Natural and Genetic Engineering of the Heat-Shock Protein Hsp70 in Drosophila melanogaster: Consequences for Thermotolerance

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Natural and Genetic Engineering of the Heat-Shock Protein Hsp70 in
Drosophila melanogaster: Consequences for Thermotolerance

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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...
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 ephemeral nature 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 McKechnie, 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.
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 (Parsons 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).
**hsp70 genome**

**Distribution**

Various; often limited geographically or ecologically

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

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

**orena**

**erecta**

**teissieri**

**yakuba**

**mauritiana**

**sechellia**

**simulans**

**melanogaster**

![Diagram of hsp70 genome and distribution](image)

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**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 thermostolerance, 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 thermostolerant 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 thermostolerance 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-
leagues have modified this technique to create allelic 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, 1997c), usually tolerate 36° pretreatment well, and naturally encounter these (and more extreme) temperatures in necrotic fruit (Feder et al., 1997a). 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

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Fig. 4. Body temperatures and levels of Hsp70 in Drosophila larvae within necrotic apples undergoing experimental insolation (Feder et al., 1997a). 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.
Collectively, these results demonstrate that experimental manipulation of the copy number of \textit{hsp70} is sufficient to affect inductive thermotolerance, and suggest that \textit{Hsp70} can be limiting for thermotolerance. While these findings corroborate considerable earlier work with \textit{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 \textit{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 \textit{Hsp70} expression in wild-type larvae (Feder \textit{et al.}, 1997a). Definitive confirmation of the fitness consequences of engineered variation in \textit{hsp70} copy number must await experiments in natural environments, which are presently slated for execution.

\textbf{FROM GROSS PHENOTYPE TO CRITICAL LESION}

Presumably, \textit{Drosophila} larvae succumb to high temperature when some critical components fail, and \textit{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 \textit{Hsp70}-mediated survival in \textit{Drosophila} are unclear, and thus a complete explanation for \textit{Hsp70}-mediated thermotolerance in \textit{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 \textit{et al.}, 1993). ADH undergoes thermal inactivation at temperatures that \textit{Drosophila} larvae experience in nature (Sampsell and Barnette, 1985; Feder \textit{et al.}, 1997a). To examine the consequences of \textit{hsp70} copy number for thermal inactivation and recovery of ADH,
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°C) or severe (38.5°C) 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°C
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 ceca; 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
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 effective 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...
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 mM 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.

Heat shock. Larva-to-adult mortality was higher in extra copy larvae than in excision larvae when both were cultured at 25°C. 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 cactiophilic 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 transcrip-
tional regulation, activation of the heat shock response appears organism-spe-
cific, as expression of the principal human Hsp transcription factor, HSF1, in Drosoph-
ila cells causes its activation temperature to shift from that of human cells to that char-
acteristic of Drosophila (Wu, 1995).

Although systematic studies of Hsp variation among populations in any Drosophila 
species are not yet available, Krebs and Feder (1997b) characterized Hsp70 expres-
sion 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 sur-
vivorship when maintained at 25°C, a non-
stressful temperature. This pattern of natu-
ral variation corresponds to that discovered 
for the genetically engineered extra hsp70 
copy and excision lines (Krebs and Feder, 
1997a). In 1996, 28 isofemale lines were 
newly established from the same popula-
tion. In these lines, Hsp70 levels of 3 de-
velopmental stages (1st-instar larvae, 3rd-
instar larvae, and adults) were correlated af-
	er 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 influ-
ences Hsp expression in D. melanogaster, ob-
servering a positive association. These stud-
ies, however, were undertaken before the 
diversity of Hsp families had become ap-
parent and so did not distinguish among 
family members. At the gene sequence lev-
el, (McColl et al., 1996) subjected Dro-
sophila strains to artificial selection on 
knock-down temperature and performed de-
naturing gradient gel electrophoresis of two 
heat-shock genes, hsr-omega and hsp68 (a 
single-copy hsp70 family member). Both 
genesis underwent changes in sequence in re-
sponse 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. mel-
anogaster (Fig. 3). A common ancestor of 
drosophilids apparently underwent an ini-
tial duplication of the hsp70 gene (as shown 
in Fig. 3), which is now shared by all spe-
cies 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; Par-
dali et al., 1996). A common ancestor of 
either the melanogaster sub-group or the 6 
most derived species in the subgroup ap-
parently underwent a duplication of this en-
tire 2-copy cassette, with the second cas-
sette residing at a distinct chromosomal lo-
cus (Leigh-Brown and Ish-Horowicz, 1981). Only in D. melanogaster have hsp70 
copies proliferated in the midst of the sec-
don locus (Leigh-Brown and Ish-Horowicz, 
1981). The occurrence of the gene dupli-
cation is roughly correlated with the varia-
tion in the ecology and distribution of dro-
sophilids that Lachaise et al. (1988) sum-
marized. Although many exceptions to the 
following generalizations are evident in this 
large and speciose group, drosophilid spe-
cies 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 cosmopol-
itan in distribution, exploit a diversity of 
niches/hosts and possess distinctive hsp70 
genomes. Within the melanogaster sub-
group, in which the basel pattern is ecologi-
cal/biogeographic limitation, apparently D. 
yakuba also has independently diverged to 
achieve some ecological/biogeographic bread-
th.

**CONCLUSION**

Research on the typical model organisms 
may require integrative and comparative bi-
ologists to overcome their tendency to em-
brace seemingly more exotic species and 
their corresponding habitats. Nonetheless, 
as illustrated above, the standard model or-
organisms 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 familiar 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, and 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 exploited 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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