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# Natural and Genetic Engineering of the Heat-Shock Protein Hsp70 in *Drosophila melanogaster:* Consequences for Thermotolerance[1]

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SYNOPSIS. Larvae of the fruit fly, *Drosophila melanogaster,* live within necrotic fruit, a challenging environment in which larvae can experience severe thermal stress. One response to thermal stress, the expression of heat-shock proteins (Hsps), has evolved distinctively in this species; the gene encoding Hsp70 has undergone extensive duplication and accounts for the bulk of Hsps that are expressed upon heat shock. Genetic engineering of *hsp70* copy number is sufficient to affect thermotolerance at some (but not all) life stages. Increases in Hsp70, moreover, can protect intact larvae against thermal inactivation of the enzyme alcohol dehydrogenase and thermal inhibition of feeding. Deleterious consequences of high levels of Hsp70, however, may limit further evolutionary proliferation of *hsp70* genes. These findings illustrate how the perspectives of integrative and comparative biology, if applied to even well-studied model organisms, can lead to novel findings.

## INTRODUCTION

Carl Gans (1978) entitled his presidential address to the American Society of Zoologists "All animals are interesting!" In this address, he decried the tendency of some biological scientists to focus on popular model organisms (*e.g., Drosophila melanogaster, Escherichia coli, Caenorhabditis elegans, Saccharomyces cerevisiae,* mammalian cells in culture, and so on) and to eschew the insights that might be forthcoming from biological diversity in general and exotic species in particular. Today, many members of the Society of Integrative and Comparative Biologists, successor to the American Society of Zoologists and the publisher of *American Zoologist,* perpetuate the outlook symbolized by Gans's title and have accordingly engendered enormous scientific progress. In so doing, however, many have tended to regard the popular model organisms as mundane, not particularly interesting, or not "real" organisms. My intent here is to emphasize by example

the first word in Gans's title. The features that have made the favored model organisms popular are evolutionary solutions to significant environmental problems; these problems and solutions are every bit as interesting as those of more exotic species and deserve the same respect.

Among the most maligned of the standard model organisms is *Drosophila melanogaster,* the fruit fly. Kohler (1994) has recounted how generations of laboratory culture have stripped away many *Drosophila* traits that inconvenience laboratory experimentation. Nonetheless, *Drosophila* flourish outside the laboratory, where it is a human commensal and exploits necrotic fruit in the wild (Ashburner, 1989). The particular challenges of the necrotic fruit habitat may be responsible for some of the distinctive features of *Drosophila.* Several stages of the life cycle, (eggs, embryos, three larval instars, and perhaps pupae) obligately occur on or within necrotic fruit. Necrotic fruit is a rich but ephemeral food source for many organisms. Unlike "true" fruit flies (*Rhagoletis*), many other insects, and many non-insect frugivores, however, *Drosophila* are unable to breach the intact epidermis of necrotic fruit. *Drosophila* thrives when fruit necrosis is advanced. These characteristics of *Drosophila* limit

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their exploitation of any given fruit to the brief time from when other frugivores or mechanical injury breach a fruit's epidermis to when desiccation, dissolution, or consumption by frugivores exhaust the edible fruit. The emphemerality of this habitat may have led to the unusually rapid life cycle of *Drosophila*. Embryogenesis occurs at the physical limit of replication fork formation and movement (Karr and Mittenthal, 1992), and the time from oviposition to pupation is brief, 5–6 days at 25°C. The larvae themselves live in a semi-fluid with high concentrations of alcohols, ketones, and other organic substances, which can be nutritive at low concentration but toxic at high concentrations (McKenzie and Mc-Kechnie, 1979; McKechnie and Morgan, 1982). Biochemically, larvae deploy a suite of mechanisms for detoxifying ethanol and utilizing its breakdown products (Geer *et al.*, 1993). Necrotic fruit also supports a rich microbial flora, which can be a significant source of protein to the growing larva (Begon, 1983). When the fruit is sufficiently fluid, the larvae respire intermittently; they remain within the fluid but periodically project a pair of ventral spiracles into the atmosphere to breathe. Eggs, which are deposited on the surface of the necrosis, have a pair of projections that keep the egg from drowning; the projections themselves are especially extensive in *Drosophila* species that typically lay on more fluid media. Although these and many other features of *Drosophila* have come to light as by-products of genetic and/or molecular investigations, they are "interesting" (Gans, 1978) in their own right and ripe for future study.

## THE THERMAL ECOLOGY OF NON-ADULT DROSOPHILA MELANOGASTER

While adult *Drosophila* are minuscule, they can travel many kilometers each day (Coyne *et al.*, 1982). With both such locomotor abilities and small size, adult *Drosophila* may prospectively seek out equable microhabitats in the most hostile of environments. Whether adult *Drosophila* routinely avoid temperature stress is unknown, for current technology does not permit the long-term monitoring of body temperatures of unrestrained adult *Drosophila* in the wild. Physiological experimentation (Huey *et al.*, 1992) suggests that sustained flight is not feasible at temperatures much above 35°C (and possibly even at much lower temperatures), which ought to restrict activity at certain times of day and year.

In contrast to the adults, eggs and all but very late larvae are restricted to necrotic fruit. Pupae have not been studied systematically in the wild, although they sometimes occur on the fruit or form some distance away in soil (Sokolowski, 1985), prospectively in relation to thermal and hydric conditions immediately before pupation. When on or in necrotic fruit, non-adult *Drosophila* have negligible thermal inertia and are thus subject to the prevailing temperatures of the host fruit. This dynamic can and does impose massive high temperature stress on *Drosophila* living within or on sunlit necrotic fruit (Feder, 1996; Feder, 1997; Feder *et al.*, 1997a; Feder *et al.*, 1997b; Feder and Krebs, 1997):

- On summer days, necrotic fruit can heat to >36° after 60–90 minutes of insolation. Temperatures >40° are not uncommon.
- Fruit color, mass, and water loss affect heating kinetics but are not sufficient to mitigate temperatures harmful to *Drosophila*.
- Temperatures within a fruit are not sufficiently diverse for larvae to thermoregulate behaviorally. Accordingly, larvae cannot entirely avoid high temperatures that may be present.
- Ovipositing females do not avoid fruit unless it is warm at the time of oviposition, suggesting that at many times females oviposit on fruit that may overheat.
- *Drosophila* larvae and pupae actually infest necrotic fruit that experiences high temperatures on summer days, as is evident from acute measurements of larvae and pupae in situ in an orchard in Indiana (Fig. 1).

Such high temperatures can harm non-adult *Drosophila* (David *et al.*, 1983; Ashburner, 1989; Feder and Krebs, 1997), as the discovery of dead larvae in the field (Fig. 1) and the effects of temperature on physiological function in the species (Fig.
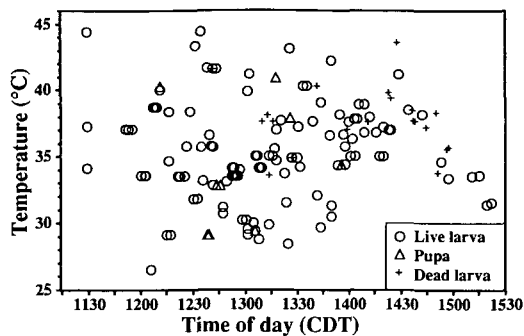
FIG. 1. Temperatures of *Drosophila* larvae and pupae in necrotic apples in an orchard in LaPorte, Indiana, on 27 August and 12–13 September 1994 (Feder *et al.*, 1997a). Apples were not disturbed by investigators before temperature measurements were made.



FIG. 2. Effect of temperature on traits related to fitness in *Drosophila* melanogaster (Feder and Krebs, 1997). Data are from Parsons (1978), David *et al.* (1983), Ashburner (1989), Huey *et al.* (1992), Krebs and Loeschcke (1994), and Feder *et al.* (1997a).

2) suggest. Growth, reproduction, and other physiological processes that underlie fitness occur most rapidly at approximately 27°C or slightly below; at higher temperatures, these functions deteriorate in direct proportion to the excess in temperature. 30°C is an ecological upper limit; unless *Drosophila* can be at temperatures <30°C for some time, their populations cannot persist in nature (Parsons, 1978). Temperatures >30°C compromise reproduction, and temperatures >37–38°C can kill quickly.

These features place *Drosophila* in elite company with respect to natural high temperature stress in animals. Most other animals either exploit environments without high temperature stress, can tolerate the full range of temperatures they experience, and/or can regulate their body temperatures (or at least avoid extreme temperatures) physiologically or behaviorally (Huey, 1991). Only rarely do animals occur in hyperthermic microhabitats from which they have no escape; other examples include aquatic organisms in natural or artificial thermal effluents, sessile animals in the intertidal, and parasites in febrile hosts. In these circumstances, mechanisms that enhance thermotolerance can be at a premium.

THE HEAT-SHOCK RESPONSE AND HEAT-SHOCK PROTEINS IN *DROSOPHILA*

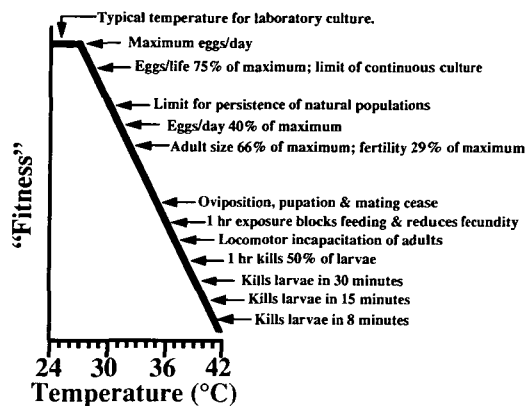Many mechanisms enable organisms to cope with obligate hyperthermia. A principal challenge in investigating any one of these mechanisms is to distinguish its effect from all others that may be acting (Feder, 1996). We chose to examine the heat-shock protein Hsp70 because molecular and genetic tools can make this distinction. Nonetheless, in keeping with Gans' dictum, we emphasize here the interesting nature of the heat-shock response in *Drosophila* and its underlying genetic mechanisms.

In response to heat and other stresses, nearly all organisms express heat-shock proteins (Hsps), highly-conserved proteins that contribute to stress tolerance by functioning as molecular chaperones (Feder *et al.*, 1995). Hsp-inducing stresses typically disrupt either the native conformation of cellular proteins or the mechanisms that ordinarily maintain these conformations (Parsell and Lindquist, 1993). In non-native conformations, proteins expose residues that ordinarily are internal, often those with hydrophobic side-groups; such exposed residues can become nuclei for inappropriate aggregation of non-native proteins. The result of this aggregation is at best diminution of the pool of functional protein and at worst outright toxicity. In their role as molecular chaperones, heat-shock proteins are able to recognize and bind non-native proteins at their exposed sites, thereby shielding the bound sites from inappropriate interactions with other sites, and to release the bound proteins in controlled fashion, there-

by allowing the proteins to refold to their native conformation or be targeted for degradation (Hartl, 1996). Hsps play similar roles even in the absence of stress. For example, Hsps accompany growing peptide strands to prevent inappropriate folding before peptide synthesis is complete, prevent peptides destined for mitochondria from folding before they can be imported, and inactivate steroid receptors by binding to them (Feder et al., 1995).

The principal families of Hsps are distinguished by the size of their monomers: 60, 70, 90, and 100-kD, and small Hsps. In eukaryotes, some families comprise several cell-compartment-specific members (e.g., distinct mitochondrial, endoplasmic reticulum, and nuclear-cytoplasmic Hsp70s). Some Hsps are expressed constitutively; others increase expression with stress or are expressed only during or after stress (Morimoto et al., 1994). The Hsp100 and Hsp70 families are especially well-established as contributing to cellular and/or organismal thermotolerance (Parsell and Lindquist, 1993). Most eukaryotes that have been examined in this regard express members of both families, and the two families can partially complement one another in yeast (Sanchez et al., 1993).

Drosophila melanogaster is distinctive with respect to both Hsp families. This species is not known to express an Hsp100 family member, although a yeast Hsp104 cDNA probe hybridizes to two differently-sized Drosophila mRNAs at low stringency (Sanchez and Lindquist, 1990; Feder and Lindquist, 1992). Perhaps in relation to the absence of an Hsp100, the Hsp70 system has undergone extensive specialization in Drosophila. The gene for the inducible cytoplasmic-nuclear Hsp70 family member, Hsp70 itself, has undergone extensive duplication during evolution (Fig. 3). Unlike most other Hsp70 family members and many other Hsps, which single genes encode, at least 5 nearly identical genes at two chromosomal loci (87A and 87C) encode Hsp70 in the haploid genome (Leigh-Brown and Ish-Horowicz, 1981). Many aspects of this system are specialized for intense expression upon heat shock (Lindquist, 1993). The genome has undergone

massive polytenization in most larval cells. The RNA polymerase is constitutively engaged to the promoter region, eliminating the time required for transcriptional engagement upon heat shock. Whereas many mRNAs become unstable at high temperatures, hsp70's 5' untranslated region stabilizes its mRNA at high temperatures. The hsp70 gene lacks introns, which eliminates temperature-sensitive and time-consuming splicing. Thus, within an hour of the start of heat shock, Hsp70 can go from being absent in Drosophila cells to accounting for 1% of total cellular protein—one of the most rapid and intense instances of gene expression (Lindquist, 1993). Drosophila larvae exhibit similar rates of Hsp70 expression in necrotic fruit in orchards in response to natural thermal stress (Fig. 4).

Biochemical and structural studies of Drosophila Hsp70 are few, but the extraordinarily high conservation of Hsp70 primary sequence among eukaryotes suggests that Drosophila Hsp70 will share many features of Hsp70 family members of other species, whose biochemistry has been studied in detail (Morimoto et al., 1994). Hsp70s have three domains: At the N-terminus is a nucleotide-binding domain, whose structure resembles that of the unrelated protein actin and whose association with and hydrolysis of adenine nucleotides regulates the binding and release of non-native proteins. Next is the non-native protein binding domain itself, whose structure is only now becoming characterized. At the C-terminus is a regulatory domain. The activities of Hsp70s clearly culminate in beneficial effects at the level of the intact cell and organ. Overexpression and introduction of exogenous Hsp70 improves the thermotolerance of various mammalian cell types in culture, protects cells against ultraviolet radiation, protects whole mammalian hearts against post-ischemic trauma, and improves the inducible thermotolerance of Drosophila cells in culture (reviewed in Feder and Krebs, 1997). Introduction of anti-Hsp70 antibodies disrupts transcription and the development of tolerance to ischemia and heat (reviewed in Feder and Krebs, 1997).

## hsp70genome    Distribution

Groups other than *melanogaster*
(e.g., *willistoni* and *obscura*).

Various; often limited
geographically or
ecologically

*montium* group
(e.g., *vulcana* and *auraria*)

Various; often limited
geographically or
ecologically

*orena*    ?????????????    1 small population

*erecta*    ?????????????    Tropical Africa;
*Pandanus* fruit

*teissieri*    Tropical Africa;
forest specialist

*yakuba*    African savanna;
generalist

*mauritiana*    Island endemic;
generalist

*sechellia*    ?????????????    Island;*Morinda* fruit

*simulans*    Cosmopolitan

87A7    87C1    Cosmopolitan
*melanogaster*

≥1 COPIES INSERTED HERE

*melanogaster* sub-group
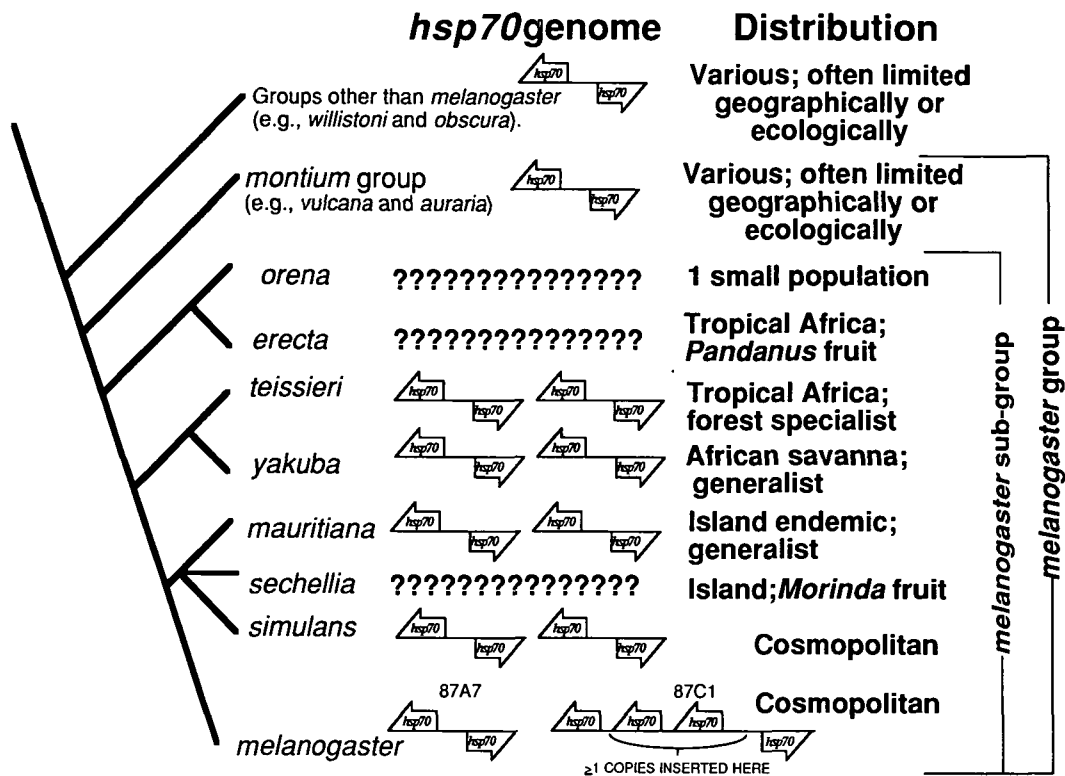
*melanogaster* group

FIG. 3.   Size and organization of the *hsp70* genome in relation to phylogeny and distribution of *Drosophila* (unpublished analysis of B. Bettencourt). *hsp70* genes occur as tandem repeats of two or more copies at one or two chromosomal loci; intervening sequence separates genes at each locus (Peters *et al.*, 1980; Leigh-Brown and Ish-Horowicz, 1981; Molto *et al.*, 1987; Bonorino *et al.*, 1993; Molto *et al.*, 1993; Molto *et al.*, 1994; Pardali *et al.*, 1996). The phylogeny is that of Lachaise *et al.* (1988) as modified by Russo *et al.* (1995). Information on distribution and ecology is from Lachaise *et al.* (1988).

## TESTING FITNESS CONSEQUENCES OF HSP70

While *Drosophila melanogaster* is interesting in its own right, it is especially exciting because its genetic and molecular tractability (Goldstein and Fyrberg, 1994) permit experimentation that is presently impossible in most other complex eukaryotes. The present issue is: Given the number and diversity of traits that underlie organismal thermotolerance, how significant is Hsp70 or any other single trait? Answering this question through standard comparative biology is exceedingly problematic because traits co-vary (Feder, 1996; Feder and Krebs, 1997). A thermotolerant organism, for example, can sometimes be shown to express more Hsp70 than a less tolerant organism, but demonstrating that Hsp70 per se and not some other traits is responsible

for the enhanced thermotolerance may well be impossible in a comparison of natural species, populations, individuals, or higher taxa. What is needed is a means of manipulating a single trait or traits experimentally against an otherwise constant background.

Genetic and molecular modifications are such means. One exemplary technique exploits a mobile genetic element of *Drosophila*, the P element. In the wild-type genome, regions of DNA flanked by P elements can "jump" from place to place. By flanking a gene of interest with P elements through standard techniques of molecular cloning and by injecting the resultant construct into a developing egg, the gene can be introduced into the germ-line of *Drosophila* (Cooley *et al.*, 1988; Goldstein and Fyrberg, 1994). Susan Lindquist and col-
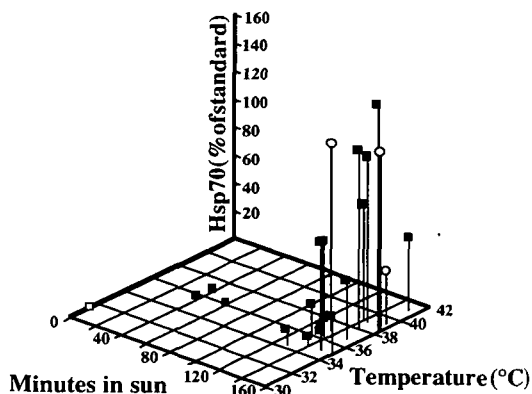
FIG. 4. Body temperatures and levels of Hsp70 in *Drosophila* larvae within necrotic apples undergoing experimental insolation (Feder *et al.*, 1997*a*). Apples previously undisturbed by the investigators were transferred from deep shade to sunlit grass. At various times afterwards, temperatures of indwelling larvae were determined and larvae were frozen in liquid nitrogen for subsequent determination of Hsp70 levels. Solid squares: living larvae; open circles: dead larvae. An Hsp70 level of 100 corresponds to that of lysates of *Drosophila melanogaster* Schneider 2 cells given a 60 min heat shock at 36.5°C and 60 min recovery at 25°C before lysis.

leagues have modified this technique to create allellic series in which pairs of strains share a site of transgene integration but differ in the number of transgene copies (Welte *et al.*, 1993). Thus, they created pairs of "extra copy" strains, with 12 transgenic copies of *hsp70*, and corresponding "excision" strains with only the 10 wild-type copies. The identical site of transgene integration in each pair of strains controls for positional or insertional mutagenesis, in which the insertion of the transgene into the chromosome may be chance disrupt another gene. Of course, members of each extra copy and excision pair also differ in the size of the transgene insert, which can yield a phenotype by differentially isolating two interacting genetic elements (Stearns and Kaiser, 1996). The likelihood of two independent transformations yielding the same phenotype as a sole function of insert size, however, is negligibly small; demonstrating similar phenotypes in at least two pairs of extra copy and excision strains effectively excludes insert size as a contributory variable (Welte *et al.*, 1993). The end result, then, are pairs of *Drosophila* strains that

differ only in the number of *hsp70* gene copies. These strains differ correspondingly in the rate and magnitude of Hsp70 expression (Fig. 5B); do they likewise differ in thermotolerance?

Investigators of the heat-shock response have typically used experimental regimes involving a defined exposure to a relatively severe heat shock, either with or without pretreatment; *i.e.*, prior exposure to a mild, Hsp-inducing heat shock. Basal or constitutive thermotolerance, evident in the absence of pretreatment, may reflect thermotolerance mechanisms that do not require induction (but see Krebs *et al.* (1996)), whereas inducible thermotolerance, evident only after pretreatment, reflects thermotolerance due to Hsps and other rapidly-induced mechanisms. Typical experiments might involve pretreatment regimes of less than 2 hours, and thus resemble heat hardening more closely than acclimation in the parlance of traditional ecophysiology. The following experiments used 36° as a pretreatment temperature and 38.5–41°C as heat-shock temperatures. *Drosophila* express Hsp70 most strongly during or after exposure to 36° (Krebs and Feder, 1997*c*), usually tolerate 36° pretreatment well, and naturally encounter these (and more extreme) temperatures in necrotic fruit (Feder *et al.*, 1997*a*). Welte *et al.* (1993) reported that pretreatment dramatically improved the thermotolerance of both extra-copy and excision embryos, but much more so in extra-copy embryos. The enhanced thermotolerance in the extra-copy strain was evident between 6 and 12 hr of embryonic development, a time after cellularization and during gross morphogenesis and maturation. Embryos of this age, however, are not known to undergo thermal stress in the wild, as are larvae and pupae. Feder *et al.* (1996) reported that in wandering 3rd-instar larvae, Hsp70 levels differ most greatly between extra-copy and excision strains at 1 hr after pretreatment (Fig. 5B). At this time, the thermotolerance of excision larvae had increased to approximately 150% of control levels, whereas the thermotolerance of extra-copy larvae increased to approximately 350% (Fig. 5C). Similarly, Feder *et al.* (1996) examined the eclosion of adults
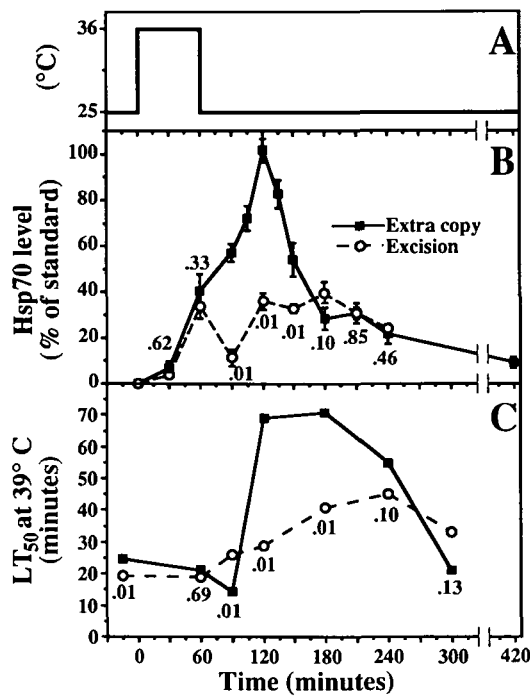
FIG. 5. The relationship between Hsp70 levels and thermotolerance in wandering phase 3rd-instar larvae of the extra-copy and excision strains (Feder et al., 1996). A. Temperature during the experiment. B. Hsp70 concentrations. Data are plotted as in Figure 1D. C. Thermotolerance. At each time point, larvae were transferred to 39° and the $LT_{50}$ was determined. The number underneath each pair of values is the $P$ value resulting from a logrank test comparing the survival times of extra-copy and excision larvae. Each point represents 13–18 larvae.

from pupae that were heat shocked after pupation; pretreatment enormously increased eclosion success, and more so in the extra-copy strain.

Collectively, these results demonstrate that experimental manipulation of the copy number of *hsp70* is sufficient to affect inducible thermotolerance, and suggest that Hsp70 can be limiting for thermotolerance. While these findings corroborate considerable earlier work with *Drosophila* cells in culture (see above), they materially extend the earlier work by demonstrating that such effects are manifest even against the background of all the other thermotolerance mechanisms that complex multicellular eukaryotes deploy. Can the results also establish that variation in Hsp70 is consequential

for fitness in the wild? The experimental temperature regimes are similar to natural ones, and the results are readily extrapolatable to differential survival of natural thermal stress. More ecologically realistic pretreatment regimes, involving gradually increasing temperatures rather than abrupt changes, do not materially affect rates of Hsp70 expression in wild-type larvae (Feder et al., 1997a). Definitive confirmation of the fitness consequences of engineered variation in *hsp70* copy number must await experiments in natural environments, which are presently slated for execution.

## FROM GROSS PHENOTYPE TO CRITICAL LESION

Presumably, *Drosophila* larvae succumb to high temperature when some critical components fail, and Hsp70 improves thermotolerance by protecting these components from heat damage, enhancing the recovery of damaged components, or both. Although numerous cellular and organismal components could behave in this way, the exact identity of those components critical for death or Hsp70-mediated survival in *Drosophila* are unclear, and thus a complete explanation for Hsp70-mediated thermotolerance in *Drosophila* is still lacking. Clearly, many components are poor candidates for critical lesions; many proteins are stable at temperatures above those sufficient for heat death, and several aspects of growth and reproduction fail at temperatures below that of outright death.

A first approach to identifying putative critical lesions has been to focus on temperature-sensitive traits that fail at appropriate temperatures. Among these is the enzyme alcohol dehydrogenase (ADH), whose geographical variation in thermal sensitivity has previously attracted considerable attention (Chambers, 1988). ADH activity is closely linked to ethanol tolerance in larvae and their ability to exploit ethanol as a nutrient (Geer et al., 1993). ADH undergoes thermal inactivation at temperatures that *Drosophila* larvae experience in nature (Sampsell and Barnette, 1985; Feder et al., 1997a). To examine the consequences of *hsp70* copy number for thermal inactivation and recovery of ADH,
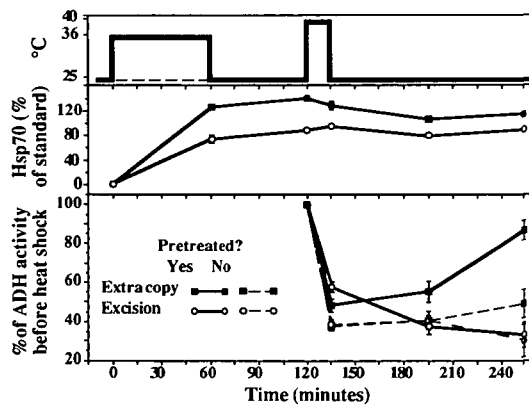
Fig. 6.   Effect of *hsp70* copy number on reactivation of alcohol dehydrogenase in whole 2nd-instar larvae (Feder and Krebs, 1997). Top: Groups of larvae were exposed to 40°C for 15 min after either being exposed to 36°C for 1 hour (pretreated) or being kept at 25°C (unpretreated). Middle: Hsp70 levels in pretreated larvae of the extra-copy and excision strains. Data are plotted as in Figure 4. Unpretreated larvae expressed no Hsp70 before or during heat shock. Bottom: Alcohol dehydrogenase (ADH) activity of whole larvae. ADH activity was determined from crude homogenates according to (McKechnie and Geer, 1984), standardized by μg protein in the homogenate, and expressed as a percentage of the ADH activity determined before heat shock. Means are plotted ± one standard error.

we exposed second-instar larvae to a heat shock that reduced ADH activity by 40% (Fig. 6). Hsp70 was present at the highest levels during the heat shock in larvae of the extra-copy strain that had been pretreated at 36°C beforehand. ADH activity recovered to near-initial levels during the 2 hours after heat shock in these larvae, but not in extra-copy larvae that were not pretreated before heat shock or in excision larvae with or without pretreatment. The greater levels of Hsp70 in pretreated larvae of the extra-copy strain could yield this pattern by protecting de novo synthesis of ADH after the heat shock and/or by reactivating pre-existing ADH unfolded by the heat shock.

A second approach to identifying putative critical lesions has been to expose larvae to appropriate temperatures and survey which components fail or sustain damage. Hsp70 itself is one useful marker of cellular stress or damage. Another is trypan blue, a vital dye that healthy cells exclude; cells that stain with trypan blue are moribund or dead. Whole larvae were exposed to either

mild (36°) or severe (38.5°) heat shock for 1 hour, and then examined for either Hsp70-specific immunofluorescence or trypan blue staining at various times thereafter (Krebs and Feder, 1997c). Several tissue-specific patterns ensued: Brain, salivary gland, imaginal disks and hindgut cuticle expressed Hsp70 intensely within the first hr after heat shock, whereas gut tissues, fat body, and Malpighian tubules did not express Hsp70 until 4–22 hr after heat shock. Mild heat shock increased trypan blue staining only slightly. Severe heat shock, by contrast, increased trypan blue staining markedly in many tissues, but in inverse relationship to the time each tissue required for Hsp70 expression. Gut, for example, which showed a prolonged delay in Hsp70 expression after heat shock, stained intensely with trypan blue (Fig. 7), suggesting that it is especially thermosensitive. Coincidentally, the bulk of larval death after a 38.5°C heat shock occurs not immediately but during the ensuing days (Krebs and Feder, 1997a), a time course consistent with starvation and dehydration due to gut failure.

The exceptional sensitivity of the larval gut to thermal damage prompted a more detailed examination of gut function and protective effects of Hsp70. This study exploited a second genetically engineered mutant of Drosophila, mths70a, which has a P element insertion of the hsp70 coding region under control of the metallothionein promoter. Rearing mths70a larvae on medium that is 2 mM copper causes the gut (but not other tissues) to express Hsp70 (Fig. 8). The consequences of pretreatment in general and Hsp70 expression in particular are evident in feeding rates of Drosophila larvae after heat shock (Fig. 9). Larval ingestion rates can be measured by placing larvae in a yeast slurry containing FD&C #1, a dye that does not pass the gut wall in Drosophila, but no copper. In control larvae, FD&C #1 content is a linear function of feeding time for approximately 90 minutes after placement in the dyed slurry. Heat shock (38.5°C for 1 hr) inhibits feeding for as much as 20 hr afterwards. In both wild-type (data not shown) and mths70a larvae reared on medium without copper, an hsp-inducing pretreatment of 1 hr at 36°
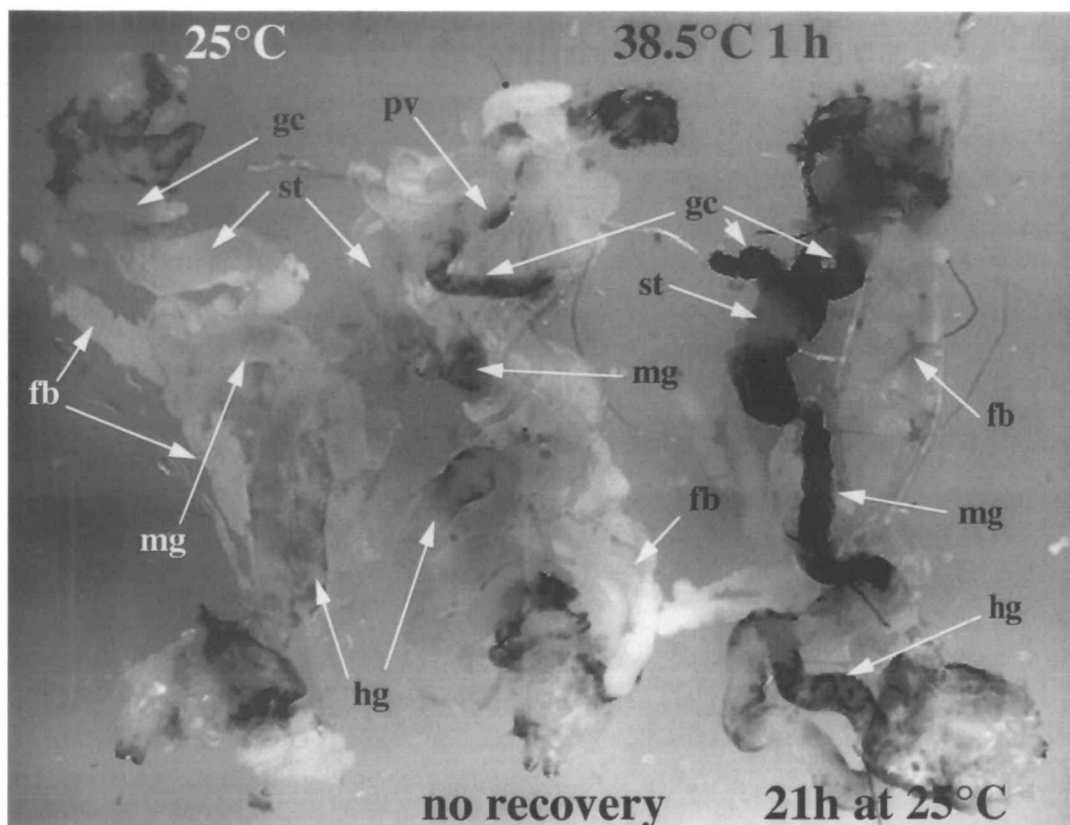
FIG. 7. Variation in trypan blue staining of larvae either untreated, exposed to 38.5°C for 1 hr, or exposed to 38.5°C for 1 hr and maintained at 25°C until the following day: fb, fat body; pv, proventriculus, gc, gastric caeca; st, stomach; mg, midgut; and hg, hindgut (Krebs and Feder, 1997c).

significantly protects larvae against the effect of heat shock on ingestion. This effect, however, is clearly attributable neither to Hsp70 nor to Hsps in ensemble, because pretreatment may enhance thermotolerance through mechanisms other than Hsps. Accordingly, this experiment was repeated with mths70a larvae reared on 2 mM copper medium. Rearing on copper affected neither the feeding rates of larvae maintained at 25° nor the protective effect of pretreatment on feeding after heat shock. In the absence of pretreatment, however, heat-shocked mths70a larvae reared on 2 mM copper showed significantly greater feeding rates than their counterparts reared on 0 mM copper, suggesting that Hsp70 expression is specifically responsible for some component of protection against thermal damage.

The preceding experiments are interesting both in their own right and as examples of the insights available from study of a genetically tractable organism. Numerous Drosophila strains are readily available with well-characterized phenotypes for many genes of potential interest, and can sometimes be created to accommodate a particular experimental design. Through judicious comparison and manipulation of these strains, the experimentalist can analyze the consequences of individual genes for physiological function and fitness with unparalleled rigor.

HSP70: TOO MUCH OF A GOOD THING?

The evolutionary proliferation of hsp70 genes in D. melanogaster is exceptional. On the one hand, this proliferation is understandable in light of the occurrence of
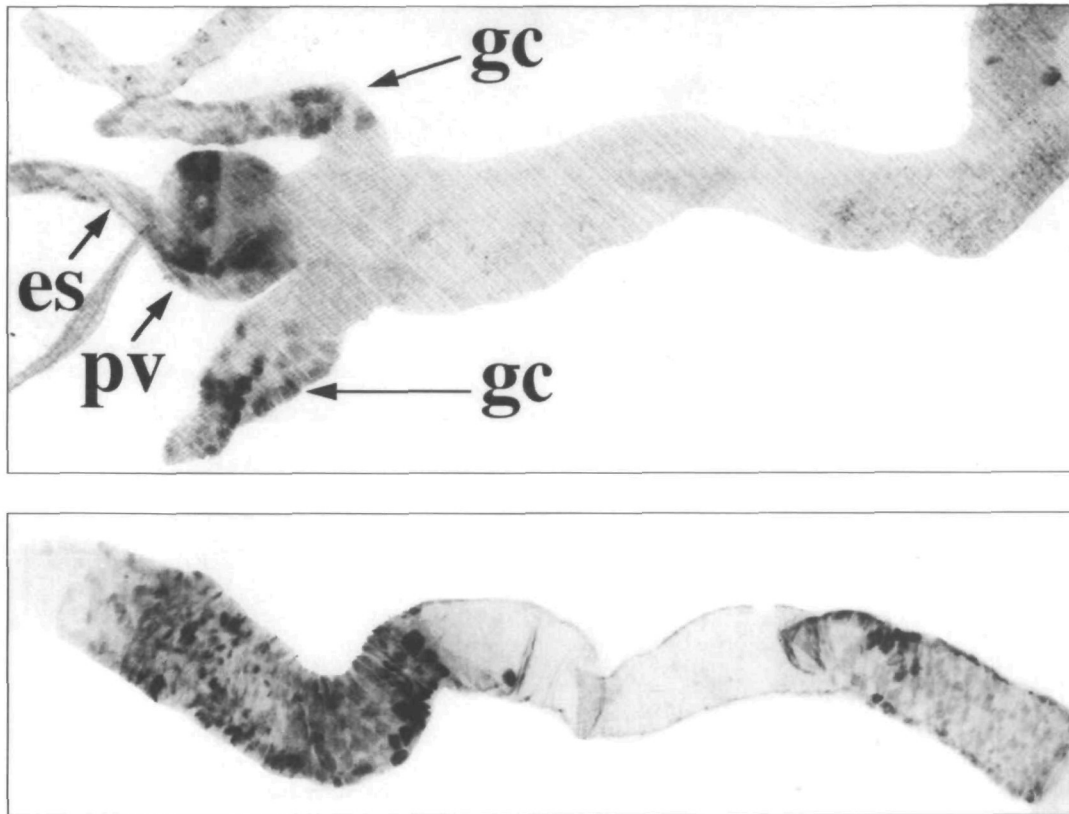
FIG. 8. Hsp70-specific immunofluorescence in larvae of a strain transformed with a *metallothionein* promoter—*hsp70* coding region—*Adh* 3' construct, and reared with 2 mm $CuCl_2$ in the culture medium. Top: Esophagus, proventriculus, and proximal foregut. Bottom: Region of foregut distal to top image. Larvae of other strains reared on identical medium and *mths70a* larvae reared on medium without $CuCl_2$ show no detectable staining. Image has been inverted and digitally enhanced to emphasize staining. Abbreviations as in Figure 7.

thermal stress in the necrotic fruit habitat of this species (see above), the consequences of Hsp70 expression for thermotolerance, and the absence of an Hsp100 family member. On the other hand, Hsp70 can be deleterious in some circumstances, and thus the wild-type *hsp70* copy number could result from an evolutionary trade-off of the advantages and disadvantages of high Hsp70 expression. Hsp70 expression is strongly repressed in the absence of heat shock and once recovery from heat shock is complete (Lindquist, 1993). Constitutive expression of Hsp70 in *Drosophila* cells inhibits their proliferation, and this inhibition ceases once Hsp70 is sequestered into intracellular granules (Feder *et al.*, 1992). Possible mechanistic explanations for these patterns are that (1) Hsp70 is such an ef-

fective molecular chaperone that it binds other proteins promiscuously when its proper targets are not present (Krebs and Feder, 1997a); and/or (2) expression of Hsp70 and other Hsps is so massive that it usurps cellular resources required for other processes (Coleman *et al.*, 1995).

Regardless of its underlying mechanisms, a putative trade-off of positive and negative consequences of Hsp70 expression emerged when excision larvae outperformed extra copy larvae in several experiments (Krebs and Feder, 1997a). For example, after a heat shock that larvae initially survived, excision larvae grew to adulthood more successfully than extra copy larvae, which have significantly higher Hsp70 levels after heat shock than do the excision larvae. Such patterns ensue even in the absence of
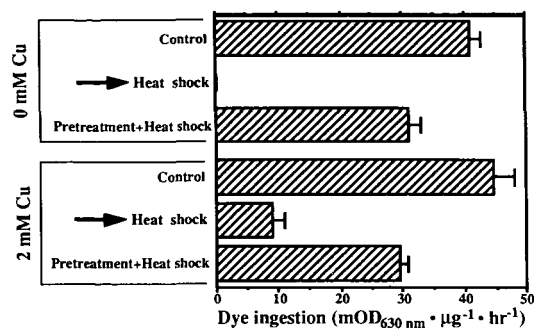
FIG. 9. Effect of heat shock, pretreatment, and pre-induction of Hsp70 on feeding rates of 3rd-instar larvae transformed with a *metallothionein* promoter—*hsp70* coding region—*Adh* 3' construct. Larvae were reared either on 0 or 2 m*M* copper in laboratory medium, and then assigned to the following treatments: Control, sham transfer at constant 25°C; Pretreatment, 36°C for 50 minutes and 25°C for 1 hr; Heat shock, 38.5°C for 50 min. Larvae were fed yeast paste without copper for 16 hr after the end of treatment, and then transferred to a yeast slurry made with 1% FD&C Blue #1 dye in water. This dye does not transit the gut wall, and thus serves as a marker for ingestion (Edgecomb *et al.*, 1994). After 75 min in the dyed yeast, groups of larvae were homogenized and analyzed for dye-specific absorbance at 630 nm and total protein content. Dye ingestion per hour was standardized by μg protein in the homogenate. Means ± standard error are plotted.
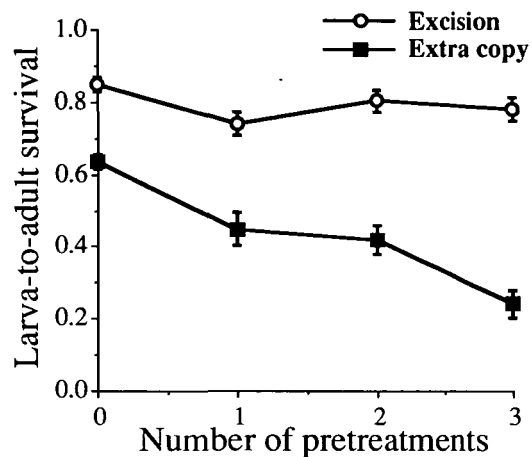


FIG. 10. Larva-to-adult survival following exposure of recently hatched first instar larvae of the excision and extra-copy strains to one of four treatment conditions: pretreatment once (2–4 hr after hatching), twice (after hatching and 24 hr later), or 3 times (as for "twice" and another 24 hr later). The untreated control was held at constant 25°, which was the environment in which individuals developed except when undergoing treatment (Krebs and Feder, 1997a).

heat shock. Larva-to-adult mortality was higher in extra copy larvae than in excision larvae when both were cultured at 25°. In another experiment, 0–3 pretreatments (each 36°C for 1 hr, which itself causes no acute mortality) were administered to excision and extra copy larvae (Fig. 10). In excision larvae, pretreatment frequency did not affect larva-to-adult mortality, while in extra copy larvae, larva-to-adult mortality was proportional to the number of pretreatments administered.

## THE EVOLUTION OF THE HSP70 SYSTEM IN DROSOPHILA

Although Hsps are among the most highly conserved and most primitive of all proteins (Munson *et al.*, 1993; Gupta and Singh, 1994), evolution has extensively modified the conditions under which Hsps are expressed. For example, the temperatures at which Hsps are induced have evolved to correspond to temperatures that are stressful for a given species or cell type. Thus, Antarctic organisms begin to express

Hsps when heated to temperatures <10°C (Vayda and Yuan, 1994), some hyperthermophiles do not express Hsps until temperatures exceed 60°C (Trent *et al.*, 1990; Ohta *et al.*, 1993; Polla *et al.*, 1993; Trent *et al.*, 1994), and hypothermic regions of mammals (*e.g.*, testis) express Hsps at lower temperatures than normothermic organs (Sarge, 1995; Sarge *et al.*, 1995).

Because knowledge and experimental techniques are so advanced for both the general biology of *Drosophila* and their Hsps, *Drosophila* are an obvious model system for elucidating variation in Hsp expression among closely related species and within a single species. At the protein level, maximum Hsp70 expression in two cactiphilic species, *D. arizonae* and *D. mojavensis*, occurs at higher temperature than in *D. melanogaster* (S. Lindquist, personal communication cited by Huey and Bennett (1990)). Similarly, maximal expression occurs in a species of Nearctic origin (*Drosophila ambigua*) at lower temperatures than in other *Drosophila* species with tropical origins (Gehring and Wehner, 1995). Additional experimentation, however, is required to establish that this variation reflects

environmental adaptation of each species rather than their phylogeny. At the level of transcriptional regulation, activation of the heat shock response appears organism-specific, as expression of the principal human Hsp transcription factor, HSF1, in *Drosophila* cells causes its activation temperature to shift from that of human cells to that characteristic of *Drosophila* (Wu, 1995).

Although systematic studies of Hsp variation among populations in any *Drosophila* species are not yet available, Krebs and Feder (1997*b*) characterized Hsp70 expression and thermotolerance for 20 isofemale lines that were established in 1995 from the orchard population represented in Figure 1. Larvae of these lines varied more than 2-fold in Hsp70 levels after a 36°C heat shock. Lines with the highest Hsp70 levels had the highest tolerance of stressful temperatures but the lowest larva-to-adult survivorship when maintained at 25°C, a non-stressful temperature. This pattern of natural variation corresponds to that discovered for the genetically engineered extra *hsp70* copy and excision lines (Krebs and Feder, 1997*a*). In 1996, 28 isofemale lines were newly established from the same population. In these lines, Hsp70 levels of 3 developmental stages (1st-instar larvae, 3rd-instar larvae, and adults) were correlated after a standard heat shock, but survival of adults after exposure to thermal stress was not correlated with expression of Hsp70, signifying that inter-individual variation may be conserved during development.

Other heat shock proteins may covary with thermal environment. Alahiotis and colleagues (*e.g.*, Stephanou *et al.* (1983)) discovered that artificial selection influences Hsp expression in *D. melanogaster*, observing a positive association. These studies, however, were undertaken before the diversity of Hsp families had become apparent and so did not distinguish among family members. At the gene sequence level, (McColl *et al.*, 1996) subjected *Drosophila* strains to artificial selection on knock-down temperature and performed denaturing gradient gel electrophoresis of two heat-shock genes, *hsr-omega* and *hsp68* (a single-copy *hsp70* family member). Both

genes underwent changes in sequence in response to selection.

At the gene and chromosome level, the confluence of phylogenetic analysis and studies of *hsp70* genes in particular species may reveal the historical sequence leading to the distinctive *hsp70* genome of *D. melanogaster* (Fig. 3). A common ancestor of drosophilids apparently underwent an initial duplication of the *hsp70* gene (as shown in Fig. 3), which is now shared by all species examined to date (Peters *et al.*, 1980; Leigh-Brown and Ish-Horowicz, 1981; Molto *et al.*, 1987; Bonorino *et al.*, 1993; Molto *et al.*, 1993; Molto *et al.*, 1994; Pardali *et al.*, 1996). A common ancestor of either the *melanogaster* sub-group or the 6 most derived species in the subgroup apparently underwent a duplication of this entire 2-copy cassette, with the second cassette residing at a distinct chromosomal locus (Leigh-Brown and Ish-Horowicz, 1981). Only in *D. melanogaster* have *hsp70* copies proliferated in the midst of the second locus (Leigh-Brown and Ish-Horowicz, 1981). The occurrence of the gene duplication is roughly correlated with the variation in the ecology and distribution of drosophilids that Lachaise *et al.* (1988) summarized. Although many exceptions to the following generalizations are evident in this large and speciose group, drosophilid species are very often restricted to a narrow ecological niche, particular host plant, and/ or are limited in geographic range. The two most dramatic exceptions are *D. simulans* and *D. melanogaster*; which are cosmopolitan in distribution, exploit a diversity of niches/hosts and possess distinctive *hsp70* genomes. Within the *melanogaster* subgroup, in which the basal pattern is ecological/biogeographic limitation, apparently *D. yakuba* also has independently diverged to achieve some ecological/biogeographic breadth.

## Conclusion

Research on the typical model organisms may require integrative and comparative biologists to overcome their tendency to embrace seemingly more exotic species and their corresponding habitats. Nonetheless, as illustrated above, the standard model or-

ganisms can be interesting in their own right, and may offer an unexpected advantage to the integrative and comparative biologist: Model organisms often engender standard approaches to their analysis, which can exclude other approaches, such as those of integrative and comparative biology. Thus, when integrative and comparative biologists study a model organism, they may bring with them concepts and paradigms that may be ordinary in their field of origin but are unfamiliar to the organism's usual community of investigators. The present investigation, for example, has several components (e.g., determinations of thermal regimes in necrotic fruit containing Drosophila, differential sensitivity of Drosophila tissues to heat shock, natural intrapopulation variation in Hsp70 expression) familiar in ecological and evolutionary physiology but largely unprecedented in the Drosophila/heat-shock communities. By the same token, integrative and comparative biologists seldom have explotied genetically engineered mutants. Accordingly, integrative and comparative biologists may have much to offer and much to gain in collaborations with those who primarily study the less "interesting" (Gans, 1978) organisms.

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## References

Ashburner, M. 1989. Drosophila: A laboratory manual. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

Begon, M. 1983. Yeasts and Drosophila. In M. Ashburner, H. L. Carson, and J. N. Thompson (eds.), The genetics and biology of Drosophila 3b, pp. 345–384. Academic Press, Inc., London.

Bonorino, C. B., E. S. T. Couto, E. Abdelhay, and V.

L. Valente. 1993. Heat shock genes in the willistoni group of Drosophila: Induced puffs and proteins. Cytobios 73:49–64.

Chambers, G. K. 1988. The Drosophila alcohol dehydrogenase gene-enzyme system. In E. W. Caspari and J. G. Scandalois (eds.), Advances in genetics, pp. 39–107. Academic Press, New York.

Coleman, J. S., S. A. Heckathorn, and R. L. Hallberg. 1995. Heat-shock proteins and thermotolerance: Linking ecological and molecular perspectives. Trends Ecol. Evol. 10:305–306.

Cooley, L., R. Kelley, and A. C. Spradling. 1988. Insertional mutagenesis of the Drosophila genome with single P elements. Science 239:1121–1128.

Coyne, J. A., I. A. Boussy, T. Prout, J. S. Jones, S. Bryant, and J. A. Moore. 1982. Long-distance migration of Drosophila. Am. Nat. 119:589–595.

David, J. R., R. Allemand, J. Van Herrewege, and Y. Cohet. 1983. Ecophysiology: Abiotic factors. In M. Ashburner, H. L. Carson, and J. N. Thompson (eds.), The genetics and biology of Drosophila 3d, pp. 105–170. Academic Press, Inc., London.

Edgecomb, R. S., C. E. Harth, and A. M. Schneiderman. 1994. Regulation of feeding behavior in adult Drosophila melanogaster varies with feeding regime and nutritional state. J. Exp. Biol. 197: 215–236.

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

Feder, M. E. 1996. Ecological and evolutionary physiology of stress proteins and the stress response: the Drosophila melanogaster model. In I. A. Johnston and A. F. Bennett (eds.), Animals and temperature: Phenotypic and evolutionary adaptation, pp. 79–102. Cambridge University Press, Cambridge.

Feder, M. E. 1997. Necrotic fruit: A novel model system for thermal ecologists. J. Thermal Biol. 22:1–9.

Feder, M. E., N. Blair, and H. Figueras. 1997a. Natural thermal stress and heat-shock protein expression in Drosophila larvae and pupae. Funct. Ecol. 11: 90–100.

Feder, M. E., N. Blair, and H. Figueras. 1997b. Oviposition site selection: Unresponsiveness of Drosophila to cues of potential thermal stress. Anim. Behav. 53:585–588.

Feder, M. E., N. V. Cartaño, L. Milos, R. A. Krebs, and S. L. Lindquist. 1996. Effect of engineering hsp70 copy number on Hsp70 expression and tolerance of ecologically relevant heat shock in larvae and pupae of Drosophila melanogaster. J. Exp. Biol. 199:1837–1844.

Feder, M. E. and R. A. Krebs. 1997. Ecological and evolutionary physiology of heat-shock proteins and the stress response in Drosophila: Complementary insights from genetic engineering and natural variation. In R. Bijlsma and V. Loeschcke (eds.), Stress, adaptation, and evolution, p. 155–173 Birkhäuser Verlag, Basel. (In press).

Feder, M. E. and S. L. Lindquist. 1992. Evolutionary loss of a heat shock protein. Amer. Zool. 32:51A.

Feder, M. E., D. A. Parsell, and S. L. Lindquist. 1995. The stress response and stress proteins. *In* J. J. Lemasters and C. Oliver (eds.), *Cell biology of trauma*, pp. 177–191. CRC Press, Boca Raton, FL.

Gans, C. 1978. All animals are interesting! Amer. Zool. 18:3–9.

Geer, B. W., P. W. H. Heinstra, and S. W. McKechnie. 1993. The biological basis of ethanol tolerance in *Drosophila*. Comp. Biochem. Physiol. 105B:203–229.

Gehring, W. J. and R. Wehner. 1995. Heat shock protein synthesis and thermotolerance in *Cataglyphis*, an ant from the Sahara desert. Proc. Natl. Acad. of Sci. U.S.A. 92:2994–2998.

Goldstein, L. S. B. and E. A. Fyrberg. (Eds.) 1994. Drosophila melanogaster: *Practical uses in cell and molecular biology. Methods in cell biology*. Academic Press, San Diego.

Gupta, R. S. and B. Singh. 1994. Phylogenetic analysis of 70 kD heat shock protein sequences suggests a chimeric origin for the eukaryotic cell nucleus. Current Biol. 4:1104–1114.

Hartl, F. U. 1996. Molecular chaperones in cellular protein folding. Nature 381:571–580.

Huey, R. B. 1991. Physiological consequences of habitat selection. Am. Nat. 137:S91–S115.

Huey, R. B. and A. F. Bennett. 1990. Physiological adjustments to fluctuating thermal environments: An ecological and evolutionary perspective. *In* R. I. Morimoto, A. Tissieres and C. Georgopoulos (eds.), *Stress proteins in biology and medicine*, pp. 37–59. Cold Spring Harbor Lab. Press, Cold Spring Harbor, NY.

Huey, R. B., W. D. Crill, J. G. Kingsolver, and K. E. Weber. 1992. A method for rapid measurement of heat or cold resistance of small insects. Funct. Ecol. 6:489–494.

Karr, T. L. and J. E. Mittenthal. 1992. Adaptive mechanisms that accelerate embryonic development in *Drosophila*. *In* J. E. Mittenthal and A. Baskin (eds.), *Principles of organization in organisms*, pp. 95–108. Addison-Wesley.

Kohler, R. E. 1994. *Lords of the fly: Drosophila genetics and the experimental life*. University of Chicago Press, Chicago.

Krebs, R. A. and M. E. Feder. 1997a. Deleterious consequences of Hsp70 overexpression in *Drosophila melanogaster* larvae. Cell Stress & Chaperones 2:60–71.

Krebs, R. A. and M. E. Feder. 1997b. 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, R. A. and M. E. Feder. 1997c. Tissue specific variation in Hsp70 expression and thermal damage in *Drosophila melanogaster* larvae. J. Exp. Biol. 200:2007–2015.

Krebs, R. A., V. La Torre, V. Loeschcke, and S. Cavicchi. 1996. Heat-shock resistance in *Drosophila* populations: Analysis of variation in reciprocal cross progeny. Hereditas 124:47–55.

Lachaise, D., M. L. Cariou, J. R. David, F. Lemeunier,

L. Tsacas, and M. Ashburner. 1988. Historical biogeography of the *Drosophila melanogaster* species subgroup. Evolutionary Biol. 22:159–225.

Leigh-Brown, A. J. and D. Ish-Horowicz. 1981. Evolution of the 87A and 87C heat-shock loci in *Drosophila*. Nature 290:677–682.

Lindquist, S. 1993. Autoregulation of the heat-shock response. *In* J. Ilan (ed.), *Translational regulation of gene expression 2*, pp. 279–320. Plenum Press, New York.

McColl, G., A. A. Hoffmann, and S. W. McKechnie. 1996. Response of two heat shock genes to selection for knockdown heat resistance in *Drosophila melanogaster*. Genetics 143:1615–1627.

McKechnie, S. W. and B. W. Geer. 1984. Regulation of alcohol dehydrogenase in *Drosophila melanogaster* by dietary alcohol and carbohydrate. Insect Biochem. 14:231–242.

McKechnie, S. W. and P. Morgan. 1982. Alcohol dehydrogenase polymorphism of *Drosophila melanogaster*: Aspects of alcohol and temperature variation in the larval environment. Aust. J. Biol. Sci. 35:85–93.

McKenzie, J. A. and S. W. McKechnie. 1979. A comparative study of resource utilization in natural populations of *Drosophila melanogaster* and *D. simulans*. Oecologia 40:299–309.

Molto, M. D., M. J. Martinez-Sebastian, and R. de Frutos. 1994. Phylogenetic relationships between *Drosophila subobscura*, *D. guanche* and *D. madeirensis* based on Southern analysis of heat shock genes. Hereditas 120:217–223.

Molto, M. D., L. Pascual, and R. de Frutos. 1987. Puff activity after heat shock in two species of the *Drosophila obscura* group. Experientia 43:1225–1227.

Molto, M. D., L. Pascual, M. J. Martinez-Sebastian, and R. de Frutos. 1993. Heat shock proteins in three related *Drosophila* species belonging to the *obscura* group. Experientia 49:54–56.

Morimoto, R. I., A. Tissieres, and C. Georgopoulos, (eds.) 1994. *Heat shock proteins: Structure, function and regulation*. Cold Spring Harbor Lab. Press, Cold Spring Harbor, NY.

Munson, D., R. Obar, G. Tzertzinis, and L. Margulis. 1993. The 'tubulin-like' S1 protein of *Spirochaeta* is a member of the hsp65 stress protein family. Biosystems 31:161–167.

Ohta, T., K. Honda, K. Saito, H. Hayashi, H. Tano, T. Hamamoto, and Y. Kagawa. 1993. Heat shock promoter of thermophilic chaperonin operon. Biochem. Biophys. Res. Commun. 191:550–557.

Pardali, E., E. Feggou, E. Drosopoulou, I. Konstantopoulou, Z. G. Scouras, and P. Mavragani-Tsipidou. 1996. The Afrotropical *Drosophila montium* subgroup: Balbiani ring 1, polytene chromosomes, and heat shock response of *Drosophila vulcana*. Genome 39:588–597.

Parsell, D. A. and S. Lindquist. 1993. The function of heat-shock proteins in stress tolerance: Degradation and reactivation of damaged proteins. Ann. Rev. Genet. 27:437–496.

Parsons, P. 1978. Boundary conditions for *Drosophila*

resource utilization in temperate regions, especially at low temperatures. Am. Nat. 112:1063–1074.

Peters, F. P., N. H. Lubsen, and P. J. Sondermeijer. 1980. Rapid sequence divergence in a heat shock locus of *Drosophila*. Chromosoma 81:271–280.

Polla, B. S., S. Kantengwa, G. J. Gleich, M. Kondo, C. M. Reimert, and A. F. Junod. 1993. Enhanced thermotolerance and temperature-induced changes in protein composition in the hyperthermophilic archaeon ES4. J. Bacteriol. 175:2839–2843.

Russo, C. A. M., N. Takezaki, and M. Nei. 1995. Molecular phylogeny and divergence times of drosophilid species. Mol. Biol. Evol. 12:391–404.

Sampsell, B. M. and V. C. Barnette. 1985. Effects of environmental temperatures on alcohol dehydrogenase activity levels in *Drosophila melanogaster*. Biochem. Genet. 23:53–59.

Sanchez, Y. and S. L. Lindquist. 1990. HSP104 required for induced thermotolerance. Science 248: 1112–1115.

Sanchez, Y., D. A. Parsell, J. Taulien, J. L. Vogel, E. A. Craig, and S. Lindquist. 1993. Genetic evidence for a functional relationship between *hsp104* and *hsp70*. J. Bacteriol. 175:6484–6491.

Sarge, K. D. 1995. Male germ cell-specific alteration in temperature set point of the cellular stress response. J. Biol. Chem. 270:18745–18748.

Sarge, K. D., A. E. Bray, and M. L. Goodson. 1995. Altered stress response in testis. Nature 374:126.

Sokolowski, M. B. 1985. Genetics and ecology of *Drosophila melanogaster* larval foraging and pupation behaviour. J. Insect Physiol. 31:857–864.

Stearns, S. C. and M. Kaiser. 1996. Effects on fitness components of P-element inserts in *Drosophila melanogaster*: Analysis of tradeoffs. Evolution 50:795–806.

Trent, J. D., M. Gabrielsen, B. Jensen, J. Neuhard, and J. Olsen. 1994. Acquired thermotolerance and heat shock proteins in thermophiles from the three phylogenetic domains. J. Bacteriol. 176:6148–6152.

Trent, J. D., J. Osipiuk, and T. Pinkau. 1990. Acquired thermotolerance and heat shock in the extremely thermophilic archaebacterium *Sulfolobus* sp. strain B12. J. Bacteriol. 172:1478–1484.

Vayda, M. E. and M. L. Yuan. 1994. The heat shock response of an antarctic alga is evident at 5 degrees C. Plant Mol. Biol. 24:229–233.

Welte, M. A., J. M. Tetrault, R. P. Dellavalle, and S. L. Lindquist. 1993. A new method for manipulating transgenes: Engineering heat tolerance in a complex, multicellular organism. Current Biol. 3: 842–853.

Wu, C. 1995. Heat shock transcription factors: Structure and regulation. Ann. Rev. Cell Dev. Biol. 11: 441–469.