NOTES AND COMMENTS

THERMAL SENSITIVITY OF *DROSOPHILA MELANOGASTER* RESPONDS RAPIDLY TO LABORATORY NATURAL SELECTION

RAYMOND B. HUEY
Department of Zoology NJ-15, University of Washington, Seattle, WA 98195 USA

AND

LINDA PARTRIDGE, KEVIN FOWLER
Department of Zoology, University of Edinburgh, West Mains Road, Edinburgh 3H9 3JT, UK

Rates of physiological processes are strongly influenced by body temperature (Hochachka and Somero, 1984; Prosser, 1986), and this thermal sensitivity can profoundly affect the behavior, ecology, and evolution of ectotherms (Heinrich, 1981; Huey, 1982; David et al., 1983). Not surprisingly, the evolution of thermal sensitivity has long been a central subject in physiological ecology (reviewed in Huey, 1982; Huey and Kingsolver, 1989). Nevertheless, two fundamental evolutionary issues remain inadequately resolved:

(i) How rapidly can thermal sensitivity evolve? In other words, is thermal sensitivity evolutionarily conservative or labile (Morrison and Milkman, 1978; Hertz et al., 1983; Bennett et al., 1990)?

(ii) What genetic constraints limit evolutionary responses? For example, do genetic correlations exist between performances at intermediate and extreme temperatures (Huey and Hertz, 1984; Huey and Bennett, 1987; Lynch and Gabriel, 1987; Bennett et al., 1990)?

Comparative studies have been the primary method for investigating evolutionary questions in thermal physiology (Prosser, 1986). Such studies effectively document patterns that are the result of evolution over geological time (Huey, 1987; Harvey and Pagel, 1991); but they can sometimes yield ambiguous answers to the above questions, especially as regards contemporary evolutionary dynamics (see Discussion).

Selection experiments are a complementary tool for studying microevolutionary issues (Falconer, 1981; Stearns, 1989; Rose et al., 1990). Selection experiments, which can document the replicated and controlled dynamics of evolution in real time, hold considerable potential for investigating the microevolution of thermal sensitivity (White et al., 1970; Morrison and Milkman, 1978; Stephanou and Alahiotis, 1983; Huey and Kingsolver, 1989; van Delden and Kamping, 1989; Huey and Bennett, 1990; Bennett et al., 1990).

We used "laboratory natural selection" (Rose et al., 1990) to probe the evolution of thermal sensitivity of *Drosophila melanogaster.* We raised replicate lines of flies (founded from a common stock) in population cages for over four years under selection regimes of 16.5°C or 25°C—"intermediate" temperatures that are well within the vital range for this species. Parallel divergence of these replicates within thermal regimes suggests adaptation by natural selection to the different thermal environments, as might occur in natural populations subject to an abrupt environmental change. We then investigated two questions. First, had the thermal sensitivity of performance (e.g., of development rate) at intermediate temperatures diverged? If so, then thermal sensitivity can evolve rapidly. Second, did natural selection at intermediate temperatures influence tolerance of extreme temperature? If so, then a genetic correlation exists between performance at intermediate and at extreme temperatures.

Our project extends earlier work on the effects of natural selection under different thermal regimes on heat tolerance (Kilias and Alahiotis, 1985; Stephanou and Alahiotis, 1983), the thermal dependence of development time (Lints and Bourgois, 1987), and on genetic polymorphisms (van Delden and Kamping, 1989) of unreplicated populations of *D. melanogaster.* Natural selection in different thermal regimes has also been used to study the evolution of body size (Anderson, 1973; Powell, 1974; Caricchi et al., 1989) and life history (Mourad, 1965) in *Drosophila.*

The imminent prospect of global climate warming via the greenhouse effect (Dobson et al., 1989) gives renewed urgency to studies of organismal thermal sensitivity (Parsons, 1989; Bennett et al., 1990; Holt, 1990). By altering the physiological performance of ectotherms, rapid global warming may seriously disrupt populations and communities. This scenario assumes, however, that organismal thermal sensitivity will be incapable of adapting to rapidly changing temperatures. Selection studies (Bennett et al., 1990) such as the present one test this assumption.

MATERIALS AND METHODS

Populations were founded from a large outbred stock collected by Dr. G. Wilkinson in Brighton, England in June 1984. This stock was maintained on Lewis medium (sucrose-yeast) in a large population cage at 25°C (12L:12D) until January 1985. The stock was then subdivided to found the two temperature groups, each with three replicates. Founding densities were c. 2,000 adults. Humidity was uncontrolled, but high-humidity refugia (and food) were available in media bottles (12 bottles

at 25°C, 18 at 16.5°C, rotated on staggered four- or six-week schedules, respectively).

Large populations were maintained with overlapping generations in population cages for over four years at either 16.5°C or 25°C. [Note: Low-temperature flies were held for about 1 year at 18°C before transfer to 16.5°C.] In effect, the flies were subject to natural selection (Rose et al., 1990) for growth, competition, and reproduction at low versus high temperature. Densities of larvae and adults were uncontrolled but crowded. No dramatic fluctuations in populations were noted. The exact number of generations of selection is unknown. However, based on development times obtained from our experiments, the low-temperature and high-temperature flies must have experienced fewer than 60 and 100 generations, respectively, when the development-time experiment was conducted, and fewer than 66 and 110 generations by the heat-tolerance experiments.

Development Time

We measured the thermal dependence of development times in April 1989. As a precaution against transgenerational effects, we obtained parental flies for both lines by raising them from eggs at 25°C. [Note: this procedure causes one generation of reverse selection on the low-temperature line (L. Mueller, pers. comm.). Nevertheless, we still observed significant divergence between lines (see below).] When these flies were about 7 days old (as adults), they were sorted into vials (10 males and 10 females per vial) and maintained overnight with fresh food and live yeast (prelay 1). The following morning, flies were transferred to fresh vials (prelay 2) for three hr, then transferred to fresh vials twice in succession for 2 hr each (first and second egg collection), and finally transferred to new vials (overnight prelay). The complete sequence was repeated the following day. On both days, eggs from the two collections were raised at either 16.5°C or at 25°C until eclosion was complete. We checked vials twice daily and removed and counted newly emerged males and females. We used 17 to 18 vials per replicate per temperature treatment per day (432 vials total). Egg density was uncontrolled (see below), and the number of emerging flies from each vial (a measure of crowding) averaged 53.4 (range 8 to 139).

To compare statistically the thermal sensitivity of development times of the lines (selection regimes), we used an analysis of variance, with three replicates nested within each of two selection regimes. The complete model examined the effects of selection regime ($df = 1$), temperature ($df = 1$), day ($df = 1$), replicates within selection regime ($df = 4$), selection regime-by-temperature interaction ($df = 1$), and temperature-by-replicate within selection regime interaction ($df = 4$); with the number of emerging flies ("crowding" and crowding squared) used as covariates (each $df = 1$). [A curvilinear effect of crowding on development time was suggested by exploratory data analysis.] Because average development times of males and females (corrected for crowding) within vials were correlated ($r = 0.51$) and thus non-independent, we fit separate models for males and females. R^2 values were >0.98 for both models. We report statistical inferences based on Type III sums of squares (amount explained by a variable after adjusting for other variables), but conclusions would be unaffected by using Type I sums of squares. Data in Table 1 are point estimates, which represent the average development time (across vials, days, and replicates) for each sex and selection regime, adjusted for mean crowding. In addition to the results discussed below, "day" and "replicates within selection regime" significantly affected (P's < 0.001) development times of both females and males.

The key statistic here involves the significance level of the selection regime-by-temperature interaction, not the significance level of the selection regime. Absolute development times probably reflect natural selection in response to differing competitive environments (Mueller, 1988), which were uncontrolled, as well as to differing thermal environments.

An alternative way to analyze our data would be to use a microevolutionary rate test (Lande, 1977; see also Turelli et al., 1988) that tests the hypothesis that genetic drift alone can account for the observed divergence among lines. Lande's test requires estimates of heritabilities, the number of generations, and the effective population size, as well as the observed phenotypic and between-population variance. Some of our parameter estimates—especially of effective population size—would be crude at best. Instead of estimating these quantities, we choose to make an empirically based estimate of the drift between lines. Our analysis does this by using the variance component between replicates within selection regimes. In the present case, this should be equivalent to Lande's test if one took into account the errors in the required parameters (J. Felsenstein, pers. comm.).

Heat Tolerance

We measured the heat tolerance of the populations in September 1989. Larvae from both selection regimes were reared at standardized densities (c. 55 larvae per vial) at 25°C and were never exposed to anesthesia. During photophase, adults of both sexes (about 4 to 7 days after eclosion) were transferred to open vials (but flies were restricted to the bottom half of the vials by a mesh screen) for 1 hr, and then lowered into a covered Haacke water bath (39.5°C) for 30 min. The bottom half of each vial was positioned well below the surface of the water. Relative humidities (near saturation) and temperatures inside the vials should equilibrate quickly. We measured heat tolerance for flies in six blocks of 12 vials, with two vials per replicate per block (total 72 vials, with 12 per replicate), randomized for position. [Blocking controls for between-block variation in survival probabilities. In our experiments, survival varied significantly among blocks, because the circulator became partially clogged between blocks 3 and 4.] Following heat shock, vials were immediately cooled; and flies were transferred to vials at 20°C with media. After 20 hr, we began scoring the proportions of females and of males in each vial that survived (i.e., were able to walk when prodded).

Heat tolerance data (logit scale) were analyzed with logistic regression, which is the maximum likelihood estimation for a binomial model; this approach avoids the limitations of arcsine square-root transformations (Weisberg, 1985). To compare overall heat resistance, we compared two nested models (replicates nested within lines). The larger model measured the effects of selection regime ($df = 1$), sex ($df = 1$), block ($df = 5$),

TABLE 1. Point estimates (see Materials and Methods, ± standard errors) for development times (in days, corrected for density) of the 16.5°C versus 25°C lines of *Drosophila melanogaster* at two temperatures. A line-by-temperature interaction is evident: both females and males of the 16.5°C-line developed relatively quickly at low temperature but relatively slowly at high temperature.

Dev. temp.	Selection regime	Replicate	Development time (days)	
			Females	Males
16.5°C	16.5°C	1	23.0 ± 0.03	23.6 ± 0.03
		2	22.8 ± 0.03	23.4 ± 0.03
		3	23.2 ± 0.03	23.9 ± 0.03
		avg.	**23.00 ± 0.019**	**23.64 ± 0.019**
	25°C	1	23.4 ± 0.04	24.2 ± 0.04
		2	23.4 ± 0.04	24.2 ± 0.03
		3	23.7 ± 0.04	24.2 ± 0.03
		avg.	**23.51 ± 0.020**	**24.26 ± 0.020**
25°C	16.5°C	1	9.6 ± 0.03	9.8 ± 0.03
		2	9.4 ± 0.03	9.6 ± 0.03
		3	9.5 ± 0.03	9.7 ± 0.03
		avg.	**9.49 ± 0.020**	**9.70 ± 0.020**
	25°C	1	9.4 ± 0.04	9.6 ± 0.04
		2	9.3 ± 0.03	9.5 ± 0.03
		3	9.4 ± 0.04	9.6 ± 0.04
		avg.	**9.35 ± 0.021**	**9.57 ± 0.021**

replicates within selection regimes ($df = 4$), vials nested within block and replicates ($df = 36$) along with several interactions (sex-by-block, block-by-selection regime, sex-by-replicate, and block-by-replicate). The smaller model was identical, except that the effect of selection regime and its interaction with block were excluded. The likelihood was computed for these two models, and twice the log of the ratio of the likelihoods compared with the chi square distribution ($df = 6$).

RESULTS

Development times (time to eclosion) of both selection regimes were more than twice as long at low temperature than at high temperature (Table 1; temperature effect, P's ≪ 0.001). The effects of temperature on development times differed significantly, however, between selection regimes (selection regime-by-temperature interaction, P's < 0.0001). For example, low-temperature flies developed about ½ day faster than did high-temperature flies at 16.5°C. The converse was true at 25°C, but the time difference was only a few

hours (Table 1). Replicates within selection regimes also showed significant heterogeneity (P's < 0.001) but were largely non-overlapping between selection regimes. Overall, the thermal dependence of development time appears to have evolved during four years of natural selection in the laboratory.

High-temperature flies had significantly higher survival of heat shock (30 min at 39.5°C) than did low-temperature flies (Table 2, P < 0.05). However, average differences in percent survival between selection regimes are minor (Table 2, 2.8% for females, 4.0% for males) and smaller than the differences among replicates within lines (maximum = 11.9%, Table 2). Replicate identity had a significant effect (P ≪ 0.0001). Averages for the replicates showed some overlap between lines, suggesting that not all replicates showed parallel responses to the selection regimes.

DISCUSSION

The thermal sensitivity of development time and possibly the heat resistance of *D. melanogaster* evolved

TABLE 2. Proportion of flies surviving heat shock (30 min at 39.5°C). Average ± standard error (average number per sex per vial). Flies from the high-temperature line had slightly higher heat tolerance than did flies from the low-temperature line.

Selection regime	Replicate	Proportions surviving	
		Males	Females
16.5°C	1	0.131 ± 0.0206 (22.3)	0.395 ± 0.0305 (21.3)
	2	0.037 ± 0.0110 (24.8)	0.406 ± 0.0310 (20.9)
	3	0.049 ± 0.0133 (22.0)	0.294 ± 0.0281 (21.8)
	avg.	**0.071 ± 0.0089** (23.0)	**0.364 ± 0.0174** (21.4)
25°C	1	0.085 ± 0.0177 (20.7)	0.355 ± 0.0302 (20.9)
	2	0.146 ± 0.0229 (19.9)	0.387 ± 0.0331 (18.1)
	3	0.069 ± 0.016 (20.7)	0.474 ± 0.0329 (19.2)
	avg.	**0.099 ± 0.0110** (20.4)	**0.404 ± 0.0185** (19.4)

quickly in response to laboratory natural selection on replicate populations exposed to 16.5°C versus 25°C. Flies from the low-temperature line developed about ½ day faster than did high-temperature flies at 16.5°C, whereas high-temperature flies developed a few hours faster than the low-temperature flies at 25°C (Table 1). [This qualitative pattern has recently been substantiated by an independent analysis of development times of these lines raised at standard densities (B. Barrie and L. Partridge, unpubl. data).] Because eggs used in these experiments were obtained from parents raised for one generation at a "common garden" temperature, differences between populations in the thermal sensitivity of development time are likely genetic; but standard genetic crosses will be necessary to validate this assumption (e.g., Stephanou and Alahiotis, 1983).

The observed divergence was rapid (i.e., in less than 4.5 yr). This accounts at most (Materials and Methods) to 66 generations of selection at low temperature and 110 generations at high. However, because we did not measure the thermal dependence of development time at intervals from the establishment of the selection regimes, we cannot determine whether the observed divergence reflects evolution in one or in both regimes.

Development time is a key determinant of the rate of population growth (Charlesworth, 1980). Accordingly, the observed change in the thermal sensitivity of development time (Table 1) presumably reflects natural selection on the ability of flies to reach maturity quickly under a given thermal and competitive regime. Nevertheless, although natural selection experiments are ecologically realistic and can be used effectively to monitor the results of selection, they do obscure the exact focus or foci of selection (Velde and Scharloo, 1988; Rose et al., 1990). For example, lines of *D. melanogaster* and of *D. pseudoobscura* maintained for several years at low versus high temperature diverged in a variety of traits (body size, sexual isolation, oviposition rhythm, sterility, development time, allozyme and inversion frequencies as well as in heat resistance and heat-shock-protein response: Mourad, 1965; Anderson, 1973; Powell, 1974; Kilias and Alahiotis, 1985; Stephanou and Alahiotis, 1983; Lints and Bourgois, 1987; Cavicchi et al., 1989; van Delden and Kamping, 1989). Accordingly, the selective paths leading to an observed change in the thermal sensitivity of development time might well reflect direct or indirect selection. Resolution will require the use of artificial selection experiments (Velde and Scharloo, 1988; Stearns, 1989; Rose et al., 1990), which selectively delimit which phenotypes breed.

Tolerance to heat shock also appears to have evolved. Overall, the high-temperature flies had slightly higher heat resistance than did the low-temperature flies. However, the statistical significance of this difference is marginal ($P < 0.05$). Moreover, the proportion of flies surviving differed more among replicates within lines than between selection regimes (Table 2), suggesting an important role of genetic drift as well. In any case, because the flies had not knowingly been subjected to direct selection on heat resistance while in the laboratory, our results imply that natural selection on performance at intermediate temperatures (i.e., at 16.5°C or 25°C) may sometimes lead to a genetically correlated response (Falconer, 1981) in resistance to extreme temperature. However, the specific physio-

logical basis underlying this apparent genetic correlation is unknown.

Several studies have previously demonstrated rapid genetic responses of the thermal sensitivity of small organisms to temperature (reviews in David et al., 1983; Parsons, 1987; Huey and Kingsolver, 1989). Tolerance to heat shock responds rapidly to artificial selection in *D. melanogaster* (Morrison and Milkman, 1978; Stephanou and Alahiotis, 1983), a parasitic wasp (White et al., 1970), and a fish (Shah, 1985). Moreover, populations of fish exposed to heated effluents from nuclear power plants have higher upper lethal temperatures than do nearby populations from cooler ponds (Holland et al., 1974). Thermal sensitivity of flagellates (Dallinger, 1887) and the fitness of *E. coli* (Bennett et al., 1990) also evolves quickly in response to natural selection at high temperatures in the laboratory.

Our results are similar to those reported for *D. melanogaster* that were exposed to seven years of natural selection at 25° versus 14°C ("Greek flies," Stephanou and Alahiotis, 1983) or 6 years at 21°, 25°, or 28°C ("Oregon-R" stock, Lints and Bourgois, 1987). Some minor differences are, however, apparent. With the Greek flies, the difference between lines in heat tolerance was much more marked (survival of 31% vs. 57%) than in our Brighton flies (Table 2). With the Oregon-R flies, the 21°C line developed more quickly at 25°C than did the 25°C line, contrary to our results (Table 1; B. Barrie and L. Partridge, unpubl. data). These different responses might reflect geographic differences in the initial genetic composition of these flies, differences in duration of selection, subtle differences in experimental procedures, or random error. Moreover, because replicate populations within selection regimes show significant heterogeneity in thermal sensitivity (above), the differences might also reflect the lack of replication in previous studies.

Comparative Studies Versus Selection Experiments

Comparative studies analyze the net results of long-term selection. In the absence of fossil data, they are the only way to investigate how traits have actually diverged in nature (Harvey and Pagel, 1991). Nevertheless, they can have difficulty establishing cause and effect (Huey and Bennett, 1986; but see Harvey and Pagel, 1991). In particular, as argued below, comparative studies may be ambiguous for analyzing some questions concerning the evolution of thermal sensitivity.

Comparative studies can estimate rates of evolution of thermal sensitivity if divergence times are known (Haldane, 1949). However, because only *average* rates of change can be calculated (Huey, 1982, 1987), maximal rates of change will be underestimated if evolutionary change is episodic (Wake et al., 1983).

Comparative studies have occasionally attempted to infer genetic correlations involving thermal sensitivity (Huey and Bennett, 1987; Huey and Hertz, 1984). However, such inferences are unreliable: an interspecific correlation between two traits can reflect either a common genetic correlation or correlated selection pressures on those traits (Felsenstein, 1988 p. 452; Zeng, 1988).

In contrast, selection experiments generate direct estimates of maximal rates of evolution and of genetic

correlations (Falconer, 1981; Stearns, 1989; Rose et al., 1990). Moreover, selection experiments can be replicated (Cohan and Hoffmann, 1989) giving us the ability to replay the "tape of evolution" (Gould, 1989) under precisely controlled conditions. Nevertheless, selection experiments have their limits. Population cages are artificial environments (Rose et al., 1990), and they may prevent organisms from using normal behavioral adjustments that might alter evolutionary outcomes. Accordingly, selection experiments indicate what might happen in nature, but not necessarily what will happen.

Comparative approaches and selective experiments are thus complementary: they address similar evolutionary questions, but from a different perspective. Each has strengths that complement the weaknesses of the other. Ultimately, our understanding of evolution can only be enhanced by incorporating data from both approaches.

Implications

Small organisms with short generation times clearly have the capacity to respond genetically and rapidly to shifts in the thermal environment. Accordingly, the physiological and ecological consequences of global climate warming may be influenced by evolution of thermal sensitivity, at least in rapidly reproducing species (Parsons, 1989; Bennett et al., 1990; Holt, 1990). Nevertheless, large organisms (e.g., trees, many vertebrates), which have long generation times, are unlikely to make sufficiently rapid genetic adjustments and may thus experience stress and extinction. Because of these effects on large organisms, global climate warming could still have severe — if primarily indirect — effects even on rapidly evolving organisms.

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