Thermal Sensitivity of *Drosophila melanogaster*: Evolutionary Responses of Adults and Eggs to Laboratory Natural Selection at Different Temperatures

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ABSTRACT

We compared aspects of the thermal sensitivity of replicated lines of *Drosophila melanogaster* that had been evolving by laboratory natural selection at three selection temperatures: 16.5°C (10+ yr), 25°C (9+ yr), or 29°C (4+ yr). The 16.5°C and 25°C lines are known to have diverged in fitness at 16.5°C versus 25°C and also in heat tolerance. We designed new experiments to explore further possible shifts in thermal sensitivity of these lines. The optimal temperature for walking speed of adults was positively related to selection temperature, but differences among lines in thermal sensitivity of walking speed were small. Performance breadth was inversely related to selection temperature. Tolerance of adults to an acute heat shock was also positively related to selection temperature, but tolerance to a cold shock was not. Thus, fitness at moderately high temperatures is genetically coupled with tolerance of extreme high (but not of low) temperature. Knock-down temperature and walking speed at high temperature, however, were independent of selection temperature. In contrast to adults, eggs from different lines had similar heat and cold tolerance. Thus, long-term natural selection has led to divergence in thermal sensitivity of some (but not of all) traits and may have had more of an impact on adults than on eggs. Attempts to predict evolutionary states in nature are, however, complicated because of the observed genetic correlations and the simple selection scheme.

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Introduction

Temperature has profound effects on the short-term performance capacities of an ectotherm and ultimately on its fitness (Huey and Stevenson 1979). Extreme low or high temperatures are lethal, and within the viable limits, performance (or absolute fitness) increases gradually with temperature up to an optimum before dropping precipitously as temperature approaches the upper lethal limit. How the thermal sensitivity of an ectotherm evolves in response to environmental change has long been a favorite topic of comparative physiologists. The vast majority of the resulting studies have used between species (Prosser 1986) or between population (Garland and Adolph 1991) comparisons to deduce historical evolutionary patterns. Recently, however, evolutionary physiologists have begun to use selection experiments on laboratory stocks (Rose et al. 1987) to elucidate short-term evolutionary patterns (Stephanou and Alahiotis 1983; Service et al. 1985; Cavicchi et al. 1989; Hoffmann and Parsons 1989, 1991; Bennett et al. 1990; Huey et al. 1991; Graves et al. 1992; Neat et al. 1995; Loeschcke and Krebs 1997). Such selection experiments are an effective way of uncovering traits that are genetically correlated with traits that are direct targets of selection; an awareness of such correlated traits is crucial to understanding or predicting evolutionary trajectories (Arnold 1987; Falconer 1989).

Laboratory natural selection experiments in particular are a practical and relatively natural way to explore the genetic potential for evolutionary change (Rose et al. 1987). With respect to temperature, an appropriate experimental design involves subdividing a stock of organisms and culturing lines in large, replicated populations at different temperatures for extended periods. If the selection temperatures are different enough to influence the performance and fitness of the organisms, and if the genetic architecture underlying thermal sensitivity is permissive to evolutionary change, then the thermal sensitivity of each line will adapt to the selection temperature, barring interactions caused by conflicting selection on genetically correlated traits. Of course, the selected populations must be large and replicated to reduce the risk that observed evolutionary shifts are inadvertent consequences of genetic drift or of inbreeding (Cohan 1984; Falconer 1989).

Here we compare the thermal physiology of replicated lines of *Drosophila melanogaster* that have been evolving independently by laboratory natural selection for several years at three

constant temperatures (16.5°C from 1984 to 1995, 25°C from 1985 to 1995, and 29°C from 1990 to 1995; three replicates per selection temperature; see Cohan 1984). These selection temperatures are well within the range of temperatures tolerated by this species during acute exposures (roughly −3°C to +39°C; Parsons 1978; David et al. 1983) but different enough from each other to have major effects on the performance and fitness of flies (David et al. 1983; Partridge et al. 1994*b*). With these selected lines, we addressed several issues.

First, have the lines diverged in thermal sensitivity at temperatures within the viable limits? Specifically, has the thermal sensitivity of performance begun to adapt to selection temperature (Bennett et al. 1990, 1992; Huey et al. 1991)? If so, then flies from 29°C lines, for example, should have the highest performances at high test temperatures but the worst at low test temperatures, relative to flies from the other selection lines.

Second, has laboratory natural selection at nonextreme temperatures led to a genetically correlated divergence in tolerance of extreme heat or cold (Stephanou et al. 1983; Huey et al. 1991; Loeschcke and Krebs 1994; Cavicchi et al. 1995), even though the lines have not been exposed to extreme temperatures in the laboratory? If such a change in tolerance has occurred, then flies from 29°C lines should, for example, have the greatest tolerance of extreme heat.

Third, has laboratory natural selection similarly influenced the thermal sensitivity of different life stages (Tucić 1979; Krebs and Loeschcke 1995; Loeschcke and Krebs 1997; see also Coyne et al. 1983)? If so, both eggs and adults from the selection lines should show parallel patterns of thermal sensitivity.

Several previous studies have used laboratory natural selection of *Drosophila* at different temperatures to explore various evolutionary issues. In the 50's, M. Vetukhiv established lines of *Drosophila pseudoobscura* at three selection temperatures, and his stocks were used in a pioneering series of studies of divergence in body size and in life history traits (Mourad 1965; Anderson 1966; Kitagawa 1967; Ehrman 1969; Powell 1974). Beginning in the late 70's, Cavicchi and colleagues (Cavicchi et al. 1978, 1985, 1989, 1991, 1995) began to document marked divergence in life history, body size and shape, and heat tolerance of replicated lines of *D. melanogaster* that had been evolving for up to 15 yr at three temperatures. Similarly, Alahiotis and colleagues studied two temperature lines of *D. melanogaster* and showed divergence in (and also established the chromosomal basis of) survival of a heat shock as well as in synthesis of heat shock proteins (Alahiotis and Stephanou 1982; Stephanou and Alahiotis 1983; Stephanou et al. 1983). Partridge and colleagues have previously examined aspects of the particular lines studied herein. For example, Huey et al. (1991) showed that the 16.5° and 25°C lines had diverged (after 4+ yr) in the thermal dependence of growth rate and in tolerance of extreme heat. Similarly, Partridge and colleagues (Partridge et al. 1994*a,* 1994*b,* 1995; James and Partridge 1995; Neat et al. 1995; Azevedo et al. 1996) have studied the divergence of the 16.5° and 25°C lines in terms of body size and several life-history traits, including fitnesses at the two selection temperatures. It is important that they have shown that each line has relatively high fitness at its own selection temperature (Partridge et al. 1995).

This new study extends our prior research in several ways. In particular, we have obtained data on the effects of a broad range of temperatures on all three selection lines and on both adults versus eggs for some traits. Further, we employ a powerful statistical approach (orthogonal polynomials, see Material and Methods) that allows us to extract information concerning possible linear and quadratic responses to selection temperature. Our analyses show (1) that relatively little evolution of temperature sensitivity has occurred, though some traits apparently are adapting to selection temperature, (2) that such natural selection has had a correlated impact on the performance of flies at temperatures well outside the range of temperatures experienced by flies during selection, but (3) that adults and eggs do not always show parallel responses.

Material and Methods

Stocks

As detailed in Huey et al. (1991) and Partridge et al. (1994*b*), a large stock of *Drosophila melanogaster* was collected in a fruit market in Brighton, England, in June 1984, and maintained in a single mass culture at 25°C for about 1 yr. The stock was then subdivided, and three replicate populations were established at both 18°C and at 25°C. After about a year, the 18°C stocks were transferred to 16.5°C and then maintained at that temperature. In late 1990, each of the three 25°C replicates were subdivided to establish three replicate populations at 29°C. Flies were cultured in large population cages (overlapping generations) without control over larval or adult density.

In the spring of 1994, samples of all replicates were transferred to the appropriate temperature regime at the University of Washington (except that the 16.5°C flies were maintained at 18°C). Larval density was controlled during culturing (ca. 50 eggs per vial). Our present studies were conducted in February 1995. Thus, flies had been evolving at 16.5°–18°C for 9+ yr, at 25°C for 10+ yr, and at 29°C for 3+ yr. The number of generations at each temperature is unknown, but we would estimate that the flies were tested after approximately 100 generations at 16.5°–18°C, approximately 275 generations at 25°C, and approximately 150 generations at 25°C followed by approximately 100 generations at 29°C.

To control for the possibility of confounding effects of developmental and parental temperatures (Maynard Smith 1957; Crill et al. 1996) in all experiments, we compared all stocks only after culturing them at controlled larval density (above) for two generations at a common temperature of 25°C. Any differences among lines should mainly reflect genetic differences.

Thermal Dependence of Walking Speed

We measured the impact of temperature on the walking speed of adult flies. Speed is often used as a surrogate for overall organismal performance, but it can also directly influence survival (Christian and Tracy 1981; Hertz et al. 1983). Moreover, because speed is correlated with male mating success in *D. melanogaster* (Partridge et al. 1987) and is markedly affected by temperature (Crill et al. 1996), the thermal dependence of speed might well be altered by laboratory natural selection at different temperatures. Details of procedures follow Gilchrist (1996) and Crill et al. (1996). A total of 108 individuals were tested (six males and six females from each of the nine lines). In brief, we indexed walking speed (cm/s) by knocking down a fly in a narrow plastic test tube (13 × 100 mm) and measuring the time required to walk up the tube to a height of 6.0 cm. Each fly was generally tested once (but see Crill et al. 1996, p. 1208) at the following sequence of temperatures: 15°, 30°, 20°, 10°, 25°, 35°, and 40°C.

We extracted several statistics from the above data for each individual. The optimal temperature was defined as the single temperature at which an individual walked fastest. The maximal speed was the speed at an individual's optimal temperature. Performance breadth is an index of the degree of thermal specialization of performance and is calculated following Gilchrist (1996).

Knock-Down Temperature (Adults)

Knock-down temperature is a measure of a fly's ability to hold on to a substrate at extreme high temperature, but it is not a measure of the ability of a fly to survive an extreme heat shock (see below). Procedures follow Huey et al. (1992) and Crill et al. (1996). To score knock-down temperature, we transferred adult flies (ca. 1,000 flies at a time, age = 3–4 d) to a glass column (with internal baffles) that was surrounded by a water bath (30°C). Then the temperature in the water bath was heated (ca. 1°C/min), thereby heating the flies inside the column. Eventually, flies were incapacitated by the heat and fell into a collecting tube below. We recorded the numbers of male and of female flies that fell out in 0.5°C intervals.

We ran a single batch for each of the nine sets (i.e., three replicate lines from each of the selection regimes) of flies and analyzed the mean values for males and for females within each set. This is a very conservative procedure, but it compensates for the nonindependence of scores for flies in each batch (see Crill et al. 1996).

Heat Tolerance (Adults and Eggs)

Heat tolerance of adults was scored as the percentage of flies that survived a short-term heat shock (Huey et al. 1991). Flies were maintained at the common temperature of 25°C until 3–5 d of adult age. To separate flies into groups of roughly 50 flies (mixed sexes), we lightly anesthetized flies with CO_2 and then returned them to 25°C for 1 d. Light CO_2 anesthesia influences heat tolerance of *D. melanogaster* even after 1 d (Smith and Huey 1991), but any such effect should be shared by all lines. Vials with flies were then submerged (saturated humidity) into a hot water bath for 0.5 h and then immediately transferred to vials with media at 25°C. We used two different heat shocks (38.0° and 38.5°C). Heat tolerance of *Drosophila* varies somewhat from day to day (Coyne et al. 1983), and heat shock tests at multiple temperatures increase the chance of obtaining useful data. The following day we scored the percentage of flies that were alive (by means of a reflex response to touch). We ran five vials per replicate per line and subsequently analyzed the percentage (arcsine square-root transform) of male and of females flies that survived in each vial.

For studies of egg tolerance to heat, we collected eggs from a large (>200) sample of females approximately 16 h after laying (Welte et al. 1993). For each replicate within each selection temperature, we set up eight vials with 30 eggs/vial. The vials from each replicate were bound together with a weighted vial and submerged in a water bath at 38.0°C. Then one vial from each set was removed at 15, 30, 35, 60, 75, 90, 105, and 120 min and placed in an incubator at 25°C until all adults had emerged. We scored the proportion of eggs that yielded adults (number of emerging adults/30 eggs) and then used logistic regression (logit transformation) to estimate the LT_{50}, which is the exposure time at 38°C that results in 50% of all eggs producing adults. Despite the number of vials scored, the use of LT_{50} reduces the data to a single number for each replicate, so the power to detect a significant difference among selection temperatures is limited.

Cold Tolerance (Adults and Eggs)

Cold tolerance of adults was scored as the percentage of flies that survived a cold shock. We prepared vials of flies (as above) and transferred them to a bath (ethylene glycol and water) at −3.5°C or −4.0°C. After 1 h, we removed the vials and transferred them to 25°C for 24 h before scoring the percentages of flies (males, females) in each vial that were alive.

For the studies of egg cold tolerance, eight vials of 30 eggs each were prepared for each replicate, as described above for egg heat tolerance. Vials were then submerged at −4.0°C. One vial from each line was removed at 30-min intervals (between 30 and 240 min) and then transferred to 25°C until all adults had emerged. After computing the percentage of eggs that produced adults for each vial, we used logistic regression to estimate the LT_{50}, as above. Power is limited, for reasons given above.

Statistical Analyses

The primary issues of interest here are the potential impact of selection temperature (i.e., temperature at which the lines have

been evolving) and of sex. Both are fixed effects. However, selection temperature is an ordered factor, and our basic hypothesis is that thermal sensitivity is directly related to selection temperature. Traditional ANOVA is inappropriate for evaluating that basic hypothesis because such an ANOVA determines only whether heterogeneity exists among groups and not whether that heterogeneity is ordered. As a result, power is sacrificed (Rice and Gaines 1994). In contrast, orthogonal polynomial analysis is a type of ANOVA that is designed specifically to address ordered factors (Sokal and Rohlf 1981). This analysis generates contrast functions that assess the significance of both linear (first-contrast) and quadratic (second-contrast) terms. Linear and quadratic terms are of interest as they indicate whether a given trait such as heat tolerance is linearly or curvilinearly related to selection temperature. A polynomial regression can also assess linear and quadratic patterns but is appropriate only when the independent variables are continuous.

In conducting the orthogonal polynomial tests, we initially treated replicates within selection lines as fixed effects in the various analyses. If the replicate variance was significant, we treated the lines as random blocks and conservatively used the estimated mean square term as the error term.

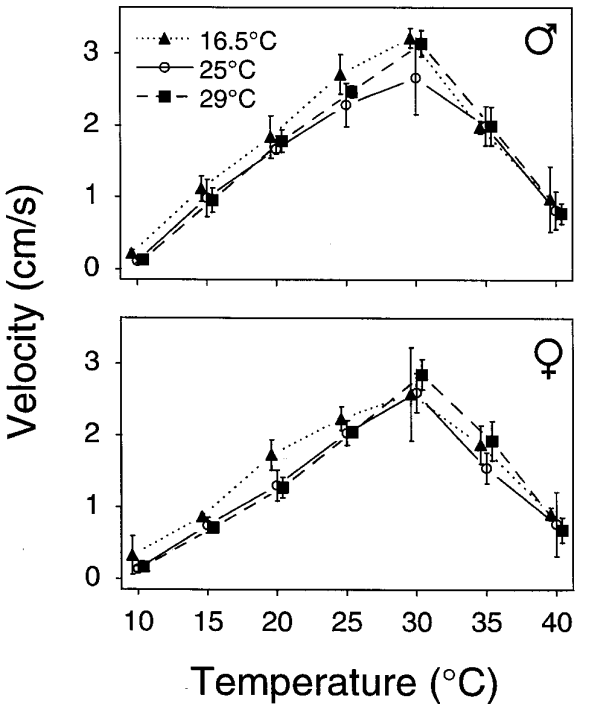

Figure 1. Walking speed (cm/s) of three lines of *Drosophila melanogaster* that have been evolving at 16.5°, 25°, or 29°C. Data for males are shown in the upper panel, and those for females are shown in the lower. Values plotted are means (±1 SD) for the speeds of three replicates at each temperature line.

Results

Thermal Sensitivity of Locomotion

The basic patterns for walking speed as a function of temperature were similar among lines at different selection temperatures (Fig. 1; Table 1). Even so, the lines have diverged slightly in thermal sensitivity. The temperature at which flies walked fastest was weakly but positively related to selection temperature ($P = 0.036$); flies evolving at 29°C had the highest optimal temperature (average of males and females = 30.1°C), whereas flies evolving at 16°C and at 25°C had lower optimal temperatures (average of males and females = 28.6° and 28.2°C, respectively). Orthogonal polynomials also detected a significant curvilinear pattern ($P = 0.01$), reflecting the divergence of the 29°C flies from the other groups. Neither sex ($P = 0.2$) nor replicate within treatment ($P = 0.24$) had significant effects on optimal temperature (Table 1).

Differences in speed among lines were conspicuous only at low to moderate temperatures (Fig. 1). At 15°, 20°, and 25°C, but not 10°C, speed was inversely related to selection temperature (Fig. 1; $P < 0.04$). Flies from the 16.5°C line generally had the fastest speeds, whereas flies the other two lines were similar to each other. At high temperatures (30°, 35°, and 40°C), however, speed was unrelated to selection temperature (linear polynomial terms, $P > 0.09$).

Performance breadths were inversely related to selection temperature (linear polynomial term, $P = 0.029$; Fig. 1; Tables 1, 2), mainly because speeds of the 16.5°C flies were less depressed at low temperatures than were those of the other lines. Neither sex ($P = 0.33$) nor replicate within temperature line ($P = 0.59$) had significant effects on performance breadth (Table 2).

Maximal speed was unrelated to selection temperature (Fig. 1; Tables 1, 2). Replicates within temperature lines did not differ significantly ($P = 0.27$; Table 2), but males were significantly faster than females (3.0 vs. 2.7 cm/s, $P = 0.02$, Table 2).

Knock-Down Temperature

Contrary to expectation, knock-down temperature was independent of line temperature (Tables 1, 3). Replicates varied significantly within lines ($P = 0.002$), but no other factor or interaction was significant (Table 3).

Heat Tolerance (Adults and Eggs)

As expected (Stephanou and Alahiotis 1983; Huey et al. 1991; Cavicchi et al. 1995), the probability that adults would survive a heat shock increased directly with line temperature (Table 1). Orthogonal polynomials detected a significant linear and positive effect of line temperature ($P = 0.009$; Table 4), but not a significant curvilinear one ($P = 0.52$). Females were more

Table 1: Performance traits of the three lines at each selection temperature

Trait and Line	Female	Male
Optimal temperature for walking speed (°C):		
16.5°C	28.61 ± 1.73	28.61 ± .96
25°C	28.89 ± .96	27.50 ± .83
29°C	30.28 ± .48	30.00 ± .83
Performance breadth (°C):		
16.5°C	11.48 ± .74	10.18 ± .79
25°C	9.24 ± 1.57	11.07 ± .68
29°C	9.18 ± 1.39	9.76 ± .31
Maximum walking speed (cm/s):		
16.5°C	2.75 ± .47	3.33 ± .07
25°C	2.74 ± .13	2.86 ± .47
29°C	2.92 ± .14	3.21 ± .28
Knock-down temperature (°C):		
16.5°C	38.43 ± .36	38.34 ± .18
25°C	37.81 ± .46	37.77 ± .50
29°C	38.06 ± .31	38.15 ± .52
Adult cold survival (%):		
16.5°C:		
−4.0°C	.74 ± .04	.70 ± .20
−3.5°C	.80 ± .09	.83 ± .13
25°C:		
−4.0°C	.74 ± .18	.61 ± .34
−3.5°C	.72 ± .13	.58 ± .19
29°C:		
−4.0°C	.73 ± .03	.57 ± .02
−3.5°C	.65 ± .14	.68 ± .10
Adult heat survival (%):		
16.5°C:		
38.0°C	.44 ± .34	.34 ± .13
38.5°C	.04 ± .04	.06 ± .05
25°C:		
38.0°C	.83 ± .13	.69 ± .09
38.5°C	.27 ± .17	.08 ± .03
29°C:		
38.0°C	.81 ± .10	.75 ± .15
38.5°C	.33 ± .21	.16 ± .05
Egg cold LT_{50} (min):		
16.5°C	6.21 ± 2.60	
25°C	6.69 ± 4.52	
29°C	10.6 ± 2.01	
Egg hot LT_{50} (min):		
16.5°C	1.54 ± .28	
25°C	.59 ± .24	
29°C	2.04 ± .44	

Note. Values are presented as mean ± 1 SD of males and females for adults and of all individuals for eggs.

heat resistant than males (44.3% vs. 31% survival, $P < 0.001$), and replicates within lines showed considerable heterogeneity ($P < 0.001$).

Flies exposed to a heat shock of 38°C were much more likely to survive than those exposed to 38.5°C (68% vs. 11%, $P < 0.001$; Tables 1, 4). Even so, the rank order of survival for the three temperature selection lines was the same at both heat-shock temperatures (29°C > 25°C > 16.5°C).

Patterns for egg heat tolerance were different than those for adults. The LT_{50} was not linearly related to selection temperature ($P > 0.05$; Tables 1, 4). A quadratic effect was significant ($P = 0.002$), reflecting the relatively low LT_{50}'s of the 25°C flies (Table 1). Variation among replicates within selection temperatures was minor ($P = 0.40$).

Cold Tolerance (Adults and Eggs)

The probability of survival of a cold shock was inversely but not significantly related to line temperature ($P = 0.31$; Tables 1, 5). Females had slightly higher survival than did males (76% vs. 61%, $P = 0.02$; Tables 1, 5). Replicates within lines showed significant heterogeneity ($P < 0.001$). Cold shock temperature (−3.5°C, −4°C) had no significant effect on cold tolerance, nor did it interact significantly with any other factor.

LT_{50}'s of eggs were unrelated to selection temperature (Fig. 1; Table 1); neither the linear ($P = 0.18$) nor the quadratic ($P = 0.35$) term was significant. The 29°C eggs had (surprisingly) the longest LT_{50}, which suggests that the lack of significant effect is not a consequence of limited power (see Material and Methods). Variation among replicates within selection temperatures was significant ($P > 0.001$; Table 5).

Discussion

Our analyses show that lines of *Drosophila melanogaster* that have been evolving at different (constant) temperatures for several years have diverged in some (but not all) thermal traits, and have done so in complex ways. In the cases in which the populations have diverged, the patterns are generally indicative of adaptation to temperature. Even so, thermal sensitivity and selection temperature are not always positively related, and patterns for adults and eggs sometimes differ. Thus, even in a simple (constant-temperature) environment, patterns of adaptation to temperature are complex.

Thermal Sensitivity of Locomotion

We used walking speed to index organismal performance (see Introduction) and measured speed of all selection lines (after two generations at 25°C) at seven temperatures between 10°C and 40°C. Partial adaptation of performance to selection temperature is suggested by the significant and positive relationship between the average optimal temperature for walking and se-

Table 2: Orthogonal polynomial analyses of the effects (linear and quadratic) of line temperature on performance traits

Trait and Effect	df	Sum of Squares	Mean Square	*F* Value	Probability (*F*)
Optimal temperature for walking speed:					
Line temperature:					
Linear	1	30.29	30.29	4.541	.036
Quadratic	1	45.18	45.18	6.773	.011
Sex	1	8.33	8.33	1.249	.266
Linear × sex	1	1.12	1.12	0.168	.683
Quadratic × sex	1	8.60	8.60	1.290	.259
Replicates in line	6	54.17	9.03	1.354	.241
Residuals	96	640.28	6.67	. . .	. . .
Performance breadth:					
Line temperature:					
Linear	1	.36	.36	4.926	.029
Quadratic	1	.01	.01	.101	.752
Sex	1	.07	.07	.945	.334
Linear × sex	1	.21	.21	2.940	.090
Quadratic × sex	1	.13	.13	1.829	.179
Replicates in line	6	.34	.06	.776	.591
Residuals	96	6.93	.07	. . .	. . .
Maximum walking speed:					
Line temperature:					
Linear	1	.00	.00	.001	.969
Quadratic	1	.19	.19	3.392	.069
Sex	1	.31	.31	5.387	.022
Linear × sex	1	.08	.08	1.321	.253
Quadratic × sex	1	.08	.08	1.365	.246
Replicates in line	6	.44	.07	1.279	.274
Residuals	96	5.50	.06	. . .	. . .

lection temperature (Fig. 1; Tables 1, 2). Even so, other aspects of the performance curves are inconsistent with a simple adaptive expectation. For example, optimal temperature for the 16.5°C and 25°C lines were very similar (Table 1). Moreover, measured differences among lines at all test temperatures were always small in magnitude (Fig. 1), and patterns sometimes contradicted adaptationist expectations. For example, even though the 16.5°C flies generally had (as expected) the fastest speeds at low test temperatures, they ran as fast if not faster than the other lines at a high temperature (Fig. 1).

Table 3: Orthogonal polynomial analysis of effects of selection temperature and sex on knock-down temperature of adults

Effect	df	Sum of Squares	Mean Square	*F* Value	Probability (*F*)
Line temperature:					
Linear	1	203.78	203.78	1.669	.244
Quadratic	1	316.68	316.68	2.594	.158
Sex	1	2.39	2.39	.315	.595
Linear × sex	1	9.78	9.78	1.290	.299
Quadratic × sex	1	1.78	1.78	.234	.646
Replicate in line	6	732.67	122.11	16.098	.002
Residuals	6	45.51	7.59	. . .	. . .

Note. The *F*-tests for the linear and quadratic effects of selection temperature use replicates-within-line temperature as the error term.

Table 4: Orthogonal polynomial analysis of effects of selection temperature and sex on heat tolerance of adults and eggs

Effect	df	Sum of Squares	Mean Square	*F* Value	Probability (*F*)
Adult heat tolerance:					
Line temperature:					
Linear	1	96.63	96.63	14.481	.009
Quadratic	1	3.11	3.11	.465	.521
Temperature	1	333.83	333.83	345.949	.000
Sex	1	21.40	21.40	22.172	.000
Linear × temperature	1	4.69	4.69	4.862	.029
Quadratic × temperature	1	2.59	2.59	2.683	.103
Linear × sex	1	2.38	2.38	2.465	.118
Quadratic × sex	1	1.39	1.39	1.441	.232
Temperature × sex	1	.02	.02	.021	.884
Replicate in line	6	40.04	6.67	6.916	.000
Residuals	164	158.25	.96	. . .	. . .
Egg heat tolerance:					
Line temperature:					
Linear	1	.12	.12	1.055	.344
Quadratic	1	3.14	3.14	28.610	.002
Residuals	6	.66	.11	. . .	. . .

Note. The *F*-tests for the linear and quadratic effects of selection temperature use replicates-within-line temperature as the error term.

It is worth noting that the observed shifts of the thermal dependence of walking performance (Fig. 1) and of fitness (Partridge et al. 1995) in response to laboratory natural selection do not constitute compensatory adaptation to selection temperature. For example, 16.5°C flies run fastest at 29°C, not at 16.5°C (Fig. 1). Similarly, the 16.5°C flies have higher absolute fitness at 25°C than at 16.5°C (Partridge et al. 1995).

It is interesting that thermal performance breadth (a measure of thermal specialization) was inversely related to selection temperature (Tables 1, 2), such that flies evolving at high temperature, for example, ran well over a relatively restricted range of temperatures. Natural selection at high temperature (29°C) appears to have led to a reduction in relative performance at low temperatures without an increase in relative performance at high temperatures (Fig. 1) or without an increase in maximal speed (see below). Therefore, natural selection at high temperature has seemingly resulted in a trade-off in performance at low temperature, but the reverse was not associated with natural selection at low temperature.

The thermal dependence of walking speed has not been investigated in previous studies with temperature selection lines of *Drosophila;* therefore, we cannot know whether our results are general for this taxon. However, adaptation of life-history traits to selection temperature has been demonstrated in previous studies of the three Cavicchi lines (Cavicchi et al. 1989) and of two of the Partridge lines (16.5°C and 25°C; Huey et al. 1991; Partridge et al. 1995) of *D. melanogaster.* For example, Partridge's 25°C lines have higher levels of fitness (i.e., intrinsic rate of increase) than the 16.5°C lines at 25°C, but the reverse is true at 16.5°C (Partridge et al. 1995).

The bacterium *Escherichia coli* has also been subjected to laboratory natural selection at different temperatures (Bennett et al. 1990, 1992; Bennett and Lenski 1993; Lenski and Bennett 1993). Adaptation to temperature is rapid and marked in *E. coli,* especially at a stressful (high) temperature (Lenski and Bennett 1993). However, adaptation is remarkably temperature specific; for example, natural selection at 42°C markedly enhances fitness at that temperature but has little impact on fitness at other temperatures (20°–37°C; Bennett and Lenski 1993; Lenski and Bennett 1993). As a result, natural selection at different temperatures has little impact on the width of the thermal niche (temperatures over which *E. coli* can maintain itself in serial dilution; Bennett and Lenski 1993).

Knock-Down Temperature

Knock-down temperature is better thought of as an index of performance at high temperature than as a measure of heat tolerance (i.e., ability to survive a heat shock, see below; see also Hoffmann et al. 1997). It is interesting that knock-down temperature was unrelated to selection temperature (Tables 1, 3), and this result is consistent with the similarity of walking speeds of all selection lines at 40°C (Fig. 1). The lack of an evolutionary response in knock-down temperature is not the result of insufficient genetic variation in this trait, as knock-

Table 5: Orthogonal polynomial analysis of effects of selection temperature and sex on cold tolerance of adults and eggs

Effect	df	Sum of Squares	Mean Square	*F* Value	Probability (*F*)
Adult cold tolerance:					
Line temperature:					
Linear	1	7.10	7.10	1.214	.313
Quadratic	1	.56	.56	.095	.768
Temperature	1	1.45	1.45	1.782	.184
Sex	1	4.45	4.45	5.501	.020
Linear × temperature	1	2.15	2.15	2.660	.105
Quadratic × temperature	1	2.20	2.20	2.721	.101
Linear × sex	1	2.24	2.24	2.775	.097
Quadratic × sex	1	1.36	1.36	1.681	.197
Temperature × sex	1	1.00	1.00	1.238	.267
Replicate in line	6	35.10	5.85	7.232	.000
Residuals	6	.66	.11	. . .	. . .
Egg cold tolerance:					
Line temperature:					
Linear	1	24.38	24.38	2.343	.177
Quadratic	1	10.48	10.48	1.006	.355
Residuals	6	62.46	10.41	. . .	. . .

Note. The *F*-tests for the linear and quadratic effects of selection temperature use replicates-within-line temperature as the error term.

down temperature responds rapidly to artificial selection (Huey et al. 1992; see also Hoffmann et al. 1997).

Because knock-down temperatures have not previously been compared in lines subject to natural selection at different temperatures, we do not know whether the observed lack of divergence among lines is general. Knock-down temperature is sensitive, however, to developmental temperature (Crill et al. 1996).

Correlated Shifts in Heat Tolerance

A key question in evolutionary physiology is whether natural selection at different (nonextreme) temperatures leads to a correlated shift in tolerance of extreme heat or cold (Huey and Kingsolver 1989, 1993; Bennett and Lenski 1993). To address this issue, we compared the heat and cold tolerance (i.e., ability to survive an acute heat or cold shock) of our temperature-selected lines at both the egg and the adult life stages.

For adult flies, the probability of surviving a heat shock (38°C or 38.5°C) was strongly and positively related to selection temperature (Tables 1, 4). Thus, tolerance of adults to extreme heat evolved as a correlated response to natural selection at nonextreme temperature, even though none of the flies had been exposed to extreme temperatures since being established in the lab (1984). This finding corroborates our earlier findings for the 16.5°C and 25°C lines after only 4+ yr of selection (Huey et al. 1991). Moreover, it is consistent with results from two previous studies with *D. melanogaster* (Stephanou and Alahiotis 1983; Stephanou et al. 1983; Cavicchi et al. 1995). In contrast, natural selection at nonextreme temperatures in *E. coli* has had no impact on the upper temperature at which the bacteria can sustain themselves in serial dilution (Bennett and Lenski 1993).

The physiological basis for the enhanced heat tolerance of lines evolving at high temperature requires investigation. Alahiotis and Stephanou (1982) suggest that heat shock proteins may be involved. Whereas some heat shock proteins are inducible after a heat shock or other stress, others are expressed constitutively even in unstressed cells and serve important roles as molecular chaperons (molecules that help a cell cope with potentially cytotoxic unfolded proteins; Parsell and Lindquist 1993; Feder et al. 1995; Feder 1996). Perhaps the expression or action of these constitutive proteins is altered by selection at relatively low (<30°C) temperatures. Other possible mechanisms of enhanced high-temperature performance include an increase in the ratio of saturated to unsaturated fatty acids in the cell membranes of the 29°C lines (Cossins and Prosser 1978) or the evolution of allozymes with greater catalytic efficiency and/or concentrations at specific temperatures (Barnes and Laurie-Ahlberg 1986).

Although laboratory natural selection influences adult heat tolerance (i.e., survival of a heat shock; Stephanou and Alahiotis 1983; Cavicchi et al. 1995; this study), it has no impact on knock-down temperature or walking speed at high temperature (see above; Tables 1, 4). Obviously, the ability to survive a heat shock must be genetically uncoupled with the ability to perform

well at high temperature. Consistent with this suggestion is the observation that artificial selection on knock-down temperature (G. W. Gilchrist and R. B. Huey, unpublished data) or on knock-down time (the time at which flies fall from a column at 38.5°–39°C; Hoffmann et al. 1997) does not lead to a correlated shift in heat tolerance.

Patterns for heat tolerance of eggs differed strikingly from that of adults (Table 1). Specifically, egg heat tolerance was not linearly related to selection temperature (Tables 1, 4). This is surprising, given the fact that eggs—unlike adults—cannot use behavior to avoid heat stress (Welte et al. 1993; Feder 1996; see also Coyne et al. 1983) and so might be expected to have genetic variation for physiological mechanisms of stress resistance. Unfortunately, heat tolerance of eggs in lines evolving at different temperatures has not been previously tested, so the generality of this pattern cannot currently be established. Nonetheless, adults and eggs might well show different responses, given that thermal resistance is only weakly correlated among developmental stages (Tucić 1979; Loeschcke and Krebs 1997).

Lack of Correlated Shifts in Cold Tolerance

The cold tolerances of adults and of eggs showed similar patterns; both were unrelated to line temperature (Tables 1, 5). Thus, natural selection at intermediate temperatures had no correlated evolutionary effect on the cold tolerance of these flies.

Tolerance of extreme cold has not been examined in previous studies of lines evolving by natural selection at different temperatures, so the generality of the apparent genetic independence of cold and heat tolerance observed here cannot be established. Only one study of artificial selection is relevant here, and this yielded inconsistent results. Selection for increased adult heat tolerance in the parasitic wasp *Aphytis* had no effect on cold tolerance, but reciprocal selection for increased adult cold tolerance slightly increased heat tolerance (White et al. 1970). Interspecific comparative analyses of lizards (Huey and Bennett 1987; Huey and Kingsolver 1993) and of fishes (Brett 1970) suggest that heat and cold tolerance do evolve independently, as least over long time scales.

Implications for Responses to Climate Change

Experimental studies using laboratory natural selection at different temperatures are relevant to debates as to whether animals have the genetic capacity to adapt to rapid global warming (Hoffmann and Blows 1993; Huey and Kingsolver 1993; Lynch and Lande 1993; Davis et al. 1995). Previous studies with several stocks of *Drosophila* and with *E. coli* (e.g., Anderson 1966; Alahiotis and Stephanou 1982; Stephanou and Alahiotis 1983; Lints and Bourgois 1987; Lenski and Bennett 1993; Cavicchi et al. 1995; Partridge et al. 1995) all show rapid evolutionary responses to sustained shifts in body temperature. Moreover, previous studies with the same stocks of flies studied herein also document rapid divergence in diverse traits (Huey et al. 1991; Partridge et al. 1994*a;* James and Partridge 1995). Consequently, the data reported in this article, combined with previous studies, suggest that rapid evolutionary responses to climate change are genetically feasible.

A demonstration that rapid evolution is genetically feasible is no guarantee that such evolution will actually occur in nature. In fact, actual evolutionary outcomes will be difficult to predict for several reasons (Cavicchi et al. 1995). First, our experiments demonstrate that natural selection at nonextreme temperatures results in important correlated responses that might influence evolutionary trajectories (Stephanou and Alahiotis 1983; Arnold 1987; Cavicchi et al. 1995). For example, tolerance (at least of adults) of extreme temperatures is altered as a correlated response, and overall locomotor performance seems to be reduced as a result of sustained evolution at a high temperature. Second, and more important, the type of fixed-temperature selection scheme used here (Material and Methods) is highly artificial (Bradshaw 1980; Brakefield and Mazzotta 1995) and does not allow for animals to use themoregulatory behaviors (Bartholomew 1964; Dunham 1993) to ameliorate the impact of a sustained temperature shift. Third, simple selection schemes may not accurately reflect the influence of density dependence or competitive or parasitic interactions (Mueller 1988; Davis et al. 1995; Joshi and Thompson 1996), all of which are operative forces in nature and will themselves be temperatures sensitive. Ultimately, studies that incorporate more complex selection regimes will be necessary to predict the probable evolution of thermal sensitivity in nature.

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