Heat-induced expression of a molecular chaperone decreases by selecting for long-lived individuals

Fabian M. Norrya,b, Volker Loeschckea,∗

aDepartment of Ecology and Genetics, University of Aarhus, Ny Munkegade, Bldg. 540, DK-8000 Aarhus C, Denmark
bDepartamento de Ecología, Genética y Evolución, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, (1428) Buenos Aires, Argentina

Received 30 October 2002; received in revised form 10 February 2003; accepted 25 February 2003

Abstract

The 70 kDa heat-shock protein (Hsp70) exhibits a broad range of chaperone functions that respond both to internal and external stresses, and its heat-induced expression both declines with age and reduces age-specific mortality rates. Here we test for changes in both longevity and the level of Hsp70-induced expression as correlated responses to selection on both heat-stress resistance and longevity in D. melanogaster. Three replicated H lines were heat-stress selected and compared to their respective non-selected controls (C lines) in the 25th generation of heat-stress selection. The direct response in heat-stress resistance was 56% in males and 38% in females. Heat-stress selection improved longevity in males at normal temperatures. All lines were subsequently subjected to one generation of truncation selection on longevity (selection intensity, i = 1.28 for H lines and 1.36 for C lines). The heat-induced Hsp70 expression seems to increase very weakly by heat-stress selection but shows a dramatic and persistent decline by selecting for long-lived flies. A mechanism of longevity selection, involving changes in Hsp70 regulation, is suggested.

© 2003 Elsevier Science Inc. All rights reserved.

Keywords: Drosophila; Hsp70; Longevity; Heat-stress resistance; Truncation selection

1. Introduction

Longevity and stress-response systems can be related in complex ways (Kirkwood, 1977; Kirkwood and Rose, 1991; Agarwal and Sohal, 1994; Lithgow et al., 1995; Lithgow and Kirkwood, 1996; Tatar et al., 1997; 1999a,b; King and Tower, 1999; Minois et al., 2001). Sub-lethal heat shock often extends life span at normal temperatures in Drosophila and other organisms, and expression of heat-shock genes might be causally related to this beneficial effect of mild-stress (Khazaeli et al., 1997; Shama et al., 1998; Hercus et al., 2003 and references therein). Heat-induced expression of Hsp70, a heat-shock protein, may reduce age-specific mortality rates (Tatar et al., 1997; Tatar, 1999a,b; Minois et al., 2001), and decreases fecundity and/or egg hatch (Krebs and Loeschcke, 1994a,b; Silbermann and Tatar, 2000) in D. melanogaster at normal temperatures after induction. This multifunctional molecule, when under inducible regulatory expression, exhibits a broad range of chaperone functions that respond to both internal and external stresses (Lindquist, 1986; Parsell and Lindquist, 1993; Morimoto et al., 1994; Wheeler et al., 1995; Feder, 1999; Feder and Hofmann, 1999), and its heat-induced expression decreases with age (Gutsmann-Conrad et al., 1999; Sørensen and Loeschcke, 2002). Although this element of the heat-shock response has been implied to be a modifier of both mortality rate (Tatar et al., 1997; Tatar, 1999a,b) and fecundity (Krebs and Loeschcke, 1994a,b; Silbermann and Tatar, 2000), Hsp70 per se may not affect mean longevity (Minois et al., 2001; Minois and Vainberg, 2002), and its possible link with genetic variation in longevity remains unknown.

Positive relationships between heat-shock resistance and longevity have also been reported for Drosophila. For example, brief exposure to elevated but sub-lethal levels of heat increased the mean life span of D. melanogaster at normal temperatures (e.g. Khazaeli et al., 1997). However, genetic variation in resistance to multiple stresses may not necessarily be associated with longevity (Harshman et al., 1999), and cold-stress selection does not increase longevity.
in *D. melanogaster* at normal temperatures (Norry and Loeschcke, 2002a). A central hypothesis that has never been tested is whether selection for extended life affects the level of heat-induced expression of genes of the heat-shock response. However, the broad-sense heritability is often moderate or high for Hsp70 expression (e.g. Krebs et al., 1998), suggesting that Hsp70 expression can respond rapidly to selection. For instance, Hsp70 inducible expression responds consistently to both natural and laboratory thermal selection in *Drosophila* (e.g. Sørensen et al., 1999; 2001; Bettencourt et al., 1999, 2002).

In an assessment of the possible consequences of artificial selection, we tested for changes in both longevity and the level of Hsp70-induced expression as correlated responses to selection on both heat-stress resistance and longevity, respectively. Three replicated lines were heat-stress selected and compared to control lines in *D. melanogaster*. Two main hypotheses are addressed. First, that longevity selection affects the level of heat-induced Hsp70 expression. This is expected if there is any strong genetic relationship between longevity and Hsp70 inducible expression. Secondly, we test whether heat-stress selection (i) increases resistance to heat stress, as expected if the heritability of the trait is greater than zero (ii) increases longevity at normal temperature, as expected if life span is genetically correlated with heat-stress resistance (Partridge et al., 1995) and (iii) increases the heat-induced Hsp70 expression, as expected if Hsp70-inducible level is a target of heat-stress selection.

### 2. Materials and methods

#### 2.1. Heat-stress selection

The base population was the laboratory tenth generation of a large sample of wild flies collected in Denmark, from which two sets of thermal regimes were initiated: (i) the control lines, in three replicates (C1, C2 and C3), which were kept at constant 25 °C at a 12 h light 12 h dark cycle, and (ii) the heat-stress lines, in three replicates, denoted H1, H2, and H3. All H lines were simultaneously selected by exposing 4-d-old heat-treated (37 °C for 75 min, about 0% of mortality) adults to a potentially lethal stress of 38.6 °C for 90 min (20 vials/line, 20 flies/vial, making a total of about 400 flies per replicated line). This selection was performed every other generation one hour after the 37 °C pre-treatment (selection intensity was between 0.27 and 0.97 per selection generation, and alternate generations were used to recover population size in H lines). All three C lines were similarly heat-treated (i.e. 37 °C for 75 min) every other generation, but were not heat-stress selected. All lines were maintained at 25 °C with 225 individuals per generation, on average, with six standard bottles per replicate line (standard bottles are 90 × 55 mm shell bottles containing 40 ml of culture medium). Thus both H and C lines shared the same thermal environment excepting the heat-stress selection treatment.

#### 2.2. Measurement and analysis of heat-stress resistance

After the last generation of heat-stress selection (i.e. the 50th generation from the start of the experiment, but the 25th generation of heat-stress selection), resistance to a heat stress (39 °C for 31 min without acclimation) was measured in 4 d-old adults. Experimental individuals were reared at a density of 35 2-h-old-larvae per vial at 25 ± 1 °C. The test was performed with 22 flies per vial and seven vials per sex and replicate line (22 flies × 7 vials × 2 sexes × 6 lines = 1848 flies). For analysis, the number of survivors in each vial (our unit of replication) was expressed as a proportion to which the arcsine square-root transformation was applied (Krebs et al., 1998). Heat-stress resistance was analyzed with an ANOVA by using heat-stress selection (i.e. H vs. C) and sex as fixed factors, and replicate within type of line as random factor.

#### 2.3. Life span and longevity selection

Experimental individuals were reared under standardized conditions of larval density at 25 °C (35 larvae per standard vial; standard vials are 95 × 20 mm shell vials containing 6 ml of culture medium). Ten vials each containing 10 males and 10 females at 1 d old were set up for each replicate line under slight CO2 anesthesia. The longevity experiment was carried out at 25 °C and a 12 h light 12 h dark cycle. The flies were simultaneously transferred to fresh vials every two days, when all vials were examined for deaths. The number of vials was gradually reduced as deaths occurred, with adults being kept at a density as close to 20 per vial as possible, with the prevailing sex ratio. Longevity data (in days) were ln-transformed and subjected to an ANOVA by using heat-stress selection (i.e. H vs. C) as fixed factor and replicate within type of line as random factor. Because of interactions involving sex, ANOVAs for each sex are shown separately. Longevity was also analyzed with a non-parametric (log-rank) test, which uses the observed and expected death from each sampling interval to calculate a X² statistics (Miller, 1981; Partridge et al., 1995). In this case, tests were made by pairing H and C replicates according to the line numbers that were assigned arbitrarily to them at the start of the experiment, with all three P-values being then combined (Partridge et al., 1995 see Sokal and Rohlf, 1981 pp. 779). When the differences were opposite in sign, the overall P-value reported was obtained by using the distribution of chi (Roper et al., 1993), and corrected for multiple comparisons (Rice, 1989). Finally, the semiparametric Cox’s Proportional Hazards Regression (CPHR) model (Cox, 1972) was used to estimate heat-stress selection effects on mortality in each sex. Survival data were arranged as a life-table with a censoring variable to run
CPHR using the STATISTICA package (StatSoft, 1999). In CPHR, independent variables can be categorical, and the population under study may consist of subpopulations so that CPHR performs a stratified analysis to adjust for subpopulation differences (replicate lines in this study). A baseline survival curve (i.e. the survival curve of a hypothetical ‘completely average’ individual) is then systematically flexed up or down by each independent variable, and the method computes a coefficient \( B \) for each of them. In this study, a negative \( B \)-value for heat-stress selection indicates that H lines are associated with lower mortality because the codes used for heat-stress selection were 1 for C and 2 for H. Age-specific mortality rate was not analyzed because sample sizes larger than ours are required to test for differences in mortality rate (also see Driver, 2001 for caution against the use of the Gompertz function).

To select for long-lived individuals from both types of lines (H and C), aged flies were transferred to fresh vials every two days following the protocol described above. The vacated vials were stored to get offspring from a total of four successive (2 d)-intervals of sampling (Fig. 1). For each replicate line, offspring of long-lived flies were pooled in a F1-generation (40 flies per replicate line) by using similar numbers (9–10) of long-lived flies from each sampling interval. Selection intensity \( i' \) depends only on the selected proportion (Falconer 1989). Estimated \( i' \)-values were averaged across sampling intervals, sexes and replicates within type of line, following the procedure described in Norry and Loeschcke (2002b). To reduce any possible cross-generational effects (e.g. Hercus and Hoffmann, 2000; Gilchrist and Huey, 2001), the F1-generation was inter se crossed within each replicate line to obtain an F2-generation of long-lived flies on which Hsp70 expression was measured. That life span increased by this longevity selection was supported by an exploratory test of C lines at 29°C. This test temperature (as compared to 25°C) allowed us to quickly know whether life time increased after longevity selection. Briefly, all three C replicates were crossed both before and after longevity selection to obtain two mass populations, namely: C-BLS (an F2-hybrid mass population obtained by crossing all the three C lines before longevity selection), and C-ALS (an F2-hybrid mass population obtained by crossing all three C lines after longevity selection). Forty vials each containing a single pair (25°C-reared flies at 1 d old) were setup for both C-BLS and C-ALS populations at the same time. Vials were examined for male’s deaths every day, and mean longevity (± SD) at 29°C was measured in males: 27.02 (6.85) in C-BLS and 32.40 (6.60) in C-ALS, the difference being highly significant (\( P < 0.005 \), log-rank test).

2.4. Heat-treated samples for measurement and analysis of Hsp70 expression

Experimental individuals were reared under standardized conditions of larval density (25 larvae/vial), aged to 4 d old and heat-treated at either 35 or 37°C for 45 min. We measured the subsequent expression of Hsp70 induced 1 h after each induction treatment for both C and H lines (heat-treated flies were immediately kept at 25°C for 1 h before being stored at −70°C). This was done simultaneously in all lines for both the F2 of long-lived flies and the F1 of young (6–7 d-old) flies (the latter representing the population mean, because at that stage one did not know if they were short- or long-lived individuals). The Hsp70 expression was measured in five independent trials (our unit of replication) of enzyme-linked immunosorbent assay (ELISA), using a whole-animal assay (15 homogenised flies; see Dahlgaard, et al. 1998 for a detailed description of our ELISA conditions). Expression of Hsp70 was measured relative to a standard control lysate made from heat-stressed adults, using the antibody 7.FB (Velazquez et al., 1980; 1983).

For analysis, Hsp70 expression data (% of standard) were ln-transformed and subjected to a multi-way, partially-hierarchical ANOVA, separately for each sex. Heat-stress selection (i.e. \( H_i \) vs. \( C_i \) lines), longevity selection, and
induction temperature (hereafter referred to as IT), are fixed factors. Replicate within stress selection is a random factor.

To explore the relationship between mean longevity and Hsp70 regulation we obtained spline surfaces by regressing mean longevity (in ln-day) on Hsp70 expression induced to both 35 and 37 °C. Data points are mean values for each replicate line before longevity selection. For males, data from ELISA shown in Fig. 2 were used. However, a new ELISA was performed for females (before longevity selection, in five independent trials in the same ELISA conditions described above), using a standard of higher expression than that used to test for differences before and after longevity selection. This allowed us to fit the spline surface within a range of 50–100% of standard in both sexes. Mean values from the ELISA performed to fit spline surface in females are (for C1, C2 C3, H1, H2, H3, respectively): 62.66, 60.42, 64.52, 62.67, 60.26, and 52.41 for IT of 35 °C; 76.63, 75.61, 84.70, 98.00, 94.65, and 84.28 for IT of 37 °C; SE values are within the same range as in Fig. 2).

All analyses were performed using the STATISTICA package (StatSoft, 1999).

3. Results

The lines had diverged after 25 generations of heat-stress selection. Survival to our experimental conditions of heat stress was, on average, 56% higher in H males than in C males and 38% higher in H females than in C females (Table 1). Indeed, the difference between H and C was significant for heat-stress resistance: (1) MS_H vs. C = 1.678, $F_{(1,4)} = 8.526^*$; (2) MSSEX = 0.170, $F_{(1,4)} = 1.591$; (3) MSREPLICATES WITHIN LINE = 0.197, $F_{(4,72)} = 3.522^*$; MSINTERACTION 1x2 = 0.079, $F_{(1,4)} = 0.735$; MSINTERACTION 2x3 = 0.107, $F_{(4,72)} = 0.056$; *$P < 0.05$ (see M&M. for ANOVA model). Life span at 25 °C tended to be higher in H lines than in C lines (Fig. 1). Although borderline significant in ANOVA, the difference in mean longevity between H and C was, on average, higher than...
Table 1
Mean resistance (proportion alive ± SD) to a heat stress (39 °C for 31 min without acclimation), and mean longevity (ln-days ± SD) of males and females at 25 °C are shown for heat-stress-selected (S) and control lines (C). Bold typeface indicates marginal mean values (± SD) for lines.

<table>
<thead>
<tr>
<th>Line</th>
<th>Resistance to heat stress</th>
<th>Longevity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Males</td>
<td>Females</td>
</tr>
<tr>
<td>C</td>
<td>0.333 (0.268)</td>
<td>0.509 (0.168)</td>
</tr>
<tr>
<td>C2</td>
<td>0.714 (0.222)</td>
<td>0.602 (0.212)</td>
</tr>
<tr>
<td>C3</td>
<td>0.798 (0.139)</td>
<td>0.648 (0.278)</td>
</tr>
<tr>
<td>C</td>
<td>0.615 (0.210)</td>
<td>0.586 (0.219)</td>
</tr>
<tr>
<td>H</td>
<td>0.933 (0.275)</td>
<td>0.955 (0.154)</td>
</tr>
<tr>
<td>H2</td>
<td>0.941 (0.201)</td>
<td>0.787 (0.175)</td>
</tr>
<tr>
<td>H3</td>
<td>1.003 (0.332)</td>
<td>0.681 (0.314)</td>
</tr>
<tr>
<td>H</td>
<td>0.959 (0.269)</td>
<td>0.808 (0.214)</td>
</tr>
</tbody>
</table>

The heat-induced Hsp70 expression tended to be higher in H lines than in C lines (see white bars, Fig. 2). Although the effect of heat-stress selection was non-significant in the overall ANOVA shown in Table 3, this effect was significant in an ANOVA for females before longevity selection (white bars, Fig. 2), using stress selection (i.e. H vs. C) and induction temperature (IT), as fixed factors, and replicates within lines as a random factor (MSH vs. C = 0.307, F1,4 = 9.37; MSIT = 0.982; F1,4 = 117.21; *P < 0.05; ***P < 0.005).

There was a significant interaction between stress selection and IT in males (Fig. 2; Table 3). The regression surface that relates mean longevity to the heat-inducible level of Hsp70 at different ITs provides an exploratory figure of the relationship between longevity and Hsp70 regulation (Fig. 3): high longevity corresponds to down-regulation at low IT (35 °C) but high expression at higher IT (37 °C).

We found the heat-induced Hsp70 expression in adult males is consistently decreased in males of the F2 of long-lived flies in both H and C lines (Fig. 2; Table 3). This longevity selection response was highly significant in males but only marginally so in females (Table 3).

After 25 generations of heat-stress selection, the H lines, including their respective lines derived from long-lived flies, were maintained without heat-stress selection for another 25 generations. To reduce any possible effects of inbreeding, all three replicate lines were subsequently intercrossed to obtain both an F1 hybrid mass population (i.e. the 28th generation after the last generation of heat-stress selection) derived from the H lines before longevity

Table 2
Proportional hazards (Cox) regression coefficients (± SE) estimated by using heat-stress selection as predictor variable in an analysis stratified to adjust for among-replicate differences (see test for details). P-values were corrected for multiple comparisons by using the sequential Bonferroni method (Rice, 1989).

**Table 3**
ANOVA on ln-(Hsp70 expression) performed to test for effects of (1) heat-stress-selection treatment (i.e. H vs. C lines), (2) replicates within stress selection treatment, (3) longevity selection treatment, and (4) induction temperature, IT. Bold typeface indicates significant P-values.

**Table 3**
ANOVA on ln-(Hsp70 expression) performed to test for effects of (1) heat-stress-selection treatment (i.e. H vs. C lines), (2) replicates within stress selection treatment, (3) longevity selection treatment, and (4) induction temperature, IT. Bold typeface indicates significant P-values.
selection (H-BLS population) and an F3-hybrid mass population derived from the H lines after longevity selection (H-ALS population). Heat-induced Hsp70 expression was measured in females and compared between H-BLS and H-ALS populations. Expression of Hsp70 was found to be lower in the H-ALS than in the H-BLS population [35 °C-induced mean expression (± SE) is: 63.34 ± 1.87, for H-BLF; 56.77 ± 2.60, for H-ALF; t = 2.16, df = 28; P < 0.05]. Thus, the reduced level of Hsp70 expression has been maintained for at least 28 generations after a single generation of truncation selection for long-lived flies. This result is consistent with the observation that between-line differences in Hsp70 expression of D. melanogaster can persist during many generations of routine culture at 25 °C (Krebs et al., 2001).

4. Discussion

The results indicate that the heat-induced Hsp70 expression exhibits substantial additive genetic variation between samples taken before and after longevity selection. Twenty-five generations of heat-stress selection improved both heat-stress resistance and longevity in males but did not increase the 35 °C-induced Hsp70 expression in this sex (though a weak increase was apparent for the 37 °C-induced Hsp70 expression). Previous work showed that heat-stress resistance and Hsp70 expression are related but only weakly in wild-type D. melanogaster populations (e.g. Dahlgaard et al., 1998; Krebs et al., 1998). Here we show that Hsp70 expression in females seems to increase very weakly by heat-stress selection but certainly decreases consistently by selection for long-lived individuals (Fig. 2). Bettencourt et al. (1999) showed that other forms of heat selection (28 °C-laboratory natural selection) reduce rather than increase Hsp70 expression, with high-temperature lines expressing 34–40% less Hsp70 than do control lines in a range of inducing temperatures. Their results when combined with ours suggest that selection for heat tolerance and selection for extended life can have either similar or different consequences on Hsp70 regulation or expression depending on the thermal selection regime (e.g. 28 °C-laboratory natural selection vs. heat-stress selection, see also Sørensen et al., 1999).

Given that Hsp70 induction may have deleterious effects on either fecundity or fertility in Drosophila (Krebs and Loeschcke, 1994a,b; Silbermann and Tatar, 2000), it is not surprising that heat-selection regimes can often result in only a weak increase in Hsp70 expression. For instance, the apparently weak response to heat selection we found for the 37 °C-induced Hsp70 expression could be underestimated because of possible side-effects of Hsp70 on either fecundity or fertility (i.e. if there is a trade-off between fecundity and Hsp70, so that stress-selected individuals with higher Hsp70 expression contributed less to the next generation than did other stress-selected individuals with lower Hsp70 expression).

Although borderline significant in ANOVA, after 25 generations of heat-stress selection a moderate (13%, on average) increase in mean longevity was found in males. This beneficial effect of heat-stress selection was confirmed by both log-rank test and Cox-regression analysis. Other positive relationships between heat-stress resistance and
longevity have been reported in previous studies, including the finding of a heat-stress tolerant phenotype of single-mutations that extend the life span of the worm Caenorhabditis elegans (Kenyon et al., 1993; Lithgow et al., 1994, 1995; Larsen et al., 1995). In addition, heat-induced over-expression of Hsp70 increased longevity in D. melanogaster (Tatar et al., 1997; but see Minois et al., 2001; Minois and Vainberg, 2002), and non-lethal levels of heat-shock extended life span in C. elegans (Lithgow et al., 1995), D. melanogaster (Khazaeli et al., 1997), and yeast (Shama et al., 1998). The main finding in the present study is that decreased rather than increased heat-inducible Hsp70 expression is the outcome of selecting for long-lived individuals (Fig. 2).

With longevity selection regimes like the present, the more successful individuals will be those that having survived to very late ages are also the most fecund (Rose 1999; Promislow and Bugbee, 2000). Perhaps this selection could partly have resulted in a purging of deleterious alleles with side-effects on heat-stress resistance and Hsp70 expression. However, it was possible for C lines but not for H lines because H lines were heat-stress selected for many generations before the start of longevity selection (i.e., H lines were not allowed to maintain and/or accumulate mutations with deleterious effects at the 4 d-old-testing age). Moreover, there was no change in heat-stress resistance before and after longevity selection in H and C lines (results not shown for heat-stress tests of 39 °C for 31 min with and without 37 °C-acclimation for the same sample sources used to measure Hsp70 expression), a result that is not consistent with the above-indicated hypothesis of removal of generally deleterious alleles.

Regulated instead of constitutive expression of Hsp has evolved in all organisms, and we know now that dramatic and persistent changes in Hsp70 expression can result from selection on longevity (Fig. 2). An extension of the theory of the disposable soma of aging suggests that induced expression of genes that regulate the processes of somatic maintenance and repair, such as hsp70, either extends longevity or reduces age-specific mortality rates (Tatar et al., 1999a and references therein). We have not re-tested this hypothesis but the possibility of strong genetic relationships between longevity and Hsp70 expression. Because expression is induced, costs and trade-offs are predicted (Hoffmann and Parsons, 1991), and our present results show that the heat-induced Hsp70 expression is reduced dramatically by selecting for long-lived flies.

A mechanism of longevity selection that involves Hsp70 regulation becomes apparent when mean longevity is plotted against Hsp70 expression induced at different ITs (Fig. 3). Presumably, short-lived individuals are very susceptible to heat stress in terms of Hsp70 induction, showing a high inducible expression at a moderate IT of 35 °C (Fig. 3). Perhaps, individuals that can tolerate high-temperature stress do not need emergency defenses at the same degree as other individuals, remain more homeotic in their protective mechanisms in the presence of stress and live longer, showing very low Hsp70 expression at moderate IT (Fig. 3). This hypothesis explains why genetic changes in the heat-induced Hsp70 expression were consistent and in the direction observed after a single generation of truncation selection on longevity. Alternative hypotheses are not supported by the selection responses observed. Firstly, because protein thermal denaturation might induce Hsp70 expression, it could be argued that thermal stability of non-Hsp proteins could increase by longevity selection, thus reducing Hsp70 expression. However, this is not supported because the heat-induced Hsp70 expression also decreased dramatically by longevity selection in H lines. Since these lines were selected for many generations of heat-stress selection before the start of longevity selection, their level of protein thermal stability should be close to an optimum (otherwise the trait may not be heritable, Bettencourt et al., 1999). There is no significant interaction between heat-stress (H vs. C) selection and longevity selection (interaction term 1 £ 3, Table 3), even though Hsp70 expression was measured at the same age as heat-stress selection (4 d old). Therefore, we can conclude that the very strong response to longevity selection we found in Hsp70 expression cannot be explained by the hypothesis of thermal stability of non-Hsp proteins. Secondly, another hypothesis that could be alternative to the Hsp70-regulation hypothesis is that longevity selection reduced the Hsp70 copy number, resulting in the patterns observed. However, no variation in Hsp70 copy number has been found on wild type chromosomes of D. melanogaster (e.g. Bettencourt et al., 1999; an extra copy was found but only in mutant stocks, B. Bettencourt, pers. comm). In addition, Hsp70 copy number is unaffected by experimental evolution (Bettencourt et al., 1999). Therefore, it is quite unlikely that only one generation of longevity selection reduced the Hsp70 copy number in both types of lines (H and C, Fig. 2). Thirdly, another trivial explanation for the lowered Hsp70 levels is that the F2 of long-lived flies are merely smaller, produce less total protein and thereby possibly less Hsp70. However, short-term longevity selection has no impact on body size in D. melanogaster (Norry and Loeschcke, 2002a and references therein). Thus, Hsp70 regulation rather than other possible correlated traits is implied in the longevity selection response we found in Hsp70 expression. Recently, Bettencourt et al. (2002) showed that Hsp70 alleles that differ in promotor/regulatory regions respond consistently to both natural and laboratory thermal selection in D. melanogaster. Flies can also express Hsps during aging (e.g. Heydary et al., 1994; King and Tower, 1999), but Hsp70 has a cost in reproduction (e.g. Krebs and Loeschcke 1994a,b). Thus, down-regulation of Hsp70 can also be a plausible consequence of selection of individuals that having survived to very late ages are also the most fecund during aging. However, it should be noted that both Hsp70 expression and fecundity strongly decrease with age
Heat-shock proteins such as Hsp70 acute tolerance to both internal and external stresses (Lindquist, 1986, 1993; Loeschcke et al., 1994; Morimoto, 1994; Parsell and Lindquist, 1993; Wheeler et al., 1995, Krebs and Loeschcke, 1994a,b; Tatar et al., 1997; Dahlgaard et al., 1998; Feder and Hofmann, 1999), but there are also negative consequences of Hsp70 over-expression for several fitness components (e.g. Krebs and Loeschcke, 1994a,b; Krebs and Feder, 1997; Silbermann and Tatar, 2000). If long-lived individuals remain more homeoetic in the presence of stress (e.g. if they have a higher temperature threshold for Hsp70 induction than short-lived individuals, Fig. 3), they might often avoid the costs of inducing the heat-shock response. Previous work showed that fecundity is reduced by heat-inducing Hsp70 expression at temperatures that can routinely be experienced by flies in nature (e.g. Krebs and Loeschcke, 1994a,b; Silbermann and Tatar, 2000). Therefore, Hsp70 can affect fitness in different and complex ways, and may have subtle underlying effects on life-history evolution (e.g. Feder and Krebs, 1997; Krebs and Loeschcke, 1994a,b; Tatar, 1999a,b; Silbermann and Tatar, 2000). In addition to such multiple effects of this protein, its inducible expression decreases by selecting for long-lived individuals, and as such can contribute to the evolution of senescence and other aspects of fitness.

Acknowledgements

We thank Trine Gammelgaard for technical assistance, Stuart (J.S.F.) Barker, Robert A. Krebs and Miriam Hercus for helpful comments on the manuscript, The Institute for Advanced Study and the Center for Environmental Stress and Adaptation Research at La Trobe University are greatly acknowledged for their hospitality to VL. This work was supported by the Carlsberg Foundation and the Danish Natural Sciences Research Council, which partially supported F.M.N.’s stay in Denmark. F.M.N.’s stay was also greatly supported by Consejo Nacional de Investigaciones Científicas y Técnicas (Argentina).

References


