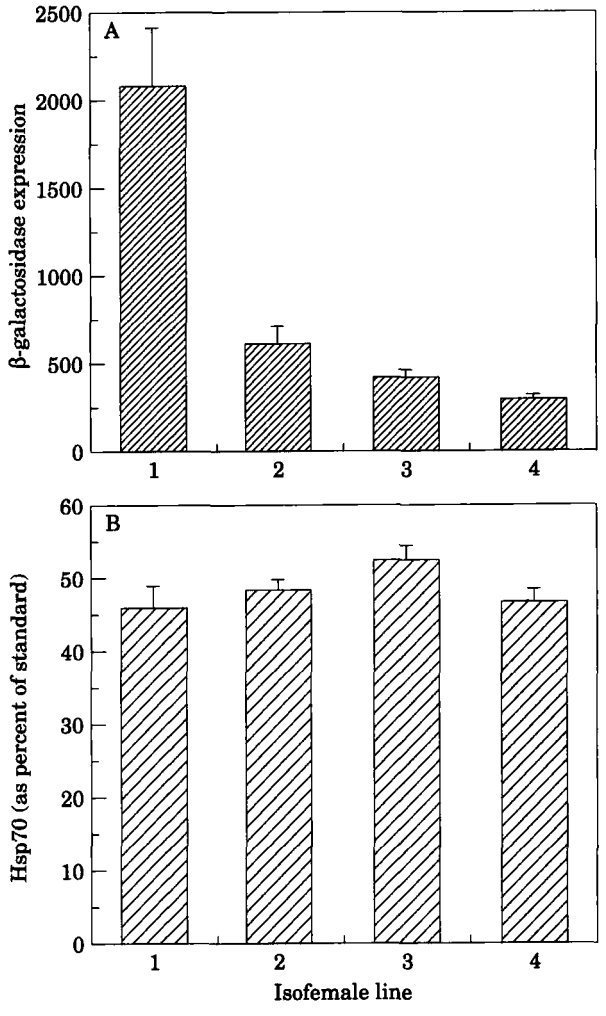

Figure 1. Mean concentration (±SE) of (A) β-galactosidase after 1 h at 36°C and 2 h at 25°C and (B) Hsp70 after 1 h at 36°C and 1 h at 25°C for 2nd-instar larvae of four isofemale lines. Protein concentrations are relative to mOD per μg soluble protein for β-galactosidase, or as a percent of the concentration of Hsp70 found for S2 *Drosophila* cells treated 1 h at 36.5°C and 1 h at 25°C.

DISCUSSION

Multiple heat treatment reduced survival in lines transformed with *hsp70-lacZ*, but differential expression of β-galactosidase cannot explain fitness variation among lines. β-galactosidase varied more than a factor of four among lines, but neither mortality nor developmental time differed substantially between the line with the highest expression and others. Although thermal stress causes many physiological changes (Huey & Bennett, 1990; Feder, 1996), short heat treatments did not lengthen developmental time of any line and, while mortality increased following heat treatment, the increase did not covary with β-galactosidase expression. Had conditions become limiting, developmental time should have varied (Bakker, 1969).

The fitness consequences of energy and nutrient reallocation depend on the

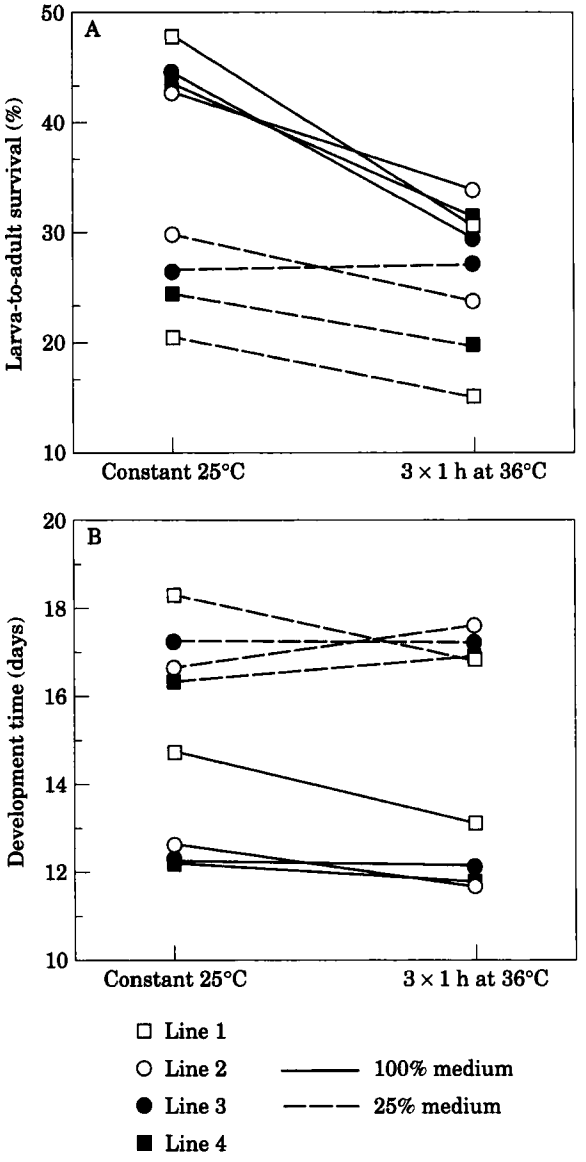

Figure 2. Mean survival and developmental time (±SE) for each of four isofemale lines either reared at constant 25°C, or at 25°C interspersed with three 1 h treatments at 36°C. Points are connected to indicate treatment responses for each line. Heat treatment induces both Hsp70 expression and expression of the inserted *hsp70-lacZ* transgene in these lines.

resource environment in which traits are measured. At times, *Drosophila* larvae live in an infinite pool of energy and nutrients. When orchard fruits are not maturing, *Drosophila* must migrate to natural refuges, which consequently may lead to virtually unlimited resources oscillating with severe larval competition on discrete and ephemeral breeding sites (Atkinson, 1979, 1985). Increasing competition or larval density decreases both the probability of any individual becoming an adult, lengthens developmental time and affects body size and other traits in those who are successful

(Barker, 1983). As the environment declines, genetic parameters responsible for variation in and covariation among these traits may also change (Gebhardt & Stearns, 1989). In our experiments, the low resource treatment effectively reduced survival and increased time of development, which is consistent with resource limitation, and high temperatures or other stresses should increase demands further (Koehn, 1991).

Koehn & Bayne (1989) and Hawkins (1991) suggest that any increase in energy demands, however small, can be a problem. With respect to heat shock, which induces rapid synthesis of Hsps, Coleman and colleagues view allocation of organismal resources as a conflict primarily between Hsp expression and other protein synthesis (Heckathorn *et al.*, 1996b). Indeed, they find that some proteins decline after heat shock and that this loss increases proportionally under conditions of low nitrogen, which Heckathorn *et al.* (1996a) interpret as a potential reallocation of amino acids for Hsp production. Free amino acid pools therefore may be insufficient for rapid production of new Hsps during the heat-shock response, but whether fitness similarly declines after this reallocation of resources is unknown. In our experiments, superimposing additional costs upon those normal for acclimation had no effect on mortality or developmental time, even when resources were especially limiting. A possible explanation for this outcome is that neither Hsp production, which can have massive costs, nor the additional expression of β-galactosidase exhausted supplies of energy and nutrients. Even inducing these proteins three times failed to increase developmental time in any of the lines.

Energy and nutrient storage may buffer the acute resource demands of thermal acclimation even for larvae on a less concentrated medium. When less food is available, slower larval development may enable similar levels of nutrient storage, as also suggested by the lack of energy limitation in growth despite seasonal variation in resource uptake in mussels (Widdows & Hawkins, 1989; Kreeger *et al.*, 1995). During heat shock in *Drosophila*, a coordinate depression in synthesis of proteins other than Hsps (Solomon *et al.*, 1991) also may spare otherwise limiting stores of amino acids for Hsp expression. Both assembly of energy stores and the consumption of resources in excess of immediate needs can themselves be costly, but these costs may be paid over an entire lifetime rather than acutely. Lengthening the time over which energetic costs are paid may reduce effects on fitness. This explanation also may apply to the recovery in growth after heat shock by larvae that over-express Hsp70 relative to those that express normal levels (Krebs & Feder, 1997a).

The only 'positive' outcome of repeated pretreatment, reduced survival, is equally well consistent with toxicity of the changes associated with thermotolerance. β-galactosidase was one of many proteins produced during heat shock of the lines, and effects could be due either to all the aggregate changes or to one in particular. Hsp70, the protein most highly induced by heat, increased similarly in all four lines. High concentrations of this protein and other family members are sufficient to raise mortality (Feder *et al.*, 1992; Krebs & Feder, 1997a), inhibit protein secretion (Dorner, Krane & Kaufman, 1988, 1992), and *in vitro*, promote protein aggregation (M. Borrelli and J. Lepock, pers. comm.). Nonetheless, raising Hsp70 levels can improve thermotolerance (Feder *et al.*, 1996) although fitness benefits after stress may trade-off with costs in its absence (Krebs & Feder, 1997c). Our results add to the growing work on the evolutionary consequences of variation in Hsp70 and inducible thermotolerance by providing additional evidence that the heat-shock response has costs to fitness as well as benefits. Moreover, at least in the present

study, limited availability of either energy or nutrients are at most a minor contributor to these costs.

ACKNOWLEDGEMENTS

We thank Yoaldena Canela and John Kim for assistance collecting data, and Jim Coleman and Scott Heckathorn for comments on the manuscript. Research was supported by National Science Foundation grants IBN94-08216 and BIR94-19545, and the Louis Block Fund of the University of Chicago.

REFERENCES

Atkinson WD. 1979. A field investigation of larval competition in domestic *Drosophila*. *Journal of Animal Ecology* **48:** 91–102.

Atkinson WD. 1985. Coexistence of Australian rainforest Diptera breeding in fallen fruit. *Journal of Animal Ecology* **54:** 507–518.

Bakker K. 1961. An analysis of factors which determine success in competition for food among larvae of *Drosophila melanogaster*. *Archives Néerlandaises de Zoologie* **14:** 200–281.

Bakker K. 1969. Selection for rate of growth and its influence on competitive ability of larvae of *Drosophila melanogaster*. *Netherlands Journal of Zoology* **19:** 541–595.

Barker JSF. 1983. Interspecific competition. In: M. Ashburner M, Carson HL, Thompson JN eds. *The Genetics and Biology of* Drosophila, vol 3c. London: Academic Press, 285–341.

Bennett AF, Lenski RE. 1997. Evolutionary adaptation to temperature. VI. Phenotypic acclimation and its evolution in *Escherichia coli*. *Evolution* **51:** 36–44.

Calow P. 1991. Physiological costs of combating chemical toxicants: ecological implications. *Comparative and Biochemical Physiology* **100C:** 3–6.

Coleman JS, Heckathorn SA, Hallberg RL. 1995. Heat-shock proteins and thermotolerance: linking ecological and molecular perspectives. *Trends in Ecology and Evolution* **10:** 305–306.

Dorner AJ, Krane MG, Kaufman RJ. 1988. Reduction of endogenous GRP78 levels improves secretion of a heterologous protein in CHO cells. *Molecular and Cell Biology* **8:** 4063–4070.

Dorner AJ, Wasley LC, Kaufman RJ. 1992. Overexpression of GRP78 mitigates stress induction of glucose regulated proteins and blocks secretion of selective proteins in Chinese hamster ovary cells. *European Molecular Biology Organization Journal* **11:** 1563–71.

Feder JH, Rossi JM, Solomon J, Solomon N, Lindquist S. 1992. The consequences of expressing hsp70 in *Drosophila* cells at normal temperatures. *Genes & Development* **6:** 1402–1413.

Feder ME. 1996. Ecological and evolutionary physiology of stress proteins and the stress response: the *Drosophila melanogaster* model. In: Johnston IA, Bennett AF, eds. *Phenotypic and Evolutionary Adaptation to Temperature*. Cambridge: Cambridge University Press, 79–102.

Feder ME. 1997. Necrotic fruit: a novel model system for thermal ecologists. *Journal of Thermal Biology* **22:** 1–9.

Feder ME, Cartaño NV, Milos L, Krebs RA, Lindquist SL. 1996. Effect of engineering *Hsp70* copy number on Hsp70 expression and tolerance of ecologically relevant heat shock in larvae and pupae of *Drosophila melanogaster*. *Journal of Experimental Biology* **199:** 1837–1844.

Feder ME, Blair N, Figueras H. 1997. Natural thermal stress and heat-shock protein expression in *Drosophila* larvae and pupae. *Functional Ecology* **11:** 90–100.

Gebhardt MD, Stearns SC. 1988. Reaction norms for developmental time and weight at eclosion in *Drosophila mercatorum*. *Journal of Evolutionary Biology* **1:** 335–354.

Hawkins AJS. 1991. Protein turnover: a functional appraisal. *Functional Ecology* **5:** 222–233.

Heckathorn SA, Poeller GJ, Coleman JS, Hallberg RL. 1996a. Nitrogen availability and vegetative development influence the response of ribulose 1,5-bisphosphate carboxylase/oxygenase, phosphoenolpyruvate carboxylase, and heat-shock protein content to heat stress in *Zea mays* L. *International Journal of Plant Science* **157:** 588–595.

Heckathorn SA, Poeller GJ, Coleman JS, Hallberg RL. 1996b. Nitrogen availability alters patterns of accumulation of heat stress-induced proteins in plants. *Oecologia* **105:** 413–418.

Hoffmann AA. 1995. Acclimation: increasing survival at a cost. *Trends in Ecology and Evolution* **10:** 1–2.
Huey RB, Bennett AF. 1990. Physiological adjustments to fluctuating thermal environments: an ecological and evolutionary perspective. In: Morimoto RI, Tissieres A, Geogopoulos C, eds. *Stress Proteins in Biology and Medicine.* Cold Spring Harbor: Cold Spring Harbor Laboratory Press, 37–59.
Junge-Berberovic R. 1996. Effect of thermal environment on life histories of free living *Drosophila melanogaster* and *D. subobscura. Oecologia* **108:** 262–272.
Koehn RK. 1991. The cost of enzyme synthesis in the genetics of energy balance and physiological performance. *Biological Journal of the Linnean Society* **44:** 231–247.
Koehn RK, Bayne BL. 1989. Towards a physiological and genetical understanding of the energetics of the stress response. *Biological Journal of the Linnean Society* **37:** 157–171.
Krebs RA, Feder ME. 1997a. Deleterious consequences of Hsp70 overexpression in *Drosophila melanogaster* larvae. *Cell Stress & Chaperones* **2:** 60–71.
Krebs RA, Feder ME. 1997b. Tissue specific variation in Hsp70 expression and thermal damage in *Drosophila melanogaster* larvae. *Journal of Experimental Biology* **200:** 2007–2015.
Krebs RA, Feder ME. 1997c. Natural variation in the expression of the heat-shock protein Hsp70 in a population of *Drosophila melanogaster*, and its correlation with tolerance of ecologically relevant thermal stress. *Evolution* **51:** 173–179.
Krebs RA, Loeschcke V. 1994. Costs and benefits of activation of the heat-shock response in *Drosophila melanogaster. Functional Ecology* **8:** 730–737.
Krebs RA, Loeschcke V. 1996. Acclimation and selection for increased resistance to thermal stress in *Drosophila buzzatii. Genetics* **142:** 471–479.
Kreeger DA, Hawkins AJS, Bayne BL, Lowe DM. 1995. Seasonal variation in the utilization of dietary protein for energy and biosynthesis by the mussel *Mytilus edulis. Marine Ecology Progress Series* **126:** 177–184.
Lindquist S. 1993. Autoregulation of the heat-shock response. In: Ilan J, ed. *Translational Regulation of Gene Expression 2*, New York: Plenum Press, 279–320.
Loomis WF, Wheeler SA. 1982. Chromatin-associated heat shock proteins of Dictyostelium. *Developmental Biology* **90:** 412–418.
Palter KB, Watanabe M, Stinson L, Mahowald AP, Craig EA. 1986. Expression and localization of *Drosophila melanogaster* hsp70 cognate proteins. *Molecular and Cellular Biology* **6:** 1187–1203.
Parsons PA. 1996. Stress, resources, energy balances, and evolutionary change. *Evolutionary Biology* **29:** 39–72.
Robertson FW. 1960. The ecological genetics of growth in *Drosophila.* 1. Body size and developmental time on different diets. *Genetical Research* **1:** 288–304.
Sanchez Y, Taulien J, Borkovich KA, Lindquist S. 1992. Hsp104 is required for tolerance to many forms of stress. *European Molecular Biology Organization Journal* **11:** 2357–2364.
Simon JA, Lis JT. 1987. A germline transformation analysis reveals flexibility in the organization of heat shock consensus elements. *Nucleic Acids Research* **15:** 2971–2988.
Simon JA, Sutton CA, Lobell RB, Glaser RL, Lis JT. 1985. Determinants of heat shock-induced chromosome puffing. *Cell* **40:** 805–817.
Solomon JM, Rossi JM, Golic K, McGarry T, Lindquist S. 1991. Changes in Hsp70 alter thermotolerance and heat-shock regulation in *Drosophila. The New Biologist* **3:** 1106–1120.
Welte MA, Tetrault JM, Dellavalle RP, Lindquist SL. 1993. A new method for manipulating transgenes: engineering heat tolerance in a complex, multicellular organism. *Current Biology* **3:** 842–853.
Widdows J, Hawkins AJS. 1989. Partitioning of rate of heat dissipation by *Mytilus edulis* into maintenance, feeding, and growth components. *Physiological Zoology* **62:** 764–784.