Acclimation and Selection for Increased Resistance to Thermal Stress in *Drosophila buzzatii*

Robert A. Krebs and Volker Loeschcke

Department of Ecology and Genetics, University of Aarhus, 8000 Aarhus C, Denmark

Manuscript received April 10, 1995
Accepted for publication October 30, 1995

ABSTRACT

Direct selection for increased resistance to a heat shock (41.9° for 90 min) was carried out using two replicate lines of *Drosophila buzzatii* that were derived from a large base population. Selected individuals were first acclimated to high temperature before selection, while control individuals were acclimated but not selected, and selection was performed every second generation. Resistance to heat shock with acclimation increased in selected lines. Without acclimation, a correlated smaller increase in heat-shock resistance was suggested. Survival of males was higher than that of females in all lines when tested with acclimation, but with direct exposure to high temperatures, survival of females was greater than that of males both in selection and control lines but not in the base population. From analysis of reciprocal cross progeny between lines, one selection line was found to possess a dominant autosomal factor that significantly increased resistance of males much more than resistance of females. Also suggestive was recessive traits on the *X* chromosome in both selection lines that increased thermotolerance. No cytoplasmic effects were found. After accounting for other effects, survival of F_1 flies was intermediate, suggesting that additive variation is present for one or more of the autosomes.

VARIATION for resistance to thermal stress is expected where individuals inhabit heterogeneous environments (DOBSON *et al.* 1989; HOLT 1990; HOFFMANN and PARSONS 1991). Genetic differences for performance of individuals at different temperatures have been commonly observed and may be related to the thermal environment of origin in Drosophila (TIMOFEEFF-RESSOVSKY in DOBZHANSKY 1937, p. 155; HOSGOOD and PARSONS 1968; LOESCHCKE *et al.* 1994; PARSELL and LINDQUIST 1994). The presence of additive genetic variation for heat-stress resistance has also been shown by significant positive responses to selection in *D. melanogaster* (MORRISON and MILKMAN 1978; KILIAS and ALAHIOTIS 1985; HUEY *et al.* 1992), *D. simulans* (JENKINS and HOFFMANN 1994) and *D. subobscura* (QUINTANA and PREVOSTI 1990), as well as in wasps (WHITE 1970) and bacteria (LEROI *et al.* 1994).

Physiological adaptation to environmental changes of a shorter duration than the generation time of the species requires plastic responses, primarily through the ability to acclimate to the new conditions. The alternative is to tolerate the ecological change without a physiological response, which could place individuals under stress if conditions become extreme. Most organisms can acclimate to high temperatures at least in part by the well known heat shock response, which is induced following exposure to temperatures 10–15° above the thermal optimum of the species (LINDQUIST 1986). However, genetic differences in acclimation responses were not identified in HOFFMANN and WATSON (1993) or in LOESCHCKE *et al.* (1994), although CAVICCHI *et al.* (1995) obtained variation among lines that had been held for many years at different constant temperatures.

Lines of Drosophila maintained under different thermal regimes for many generations may vary in survival after exposure to extreme temperatures (ALAHIOTIS and STEPHANOU 1982; HUEY *et al.* 1991; CAVICCHI *et al.* 1995), but the causes of variation may be due to different genetic and nongenetic factors. STEPHANOU *et al.* (1983) suggested that cytoplasmic factors were at least partly responsible for changes in heat shock resistance of *D. melanogaster,* while CAVICCHI *et al.* (1995) found effects due to variation on all three major chromosomes, but none due to cytoplasm. JENKINS and HOFFMANN (1994) found that offspring of *D. melanogaster* and *D. simulans* mothers exposed to heat withstood high temperatures longer before being "knocked down" than offspring of parents held at 25°, but as the effect was not passed on to the F_2 generation, they ruled out cytoplasmic inheritance. Following selection for resistance and susceptibility to short-term high temperature exposures, thermal sensitivity variation in *D. melanogaster* was localized to a factor on chromosome *2* (MORRISON and MILKMAN 1978). A natural heat-sensitive variant also was localized to a region of this chromosome (OUDMAN 1991). The heat shock proteins in *D. melanogaster* cluster on chromosome *3* (ASHBURNER and BONNER 1979), but genes that influence heat shock protein production may occur elsewhere in the genome (PARKER-THORNBURG and BONNER 1987).

Corresponding author: Robert A. Krebs, Department of Organismal Biology and Anatomy, University of Chicago, 1027 East 57th St., Chicago, IL 60637. E-mail: rkrebs@midway.uchicago.edu

Because of the well known ecology of the cactophilic *D. buzzatii* (BARKER and MULLEY 1976; BARKER *et al.* 1984), we used this species to study the genetics of high temperature tolerance. Two lines of *D. buzzatii* were subjected to direct selection for increased resistance to high temperature after acclimation to a potentially lethal thermal stress. By using direct selection, the quality of the survivors will affect their contribution to future generations and enable selection to act on many traits other than survival. Thereby, a greater level of selection will be imposed than that predicted from the proportion transferred to produce the next generation. Further, acclimation treatments were used to focus selection on resistance to the highest stresses to which individuals can withstand and still reproduce, as well as to examine the possibility of increasing resistance in acclimated individuals. Because high temperatures can reduce fecundity (KREBS and LOESCHCKE 1994a), two control lines were acclimated identically, as were the selection lines, but were not exposed to the potentially lethal stress, to better compare selection on survival after exposure to heat shock. A base population was maintained at 25°.

The goals of this study were, first, to show that selection on acclimated individuals can increase thermal resistance, second, to examine correlated changes in heat resistance without acclimation after selection with acclimation, and, third, to use crosses between lines to examine the genetic bases of these changes. Selection was performed every second generation to enable population sizes to recover, to reduce maternal effects on heat resistance, and to increase the potential opportunity for recombination to produce more resistant variants. The large numbers of individuals required to obtain sufficient numbers of offspring each selection generation and the desire to compare F_1 progeny from crosses between all lines led us to use only two replicate selection and control lines. Single generation analyses focused upon patterns of variation among parental lines and in their F_1 progeny, which were informative of the basis of genetic variation in heat-shock resistance in these lines.

MATERIALS AND METHODS

Selection: *D. buzzatii* were collected on southwestern Tenerife, Canary Islands (lat. 28°10′N), in December, 1992. Twenty-two wild-caught females were placed individually in vials of instant Drosophila medium (Carolina Biological Supply Co.) with one wild-caught male each. Approximately 10 progeny from each female were mixed to form a base population. This base population was maintained and expanded to 60 bottles of medium through four generations to collect flies for initiation of two selection lines. Each line, designated S1 and S2, was prepared from ~2000 adults. Here and in all following experiments, selection was performed only on virgin adults with 20 individuals per vial. When individuals were 3- to 4-days-old, they were acclimated to high temperature by exposure to 38° for 75 min (see KREBS and LOESCHCKE 1994a for details) and heat shocked 18–20 hr after being acclimated by exposure to 41.9° (incubator temperature) for 90 min within vials that contained agar medium and a moistened stopper to provide nearly saturated humidity. Use of virgins guaranteed that subsequent generations were produced from matings between treated individuals.

Survivors were the flies in each vial that could walk 24 hr after exposure to the stress, although in the initial generation (generation 0 in Figure 1) we found that flies that could barely walk died soon after. In all subsequent generations, selected individuals were required to walk easily, and although some died, scoring was more consistent. Surviving flies were placed within bottles of instant Drosophila medium at 25° and transferred to new bottles every 3 days until larvae were observed. About 10 days were required to obtain larvae following the first generation of selection, and the larvae present were at very low densities in the medium in this generation following selection. The total number of offspring produced by the survivors was insufficient for a new round of selection. Therefore, selection was performed every other generation.

Control lines (C1 and C2) were maintained with ~100 males and 100 females each generation, and these were reared in five bottles, ~20 pairs of adults per bottle. Virgin individuals from these lines were exposed to the acclimation treatment every second generation but were not heat shocked. There was no mortality in these lines due to acclimation to heat, and offspring production was not visibly reduced. Therefore, larval densities in control bottles were higher than those of the selection lines. However, control adults were given only 2 days to oviposit, as were selected adults in the relaxed generation, to keep larval densities low to moderate. A sample from the control lines (~500 per line) was collected in all selection generations, and they were heat shocked along with the selection lines to observe progressive divergence of lines (Figure 1), which was recorded for each generation as mean resistance of selection 1 (S1) minus control 1 (C1) and S2 minus C2.

During treatment, the stress level imposed likely varied among incubator trials within and across generations probably due either to slight variation in the final temperature reached or the rate temperature increases within the incubator (KREBS and LOESCHCKE 1994b,c), although the same "set" conditions were used. A greater stress may have contributed to only six females surviving to produce offspring in generation 3 in line S2. An extra generation therefore was required before enough flies were available to continue selection, and the selection 2 line (S2) lagged one generation behind S1 (Figure 1). After the first generation of selection, ~700–1000 virgin adult offspring of each selection line were collected, 20 per vial, for each subsequent round of selection. Probably ~10% contributed offspring to subsequent generations, and this proportion increased in later generations as resistance developed.

Changes during selection: Heat shock resistance of the base population (BP) was compared to that of the selection (S1, S2) and control (C1, C2) lines after nine selection generations. As in previous generations, virgin males and females were collected for each line and, at age 4 days, were exposed to 41.9° incubator temperature for 90 min. Additional flies of all lines and the base population were exposed to the stress without first being acclimated to determine whether selection lines still required acclimation to survive the stress. Survival without acclimation was below 1%, and these flies were discarded after scoring.

Resistance without acclimation was analyzed again following the tenth selection generation. Virgin males and females of each line (C1, C2, S1, S2) were collected and, at age 4 days, were exposed to 41.5° incubator temperature (reduced from 41.9° to increase the proportion surviving) for 90 min without first being acclimated to heat shock. Flies were heat

shocked in three blocks of replicates with six vials per sex and line in each block.

Crosses among lines: Six sets of reciprocal crosses were made among the four parental lines after selection generation 10, with one additional generation required without selection before analysis. Each cross (S1 × S2, S1 × C1, S1 × C2, S2 × C1, S2 × C2, C1 × C2) was made with 15 pairs of males and virgin females per bottle with two bottles per cross. The flies were transferred to new bottles after 2 and 4 days. Adults were discarded after 8 days. From each of these crosses and from parental lines, virgin adults of the same age were collected and exposed to 41.9° for 85 min 24 hr after first being acclimated, which differed from the usual 41.9° for 90 min 18 hr after first being acclimated. The stress method was modified to facilitate handling the larger number of flies. Twelve blocks of replicates were prepared, and in each block all crosses between lines were represented by one vial per sex and each of the parental lines by two replicates per sex, *i.e.*, 40 vials per block. All vials contained ~20 flies that were 4- to 5-days-old when heat shocked. Although the stress treatment was reduced by 5 min, surviving adults of the selection lines were used to continue selection. Additionally, survivors of the reciprocal crosses between the selection lines, now selected one generation since mixing lines, were also maintained. This hybrid line was designated H.

Retest of acclimation: Resistance to heat shock with and without the acclimation treatment was retested following the eleventh selection generation to compare these treatments when all individuals were collected under identical conditions. Virgin males and females of each parental line (C1, C2, S1, S2) were collected along with the hybrid progeny of the selection lines (line H) derived from the survivors after reciprocal crosses between lines S1 and S2 the previous selection generation. Because differences in resistance to heat shock were so large between acclimated and nonacclimated flies, adults were exposed to 41.5° incubator temperature for 90 min if not acclimated and to 41.9° for 90 min if acclimated 18 hr before exposure to the stress. Therefore, differences in survival between treatments could not be compared directly, as resistance of all lines would be much greater if acclimated than if not acclimated, regardless of the proportion surviving. For each treatment, acclimated and not acclimated, experiments were run as three blocks of replicates each containing five vials per sex for each line.

Statistical analysis: The initial test of significance of the effect of selection was made using a simple regression model for the difference in mean survivorship between each selection line and its control. This measurement was not likely to be affected greatly by random variation among blocks of replicates unless survivorship in those blocks approached 0 or 1, which would obscure differences. Such a result occurred once and the block was eliminated from the analyses. This method also reduces the problem of correlated survivorship between selected and control lines within generations due to the random variation among blocks of replicates, but *P* values, as a test of selection, may become biased by autocorrelation across generations if genetic drift affects results. Therefore, every effort was made to keep population sizes in lines large, which would enable effects to be attributable to the selection regime with greater certainty. Effects of the first generation of selection were not included because the scoring technique varied from that used subsequently, with very weak individuals judged to be alive. These died within a day. The omission of the first selection episode may have made the statistical test for selection more conservative. The response of males and females to selection was analyzed separately.

In all other analyses, the proportion of individuals surviving in each vial was arcsine-square-root-transformed, although results were very similar if actual survival values were used. Block effects were defined, and interactions between main effects and block were examined in preliminary analyses, *e.g.*, line × block and sex × block (results not shown). These effects were found not to be significant. Therefore, all interaction effects that included block were pooled in the error component of variance. The GLM procedure of SAS (SAS 1989) was used to compare effects on survival due to treatment (selection or control), line within treatment, sex, and block. Because there were only two lines in each treatment (and hence two denominator degrees of freedom), analytical power for a test of selection in single generation experiments was low. For reciprocal crosses between lines C1, C2, S1 and S2, expected differences were not random, as the two control lines and the two selection lines were predicted to be more similar. Therefore, specific types of crosses were analyzed individually, and consistency of an effect was judged by combined probability analysis.

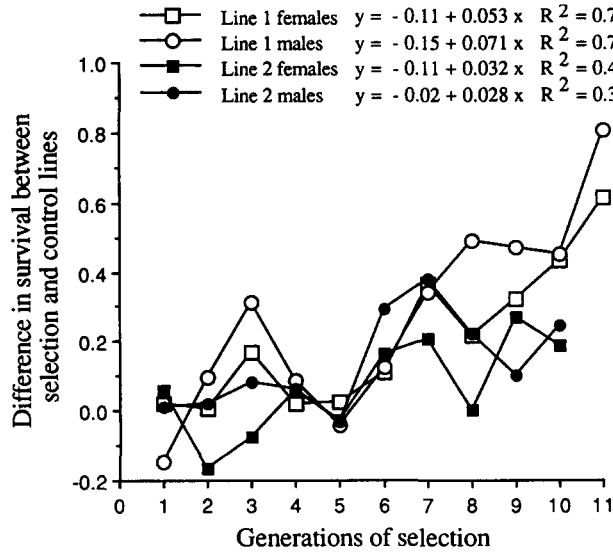

FIGURE 1.—Divergence in resistance to thermal stress (41.9° for 90 min) in acclimated males and females following direct positive selection. Presented are differences between two selection and two control lines for mean survival to the stress each generation, and the simple regression equations that predict the effect of selection generation on the difference between lines. Results for males and females are presented separately. All means were determined from ≥12 replicate vials that contained ~20 flies each. A recovery generation was provided between each selection generation, and all survivors in selection lines were used to produce the following generation of offspring. Individuals from the two control lines were acclimated every second generation but not otherwise selected.

RESULTS

Effects of selection: Differences in resistance to thermal stress between each selection line and its control line are shown in Figure 1, with the means presented after that selection generation. The differences between selection and control lines increased in both lines and was significant in both males and females of line 1 ($P < 0.001$) and for females of line 2 ($P < 0.05$). The regression coefficient for the S2 males approached significance ($0.05 < P < 0.10$). Therefore, an increase in

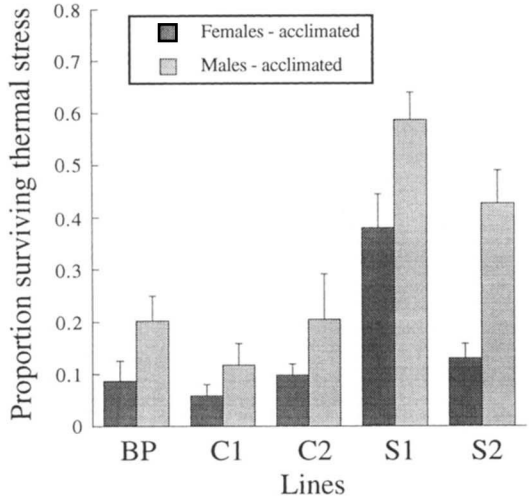

FIGURE 2.—The mean proportion (±SE) of males and females surviving exposure to 41.9° for 90 min for two selection lines after nine generations of selection, two control lines, and individuals of the base population. All flies first were acclimated to high temperatures before exposure to thermal stress.

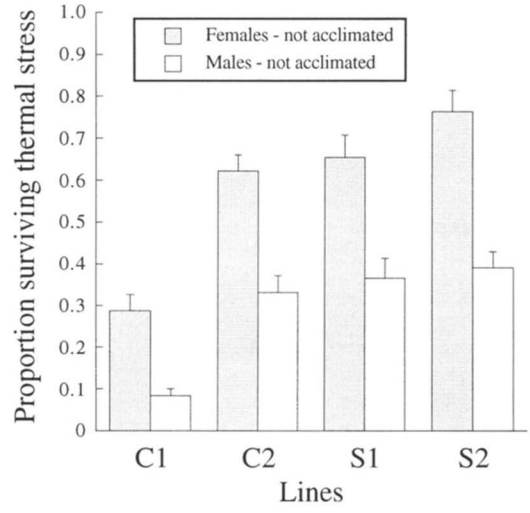

FIGURE 3.—The mean proportion (±SE) of males and females surviving exposure to 41.5° for 90 min without acclimation for two selection lines after 10 generations of selection and two control lines.

resistance to heat stress following selection was probable even if drift obscured statistical inference of P values. The negative estimates of the intercept may have been an effect of beginning analyses after one selection episode (due to the change in the scoring method thereafter) and some divergence between selection and control treatments at this generation.

Analyses of changes during selection: As Figure 1 presents only the differences between lines at each generation, mean survival after exposure of acclimated individuals to thermal stress are presented for each control and selection line, plus the mean for the base population, after nine generations of selection (Figure 2). Using a mixed-model nested design, treatment was found not to be significant in this ($F_{1,2} = 3.95$, ns) or any of the following single generation experiments presented. Significant differences were found among lines within treatments ($F_{2,144} = 16.2$, $P < 0.001$). However, significance of the selection treatment required the effect to be very much greater than variation between lines within treatments, a level that was obtained after the 16th selection generation (V. LOESCHCKE and R. A. KREBS, unpublished results). Survival of males was significantly higher than that of females, but the interaction between sex and treatment was not significant. Block effects also were significant and were partitioned as a source of variation. Mean resistance of the base population was not significantly different from either control line [using a three-line, fixed-factor analysis of variance (ANOVA)].

Following the 10th generation of selection, the two control lines and two selection lines were compared for mean survival without first being acclimated, but at a lower temperature to bring mean survival within the range (30–70%) where variances are approximately normal (Figure 3). Again treatment effects were not significant ($F_{1,2} = 1.90$), while the effect of lines within treatment was significant ($F_{3,136} = 34.8$, $P < 0.001$). t-tests among lines suggested only that the mean survival of the C1 line may be lower than that of all others. Again the effect of sex was significant, but for all lines mean survival of females was significantly higher than that of males where individuals were not acclimated to high temperature (sex effect, $F_{1,2} = 111$, $P < 0.01$). The interaction term, line × sex, was not significant.

Crosses among lines: Also following the 10th selection generation, the control lines, the selection lines and progeny of reciprocal F_1 crosses among these lines were compared for mean survival after heat shock with acclimation. The relative order of survival among parental lines was identical to that observed in the ninth selection generation; survival of S1 flies was highest followed by S2, C2 and C1 flies. Significant variation was present among these parental lines using a simple fixed factor ANOVA that contained no hypothesis of mechanism for divergence ($F_{3,175} = 85$, $P < 0.001$), and all groups differed significantly from each other when compared by Tukey's multiple comparisons test. This alternate comparison was made because how differences originated among lines was unimportant to the analysis of their genetic basis. For all lines, the proportion of acclimated males that survived was higher than that of acclimated females ($F_{1,175} = 75.5$, $P < 0.001$), and again the line × sex interaction term was not significant.

Survival of F_1 males from reciprocal crosses between the two selection lines was not different from that of males of the S1 (higher surviving) line, but F_1 male progeny differed significantly in survival from S2 males (Table 1). Survival of the F_1 females from the S1/S2

TABLE 1

Survival after heat shock for parental lines and their F_1 offspring

Male × female	Male survival	H_0:F_1 male = S1 male	H_0:F_1 male = S2 male
S1 × S1	0.96 ± 0.01		
S2 × S2	0.75 ± 0.04		
S1 × S2	0.96 ± 0.02	NS	0.0005
S2 × S1	0.93 ± 0.03	NS	0.003
Male × female	**Female survival**	**H_0:F_1 female = S1 female**	**H_0:F_1 female = S2 female**
S1 × S1	0.77 ± 0.04		
S2 × S2	0.64 ± 0.04		
S1 × S2	0.71 ± 0.07	NS	NS
S2 × S1	0.57 ± 0.09	0.035[a]	NS
Male × female	**Male survival**	**H_0:F_1 male = C1 male**	**H_0:F_1 male = C2 male**
C1 × C1	0.51 ± 0.04		
C2 × C2	0.65 ± 0.04		
C1 × C2	0.45 ± 0.08	NS	NS
C2 × C1	0.55 ± 0.08	NS	NS
Male × female	**Female survival**	**H_0:F_1 female = C1 female**	**H_0:F_1 female = C2 female**
C1 × C1	0.30 ± 0.04		
C2 × C2	0.37 ± 0.04		
C1 × C2	0.30 ± 0.06	NS	NS
C2 × C1	0.27 ± 0.07	NS	NS

Crosses were between the two selection lines (S1 and S2) and between the two control lines (C1 and C2). Presented is mean survival (±SE) separately for males and females, although statistics are for arcsine-square-root-transformed data.

[a] Combined probability that hybrid females differ from S1, NS.

TABLE 2

Survival of F_1 males and females between selection lines and controls

	Male × Female		P for H_0: means for crosses are equal
	S1 × C1	S2 × C1	
Males	0.75 ± 0.06	0.57 ± 0.06	0.034
Females	0.34 ± 0.06	0.39 ± 0.07	NS
	C1 × S1	C1 × S2	
Males	0.79 ± 0.06	0.68 ± 0.08	NS
Females	0.39 ± 0.07	0.39 ± 0.06	NS
	S1 × C2	S2 × C2	
Males	0.76 ± 0.06	0.67 ± 0.09	NS
Females	0.45 ± 0.07	0.48 ± 0.07	NS
	C2 × S1	C2 × S2	
Males	0.91 ± 0.04	0.78 ± 0.07	0.067
Females	0.48 ± 0.07	0.44 ± 0.07	NS

Mean survival (±SE) is presented, although statistics for differences between the selected line used in crosses (horizontal comparisons) are on arcsine-square-root-transformed data. Differences in means due to *X*-chromosome or cytoplasmic effects can be made by vertical comparisons between the four reciprocal crosses, with mean differences between reciprocal crosses for male offspring of 10.3 ± 2.6%, and for female offspring, 1.0 ± 2.3%. Combined probability males: $P < 0.05$.

cross was more similar to that of the S2 females, but combined probability analysis revealed no significant differences between female offspring of the two reciprocal crosses and S1 females (Table 1). Survival of F_1 progeny from crosses between control lines was more similar to that of the C1 (lesser surviving) line but was not significantly different from that of either C1 or C2 individuals (Table 1).

Comparison of survivorship of the F_1 progeny between the selection and control lines indicated several patterns of inheritance of thermal stress resistance. First, for each of the four reciprocal crosses between selection and control lines, survival of male offspring was higher when the female parent was from one of the selection lines than when the female parent was from one of the control lines, *i.e.*, where F_1 males possessed the selected *X* chromosome. These comparisons can be derived from the means presented in Table 2. The effect was significant ($P < 0.05$) only for the cross between S1 and C2 flies, although differences between reciprocal crosses were similar among all four comparisons. Mean survival was 10.3 ± 2.6% higher for males possessing the selected *X* chromosome than for those with an *X* chromosome from one of the control lines. Female progeny with female parents from one of the selection lines had an average survival that was not significantly different from progeny with female parents from a control line (means from Table 2).

Second, the rank order of differences among progeny was related to differences between the parental control lines. Progeny of crosses between either selection line and C2 (the higher surviving control) had an average 8.4 ± 1.3% higher rate of survival to a heat shock than progeny of crosses between the selection lines and C1 (eight of eight comparisons, although none were individually significant).

Third, male progeny had higher survival if the parental line was S1 rather than S2 in all four comparisons of reciprocal crosses between selection lines and controls (Table 2). This result was independent of whether the S1 parent was male or female. Comparison of survival of female offspring from crosses between S1 or S2 individuals and controls indicated that differences were small and not significant.

Retest of acclimation: Results for the retest of line and sex differences in survival to heat shock with and without acclimation, performed after the 11th generation of selection, are shown in Figure 4. As before, differences between these groups were included in Fig-

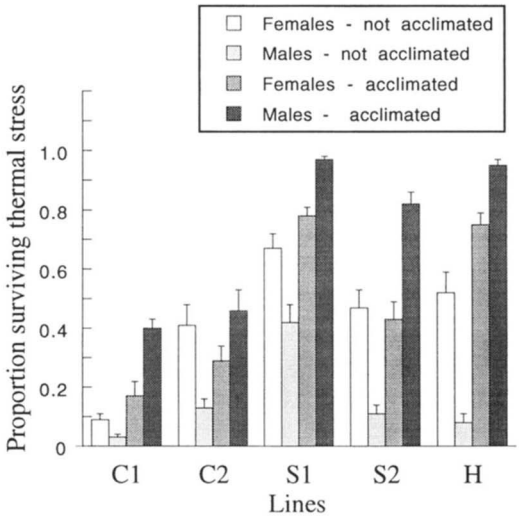

FIGURE 4.—The mean proportion (±SE) of males and females surviving exposure to thermal stress for two selection lines and two control lines after 11 generations of selection, and a hybrid line (H) that was derived by pooling surviving offspring of reciprocal crosses between the selection lines (from experiments on "crosses among lines"). Individuals either received an acclimation treatment and were exposed to 41.9°, or they were not acclimated and they were exposed to 41.5° with both temperature treatments for 90 min. Therefore, differences in survival between treatments cannot be compared directly, as resistance is much higher with acclimation.

ure 1. Five lines were compared in this analysis, S1, S2, C1 and C2, and also hybrid offspring (H) selected one generation after being produced from reciprocal crosses between the two selection lines. Because block effects were significant ($P < 0.01$) and nested within treatments, results with and without acclimation of individuals were analyzed separately. The single generation test of the effect of selection was not significant, but the direct effect using acclimation suggested substantially greater divergence among treatments ($F_{1,2} = 7.86$, which is also the ratio of divergence of lines between treatments to divergence of lines within treatments) than the indirect effect, testing differences without acclimation ($F_{1,2} = 2.5$). Pairwise comparisons of lines indicated that with acclimation, survival after thermal stress was significantly higher in the S1 and H lines, which were not different from each other, than in the S2 line. Resistance to thermal stress in these three lines was significantly higher than that of both control lines, which also were not different from each other. Without acclimation, survival was significantly higher in the S1 line than in all others, while the H, S2 and C2 lines, which were not significantly different, were significantly higher in survival than the C1 line (individual comparisons not shown).

The effect of sex on resistance again was highly significant (Figure 4), as for all lines survival of females following heat shock without acclimation greatly exceeded that of males ($F_{1,2} = 20$, $P < 0.05$), while the reverse was true where flies were acclimated, with survival of males much greater than that of females ($F_{1,2} = 74$, $P < 0.05$). This result was not found in two tests of the base population where survival to heat shock without acclimation was not significantly different between males and females. The first test was when selection lines were at generation seven, using a stress of 41.5° for 100 min (male survival, 10.3 ± 4.3%; female survival, 5.5 ± 2.9%), and the second test was after generation 11, using a stress of 41.5° for 90 min (male survival, 33.3 ± 3.6%; female survival, 39.4 ± 6.1%). Sex × line effects on survival were significant both with and without acclimation, but these effects may be attributed to smaller differences between males and females when survival either was very high (S1 and H lines with acclimation) or very low (the C1 line without acclimation).

DISCUSSION

Resistance to high temperatures increased slowly and continuously in lines subjected to selection leading to significant divergence from control lines. After 10+ generations, variation in survival after exposure to thermal stress was about eight times higher between selected and control lines than was the variation within these treatment groups, but with only two lines in each, this difference cannot be ascribed with certainty to the effect of selection. However, the high labor cost of direct selection on heat resistance, requiring ~3000 virgin individuals per selection episode, prevented our using more lines, which is generally required to conclusively separate effects of selection and drift. Despite this limitation, large enough differences were obtained among lines to enable them to be used for clarification on the basis of genetic variation in high temperature resistance.

Rapid change following selection for stress resistance has been observed by HUEY *et al.* (1992) in *D. melanogaster,* but slow divergence between selected and control lines was observed by MORRISON and MILKMAN (1978), again in *D. melanogaster,* and by QUINTANA and PREVOSTI (1990) in *D. subobscura.* These studies all used family selection techniques and carried out selection on non-acclimated individuals. Because the proportion of males and females that survive heat stress differs and fertility of *D. buzzatii* also declines following exposure to high temperatures (KREBS and LOESCHCKE 1994a), the selection differential for our lines could not be estimated. The slow divergence between lines was at least suggestive that heritability of resistance to heat stress in acclimated *D. buzzatii* individuals is low.

One concern with selection on an inducible trait, as for heat-shock resistance, is that individuals may begin to express the trait in the absence of an acclimation treatment. Following selection for survival to a desiccation stress without acclimation, HOFFMANN and PARSONS (1989) and HOFFMANN (1990) observed that a heat pretreatment increased resistance to desiccation

in *D. melanogaster* control lines but not in the lines selected for resistance. For *D. buzzatii* that had been given an acclimation treatment before exposure to stress, the heat-shock response remained inducible following strong selection for increased resistance. Costs of inducible resistance (HOFFMANN 1995) likely constrain populations from expressing the trait constitutively.

Although the acclimation treatment increases survival of individuals relative to those that are not acclimated, exposure of lines to an acclimation treatment alone did not lead to any increase in resistance to heat shock. Resistance in one control line decreased relative to that of the base population, which had been maintained at 25° and never encountered higher temperatures. Therefore, the change in the control line may have occurred from genetic drift or inbreeding, although inbreeding effects on resistance to thermal stress in *D. buzzatii* (DAHLGAARD *et al.* 1995) are not large, and control lines were maintained with 100 males and 100 females per generation. Only in the S2 selection line was inbreeding possibly a problem, where one generation bottlenecks occurred twice. These bottlenecks may have prevented the S2 line from reaching the same level of resistance as the S1 line, in which the population size never fell below 100.

Selection was not performed on the acclimation response directly but was designed to increase the resistance of acclimated males and females. The change, however, differed for the two sexes. For selected and control lines, males survived better than females among acclimated flies, and females better than males without acclimation. As acclimation improved survival of both sexes, the change was not due to damage of females by the acclimation treatment, and in a previous analysis of unselected lines, males generally performed better than females both with and without acclimation (LOESCHCKE *et al.* 1994). HUEY and BENNETT (1990) and HOFFMANN and BLOWS (1993) predicted that trade-offs with acclimation could constrain the evolution of resistance, because acclimation invokes physiological costs, at least for fecundity of *D. melanogaster* females (KREBS and LOESCHCKE 1994b). Males of that species showed no significant fitness consequences following acclimation (KREBS and LOESCHCKE 1994c). Costs of increasing the inducible response in females may have contributed to their lesser increase in resistance, or perhaps to a decrease in the ability to acclimate, a result that was supported by the change in control lines as well as in selected lines, but not in the base population.

Crosses between the two selection lines and the two control lines confirmed genetic differences among lines for resistance to thermal stress, and their bases may be explained by specific patterns of dominant, recessive and additive effects rather than by overdominance or masking of recessive deleterious alleles. For example, differences between C1 and C2 may be due to a dominant trait increasing susceptibility to heat stress, as both male and female progeny from crosses between C1 and C2 were more similar to the lower surviving line (C1).

Recessive, or at least partly recessive, *X*-chromosome effects may have contributed to variation in resistance to thermal stress. Survival of male progeny that carried the selected *X* chromosome was consistently higher than that of progeny with the control *X*. This result was similar for crosses with both the S1 and S2 lines. *X*-chromosome effects could not account for differences between either the two selection lines or the two control lines, because male progeny of reciprocal crosses between S1 and S2 or between C1 and C2 were not very different in survival.

Analysis of differences between the selection lines gave a surprising result. Male offspring from this cross were equal in resistance to the higher surviving line (S1), while survival of females was more similar to the lower surviving line (S2). An autosomally inherited variant may have occurred in the S1 line that increases survival after acclimation to thermal stress of males only or increases acclimation of males much more than that of females. Support came from dominance of the S1 line for male survival whether the S1 line was the male or female parent, while characteristics important to survival of females were similar in the two selection lines. Survival of male offspring from reciprocal crosses between selection lines and controls also was consistently higher in crosses with the S1 line than the S2 line, while for all comparisons between S1 and S2 individuals crossed with controls, survival of female offspring was similar. The presence of this genetic variant in the S1 line may be the major difference between the two selection lines, which showed much smaller differences in females than males for survival after thermal stress.

The different types of changes that occurred with selection for resistance to thermal stress support a proposal by FISHER *et al.* (1992) that multiple biochemical mechanisms influence survival after heat stress. The sex differences for resistance to thermal stress with acclimation, which were maintained following strong selection but were reversed when tested without acclimation, must be due to responses of males and females in different ways. Sex differences can occur through genetic variation on the *X* chromosome, as recessive alleles favoring resistance that segregate on the *X* chromosome will be expressed more often in males than females, causing mean survival of males to be higher. Conversely, segregating recessive alleles for a temperature-sensitive phenotype could lead to lower resistance of males than females. However, when resistance alleles become fixed, as they should under strong selection, resistance of males and females should have become more similar unless different physiological mechanisms exist in male and female *D. buzzatii* for surviving the stress.

Further support for multiple systems contributing to variation in resistance to thermal stress comes from the large differences among lines for survival with acclima-

tion, while selection lines differed little from controls without acclimation after nine selection generations (Figure 3). Two selection generations later, the higher-surviving selection line (with acclimation) had diverged from the others in resistance without acclimation, while the S2 line did not (Figure 4). Interestingly, the hybrid selection line had survival similar to the higher line with acclimation, while survival without acclimation was more like the S2 line and controls. The most likely explanation is that lines selected after acclimation to high temperatures initially respond via changes in an inducible system. After a first response is obtained, which may vary between males and females due to constraints, further changes may occur in physiological systems that are not inducible by heat.

The physiological system most commonly proposed as being important to organisms for survival to extremes of heat is the inducible heat-shock response (LINDQUIST 1993). A number of proteins are produced in much greater concentrations after only a short exposure to high temperatures than at physiologically normal temperatures. The experimental conditions that induce their production are very similar to those inducing acclimation responses that increase resistance to thermal stress in living organisms and tissue cultures (ASHBURNER 1982; PARSELL and LINDQUIST 1994). This response also occurs in nature (SPOTILA *et al.* 1989; FEDER 1996). In *Drosophila* the 70-kD heat shock protein is the one predominantly produced following exposure to high temperatures (DIDOMENICO *et al.* 1982a,b), and *Drosophila* embryos from lines that differ only for the number of copies of these genes differ for their acclimation speed and resistance to thermal stress (WELTE *et al.* 1993). Therefore it is likely that the heat-shock response has an important role for increasing survival to high temperatures in nature. However, the possible contribution of interactive physiological systems, one that is an inducible defense system and another that may be either structural or constitutively expressed, requires further study.

We thank DOTH ANDERSEN, CAMILLA HÅKANSSON and BIRGIT SØRENSEN for assistance with data collection and ARY HOFFMANN, FREDDY CHRISTIANSEN, RAY HUEY, DAVE PARKER and STUART BARKER for critical comments of the manuscript. This research was supported by grants from the Carlsberg Foundation (No. 93-0280-30) and the Danish Natural Science Research Council (No. 11-0533-01 and 94-0163-1).

LITERATURE CITED

ALAHIOTIS, S. N., and G. STEPHANOU, 1982 Temperature adaptation of *Drosophila* populations. The heat shock protein system. Comp. Biochem. Physiol. **73B:** 529–533.

ASHBURNER, M., 1982 The effect of heat shock and other stress on gene activity: an introduction, pp. 1–10 in *Heat Shock,* edited by M. ASHBURNER, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

ASHBURNER, M., and J. J. BONNER, 1979 The induction of gene activity by heat shock. Cell **17:** 241–254.

BARKER, J. S. F., and J. C. MULLEY, 1976 Isozyme variation in natural populations of *Drosophila buzzatii.* Evolution **30:** 213–233.

BARKER, J. S. F., P. D. EAST, H. J. PHAFF and M. MIRANDA, 1984 The ecology of the yeast flora in necrotic *Opuntia* cacti & of associated *Drosophila* in Australia. Microb. Ecol. **10:** 379–399.

CAVICCHI, S., D. GUERRA, V. LA TORRE and R. B. HUEY, 1995 Chromosomal analysis of heat-shock tolerance in *Drosophila melanogaster* evolving at different temperatures in the laboratory. Evolution **49:** 676–684.

DAHLGAARD, J., R. A. KREBS and V. LOESCHCKE, 1995 Heat-shock tolerance and inbreeding in *Drosophila buzzatii.* Heredity **74:** 157–163.

DAVID, J. R., R. ALLEMAND, J. VAN HERREWEGE and Y. COHET, 1983 Ecophysiology: abiotic factors, pp. 105–170 in *The Genetics and Biology of Drosophila,* Vol.3d, edited by M. ASHBURNER, H. L. CARSON and J. N. THOMPSON, JR., Academic Press, London.

DIDOMENICO, B. J., G. E. BUGAISKY and S. LINDQUIST, 1982a The heat shock response is self-regulated at both the transcriptional and posttranscriptional levels. Cell **31:** 593–603.

DIDOMENICO, B. J., G. E. BUGAISKY and S. LINDQUIST, 1982b Heat shock and recovery are mediated by different translational mechanisms. Proc. Natl. Acad. Sci. USA **79:** 6181–6185.

DOBSON, A., A. JOLLY and D. RUBENSTEIN, 1989 The greenhouse effect and biological diversity. Trends Evol. Ecol. **4:** 64–68.

DOBZHANSKY, T., 1937 *Genetics and the Origin of Species.* Columbia University Press, New York.

FEDER, M. E., 1996 Ecological and evolutionary physiology of stress proteins and the stress response: the *Drosophila melanogaster* model, pp. , in *Phenotypic and Evolutionary Adaptations to Stress,* edited by I. A. JOHNSTON and A. F. BENNETT, Cambridge University Press, Cambridge, UK (in press).

FISHER, B., P. KRAFT, G. M. HAHN and R. L. ANDERSON, 1992 Thermotolerance in the absence of induced heat shock proteins in a murine lymphoma. Cancer Res. **52:** 2854–2861.

HOFFMANN, A. A., 1990 Acclimation for desiccation resistance in *Drosophila melanogaster* and the association between acclimation responses and genetic variation. J. Insect Physiol. **36:** 885–891.

HOFFMANN, A. A., 1995 Acclimation: increasing survival at a cost. Trends Evol. Ecol. **10:** 1–2.

HOFFMANN, A. A., and M. W. BLOWS, 1993 Evolutionary genetics and climatic change: will animals adapt to global warming? pp. 165–178 in *Biotic Interactions and Global Change,* edited by P. M. KAREIVA, J. G. KINGSOLVER and R. B. HUEY, Sinauer, Sunderland, MA.

HOFFMANN, A. A., and P. A. PARSONS, 1989 Selection for increased desiccation resistance in *Drosophila melanogaster:* additive genetic control and correlated responses for other stresses. Genetics **122:** 837–845.

HOFFMANN, A. A., and P. A. PARSONS, 1991 *Evolutionary Genetics and Environmental Stress.* Oxford Science Publications, New York.

HOFFMANN, A. A., and M. WATSON, 1993 Geographical variation in the acclimation responses of *Drosophila* to temperature extremes. Am. Nat. **142:** S93–S113.

HOLT, R. D., 1990 The microevolutionary consequences of climate change. Trends Evol. Ecol. **5:** 311–315.

HOSGOOD, S. M. W., and P. A. PARSONS, 1968 Polymorphism in natural populations of *Drosophila* for the ability to withstand temperature shocks. Experientia **24:** 727–728.

HUEY, R. B., and A. F. BENNETT, 1987 Phylogenetic studies of coadaptation: preferred temperatures *vs.* optimal performance temperatures of lizards. Evolution **41:** 1098–1115.

HUEY, R. B., and A. F. BENNETT, 1990 Physiological adjustments to fluctuating environments: an ecological and evolutionary perspective, pp. 37–59 in *Heat Shock Protein in Biology and Medicine,* edited by R. I. MORIMOTO, A. TISSIÉRES and C. GEORGOPOULOS. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

HUEY, R. B., L. PARTRIDGE and K. FOWLER, 1991 Thermal sensitivity of *Drosophila melanogaster* responds rapidly to laboratory natural selection. Evolution **45:** 751–756.

HUEY, R. B., W. D. CRILL, J. G. KINGSOLVER and K. E. WEBER, 1992 A method for rapid measurement of heat or cold resistance of small insects. Funct. Ecol. **6:** 489–494.

JENKINS, N. L., and A. A. HOFFMANN, 1994 Genetic and maternal variation for heat resistance in *Drosophila* from the field. Genetics **137:** 783–789.

KILIAS, G., and S. N. ALAHIOTIS, 1985 Indirect thermal selection in *Drosophila melanogaster* and adaptive consequences. Theor. Appl. Genet. **69:** 645–650.

KREBS, R. A., and V. LOESCHCKE, 1994a Response to environmental change: genetic variation and fitness in *Drosophila buzzatii* follow-

ing temperature stress, pp. 309–321 in *Conservation Genetics,* edited by V. LOESCHCKE, J. TOMIUK and S. K.. JAIN, Birkhäuser Verlag, Basel, Germany.

KREBS, R. A., and V. LOESCHCKE, 1994b Costs and benefits of activation of the heat shock response in *Drosophila melanogaster.* Funct. Ecol. **8:** 730–737.

KREBS, R. A., and V. LOESCHCKE, 1994c Effects of exposure to short-term thermal extremes on fitness components in *Drosophila melanogaster.* J. Evol. Biol. **7:** 39–49.

KREBS, R. A., and V. LOESCHCKE, 1995 Resistance to thermal stress in adult *Drosophila buzzatii:* acclimation and variation among populations. Biol. J. Linn. Soc. (in press).

LEROI, A. M., R. E. LENSKI and A. F. BENNETT, 1994 Evolutionary adaptation to temperature. III. Adaptation of *Escherichia coli* to a temporally varying environment. Evolution **48:** 1222–1229.

LINDQUIST, S., 1986 The heat-shock response. Annu. Rev. Biochem. **55:** 1151–1191.

LOESCHCKE, V., R. A. KREBS and J. S. F. BARKER, 1994 Genetic variation for resistance and acclimation to high temperature stress in *Drosophila buzzatii.* Biol. J. Linn. Soc. Lond. **52:** 83–92.

MORRISON, W. W., and R. MILKMAN, 1978 Modification of heat resistance in *Drosophila* by selection. Nature **273:** 49–50.

OUDMAN, L., 1991 A locus in *Drosophila melanogaster* affecting heat resistance. Hereditas **114:** 285–287.

PARKER-THORNBURG, J., and J. J. BONNER, 1987 Mutations that induce the heat shock response of *Drosophila.* Cell **51:** 763–772.

PARSELL, D. A., and S. LINDQUIST, 1994 Heat shock proteins and stress tolerance, pp. 457–494 in *The Biology of Heat Shock Proteins and Molecular Chaperones,* edited by R. I. MORIMOTO, A. TISSIÉRES and C. GEORGOPOULOS. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

PARSONS, P. A., 1979 Resistance of the sibling species *Drosophila melanogaster* and *D. simulans* to high temperatures in relation to humidity: evolutionary implications. Evolution **33:** 131–136.

QUINTANA, A., and A. PREVOSTI, 1990 Genetic and environmental factors in the resistance of *Drosophila subobscura* adults to high temperature shock 2. Modification of heat resistance by indirect selection. Theor. Appl. Genet. **80:** 847–851.

SPOTILA, J. R., E. A. STANDORA, D. P. EASTON and P. S. RUTLEDGE, 1989 Bioenergetics, behavior and resource partitioning in stressed habitats: biophysical and molecular approaches. Physiol. Zool. **62**: 253–285.

STEPHANOU, G., S. N. ALAHIOTIS, C. CHRISTODOULOU and V. J. MARMARAS, 1983 Adaptation of *Drosophila* to temperature: heat-shock proteins and survival in *Drosophila melanogaster.* Dev. Genet. **3:** 299–308.

WELTE, M. A., J. M. TETRAULT, R. P. DELLAVALLE and S. LINDQUIST. 1993 A new method for manipulating transgenes: engineering heat tolerance in a complex multicellular organism. Current Biol. **3:** 842–853.

WHITE, E. B., P. DEBACH and J. GARBER, 1970 Artificial selection for genetic adaptation to temperature extremes in *Aphytes lingnanensis* (Hymenoptera: Aphelinidae). Hilgardia **40:** 161–192.

Communicating editor: L. PARTRIDGE