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CHROMOSOMAL ANALYSIS OF HEAT-SHOCK TOLERANCE IN *DROSOPHILA MELANOGASTER* EVOLVING AT DIFFERENT TEMPERATURES IN THE LABORATORY

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Abstract.—We investigated the heat tolerance of adults of three replicated lines of *Drosophila melanogaster* that have been evolving independently by laboratory natural selection for 15 yr at "nonextreme" temperatures (18°C, 25°C, or 28°C). These lines are known to have diverged in body size and in the thermal dependence of several life-history traits. Here we show that they differ also in tolerance of extreme high temperature as well as in induced thermotolerance ("heat hardening"). For example, the 28°C flies had the highest probability of surviving a heat shock, whereas the 18°C flies generally had the lowest probability. A short heat pretreatment increased the heat tolerance of the 18°C and 25°C lines, and the threshold temperature necessary to induce thermotolerance was lower for the 18°C line than for the 25°C line. However, neither heat pretreatment nor acclimation to different temperatures influenced heat tolerance of the 28°C line, suggesting the loss of capacity for induced thermotolerance and for acclimation. Thus, patterns of tolerance of extreme heat, of acclimation, and of induced thermotolerance have evolved as correlated responses to natural selection at nonextreme temperatures. A genetic analysis of heat tolerance of a representative replicate population each from the 18°C and 28°C lines indicates that chromosomes 1, 2, and 3 have significant effects on heat tolerance. However, the cytoplasm has little influence, contrary to findings in an earlier study of other stocks that had been evolving for 7 yr at 14°C versus 25°C. Because genes for heat stress proteins (hsps) are concentrated on chromosome 3, the potential role of hsps in the heat tolerance and of induced thermotolerance in these naturally selected lines is currently unclear. In any case, species of *Drosophila* possess considerable genetic variation in thermal sensitivity and thus have the potential to evolve rapidly in response to climate change; but predicting that response may be difficult.

Key words.—Acclimation, climate warming, *Drosophila melanogaster*, heat hardening, heat tolerance, genetic analysis, induced thermotolerance, temperature.

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The prospect of global climate warming during the next century (Schneider 1993) is encouraging renewed interest in studies of the evolution of physiological adaptation to temperature (Holt 1990; Hoffmann and Blows 1993). Reliable predictions concerning whether and how natural populations might respond evolutionarily to increased ambient temperatures hinge on several key issues (Hoffmann and Parsons 1991; Hoffmann and Blows 1993; Huey and Kingsolver 1993; Lynch and Lande 1993). For example, is genetic variation adequate to permit a rapid evolutionary change in thermal sensitivity? Does selection for increased fitness at a given range of temperatures lead to genetically correlated responses in other traits? Does such selection influence performance at temperatures other than the selected ones (Stephanou and Alahiotis 1983; Bennett et al. 1990; Huey and Kingsolver 1993), body size (Anderson 1973; Cavicchi et al. 1991), tolerance of other physiological stresses (Service et al. 1985; Hoffmann and Parsons 1991), acclimation capacity (Hoffmann 1990; Loeschcke et al. 1994), or even life-history traits (Hoffmann and Parsons 1991)? If so, predicting the evolutionary response to selection will be complex (Arnold 1987; Hoffmann and Blows 1993). What genetic and mechanistic bases underlie evolutionary changes in thermal sensitivity?

Experimental studies of evolution in the laboratory are an expeditious way of addressing these issues (Rose et al. 1990). With respect to thermal sensitivity, two types of laboratory selection experiments have been used. Several studies have used artificial (truncation) selection for enhanced or for reduced tolerance to extreme cold (Tucić 1979; A. A. Hoffmann pers. comm. 1994) or to heat (e.g., White et al. 1970; Morrison and Milkman 1978; Stephanou et al. 1983; Quintana and Prevosti 1990; Huey et al. 1992). These studies generally suggest that tolerance to extreme temperature responds—sometimes rapidly—to selection. However, the genetic basis of heat tolerance often varies among stocks of *Drosophila* (Morrison and Milkman 1978; Stephanou and Alahiotis 1983; Quintana and Prevosti 1991).

A complementary approach involves "laboratory natural selection" (Rose et al. 1990), in which large populations are maintained for many generations in the laboratory at different (but nonextreme) temperatures, and are thus, in effect, subject to persistent natural selection in different thermal environments (Bennett et al. 1990). In *Drosophila*, such selection can change the thermal sensitivity of fitness traits at the selected temperatures (Mourad 1965; Kilias and Alahiotis 1985; Lints and Bourgois 1987; Cavicchi et al. 1989; Huey et al. 1991), the frequency of chromosomal inversions (van Delden and Kamping 1989), and survival of extreme heat (Alahiotis and Stephanou 1982; Stephanou and Alahiotis 1983; Huey et al. 1991).

Here we investigate some aspects of the thermal sensitivity of lines of flies (*D. melanogaster*) that have been experiencing laboratory natural selection at three different temperatures (18°C, 25°C, and 28°C) for 15 yr, much longer than the comparable populations that have been investigated to date (7 yr

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for Stephanou and Alahiotis 1983; 4+ yr for Huey et al. 1991). We studied several issues. First, have the stocks diverged in tolerance of extreme heat? If so, then natural selection at nonextreme temperatures has led to a genetically correlated shift in tolerance to extreme temperature. Second, are the patterns of inter-line differences in heat tolerance consistent at different rearing temperatures? Third, does a brief heat pretreatment enhance heat tolerance ("induced thermotolerance," "heat hardening"); and do the lines differ in the threshold temperature that induces thermotolerance? Fourth, are differences in heat tolerance associated primarily with cytoplasmic (Stephanou and Alahiotis 1983) or chromosomal factors (Morrison and Milkman 1978)? If the latter, does chromosome 3 (where heat stress proteins are concentrated) play a predominant role?

The evolution of several traits in these stocks has already been investigated. For example, these temperature lines are known to have diverged in body size and shape (Cavicchi et al. 1978, 1985, 1991), as well as in the thermal sensitivity of fitness traits (e.g., female fecundity and productivity; Cavicchi et al. 1989).

Materials and Methods

The Population Origin

The founding population was of an Oregon R laboratory strain that had been mass reared for at least 20 yr at a constant temperature of 18°C. This ancestral stock was then subdivided to found three new mass populations that were maintained for an additional 15 yr: the first (A) was maintained at the ancestral 18°C temperature, but the second (B) and third (C) were established at constant 25°C and constant 28°C, respectively. Thus, flies were subject to "laboratory natural selection" (Rose et al. 1990) at constant low, intermediate, or high temperatures. Eggs were obtained from each of 55 pairs of flies from the founding population and then pooled to initiate each new population. For the next generation, each of the three temperature lines was replicated using eggs laid from the same F_1 females. Thereafter, the replicate lines were maintained independently in discrete generations for 15 yr by randomly adding 10 pairs to each of four 250-ml bottles containing 60 ml of medium (thus 40 pairs/generation/replicate), and then allowing them to lay for 2 d (details in Cavicchi et al. 1985, 1989). Natural selection was observably strong during the first year at 28°C, as C females produced few progeny per generation (Cavicchi et al. 1985, unpubl. data). However, C females eventually achieved higher fecundity, egg viability, and productivity than A females in 18°C, 25°C, or 28°C (Cavicchi et al. 1989, unpubl. data). One replicate of the A population was lost accidentally 5 yr before this study and was replaced by subdividing the surviving A stock.

Measuring Heat Tolerance

To test the effects of developmental temperature on adult heat tolerance (Hoffmann and Watson 1993), we raised flies for at least one generation at a common temperature of 18°C, 25°C, or 28°C. Then approximately 50 adult flies (age = 4–5-d old) were restrained by sponge plugs to the bottom of weighted plastic vials (without food) and heat-shocked by submerging most of the vial in a water bath at 41°C for 30 min. The flies were then transferred to vials containing fresh food and were maintained at 25°C. The percentage of flies in each vial that survived (i.e., responded to touching with tweezers) was determined 24 h later. Females and males usually were considered together (but see below) because they survived similarly in replicated experiments at different shock temperatures (females, 63%; males, 64%). Three vials for each replicate line were tested in two experimental blocks for flies that developed at 25°C, and two vials per replicate line were tested in two blocks for flies that developed at 18°C and at 28°C. Mean values reported for each replicate line represent the average percentage survival among blocks within developmental temperature. Standard errors were first computed within-blocks for each replicate line and then averaged between blocks. Humidity, which can influence heat tolerance (e.g., Maynard Smith 1956), was not specifically controlled but was probably near saturation. In any case, blocking should control for potential between-block variation in humidity. Flies were exposed to anesthesia (ether), which has a slight effect on heat tolerance (Smith and Huey 1991), but generally they were exposed 1 d before testing.

Because the above flies were tested after only a single generation at 25°C, any observed differences in heat tolerance might be influenced by cross-generational effects (Jenkins and Hoffmann 1994). Consequently, before making all of the crosses for the chromosomal substitutions (below), we wanted to determine whether the differences between the A and C lines would persist even after five generations of rearing at 25°C. (We used five generations at 25°C because five generations are required to substitute chromosomes [four generations] and to produce sufficient flies for testing [one generation].) Although this multigenerational procedure standardizes cross-generational effects, it does, of course, incorporate five generations of "convergent" selection (Quintana and Prevosti 1990) to 25°C by the A and C lines.

Because the experimental design was incomplete and unbalanced (see table 1), we were unable to use full factorial designs and instead used a series of one-way designs for analysis. Moreover, because we expected that the means of treatments being compared would have a specific order (below), we were able to used "ordered heterogeneity" tests, which are more powerful in this case than traditional, unordered ANOVA (Rice and Gaines 1994). Consequently, because we expected ($H_{A,1}$) that percentage survival of a given selected line would increase with acclimation treatments of 18°C, 25°C, or 28°C [thus, survival (acclimation at 18) < survival (25) < survival (28)], we used a new ordered-alternative test (Rice and Gaines 1994) with data for Replicate 1 from each of the three temperature lines (Replicate 2 was not sampled at all acclimation temperatures; see table 1). This test proceeds by computing the product $r_s P_c$, where r_s is the Spearman's rank correlation between the observed ranking of the acclimation treatments and that prescribed on H_A, and P_c is the complement of the P-value (i.e., $1 - P$) derived from a conventional ANOVA on percentage survival. This product will be high (thus, reject H_0) if the Spearman's rank correlation is high and if the P-value is low.

To test the a priori hypotheses ($H_{A,2}$) that percentage sur-

TABLE 1. Effect of rearing temperature (18°C, 25°C, or 28°C for one generation) on survivorship % of A, B and C populations after heat-shock at 41°C for 30 min. Two independent replicated lines (R1 and R2) are considered, but only one replicate line was tested at a rearing temperature of 18°C. At a rearing temperature of 25°C, each replicate line was represented by three samples (c. 50 flies each) in each of two blocks; at rearing temperatures of 18° and 28°C, each replication had two samples in each of two blocks. Mean values are thus based on four (18°C, 28°C) or six (25°C) vials for each replicate line. Standard errors are the average of the standard errors computed on the within-block variance.

Populations	Replicate	Rearing temperature 18°C	25°C	28°C
18°C	R1	10.0 ± 4.3	32.0 ± 8.5	67.6 ± 5.7
	R2	—	29.3 ± 2.3	72.3 ± 3.8
	avg.	10.0	30.7	69.9
25°C	R1	34.0 ± 6.0	68.0 ± 1.9	13.0 ± 2.6
	R2	—	42.1 ± 7.0	16.3 ± 1.4
	avg.	34.0	55.1	14.7
28°C	R1	90.5 ± 5.7	84.5 ± 3.9	73.0 ± 11.0
	R2	—	80.0 ± 5.6	94.0 ± 1.0
	avg.	90.5	82.3	83.5

vival increases with selection temperature within a given acclimation treatment [survival (replicate 1, line A) = survival (2, A)] < [survival (1, B) = survival (2, B)] < [survival (1, C) = survival (2, C)], we again used ordered-alternative tests with replicates nested within lines for acclimation at 25°C and at 28°C, but a simple one-way ANOVA with (replicate 1 only) for an acclimation of 18°C (replicates 2 were not tested).

To test the global significance of the three tests each of $H_{0,1}$ (thus across three acclimation treatments) and $H_{0,2}$ (across three selection lines), we used a new combined *P*-value test that computes an overall *P*-value for a series of tests addressing the same null hypothesis (Rice 1990). Data reported here are expressed as raw percentages, but all statistical tests were validated using angular-transformed data.

Induced Thermotolerance

To determine whether the temperature lines differed in the threshold temperature that induces thermotolerance (heat hardening), we randomly selected one replicate line from each of the three temperature lines and then raised each for one generation at 25°C. When the experimental flies were approximately 3–5-d old, we gave them a short pretreatment (5 min) at one of a graded series of high (but nonlethal) temperatures, returned them to 25°C for 15 min, and then heat-shocked them at 41°C for 30 min. Additional experiments, in which one of the A replicates was given heat pretreatments of 10 or 30 min, did not enhance survival above heat pretreatment of only 5 min. Control flies from each line were treated similarly but given no heat pretreatment. Percentage survival was then scored as described above. Induced thermotolerance is detectable if the percentage survival of the heat-pretreated group is elevated relative to that of the control group.

Genetic Analysis of Heat Tolerance

To determine the contribution of chromosomes 2 and 3 to heat tolerance, we measured, for example, the heat tolerance of A flies in which chromosomes 2 or 3 were substituted from the C line, and vice versa. One replicate each from the A and the C populations was selected randomly for genetic analysis. The crossing scheme, which required four generations to obtain the appropriate genotypes, is shown in figure 1. The balanced stock used for chromosome transfer is heterozygous for large inversions, and all chromosomes have dominant markers that are lethal as homozygotes (Lindsley and Zimm 1990). The balanced chromosomes were initially introduced in the Oregon population (B stock) and maintained at 25°C. Thereafter, a cross was made between 20 females of the balanced (B) stock and 20 males of the A (or C) population. The two parental stocks (P1, P2) were derived through the same number of crosses by mating females (c in fig. 1) from the First Series with males (d) from the Second Series (fig. 1). Each of the final substitution lines possessed the same mixture of chromosome 1 (approximate ratio A : B : C = 1: 4:1) as well as of chromosome 4, the Y chromosome from the original balanced stock, and the cytoplasm from the B stock. The crossing sequence was performed at 25°C.

To determine the effect of the cytoplasm and of chromosome 1 on heat tolerance, we compared the heat tolerance of female and of male offspring from reciprocal crosses between the A and C lines. Similar heat tolerance between females would indicate no cytoplasmic effect. In this case, similar heat tolerance between males would also indicate no effect of chromosome 1. An additional test of the effect of cytoplasm involved a test stock that was balanced for all three major chromosomes but had either its own cytoplasmic background or that of the C line. To obtain the appropriate stocks for these experiments, males (or females) of the balanced stock (Basc; SM1/bw^{v1}; TM2/Sb) were crossed with females (or males) of the C line and then backcrossed three times with the balanced stock. Before being tested for heat tolerance, flies in these experiments were first anesthetized with ether and separated by sex 1 d before testing. Thus, flies used in these heat tolerance assays were exclusively one sex or the other.

The relative contribution of each chromosome to heat tolerance was estimated by comparing heat tolerance of flies with different genetic constitutions. For example, the average impact of chromosome 2 was determined as the average of the absolute difference in percentage survival of 2A/3A flies versus 2C/3A flies and of 2C/3C versus 2A/3C flies. Because the cytoplasm does not influence heat tolerance in these flies (see the Results), the average impact of chromosome 1 can be estimated simply by comparing the absolute difference in percentage survival of the males from reciprocal crosses between the A and C lines (see table 3).

RESULTS

Heat Tolerance

Heat tolerance (i.e., probability of surviving a heat shock) varied with developmental temperature, but the lines differed in their sensitivity to developmental temperature (table 1). Heat tolerance of the A line (18°C) was significantly influenced by developmental temperature (one-way ANOVA, $P < 0.001$): specifically, adult heat tolerance increased with developmental temperature (r_sP_c test, $P < 0.001$) as expected

	FIRST SERIES			SECOND SERIES		
	females		*males*	*females*		*males*
1.	SM1/bw^{v1} ; TM2/Sb	x	A/A; A/A	SM1/bw^{v1} ; TM2/Sb	x	C/C; C/C
2.	SM1/A ; TM2/A	x	bw^{v1}/A; Sb/A	SM1/C ; TM2/C	x	bw^{v1}/C; Sb/C
	a. A/A ; TM2/Sb			C/C; TM2/Sb		
	b. SM1/bw^{v1} ; A/A			SM1/b2^{v1} ; C/C		
	c. A/A ; A/A			C/C ; C/C		
	d. SM1/bw^{v1} ; TM2/Sb			SM1/bw^{v1} ; TM2/Sb		
	substitution of chromosome 2					
3.	SM1/bw^{v1} ; A/A	x	C/C ; TM2/Sb	SM1/bw^{v1} ; C/C	x	A/A ; TM2/Sb
4.	SM1/C ; TM2/A	x	bw^{v1}/c : Sb/a	SM1/A ; TM2/C	x	bw^{v1}/a ; Sb/C
	C/C; A/A			A/A; C/C		
	substitution of chromosome 3					
3.	A/A : TM2/Sb	x	SM1/bw^{v1} ; C/C	C/C ; TM2/Sb	x	SM1/bw^{v1} ; A/A
4.	SM1/A ; TM2/C	x	bw^{v1}/A ; Sb/C	SM1/C ; TM2/A	x	bw^{v1}/C ; Sb/A
	A/A ; C/C			C/C ; A/A		

FIG. 1. Crossing scheme for genetic analysis of heat tolerance in *Drosophila melanogaster* that had been evolving at different temperatures in the laboratory for 15 yr. Four generations of crossing are required for the chromosome substitutions. See Materials and Methods for details.

($H_{A,1}$, see Materials and Methods). Heat tolerance of the B line (25°C) was also higher after development at 25°C versus 18°C, but, surprisingly, was much lower after development at 28°C. Consequently, although survival varied significantly among developmental temperatures ($P = 0.01$), the observed ordering did not conform to the expectation that tolerance would increase with developmental temperature ($H_{A,1}$, $P = 0.70$). Finally, heat tolerance of the C flies (28°C) neither varied significantly among developmental temperatures ($P = 0.41$), nor was it correlated with developmental temperature ($P = 0.68$). The developmental plasticity of heat tolerance seems to have been lost evolutionarily in the C line (see also Threshold Temperatures for Induced Thermotolerance, below).

In general, heat tolerance within a given acclimation treatment increased with the selection temperature (table 1). Following development at either 18°C or 25°C, heat tolerance varied positively with selected temperature as predicted ($H_{A,2}$, both $P < 0.001$): thus, C flies had the highest heat tolerance, B flies were intermediate, and the A line had the lowest tolerance. However, after development at 28°C, the C line had only slightly higher heat tolerance than the A line; the B line had inexplicably low heat tolerance, relative both to the A and C flies and also to its own survival at the lower developmental temperatures of 18°C and 25°C. Nevertheless, heat tolerance among lines conformed overall to the expected ordering, though the significance level was marginal (r_sP_c test, $P < 0.05$). Independent replicate populations within each temperature line usually showed little or only modest differentiation in heat tolerance (table 1), suggesting that replicate lines generally responded similarly to the selection regimes.

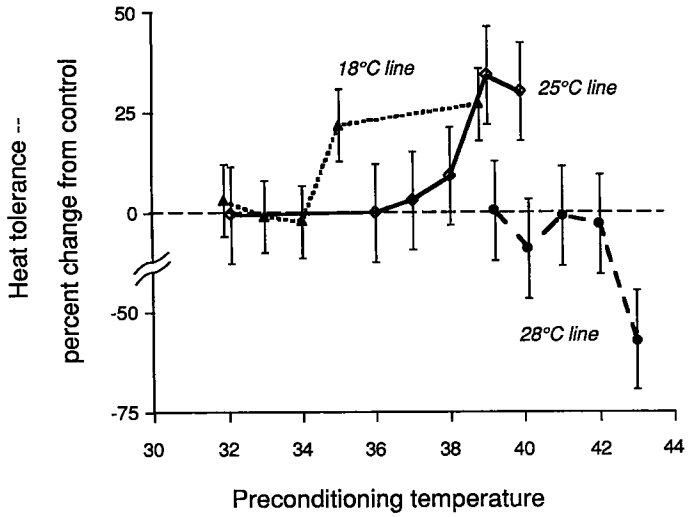

FIG. 2. Patterns of induced thermotolerance (survival relative to control: mean % change ± SE) following heat pretreatment at various temperatures. Flies that have been evolving at 18°C have a lower threshold for inducing thermotolerance than do flies evolving at 25°C. Flies evolving at 29°C seem to have lost the capacity for induced thermotolerance, but show increased thermosensitivity following heat pretreatment at 43°C.

An additional experiment corroborated the lower heat tolerance of the A line relative to the C line. Flies (three vials/line for each replicate line) were heat shocked at a graded series of temperatures after one generation at 25°C. The A flies had 100% survival at 39°C, 38% survival at 40°C, 13% survival at 41°C, and 0% survival at 42°C. In contrast, C flies had 100% survival at 40°C, 88% at 41°C, and 0% at 42°C. Thus, C flies were much more likely to survive a heat shock of 40°C or 41°C than were the A flies.

Because the above flies were tested after a single generation at a common temperature (25°C), heat differences among lines might be influenced by cross-generational effects (W. Crill pers. comm. 1993). Consequently, we compared heat tolerance of the A and C lines after five generations at 25°C (see Materials and Methods). C flies (Replicate 1 = 91.0 ± 3.0, R2 = 88.0 ± 3.0) remained much more tolerant of a 30-min heat shock at 41°C ($P < 0.001$) than were the A flies (R1 = 7.0 ± 3.0, R2 = 3.0 ± 2.0). In fact, the differences between lines appear even more pronounced than after only one generation at 25°C (see table 1).

Threshold Temperatures for Induced Thermotolerance

Induced thermotolerance (i.e., greater survival in the heat pretreated vs. control flies) in the A line occurred when the pretreatment temperature was 35°C or higher (fig. 2). In contrast, the B line showed no induced thermotolerance until the pretreatment temperature was raised to 39°C. Thus, the B line seemingly had a higher threshold temperature than the A line. In both lines, heat pretreatment improved survival by about 20% to 30% relative to the control flies. In the C line, however, no induced thermotolerance was detected at any temperature (fig. 2). Moreover, the C flies showed marked loss of thermotolerance ("thermosensitivity"; Yocum and Denlinger 1993) following a heat pretreatment of 43°C, such that they actually survived a subsequent heat shock much less well than did their control.

TABLE 2. Percent survival (± SE) after a heat-shock (41°C for 30 min) of the chromosome substitution lines. Mean values refer to three replicated experimental blocks, each with two vials per replicate line. P1 and P2 are the two parental stocks derived from the First and Second series of crosses (see fig. 1).

	Chromosome constitution		
	2	3	% survivorship
P1	A	A	38 ± 3.5
	C	A	75 ± 6.4
	A	C	57 ± 4.0
P2	C	C	83 ± 4.0
	A	C	64 ± 3.5
	C	A	59 ± 5.0

Genetic Analysis

Chromosomes from the C lines significantly enhanced heat tolerance relative to those from the A lines (table 2, substitution lines nested within series, $P < 0.001$). Chromosomes 2 and 3 both contributed to heat-shock tolerance (table 2, $P < 0.01$). The impact of chromosome 2 and 3 was clear in the P1 series: heat tolerance of flies with chromosomes 2A and 3A was substantially lower than that of flies with chromosomes 2C and 3A or with chromosomes 2A and 3C. Similarly, heat tolerance was higher in P2 flies with chromosomes 2C and 3C than with chromosomes 2A and 3C or with chromosomes 2C and 3A, although the impact was somewhat less strong than in the P1 series. The effect of chromosomes 2 and 3 was similar ($P > 0.05$, table 3) in P2 flies, but chromosome 2 appeared to contribute more to survivorship than did chromosome 3 ($P < 0.05$) in the P1 flies.

Heat tolerance was not influenced by the cytoplasm. Heat tolerance of hybrid females from reciprocal crosses between A and C populations was independent of line of the mother ($P > 0.05$, table 3). Moreover, heat tolerance of flies of the balanced stock was unaffected by balanced versus C cytoplasm (above).

Given that the cytoplasm has no detectable effect on heat tolerance (above), we can gain insight into the importance of chromosome 1 by comparing the heat tolerance of males from the reciprocal cross between A and C populations (table 3). Survival was much greater in males carrying a 1C chromosome than those carrying a 1A chromosome ($P < 0.01$). We did not test the role of chromosome Y. Males carrying chromosome 1A also carried YC, and those carrying 1C carry YA. If, however, the Y chromosome were to have a detectable

TABLE 3. Percentage survival (± SE) after heat-shock (41°C for 30 min) of male and of female progeny from reciprocal crosses between A and C populations. Mean values refer to three experimental blocks each with two vials per sex. Flies were crossed after one generation at 25°C.

Crosses	Females	Males
A × C	27 ± 3.7	8 ± 2.0
C × A	32 ± 5.0	31 ± 3.5

effect, then the difference in survivorship between males from reciprocal crosses would disappear or at least be very small.

Averaged across substitution lines, chromosomes 1, 2, and 3 appear to have had an equivalent impact on heat tolerance (table 4). In other words, substitution of either a 1C, 2C, or 3C chromosome each increased heat tolerance by a similar amount (22 to 28%: L.S.D. = ± 13).

Discussion

Heat Tolerance

Evolutionary divergence in heat-shock tolerance of replicated lines of an Oregon R stock of *Drosophila melanogaster* was marked after 15 yr of laboratory natural selection at 18°C, 25°C, and 28°C. For flies reared for one generation at either 18°C or 25°C, heat tolerance (41°C for 30 min) was greatest in the 28°C line, intermediate for the 25°C line, and lowest in the 18°C line (table 1). This pattern is seemingly not an artifact of a possible cross-generational effect: the 28°C flies still had much greater heat tolerance than did the 18°C flies even after five generations of "convergent" evolution at 25°C (above). Except for the 28°C line, which seems to have lost an acclimation response (table 1), replicate lines generally showed higher heat tolerance in response to higher rearing temperature. However, following rearing for one generation at 28°C, which is a high temperature for raising *D. melanogaster* (Cavicchi et al. 1985), the thermal tolerance of lines was 28°C >18°C > 25°C (table 1).

The observed differences among lines probably reflect evolution of heat tolerance primarily in the 25°C and 28°C lines. All of these lines were founded from a common ancestor that had already been evolving at 18°C for at least 20 yr and probably were well adjusted to that temperature. The 25°C and 28°C lines have also undergone many more generations of selection during the past 15 yr than have the 18°C lines. Moreover, selection was conspicuous immediately after the founding of the 28°C line (Cavicchi et al. 1985). About half of the founding isofemale lines became extinct within the first few generations at 28°C. Even so, some continued adaptation of the 18°C flies is likely. Several recent attempts to found new 25°C and 28°C populations directly from the 18°C line have failed: thus the 18°C flies may now be more specialized with respect to temperature than they were 15 yr ago. Similarly, fitness at 37°C continued evolving in *E. coli* even after 2000 generations of laboratory natural selection (Bennett et al. 1992).

How does the difference in heat tolerance between lines change with time? Comparing data from studies that used different stocks, selection temperatures, and heat-shock protocols is obviously risky, but a comparison of patterns is nevertheless of interest. After only 4+ yr of laboratory natural selection (Huey et al. 1991), United Kingdom flies evolving at 25°C had survival rates after heat shock that were only about 3% greater than those of flies evolving at 16.5°C. After 7 yr of natural selection (Stephanou and Alahiotis 1983), Greek flies evolving at 25°C had survival rates that were 27% greater than those of flies evolving at 14°C. After 15 yr of natural selection, our Oregon R flies evolving at 25°C had survival rates that averaged 24% greater than those of our 18°C flies (rearing temperatures of 18°C and 25°C only). These patterns encourage a study of the time course of evolutionary divergence in heat tolerance within replicated stocks.

Previous studies with *Drosophila* have demonstrated that laboratory natural selection at nonextreme temperatures is associated with evolutionary changes in tolerance of an extreme temperature (e.g., Stephanou and Alahiotis 1983; Huey et al. 1991), and our present findings are consistent with that observation. Thus, a genetic correlation (Arnold 1987) between performance at intermediate temperature and survival of extreme heat may be general for *D. melanogaster*. However, this pattern may not be universal. For example, laboratory natural selection in *E. coli* results in remarkably temperature-specific changes in fitness (Bennett et al. 1992; Bennett and Lenski 1993).

What mechanistic bases underlie the correlated effect of evolution of *Drosophila* at intermediate temperature on tolerance of extreme temperature? Our data are purely phenomenological and thus provide no direct evidence on potential mechanistic bases. Moreover, any suggestions are speculative because the physiological causes of mortality of *Drosophila* at extreme temperature are not even known. Nevertheless, increased heat tolerance might be associated with changes in inversion and allozyme frequencies (van Delden and Kamping 1989; Quintana and Prevosti 1991) or with increased enzyme thermostability (Heinrich 1981; Hochachka and Somero 1984). In addition, stocks evolving at warm temperature might maintain higher constitutive levels of heat stress proteins (see Koban et al. 1991) or be able to mobilize more heat stress proteins in response to heat shock, as suggested by studies of Alahiotis and Stephanou (1982).

Induced Thermotolerance

Brief exposure of organisms to a high but nonlethal temperature results in a transient increase in tolerance of extreme high temperature (induced thermotolerance, heat hardening) in many animals and plants (Hutchison and Maness 1979; Nover 1991). Differentiation in induced thermotolerance has not previously been studied in temperature-selected lines of *Drosophila*. However, the magnitude of induced thermotolerance did not differ among isofemale lines of *D. buzzatii* (Loeschcke et al. 1994).

Both the 18°C and the 25°C lines show induced thermotolerance, and the percentage increase in survival associated induced thermotolerance was similar in both groups (about 20% to 30%, fig. 2). The heat tolerance of the heat-pretreated 18°C flies increased roughly to the level of 25°C flies without heat pretreatment (table 1, for rearing at 25°C). Similarly, heat pretreatment increased the heat tolerance of the 25°C flies roughly to that of the 28°C flies without heat pretreatment. However, the threshold temperature that induces thermotolerance is lower in the 18°C flies than in the 25°C flies (35°C vs. 39°C; fig. 2).

Interestingly, 28°C flies did not respond to heat pretreatment (fig. 2) and have thus seemingly lost the ability to show induced thermotolerance. Of course, we cannot exclude the possibility that different (e.g., longer) pretreatments induce thermotolerance in the 28°C lines; but, as noted above, heat

TABLE 4. Contribution of chromosomes to survival of a heat-shock.

Chromosome			
1	2	3	% contribution
C			+23
	C		+37
		C	+19
	A		−19
		A	−24
Average contribution			
Chromosome 1			23
Chromosome 2			28
Chromosome 3			22

(L.S.D., ±13).

pretreatments of the A flies for 10 or 30 min did not increase survival relative to a heat pretreatment of only 5 min. Natural selection at 28°C therefore results in an evolutionary gain in tolerance of extreme high temperature, but in an evolutionary loss of an acclimation response (above, table 1) as well as of induced thermotolerance (fig. 2).

The mechanistic basis underlying the above evolutionary changes in induced thermotolerance would be of considerable interest. Heat shock ("stress") proteins would be a likely initial target for study (Lindquist and Craig 1988; Huey and Bennett 1990; Nover and Hightower 1991). For example, Greek flies evolving at 25°C had greater capacity for hsp 70 synthesis than did flies evolving at 14°C (Alahiotis and Stephanou 1982; Alahiotis 1983; Stephanou et al. 1983). Nevertheless, because chromosomes in addition to chromosome 3 (where hsp genes are concentrated) contribute to heat tolerance in our flies (below), more than heat-stress proteins must be involved.

Genetic Basis of Heat Tolerance

Chromosomes 1, 2, and 3 all appear to contribute about equally to heat tolerance, at least when averaged over genetic backgrounds (table 4). In contrast, the cytoplasm had little impact on heat tolerance either in reciprocal crosses between the 18°C and 28°C flies (females, table 3) or in flies with balanced chromosomes, but with cytoplasm from the 28°C stock (36 ± 3.4%) versus that from the balanced stock (36 ± 8.0%).

The clear importance of chromosomal factors to heat tolerance in our study contrasts with findings of Stephanou and Alahiotis (1983), who analyzed the heat tolerance of flies from Greece that had evolved at 14°C versus 25°C for 7 yr. They found that the cytoplasm was primarily responsible for differences in heat tolerance, but that chromosomes 1, 2, and 3 were involved secondarily. The difference in patterns between these two studies might reflect differences in the selected temperatures (14°C and 25°C vs. 18°C and 28°C), stocks (Greece vs. Oregon R), selection duration (7 vs. 15 yr), or, of course, random factors.

The 18°C and 28°C lines used in the present study have previously been analyzed genetically (Cavicchi et al. 1989, 1991) with respect to body dimensions (wing, thorax, head, tibia size) and to fitness traits (number of offspring per pair, female fertility). Those comparisons were made after 8 yr of laboratory natural selection. Divergence in body dimensions was associated primarily with chromosomes 2 and 3 as well as the cytoplasm, whereas divergence in fitness traits was associated with chromosome 3 and with the cytoplasm. After 15 yr of natural selection (above), divergence in heat tolerance depended on chromosomes 1, 2, and 3, but seemingly not on the cytoplasm.

Implications for Evolution in Natural Populations

Concerns over global warming during the next century provide renewed impetus to understand the evolution of thermal sensitivity. Reliable predictions will require insight into several issues (see the introduction). Is the amount of genetic variation in thermal sensitivity adequate for a rapid response to selection? To what extent will genetic correlations potentially constrain or complicate the potential responses to selection? What will be the actual patterns of selection during climate change? Could behavioral compensation ameliorate the intensity of selection on thermal sensitivity?

Laboratory studies of evolution are a powerful way of addressing such issues over short time scales (Rose et al. 1990; Hoffmann and Parsons 1991; Hoffmann and Blows 1993). For example, both artificial and natural selection experiments provide a clear answer to the first question (above): all ectotherms studied thus far possess substantial genetic variation in thermal sensitivity and thus have the genetic potential to respond evolutionarily to climate change (e.g., White et al. 1970; Morrison and Milkman 1978; Tucić 1979; Stephanou and Alahiotis 1983; Lints and Bourgois 1987; Cavicchi et al. 1991; Huey et al. 1991; Bennett et al. 1992; Bennett and Lenski 1993; Hoffmann and Blows 1993; Hoffmann pers. comm. 1994; Loeschcke et al. 1994). Moreover, recent theoretical models suggest that this potential—if realized in nature—may profoundly influence the probability of population persistence (Huey and Kingsolver 1993; Lynch and Lande 1993). Nevertheless, laboratory studies are highly artificial: no natural population will experience repeated truncation selection each generation; nor will any natural population experience a sudden and persistent climate shift to a constant temperature.

Laboratory studies also provide some insight into the second question (above), concerning the significance of genetic correlations. For example, laboratory natural selection at different temperatures affects diverse organismal traits such as body size and shape, reproductive isolation, sterility, fecundity, developmental time, oviposition rhythm, allozyme and inversion frequencies, and heat tolerance (Mourad 1965; Anderson 1973; Cavicchi et al. 1985, 1989, 1991; Kilias and Alahiotis 1985; van Delden and Kamping 1989; Huey et al. 1991). Because so many traits respond (directly or indirectly) to temperature, any that short-term evolutionary response to climate change in nature will be difficult to predict (Arnold 1987; Hoffmann and Parsons 1991).

Laboratory studies do not, however, illuminate the final two questions (selection pressures, behavioral compensation). Unfortunately, few field studies have investigated selection on thermal sensitivity (e.g., Christian and Tracy 1981; Powers 1987). Moreover, although biophysical models of

heat flux readily predict the thermoregulatory consequences of various behavioral options, those models cannot predict which particular option(s) an animal might in fact use (c.f. Dunham 1993).

In conclusion, ectotherms have the genetic capacity to evolve to climate change. However, attempts to predict evolutionary trajectories will be substantially complicated by strong genetic correlations involving diverse traits and by lack of information on selection in nature and on the pattern of behavioral compensation.

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Literature Cited

Alahiotis, S. N. 1983. Heat shock proteins. A new view on the temperature compensation. Comparative Biochemistry and Physiology 75B:379–387.

Alahiotis, S. N., and G. Stephanou. 1982. Temperature adaptation of *Drosophila* populations. The heat shock proteins system. Comparative Biochemistry and Physiology 73B:529–533.

Anderson, W. W. 1973. Genetic divergence in body size among experimental populations of *Drosophila pseudoobscura* kept at different temperatures. Evolution 27:278–284.

Arnold, S. J. 1987. Genetic correlation and the evolution of physiology. Pp. 189–215 *in* M. E. Feder, A. F. Bennett, W. W. Burggren, and R. B. Huey, eds. New directions in ecological physiology. Cambridge University Press, Cambridge.

Bennett, A. F., and R. E. Lenski. 1993. Evolutionary adaptation to temperature. II. Thermal niches of experimental lines of *Escherichia coli*. Evolution 47:1–12.

Bennett, A. F., K. M. Dao, and R. E. Lenski. 1990. Rapid evolution in response to high temperature selection. Nature 346:79–81.

Bennett, A. F., R. E. Lenski, and J. E. Mittler. 1992. Evolutionary adaptation to temperature. I. Fitness responses of *Escherichia coli* to changes in its thermal environment. Evolution 46:16–30.

Cavicchi, S., G. Giorgi, and M. Mochi. 1978. Investigation on early divergence between populations of *Drosophila melanogaster* kept at different temperatures. Genetica 48:81–87.

Cavicchi, S., D. Guerra, G. Giorgi, and C. Pezzoli. 1985. Temperature-related divergence in experimental populations of *Drosophila melanogaster*. I. Genetic and developmental basis of wing size and shape variation. Genetics 109:665–689.

Cavicchi, S., V. Guerra, V. Natali, C. Pezzoli, and G. Giorgi. 1989. Temperature-related divergence in experimental populations of *Drosophila melanogaster*. II. Correlation between fitness and body dimensions. Journal of Evolutionary Biology 2:235–251.

Cavicchi, S., G. Giorgi, V. Natali, and D. Guerra. 1991. Temperature-related divergence in experimental populations of *Drosophila melanogaster*. III. Fourier and centroid analysis of wing shape and relationship between shape variation and fitness. Journal of Evolutionary Biology 4:141–159.

Christian, K. A., and C. R. Tracy. 1981. The effect of the thermal environment on the ability of hatchling Galapagos land iguanas to avoid predation during dispersal. Oecologia 49:218–223.

Dunham, A. E. 1993. Population responses to environmental change: physiologically structured models, operative environments, and population dynamics. Pp. 95–119 *in* Kareiva et al. 1993.

Heinrich, B. 1981. Ecological and evolutionary perspectives. Pp. 235–302 *in* B. Heinrich, eds. Insect thermoregulation. Wiley, New York.

Hochachka, P. W., and G. N. Somero. 1984. Biochemical adaptation. Princeton University Press, Princeton, N.J.

Hoffmann, A. A. 1990. Acclimation of desiccation resistance in *Drosophila melanogaster* and the association between acclimation responses and genetic variation. Journal of Insect Physiology 31:885–891.

Hoffmann, A. A., and M. W. Blows. 1993. Evolutionary genetics and climate change: will animals adapt to global warming? Pp. 165–178 *in* Kareiva et al. 1993.

Hoffmann, A. A., and P. A. Parsons. 1991. Evolutionary genetics and environmental stress. Oxford University Press, Oxford.

Hoffmann, A. A., and M. Watson. 1993. Geographical variation in acclimation responses of *Drosophila* to temperature extremes. American Naturalist 142:S93–S113.

Holt, R. D. 1990. The microevolutionary consequences of climate change. Trends in Ecology and Evolution 5:311–315.

Huey, R. B., and A. F. Bennett. 1990. Physiological adjustments to fluctuating thermal environments: an ecological and evolutionary perspective. Pp. 37–59 *in* R. Morimoto, A. Tessieres, and C. Georgopoulous, eds. Stress proteins in biology and medicine. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.

Huey, R. B., and J. G. Kingsolver. 1993. Evolution of resistance to high temperature in ectotherms. American Naturalist 141: S21–S46.

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. Functional Ecology 6:489–494.

Hutchison, V. H., and J. D. Maness. 1979. The role of behavior in temperature acclimation and tolerance in ectotherms. American Zoologist 19:3677–384.

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

Jones, J. S., J. A. Coyne, and L. Partridge. 1987. Estimation of the thermal niche of *Drosophila melanogaster* using a temperature-sensitive mutant. American Naturalist 130:83–90.

Kareiva, P. M., J. G. Kingsolver, and R. B. Huey, eds. Biotic interactions and global change. Sinauer, Sunderland, Mass.

Kilias, G., and S. N. Alahiotis. 1985. Indirect thermal selection in *Drosophila melanogaster* and adaptive consequences. Theoretical and Applied Genetics 69:645–650.

Koban, M., A. A. Yup, L. B. Agellon, and D. A. Powers. 1991. Molecular adaptation to environmental temperature: heat-shock response of the eurythermal teleost *Fundulus heteroclitus*. Molecular and Marine Biology and Biotechnology 1:1–17.

Lindquist, S., and E. A. Craig. 1988. The heat-shock proteins. Annual Review of Genetics 22:631–677.

Lindsley, D. L., and G. Zimm. 1990. The genome of *Drosophila melanogaster*. Part 4: Genes L-Z, balancers, transposable elements. Drosophila Information Service 68:1–382.

Lints, F. A., and M. Bourgois. 1987. Phenotypic and genotypic differentiation in cage populations of *Drosophila melanogaster*. Génétique, Sélection, Evolution 19:155–170.

Loeschcke, V., R. A. Krebs, and J. S. F. Barker. 1994. Genetic variation for resistance and acclimation to high temperature stress in *Drosophila buzzatii*. Biological Journal of the Linnean Society (London) 52:83–92.

Lynch, M., and R. Lande. 1993. Evolution and extinction in response to environmental change. Pp. 234–250 *in* Kareiva et al. 1993.

Maynard Smith, J. 1956. Temperature tolerance and acclimatization in *Drosophila subobscura*. Journal of Genetics 85–96.

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

Mourad, A. E. 1965. Genetic divergence in M. Vetukhiv's experimental populations of *Drosophila pseudoobscura*. 2. Longevity. Genetical Research 6:139–146.

Nover, L. 1991. Induced thermotolerance. Pp. 409–452 *in* L. Nover, ed. Heat shock response. CRC Press, Boca Raton, Fla.

Nover, L., and L. Hightower. 1991. Introduction. Pp. 1–4 *in* L. Hightower and L. Nover, eds. Heat shock and development. Springer, Berlin.

Powers, D. A. 1987. A multidisciplinary approach to the study of genetic variation within species. Pp. 102–130 *in* M. E. Feder, A. F. Bennett, W. W. Burggren, and R. B. Huey, eds. New directions in ecological physiology. Cambridge University Press, Cambridge.

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. Theoretical and Applied Genetics 80:847–851.

———. 1991. Genetic and environmental factors in the resistance of *Drosophila subobscura* adults to high temperature shock III. Chromosomal-inversion and enzymatic polymorphism variation in lines selected for heat shock resistance. Genetica 84:165–170.

Rice, W. R. 1990. A consensus combined *P*-value test and the family-wide significance of component tests. Biometrics 46: 303–308.

Rice, W. R., and S. D. Gaines. 1994. The ordered-heterogeneity family of tests. Biometrics 50:1–7.

Rose, M. R., J. L. Graves, and E. W. Hutchinson. 1990. The use of selection to probe patterns of pleiotropy in fitness-characters. Pp. 29–42 *in* F. Gilbert, ed. Insect life cycles: genetics, evolution, and co-ordination. Springer, London.

Schneider, S. 1993. Scenarios of global warming. Pp. 9–23 *in* Kareiva et al. 1993.

Service, P. M., M. D. Hutchison, M. D. MacKinley, and M. R. Rose. 1985. Resistance to environmental stress in *Drosophila melanogaster* selected for postponed senescence. Physiological Zoology 58:380–389.

Smith, M. T., and R. B. Huey. 1991. Ether and CO_2 affect heat tolerance in *Drosophila melanogaster*. Drosophila Information Service 70:215.

Stephanou, G., and S. N. Alahiotis. 1983. Non-Mendelian inheritance of "heat-sensitivity" in *Drosophila melanogaster*. Genetics 103:93–107.

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*. Developmental Genetics 3:299–308.

Tucić, N. 1979. Genetic capacity for adaptation to cold resistance at different developmental stages of *Drosophila melanogaster*. Evolution 33:350–358.

van Delden, W., and A. Kamping. 1989. The association between the polymorphisms at the Adh and αGpdh loci and the In(2L)t inversion in *Drosophila melanogaster* in relation to temperature. Evolution 43:775–793.

White, E. B., P. DeBach, and M. J. Garber. 1970. Artificial selection for genetic adaptation to temperature extremes in *Aphytis lingnanensis* Compere (Hymenoptera: Aphelinidae). Hilgardia 40: 161–192.

Yocum, G. D., and D. L. Denlinger. 1993. Induction and decay of thermosensitivity in the flesh fly, *Sarcophaga crassipalpis*. Journal of Comparative Physiology B163:113–117.

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