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# Changes in thermotolerance and Hsp70 expression with domestication in *Drosophila melanogaster*

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## Abstract

To examine how the duration of laboratory domestication may affect *Drosophila* stocks used in studies of thermotolerance, we measured expression of the inducible heat-shock protein Hsp70 and survival after heat shock in *D. melanogaster* strains recently collected from nature and maintained in laboratory culture for up to 50 or more generations. After an initial increase in both Hsp70 expression and thermotolerance immediately after transfer to laboratory medium, both traits remained fairly constant over time and variation among strains persisted through laboratory domestication. Furthermore, variation in heat tolerance and Hsp70 expression did not correlate with the length of time populations evolved in the laboratory. Therefore, while environmental variation likely contributed most to early shifts in strain tolerance and Hsp70 expression, other population parameters, for example genetic drift, inbreeding, and selection likely affected these traits little. As long as populations are maintained with large numbers of individuals, the culture of insects in the laboratory may have little effect on the tolerance of different strains to thermal stress.

## Introduction

Laboratory evolution is a valuable tool to elucidate the processes and outcomes of evolution because it complements comparative analyses and field studies while it provides the high level of environmental control necessary to establish subtle effects (Gibbs, 1999). A necessary component of laboratory evolution, however, is the domestication of the study organism. The laboratory itself is a novel environment that imposes unpredictable stresses and it may influence the genetic architecture of populations (Matos *et al.*, 2000). Studies of domestication to the laboratory, therefore, are necessary to verify whether an experimental system may serve as a proxy for natural populations. Although many laboratory evolution studies include controls that do not undergo exposure to the experimental environment, few control for domestication itself or examine its influence (Harshman & Hoffmann, 2000). This deficiency looms large because laboratory and natural systems do not always yield similar results (Gibbs, 1999).

Here we use heat tolerance and expression of the inducible heat-shock protein, Hsp70, to examine physiological evolution and/or stasis in populations of *Drosophila* during laboratory domestication. We chose the first trait because repetitive exposure to physiological stresses is sufficient for evolution of tolerance in the laboratory (White *et al.*, 1970; Stephanou *et al.*, 1983; Huey *et al.*, 1991; Huey *et al.*, 1992; Cavicchi *et al.*, 1995; Travisano *et al.*, 1995; Krebs & Loeschcke, 1996; Hoffmann *et al.*, 1997; Bennett & Lenski, 1999), although in species with complex development like *Drosophila*, changes may be specific to particular stages of development (Krebs & Loeschcke, 1995; Loeschcke & Krebs, 1996). The second trait, expression of Hsp70, is both correlated with species' natural environmental stress

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regimes (e.g. Ulmasov *et al*., 1992; Vayda & Yuan, 1994; Gehring & Wehner, 1995; Feder, 1996; Hightower *et al*., 1999; reviewed in Feder & Hofmann, 1999) and is responsible for a significant fraction of inducible thermotolerance in *D. melanogaster* (Krebs & Feder, 1997a). The induction of Hsp70, however, can be deleterious in the absence of stress (Krebs & Loeschcke, 1994; Krebs & Feder, 1997a,b). Thus both traits should evolve and remain variable where populations persist in a heterogeneous environment.

The same outcome is not expected for populations evolving in the laboratory at approximately constant and nonstressful temperatures. Instead, populations may become less stress resistant or drift in their response to stress. In fact, Hsp70 expression and induction of thermotolerance both may vary in lines reared many years at different constant temperatures (Stephanou *et al*., 1983; Huey *et al*., 1991; Bettencourt *et al*., 1999). Unknown, however, is whether the conditions of the common laboratory rearing environment actually select for or against expression of Hsp70 and/or against thermotolerance, or if these traits fluctuate randomly in the absence of stress, especially in the early generations after the flies are brought into the laboratory. One additional possibility is that flies brought to the lab change in tolerance as an environmental response to the new rearing conditions. Thus, we examined these alternative outcomes discretely in populations of *Drosophila* at various times after field capture and establishment in laboratory culture.

## Materials and methods

### Collection and maintenance of natural lines

We reared *Drosophila* from necrotic fruit collected at Garwood Orchards, LaPorte, Indiana, USA, and at Patterson's Farm, in Kirtland, Ohio, USA. Collections were made during the summers (July–August) of 1995–98. Daily maxima averaged 28 °C in these areas, but sun exposed fruit can often be hot enough to kill larvae (Feder, 1996). Necrotic fruit was returned to the laboratory and placed in large population cages maintained at room temperature. Adult flies emerged from the fruit after four or more days postcollection, indicating that most individuals were captured as larvae. Emerging adults were aspirated from the cage each day prior to mating, anaesthetized with $CO_2$, and one male and one female were placed as pairs in vials containing 12 mL of yeast, cornmeal, molasses and agar medium. Male offspring were used to identify *D. melanogaster* from *D. simulans*, the latter of which is much less common at both sites. Vials that produced no offspring, whether from interspecific pairing or infertility were discarded. For each collection, offspring from about 20 pairings were pooled to form a mass population, which was reared at 22–25 °C in four half-pint bottles under a discreet generation transfer protocol. Strains were named according to collection state and year: Ind95, Ind96, Ind97, Ind98 and Oh98. New generations were set-up every 22–23 days, which correspond to 16 generations year$^{-1}$. Periodically after collection, strains were assayed for thermotolerance and for expression of Hsp70 as described below.

### Preliminary tests on domestication

Initially we assessed changes in thermotolerance and Hsp70 expression in larvae and adults from two natural lines, Ind96 after 4–6 generations and Ind95 after 22–24 generations of captive rearing. For larval thermotolerance assays, treatment temperatures were varied to impose a range of stress levels: 39.2 °C for 1 h without pretreatment, or 1 h stresses of 40.2, 40.5, or 41.0 °C with a pretreatment of 1 h at 36 °C and 1 h recovery at 25 °C before exposing individuals to stress. For all treatments, plus an untreated control, 40 1st-instar larvae were transferred to each of 10 vials per population. Larval thermotolerance was assayed as the proportion of individuals that survived to adulthood. Tolerance for 4–5 day-old adults (20 individuals transferred to each of 10 vials per treatment) was assayed at a stress of 39.5 °C for 1 h, with data recorded as the proportion of individuals able to walk the following day. Adults received a lower temperature because young larvae tolerate stress much better than do adults (Krebs *et al*., 1998).

Hsp70 expression was also determined for 1st-instar larvae exposed to 36 °C for 1 h followed by a 1-h recovery at 25 °C (five sample tubes each with about 40 larvae). Adult Hsp70 expression was assayed following 1 h exposures at 35, 36, 37, 38, 39 or 40 °C and 1 h at 25 °C (also 5 tubes temperature$^{-1}$ but with only 2 adults tube$^{-1}$).

### Short-term change in thermotolerance and Hsp70 expression

We assayed thermotolerance and Hsp70 expression in 5-day-old adults reared from fruit collected at an orchard and from four replicate offspring lines at generations 1, 2, 3, 6 and 9 assayed at the same age. Each subculture was maintained in three bottles from which about 100 adult males (in groups of 15–20) from each offspring strain × generation combination were exposed to one of the following treatments: (a) controls (untreated), (b) pretreatment at 36 °C for 1 h, but with no further stress (c) direct exposure to 39 °C for 1 h without pretreatment and (d) pretreatment, 1 h recovery at 25 °C and then exposure to the 39 °C stress. Again, we designated thermotolerance as the proportion of individuals that could walk the following day.

For analysis of Hsp70 expression, 20 5-day-old males from each generation × strain combination were exposed to 36 °C for 1 h followed by a 1-h recovery at 25 °C, and then frozen. All protein analyses were run after the ninth

generation. Therefore, we randomized flies from all line × generation samples within the Hsp70 assays to eliminate variation among enzyme linked immunoabsorbent assays (ELISA) as a confounding effect on laboratory generation.

### Long-term change in thermotolerance and Hsp70 expression

To examine change over extended time periods, all five mass populations listed above, which varied in lab culture duration from 8 to 55 generations, were analysed for Hsp70 expression and thermotolerance of larvae and of adults. Hsp70 expression was determined for larvae/flies exposed to 36 °C for 1 h followed by a 1-h recovery at 25 °C and then frozen in liquid nitrogen. Thermotolerance of 1st-instar larvae was assessed after a pretreatment (36 °C for 1 h, followed by 1 h at 25 °C), and two experimental treatments: either a stress of 1 h at 40.5 °C or at 41.0 °C. Adults, tested at 4 days of age were similarly pretreated, but stressed by a 1-h exposure at 39 °C, with tolerance assayed as performed previously. All assays of tolerance and of Hsp70 randomized individuals from the different source populations within every experimental block of replicates.

### Measuring Hsp70

For analysis of Hsp70 expression, larvae and flies were first transferred to microfuge tubes, which were immersed in water baths for 1 h at 36 °C to induce maximal expression (Krebs & Feder, 1997b), then placed for 1 h at 25 °C and finally in liquid $N_2$. To obtain 1st-instar larvae for these measurements, adults of the previous generation were transferred to oviposition chambers containing a removable dish filled with medium. Eggs were laid on and larvae were collected from this surface. Adults were transferred after anaesthetizing in $CO_2$, which does not induce the stress response (Smith & Huey, 1991; Krebs *et al*., 1995), and placing two flies within each cryotube. All tubes contained 10 $\mu$L phosphate-buffered saline (PBS) to prevent desiccation during heat shock. Hsp70 concentration in a lysate prepared from the contents of each tube was determined by ELISA, which has been described elsewhere (Welte *et al*., 1993; Feder *et al*., 1996). The ELISA signal is proportional to Hsp70 concentration in the lysates and it is expressed as a percentage of a standard signal, which in the short term experiments was that for a lysate of *Drosophila* S2 cells in tissue culture that have been exposed to 36.5 °C for 1 h and 25 °C for 1 h before lysis. Experiments to compare expression in long-reared populations utilized a pooled homogenate of similarly heat treated larvae to compare populations over time (Krebs, 1999). Because Hsp70 concentrations are not easily measured, comparison of the actual amounts expressed in the different sets of experiments was not possible.

## Results

### Preliminary experiments

The preliminary experiments suggested that both thermotolerance and Hsp70 expression varied as populations first became domesticated under constant laboratory temperatures. However, Hsp70 concentrations in larvae varied significantly across three generations of tests that compared the two lines ($F_{2,33} = 27.0$, $P < 0.01$) and these lines reversed in relative expression of Hsp70. The Ind96 strain at generation four expressed more Hsp70 than did the older Ind95 strain but by generation 6, these individuals expressed less Hsp70 than did larvae from the Ind95 strain (strain–date interaction effect was significant, $F_{2,33} = 7.2$, $P < 0.01$). Variation in thermotolerance roughly followed change in Hsp70 expression, with Ind96 flies surviving stress better at first, but not later, both as larvae and as adults.

### Short-term change

In the subsequently replicated experiments, thermotolerance and Hsp70 expression increased abruptly within 1–2 generations in the laboratory and thereafter the strains displayed only random temporal variation in these traits (Fig. 1). Shifts in Hsp70 expression were large

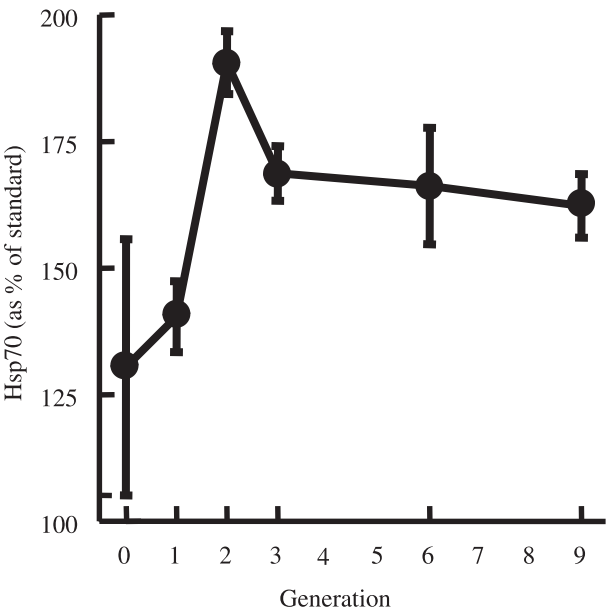



**Fig. 1** Change in the expression of Hsp70 in adults of *D. melanogaster* (strain Ind98) during their first generations in the laboratory. Generation 0 represents data on parental flies reared from rotting fruit and subsequent data are the mean ± 1 SE for results on four independent lines derived from the fruit-reared parents. All flies were treated at 36 °C for 1 h, then 25 °C for 1 h, and frozen for later analysis. Hsp70 is recorded as a percentage of a standard.

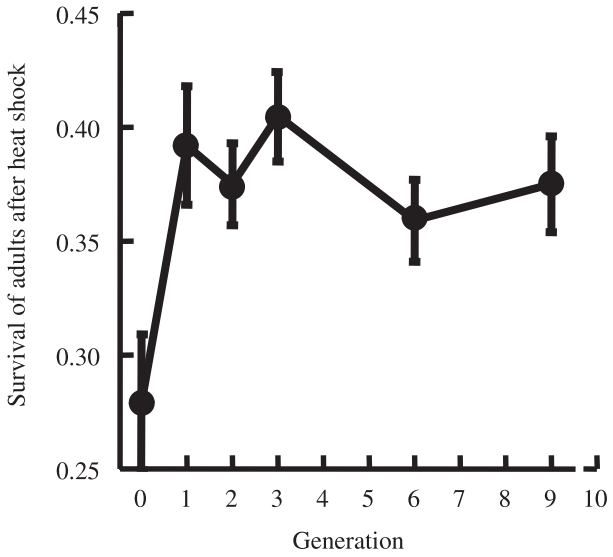



**Fig. 2** Change in the thermotolerance of adults of *D. melanogaster* (strain Ind98) during their first generations in the laboratory. Generation 0 represents data on parental flies reared from rotting fruit and these flies received heat shock at the same age as their offspring. Subsequent data are the mean ± 1 SE for results on four independent lines derived from the fruit-reared parents. All flies were pretreated at 36 °C for 1 h, then placed at 25 °C for 1 h, before they were heat shocked 1 h at 39 °C.

between parental flies (generation 0, which were flies reared from collected fruit), and those in the four independent offspring lines measured after 1, 2, 3, 6 and 9 generations of rearing at 25 °C. The differences between parents and offspring were initially small, increased markedly during the second generation, fluctuated during two additional generations, and then stabilized.

As for Hsp70, thermotolerance (which was measured as survival to a 39 °C heat shock after pretreatment) varied with time. Adults reared from fruit collected in the field tolerated heat poorly, but survival thereafter improved in generation 1 and remained fairly constant [Fig. 2, effect of generation, $F_{5,95} = 2.2$, $P = 0.06$, but tests of parental flies (generation 0) against each subsequent group were significant, $P < 0.05$, Tukey's test]. The heat shock applied killed all individuals that did not receive a pretreatment, whereas no mortality occurred in control flies or in those pretreated at 36 °C.

Important to any test of the processes underlying change with domestication is how variable or consistent expression and tolerance became among the replicate lines. All four replicate strains independently derived from the original parents changed similarly with time and differences among them were not significant for either trait. Neither were tests of interactions between replicate strain and assay date significant.

## Long-term change

Laboratory populations founded from the same natural population but in different years varied considerably in both thermotolerance and in Hsp70 expression. Larvae and adult flies from five strains reared 8–55 generations in the laboratory varied in expression of Hsp70 (Fig. 3, $F_{4,40} = 7.5$, $P < 0.001$). This variation, however, did not relate to the length of time that strains existed in the laboratory. Larvae of Ind95, the strain, reared in the laboratory the longest, produced the most Hsp70 (significantly more than did larvae of Ind96 or Ind97).

The lines also varied significantly in tolerance of severe stress (Fig. 4, $F_{4,29} = 4.5$, $P < 0.01$), which was principally caused by the inferior tolerance of the Oh98 line relative to that of the Ind96 and Ind97 lines (Tukey's studentized range test, $P < 0.05$). Therefore, the pattern of larval survival also did not relate to variation in Hsp70 expression; the Ind96 and Ind97 strains, which survived the stress in the highest proportions, produced the least Hsp70. Only the severe stress led to variation; neither in the absence of stress nor after a mild stress that killed only about 10% of the individuals, did lines vary in larva-to-adult survival.

In contrast to results in larvae, adults of these strains varied little in Hsp70 expression (Fig. 5, $F_{4,35} = 0.1$, NS), despite varying in thermotolerance (Fig. 6, $F_{4,140} = 8.70$, $P < 0.001$). Adults of the Ind96 and Ind97 strains tolerated stress less well than did adults of the other

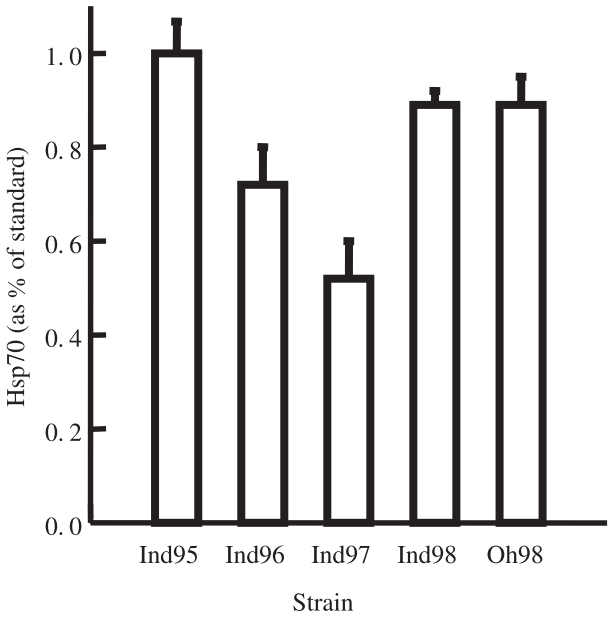
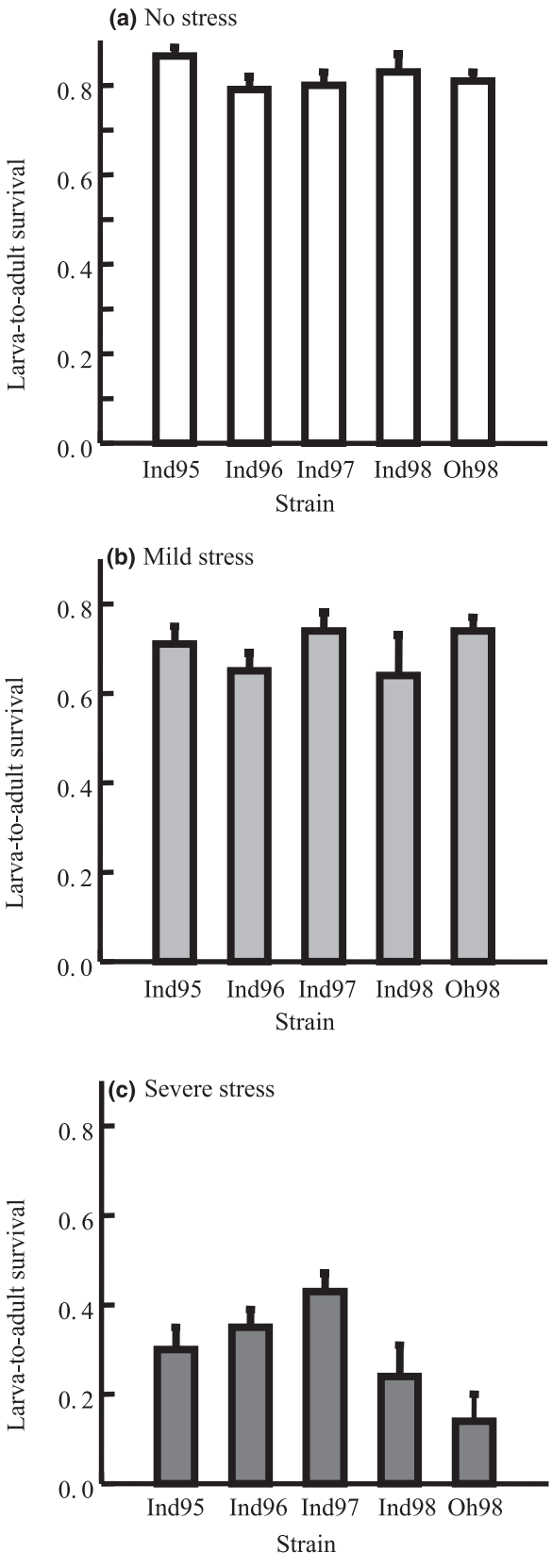



**Fig. 3** Expression of Hsp70 in 1st-instar larvae from five strains of *D. melanogaster* collected in different years. All larvae were treated at 36 °C for 1 h, then 25 °C for 1 h, and frozen for later analysis. Hsp70 was standardized at the highest mean observed within this set of experiments, and bars indicate ± 1 SE.



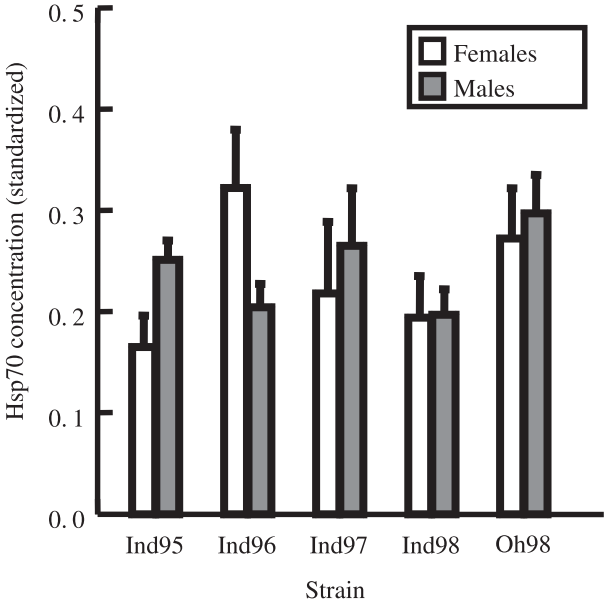




**Fig. 5** Expression of Hsp70 in adults from five strains of *D. melanogaster* collected in different years. All adults were treated at 36 °C for 1 h, then 25 °C for 1 h, and frozen for later analysis. Hsp70 was standardized at the highest mean observed within this set of experiments (that of larvae of the Ind95 strain), and bars indicate ±1 SE.

three strains and differences were significant between the Ind96 strain and all others, except Ind97 ($P < 0.05$, Tukey's test). Neither Hsp70 expression nor thermotolerance in adults varied significantly between males and females. Although a strain–gender interaction was present, it was weak (for Hsp70, $F_{4,35} = 2.2$, $P < 0.1$; for survival, $F_{4,140} = 2.9$, $P < 0.05$).

## Discussion

Both thermotolerance and Hsp70 expression varied among strains and with time in the laboratory. Such change could be caused by at least three factors. First, the laboratory could be a novel environment to which a population may adapt or domesticate (Hoffmann & Merila, 1999). Secondly, small population size and sampling may shift trait means randomly (Bijlsma *et al.*, 1999). Finally, the laboratory environment could directly affect phenotypically plastic traits. We suspect that this last factor was responsible for the dramatic change in thermotolerance and Hsp70 expression from wild-caught

**Fig. 4** Larval-to-adult survival for five strains of *D. melanogaster* collected in different years (name indicates US state and year; Ind = Indiana, Oh = Ohio). Larvae grew either in the absence of stress (a), after a mild stress of 1 h at 40.5 °C (b) or after a severe stress of 1 h at 41.0 °C (c) administered when 1st instars. Bars indicate ±1 SE.

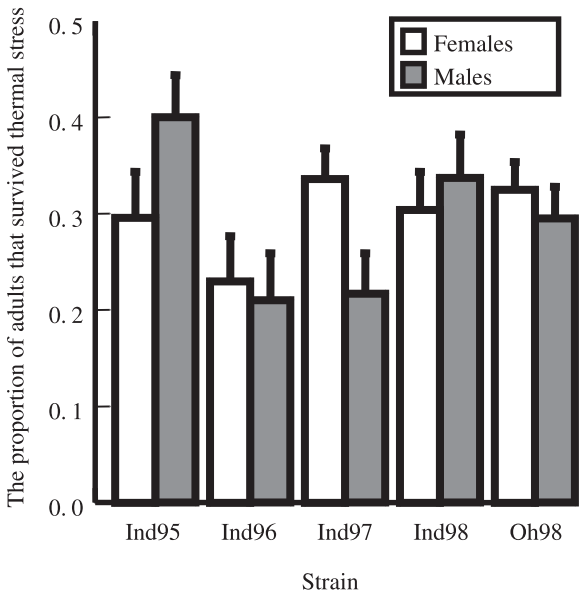



**Fig. 6** Survival after thermal stress for adults of five strains of *D. melanogaster* collected in different years. The stress treatment applied was 39.5 °C for 1 h to adults that first received a pretreatment of 1 h at 36 °C and then placed 1 h at 25 °C to recover before exposure to the stress.

flies to lab-reared flies. The laboratory medium probably provided both a better diet and a rearing environment free of disease, either of which might improve tolerance of stress. How these same variables affect Hsp70 expression is unknown. Indeed, maternal environment affects thermotolerance in *D. simulans*, which Jenkins & Hoffmann (1994) attributed to nutritional differences or variation in health. However, high larval density which reduces food availability has little effect on thermotolerance in *D. buzzatii* (Loeschcke *et al*., 1994).

After the early shifts in thermotolerance, trait means varied little with time and were similar in replicate lines. This pattern suggests that rearing flies in the absence of natural stress imposed little selection during the first 50 or more generations. These populations almost certainly would have possessed sufficient genetic variation to change because variation in stress traits is usually high in *D. melanogaster* (Parsons, 1980), as it was when estimated for both thermotolerance and Hsp70 in the founders of the first two strains, Ind95 and Ind96 (Krebs & Feder, 1997a; Krebs *et al*., 1998). Only in the preliminary experiments did we observe any substantive change beyond the first two generations, of which one was an increase and another a decrease in Hsp70 expression. Thermotolerance shifted only where Hsp70 expression declined. In these two instances, perhaps chance via genetic drift or sampling occurred despite maintenance within a discrete-generation mass-rearing regime which usually minimizes drift effects (Hoffmann & Parsons, 1988).

Many genes must underlie thermotolerance in *Drosophila* as evidenced by complex responses to selection in *D. melanogaster* (Gilchrist & Huey, 1999) and *D. buzzatii* (Krebs & Loeschcke, 1996), and from genetic interactions present in crosses among laboratory populations of both of these species (Krebs *et al*., 1996). However, Hsp70 is the only protein definitively linked to thermotolerance in *Drosophila* (Feder *et al*., 1996; Feder & Hofmann, 1999). Hsp70 is a molecular chaperone that interacts with stress-damaged proteins to inhibit their aggregation and thereby facilitate their refolding or degradation (Parsell & Lindquist, 1994). Even so, Hsp70 does not actually prevent damage from heat and is most effective in aiding recovery when present prior to stress (Krebs & Feder, 1998; Krebs, 1999). The genetic factors responsible for variation in Hsp70 expression could lie at the level of transcriptional and/or translational regulation (Bettencourt & Feder, 1999; Lerman & Feder, 1999), but gene copy number is not known to vary (Bettencourt *et al*., 1999). Likewise, functionally variant alleles of Hsp70 are not yet known.

One past observation contrasts with our conclusions here that mass reared populations change little in stress tolerance. Some *D. melanogaster* strains like Oregon R and *white*, which have inhabited laboratories for decades die at temperatures 2 °C lower than those that begin to kill recently founded wild-type strains (Welte *et al*., 1993; Krebs & Feder, 1997b). This difference in tolerance among laboratory strains of *D. melanogaster* is tantamount to survival differences of 100% in single temperature assays and is as large as that observed among species collected along major environmental gradients (Stratman & Markow, 1998; Hightower *et al*., 1999; Krebs, 1999).

The implications of our results, however, that little if any selection on Hsp70 expression and thermotolerance accompany domestication are of critical value. The differences among strains in laboratory culture also typify natural populations and such natural differences can persist during routine culture in the laboratory. Hence domestication does not automatically result in *Drosophila* that are no longer viable proxies for wild flies or have weakened in the laboratory. This outcome should hearten the authors of the numerous past, present and future experimental studies of laboratory evolution and selection, even those experimenting on older laboratory lines. The one caveat is that environmental effects may influence both traits, requiring a few laboratory generations to stabilize lines before initiating work. Once established, careful breeding should prevent the loss of thermotolerance and shifts in expression of potentially contributing proteins during the course of experiments.

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