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WITHIN- AND BETWEEN-GENERATION EFFECTS OF TEMPERATURE ON THE MORPHOLOGY AND PHYSIOLOGY OF *DROSOPHILA MELANOGASTER*

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Abstract.—We investigated the effects of developmental and parental temperatures on several physiological and morphological traits of adult *Drosophila melanogaster*. Flies for the parental generation were raised at either low or moderate temperature (18°C or 25°C) and then mated in the four possible sex-by-parental temperature crosses. Their offspring were raised at either 18°C or 25°C and then scored as adults for morphological (dry body mass, wing size, and abdominal melanization [females only]), physiological (knock-down temperature, and thermal dependence of walking speed), and life history (egg size) traits. The experiment was replicated, and the factorial design allows us to determine whether and how paternal, maternal, and developmental temperatures (as well as offspring sex) influence the various traits. Sex and developmental temperature had major effects on all traits. Females had larger bodies and wings, higher knock-down temperatures, and slower speeds (but similar shaped performance curves) than males. Development at 25°C (versus at 18°C) increased knock-down temperature, increased maximal speed and thermal performance breadth, decreased the optimal temperature for walking, decreased body mass and wing size, reduced abdominal melanization, and reduced egg size. Parental temperatures influenced a few traits, but the effects were generally small relative to those of sex or developmental temperature. Flies whose mother had been raised at 25°C (versus at 18°C) had slightly higher knock-down temperature and smaller body mass. Flies whose father had been raised at 25°C had relatively longer wings. The effects of paternal, maternal, and developmental temperatures sometimes differed in direction. The existence of significant within- and between-generation effects suggests that comparative studies need to standardize thermal environments for at least two generations, that attempts to estimate "field" heritabilities may be unreliable for some traits, and that predictions of short-term evolutionary responses to selection will be difficult.

Key words.—Acclimation, body size, coloration, developmental effects, *Drosophila melanogaster*, egg size, locomotion, maternal effects, paternal effects, temperature.

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The sins of the fathers are to be laid upon the children.
Wm. Shakespeare, *The Merchant of Venice,* Act III

The magnitude and nature of nongenetic effects on an organism's phenotype (phenotypic plasticity, norms of reaction, acclimation) have recently received considerable attention. Such nongenetic effects are relevant not only to functional biologists studying how organisms work (Somero 1995), but also to evolutionary biologists studying the dynamics of phenotypic evolution (Kirkpatrick and Lande 1989; Stearns 1989). The magnitude of phenotypic plasticity is genetically variable and can respond to selection (Gebhardt and Stearns 1988; Scheiner and Lyman 1991). However, marked phenotypic plasticity complicates attempts to predict responses to selection (Via and Lande 1985; Kirkpatrick and Lande 1989).

Most studies of phenotypic plasticity have focused on the phenotypic effects of environmental factors within an individual's lifetime. For example, many studies report on the consequences of developmental regimes (temperature, food regime, or crowding) on adult size, life span, physiological performance, or fecundity (e.g., David et al. 1983; Gebhardt and Stearns 1988). Relatively little is known, however, about cross-generational effects of parental environments. Nevertheless, such effects have been documented for diverse morphological (Falconer 1989), life history (Mousseau and Dingle 1991), and physiological (David 1962) traits. Most examples concern maternal effects, but a few papers have documented paternal effects (Giesel 1988; Mousseau and Dingle 1991).

We have investigated the within- and between-generation effects of temperature on various phenotypic traits of adult *Drosophila melanogaster*. We raised flies through two generations at all eight combinations of two paternal, two maternal, and two developmental temperatures. We developed this factorial design specifically to evaluate the relative effects of paternal, maternal, and developmental temperature (as well as of sex) on various traits. The effects of developmental temperature and of sex on many of these traits are already well established (e.g., David et al. 1983), such that some of our findings are hardly novel. However, incorporation of sex and of developmental temperature into our factorial design is nevertheless useful. Specifically, we can use information on the direction and magnitude of the effects of sex and of developmental temperature as a baseline against which to compare the effects of maternal and paternal temperatures. In contrast, had we chosen an experimental design that measured only the effects of, for example, maternal temperature, we would not be able to evaluate whether the observed effects were small or large relative to the well-known effects of sex or of developmental temperature.

The traits we scored represent morphology (body size, wing size, and degree of abdominal melanization), physiology (knock-down temperature, and thermal dependence of sprint speed), and life history (egg size). These traits poten-

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tially have important, if rather diverse, effects on fitness. For example, body size and wing size often influence social dominance, mating success, fecundity, and flight dynamics (Robertson 1957; Partridge et al. 1987a,b; Starmer and Wolf 1989). Abdominal melanization may influence heat absorption and potentially thermoregulatory capacity (Capy et al. 1988). Knock-down temperature could index probability of survival during a heat stress (Huey and Kingsolver 1993), and the thermal dependence of walking speed could affect survival or mating success (Christian and Tracy 1981; Partridge et al. 1987a). Finally, egg size can affect viability (Curtsinger 1976).

Not surprisingly, all of the traits studied here were strongly influenced by sex and developmental temperatures. However, a few traits were also influenced by maternal or paternal temperatures. Recent companion studies from our lab demonstrate that both developmental and parental temperatures influence male territorial success (Zamudio et al. 1995) and that laying and paternal (but not developmental and maternal) temperature influenced fecundity of flies early in life (Huey et al. 1995). This suite of experiments reinforces the view that adult phenotypes can sometimes be sensitive to between- as well as within-generation environmental effects. Consequently, attempts to predict short-term evolutionary responses to selection are likely to be difficult (Gupta and Lewontin 1982; Riska et al. 1985; Kirkpatrick and Lande 1989). Moreover, attempts to measure "field heritabilities" (Coyne and Beecham 1987; Riska et al. 1989; Hoffmann 1991), which assume that cross-generational effects are insignificant, may be unreliable. Our observations of significant paternal effects should encourage studies of mechanism and of the adaptive significance (or lack thereof) of such cross-generational effects.

Materials and Methods

The Flies

Flies used in this experiment originated from a large population (~1000 isofemale lines, courtesy of L. Harshman and M. Turelli) collected from Escalon, California in May 1991 and maintained at the University of California, Davis at room temperature (~22°C) 13:11 L:D cycle. We received a sample (~1000 flies) in April 1992. Thereafter, we reared flies in vials at low and controlled density (~50 eggs/vial; cornmeal, molasses, yeast, agar, tegosept) before transferring them to population cages of 2000 to 3000 individuals with discrete two-week generations at 22°C on a 12:12 L:D cycle. Present experiments were run in July through August 1992. The flies had been in captivity for about 14 months and thus should have been partially adapted to the laboratory environment (Service and Rose 1985).

Overview of Protocol

We independently manipulated paternal, maternal, and developmental temperatures of flies and then scored several traits of adults. We raised flies through two generations during which we manipulated sire's temperature (T_{sire}; 18°C or 25°C), dam's temperature (T_{dam}; 18°C or 25°C), and offspring's developmental temperature (T_{dev}; 18°C or 25°C). We selected 18°C and 25°C because these temperatures are well within the natural range of developmental temperatures for *D. melanogaster* (Parsons 1978; McKenzie and McKechnie 1979; Jones et al. 1987; Feder 1996) and these temperatures result in normal development (Economos and Lints 1986). We then crossed parental flies in the four possible sex-by-parental temperature combinations and raised the eggs at either 18°C or 25°C. Thus, eight basic treatment groups (with different thermal histories) were generated. The experiment was replicated twofold, and 16 groups of flies were monitored and scored for phenotypic traits.

The Parental Generation

To produce the parental generation, we collected eggs from the laboratory stock, transferred them into 40 vials (50 to 70 eggs/vial), and moved them to either 18°C or 25°C for development (T_{sire} and T_{dam}). Each egg-collection was limited to five hours to reduce the exposure (hence possible acclimation) of eggs to 22°C before transfer to their respective developmental temperatures. Because development rate is inversely related to temperature, we staggered these initial egg collections so that eggs raised at the two temperatures would eclose at similar times: consequently, we collected eggs destined for development at 18°C about ten days prior to collecting those destined for development at 25°C.

After the parental flies eclosed, we collected virgin flies during the middle of the eclosion period, separated males and females (light CO_2 anesthesia, 20 to 30 males or females per vial), and maintained them until ready for mating at their respective developmental temperatures (T_{sire} and T_{dam}). Because flies living at different temperatures will have different "physiological" ages (Long et al. 1980; Taylor 1981) and because parental age can influence offspring trait values (David 1962; Parsons 1964), we synchronized physiological ages of parental flies at mating. Thus 18°C parents were mated at five days of adult age, whereas 25°C parents were mated at three days of adult age (ages were scaled to relative development times at these two temperatures). Parental flies were crossed at 22°C to minimize potential natural selection at different temperatures and to ensure that all parental flies (and their eggs) experienced a small temperature shift. We repeated the entire sequence to generate two replicate groups of parents.

The Offspring Generation

We collected eggs for the offspring generation from the parental crosses (above) and transferred them to 18°C or 25°C (as above). The resulting flies belonged to eight replicated experimental groups (each with ~2000 flies) that differed in paternal, maternal, or developmental temperature (Table 1). Each of the groups of 18°C replicates eclosed after its paired 25°C replicate (see below).

We recognize two potential problems with our protocol. First, because these flies were outbred and were raised for one or two generations at different temperatures, they might have been exposed to inadvertent selection, such that differences among groups might reflect genetic as well as phenotypic effects. Such genetic differentiation should, however, be small. In pilot experiments, egg viability was invariably

TABLE 1. Morphological measurements of adult flies as a function of parental and developmental temperatures. Values given are means ± standard errors. Sample sizes are shown in parentheses. For the various temperature treatments, H = 25°C and L = 18°C.

Sex	T_{dev}	T_{dam}	T_{sire}	Dry mass (μg)	Wing length (mm)	Wing width (mm)	Melanization (% black)
F	H	H	H	396.0 ± 1.75 (20)	2.12 ± 0.002 (37)	0.98 ± 0.001 (37)	0.36 ± 0.008 (12)
F	H	H	L	393.4 ± 1.93 (20)	2.13 ± 0.001 (42)	0.99 ± 0.001 (42)	0.33 ± 0.004 (12)
F	H	L	H	392.2 ± 2.11 (20)	2.12 ± 0.002 (38)	0.98 ± 0.001 (38)	0.30 ± 0.004 (12)
F	H	L	L	393.6 ± 1.56 (20)	2.11 ± 0.001 (40)	0.98 ± 0.001 (40)	0.30 ± 0.005 (12)
F	L	H	H	396.7 ± 1.48 (20)	2.36 ± 0.002 (40)	1.08 ± 0.001 (40)	0.50 ± 0.004 (12)
F	L	H	L	411.1 ± 2.05 (20)	2.36 ± 0.002 (39)	1.07 ± 0.001 (39)	0.53 ± 0.005 (12)
F	L	L	H	428.8 ± 2.18 (20)	2.35 ± 0.002 (39)	1.08 ± 0.001 (39)	0.55 ± 0.006 (12)
F	L	L	L	444.8 ± 2.08 (20)	2.35 ± 0.002 (40)	1.07 ± 0.001 (40)	0.55 ± 0.006 (12)
M	H	H	H	270.2 ± 1.24 (20)	1.86 ± 0.002 (37)	0.89 ± 0.001 (37)	—
M	H	H	L	275.8 ± 1.55 (20)	1.87 ± 0.001 (40)	0.89 ± 0.001 (40)	—
M	H	L	H	267.7 ± 1.50 (20)	1.87 ± 0.001 (36)	0.89 ± 0.001 (36)	—
M	H	L	L	267.3 ± 1.34 (20)	1.85 ± 0.001 (38)	0.88 ± 0.001 (38)	—
M	L	H	H	254.7 ± 1.98 (20)	2.09 ± 0.002 (36)	0.98 ± 0.001 (36)	—
M	L	H	L	265.5 ± 2.14 (20)	2.10 ± 0.001 (39)	0.98 ± 0.001 (39)	—
M	L	L	H	283.8 ± 1.82 (20)	2.09 ± 0.002 (35)	0.98 ± 0.001 (35)	—
M	L	L	L	283.9 ± 2.48 (20)	2.09 ± 0.002 (34)	0.99 ± 0.001 (34)	—

high (> 90% of all eggs produced adults) at both 18°C and 25°C, such that little viability selection could have occurred during the egg stage. Moreover, the traits studied here have only moderate heritabilities (Roff and Mousseau 1987; Huey et al. 1992). Nevertheless, some selection could have occurred either if genotypes differed in their mating success or fecundity as a function of temperature, or if mating success or fecundity was genetically correlated with traits scored here (Gupta and Lewontin 1982; A. A. Hoffmann, pers. comm., 1994). Second, we set up simultaneously all eggs for the offspring generation (within a replicate) to ensure that all offspring flies would start development at the same time and in the same batch of media. Consequently, however, 25°C flies eclosed and were scored before the 18°C flies. Therefore, any observed differences between developmental-temperature groups might in part reflect measurement at different times (Coyne et al. 1983). However, because observed differences between replicates were generally insignificant (see below), our conclusions are unlikely to be seriously confounded by temporal bias.

Morphological and Life-History Measurements

Dry Body Mass

We measured the dry mass of 10 flies of each sex randomly chosen from different vials, set up for each of the 16 experimental groups (Σ = 320 flies). We dried flies at 60°C and then weighed individual flies (those with complete wings and legs) to the nearest μg.

Wing Size

We removed the right wing from 20 flies of each sex randomly selected from each experimental group (Σ = 640). We fixed the wings to glass slides (Partridge et al. 1987a), projected the images on a video monitor, and then used a digitizing program to measure the length (L3 vein) and the width (where L5 meets the wing margin) of each wing.

Abdominal Melanization

We scored the degree of melanization of the posterior portion of the abdomen (see David et al. 1990) for six females from each experimental group (Σ = 96; all males have heavily melanized abdomens). We used a camera lucida to trace the silhouettes (lateral projected view) of the three posterior tergites and of the melanized portions, and then gravimetrically estimated the percentage of the projected surface area that was melanized.

Egg Size

Flies were initially maintained at their developmental temperature for three to five days posteclosion. They were then transferred to 22°C for an additional 12 h, when we began collecting freshly laid eggs. To facilitate handling, we partially submerged eggs in a drop of tap water and thus measured fully hydrated eggs. We recorded video images of 16 to 20 eggs from each experimental group (Σ = 314) and later used a digitizing program to measure maximal length (l) and width (w) in arbitrary units of each egg.

Physiological Measurements

Thermal Dependence of Speed

On the morning of testing, 15 males and 15 females from each replicated treatment group were selected haphazardly and placed individually (without anesthesia) into plastic vials (70 × 15 mm). Speed was measured in a temperature-controlled, walk-in environmental chamber at the following sequence of temperatures 15°, 25°, 10°, 20°, 30°, 35°C. (Flies can be permanently damaged by high temperatures, so performance at 35°C had to be measured last.) Vials were placed in the chamber 15 to 20 min prior to testing to ensure that flies had equilibrated to the test temperature. Between trials, all individuals were held at 22°C.

To measure walking speed, we knocked a fly down to the bottom of a vial, and recorded the elapsed time (later converted to velocity, cm/s) until the fly reached to top of the

vial. The resultant walking speeds are voluntary but appear to be near maximal performance levels. In effect, the flies are exhibiting an escape response after being knocked off their perch. (This technique [Miquel 1976] also takes advantage of the negative geotropism of *D. melanogaster*.) Only one run per fly per temperature was used in about 95% of the tests. If, however, an individual flew rather than walked, up to two additional attempts were made. If the animal repeatedly flew, then the time taken for the last flight was recorded. At extreme temperatures some flies remained immobile for 60 sec (2% at 10°C; 14% at 35°C): these flies were assigned a velocity of 0.0 cm/s. All individuals were analyzed unless they failed to run at three or more adjacent test temperatures.

Knock-Down Temperature

We measured the "knock-down" temperature, which is the upper temperature at which flies lose coordination and fall from a glass column (see Fig. 1 in Huey et al. 1992). The apparatus used is a tall, water-jacketed glass column (with internal baffles) connected to a heated water bath. The water bath (and thus column temperature) was initially set to 30°C, which is a warm but not disabling temperature. We added about 1000 flies from a given group to the top of the column and began pumping increasingly warm water through the surrounding water jacket. The air inside the column heated at a fairly constant rate (~0.7°C/min). Because *Drosophila* are very small, their body temperature closely tracks the column's air temperature (see Appendix in Huey et al. 1992), which we monitored with a fine thermocouple.

As the column heated, a fly became progressively warmer and eventually reached its "knock-down" temperature (T_{kd}) and then fell out the column into collecting tubes, which were changed at 0.5°C intervals. We measured T_{kd} for a total of 17,254 flies. Mean knock-down temperatures are generally repeatable (Huey et al. 1992; below).

Because adult age affects heat resistance in *Drosophila* (Lamb and McDonald 1973), we standardized the physiological age of flies (as above). Specifically, we measured knock-down temperatures on flies aged 5–6 days (18°C development) or 3–4 days (25°C development). All flies were transferred to 22°C for 24 h prior to measurement so that all flies would experience the same acute temperature shift (22°C to 30°C) at the beginning of the knock-down experiment.

Statistical Analyses

To determine whether transformations were required, we inspected distributions of data and of residuals for normality. For dry body mass and knock-down temperature, no transformation was necessary. (In any case, we verified that standard transformations did not affect statistical conclusions.) Thermal performance breadths (see below) and maximal speeds were log-transformed. Percent melanization data were arcsine (square root) transformed. For both wing and egg size, lengths and widths were correlated: we analyzed the principal components (from z scores) for these two size measurements.

With knock-down temperature and the size measurements, we initially ran a full factorial analysis of variance for T_{sire}, T_{dam}, T_{dev}, sex, and replicate with all possible two-way interactions. With percent melanization and egg size, which were measured only for females, we initially used a four-factor analysis of variance, with all two-way interactions. If these initial analyses showed that replicates differed significantly, we included the replicate sum of squares in the error sum of squares (Sokal and Rohlf 1981, p. 349). Abdominal melanization was correlated with projected area of the tergites, so projected area was included as a covariate in the melanization analyses. Most analyses were performed using S-Plus (StatSci Inc. 1993).

In the measurements of knock-down temperature, about 1000 flies all from the same treatment group were tested in a given run (above), such that data for individuals within runs were probably not fully independent. Therefore, we computed and subsequently analyzed only the mean knock-down temperature for males and for females from each run. This is an extremely conservative procedure because 17,254 measurements are reduced to only 32.

The thermal dependence of sprint speed is a multivariate trait, as performance is scored over multiple temperatures. We analyzed the data two ways. First, we analyzed three performance measures that summarize the position, height, and breadth of these performance curves (see Hertz et al. 1983): T_{opt} is the observed temperature at which an individual walked fastest, u_{max} is the speed at T_{opt} (thus maximal speed), and T_{br} is an index describing the breadth of the performance curve. This latter index, which is derived from the second moment of area about a neutral axis, describes the distribution of performance about a central point, in this case about T_{opt}:

$$T_{br} = \sqrt{\sum \left[\frac{u_i(T_i - T_{opt})}{u_{max}}\right]^2} \quad (1)$$

where u_i is walking velocity at temperature T_i. (Velocities u_i were standardized to u_{max} to remove spurious correlations between T_{br} and u_{max}.)

We also used a repeated-measures ANOVA to analyze thermal performance curves. Intra-individual effects were nested inside the effects for maternal, paternal, and developmental temperatures. This term was used as the error term for the among-subjects analysis.

Results and Discussion

Dry Body Mass and Wing Size

Results

Dry body mass averaged 339 ± 4.4 μg ($\bar{x}$; ± SE. Table 1), and the full model (Table 2) accounted for most of the variation (79%) in dry mass. Sex had the dominant influence on mass ($P < 0.0001$): females were much larger than males (by 50%; Fig. 1). Developmental ($P = 0.0007$) and dam ($P = 0.003$) temperatures also influenced dry mass. Flies were also relatively heavy if they developed at low temperature or if their dam had been at low temperature (Fig. 1). However, these effects were small (e.g., development at 18°C increased dry mass by only 4% relative to development at 25°C). Sire temperature had no significant effect ($P = 0.17$).

The interaction between sex and developmental temperature was significant (Table 2, $P = 0.003$): only female dry body mass was sensitive to developmental temperature. (A

TABLE 2. ANOVA tables for morphological traits.

Source	df	Sum of sq	Mean sq	F value	Pr(F)
Mass:					
Sex	1	1,478,864	1,478,864	1087.87	< 0.001
T_{sire}	1	2554	2554	1.88	0.171
T_{dam}	1	12,152	12,152	8.94	0.003
T_{dev}	1	15,961	15,961	11.74	< 0.001
Sex:T_{sire}	1	218	218	0.16	0.689
Sex:T_{dam}	1	832	832	0.61	0.435
Sex:T_{dev}	1	12,350	12,350	9.09	0.003
T_{sire}:T_{dam}	1	160	160	0.12	0.732
T_{sire}:T_{dev}	1	1739	1739	1.28	0.259
T_{dam}:T_{dev}	1	20,448	20,448	15.04	< 0.001
Error	309	420,060	1359		
Wing size (PC1):					
Sex	1	547.886	547.886	2233.767	< 0.001
T_{sire}	1	0.058	0.058	0.236	0.628
T_{dam}	1	0.230	0.230	0.937	0.333
T_{dev}	1	471.232	471.232	1921.246	< 0.001
Sex:T_{sire}	1	0.030	0.030	0.121	0.728
Sex:T_{dam}	1	0.004	0.004	0.015	0.904
Sex:T_{dev}	1	0.030	0.030	0.120	0.729
T_{sire}:T_{dam}	1	0.280	0.280	1.141	0.286
T_{sire}:T_{dev}	1	0.000	0.000	0.002	0.965
T_{dam}:T_{dev}	1	0.115	0.115	0.467	0.494
Error	599	146.919	0.245		
Melanization:					
Abdominal area	1	0.082	0.082	19.13	< 0.001
T_{sire}	1	0.000	0.000	0.10	0.747
T_{dam}	1	0.002	0.002	0.44	0.510
T_{dev}	1	1.042	1.042	241.73	< 0.001
T_{sire}:T_{dam}	1	0.000	0.000	0.12	0.726
T_{sire}:T_{dev}	1	0.003	0.003	0.75	0.388
T_{dam}:T_{dev}	1	0.051	0.051	11.74	< 0.001
Error	88	0.379	0.004		

separate analysis only of males verified that male size was insensitive to developmental temperature [$P = 0.76$].) Dam by developmental temperature was also significant ($P = 0.0001$): developmental temperature had a marked effect only if the dam had been raised at 18°C but not at 25°C.

Wing length and width were strongly correlated ($r = 0.94$), and so we analyzed only the first principal component score (97% of variance) of z-transformed measurements. The resulting ANOVA model (Table 2) accounted for 87% of the variation. Females had much larger wings than males ($P < 0.0001$, Table 2). Developmental temperature also had a large, negative effect (Fig. 1, $P < 0.0001$). However, neither dam ($P = 0.33$) nor sire ($P = 0.63$) temperature influenced wing size. None of the two-way interactions was significant (all $P > 0.28$, Table 2).

Discussion

Developmental temperature had significant and inverse effects on dry body mass and on wing size of females, but on only wing size of males. The insignificant response of dry body mass of males may be idiosyncratic. In a separate study in our laboratory (Zamudio et al. 1995), but with a different stock of *D. melanogaster,* male dry mass was significantly increased by development at 18°C versus 25°C. In any case, the inverse relationship between body size and developmental temperature reiterates a classic pattern for *Drosophila* (David et al. 1983) and many other ectotherms (Atkinson 1994). (However, body size may be relatively small in flies that developed at extremely low temperature [Economos and Lints 1984].)

The mechanistic basis for the inverse relationship between developmental temperature and body size is of interest (Partridge et al. 1995). Development at low temperature results in slow growth and in delayed maturation at a large size in many organisms (Atkinson 1994). In contrast, development on low quality food also slows growth and delays maturation, but at a smaller—not larger—size (Gebhardt and Stearns 1988). Several reaction-norm models for size and age at maturity as functions of temperature or food quality have been proposed (Stearns and Crandall 1984; Berrigan and Charnov 1994). For example, Berrigan and Charnov (1994) analyze reaction norms in terms of a general growth model, in which larger size (e.g., resulting from low temperature development) is assumed to result in increased fecundity. However, when females of the stocks studied herein are raised at low temperature, they are relatively large (Table 1), but nevertheless do not in fact have increased fecundity, at least early in life (Huey et al. 1995; below). Consequently, an evolutionary explanation for these reaction norms in *D. melanogaster* remains elusive.

Evolutionary and developmental responses to temperature are parallel. *Drosophila* evolving by laboratory natural selection at low temperatures become genetically larger than

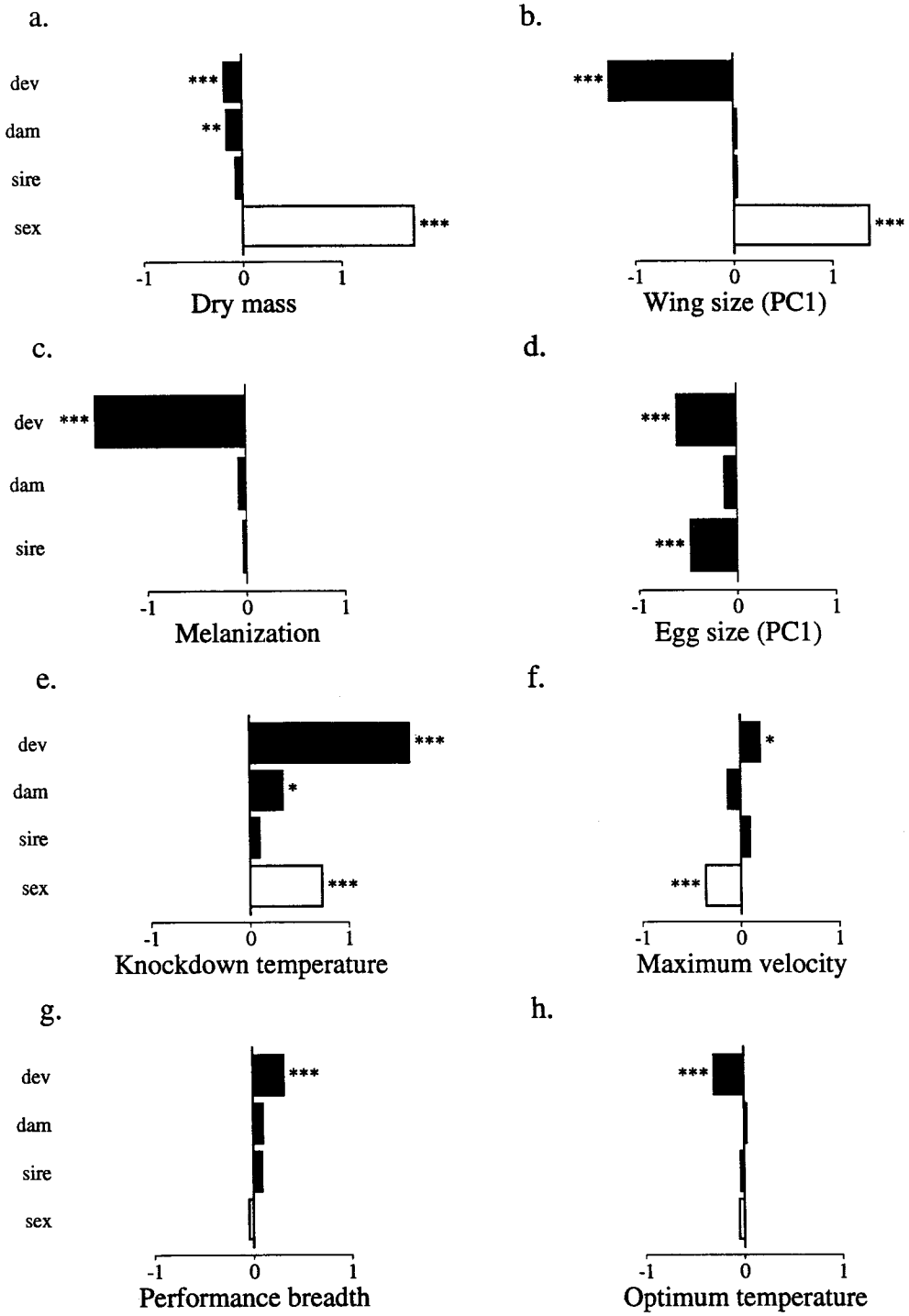

FIG. 1. Effects of treatments on eight phenotypic traits of *Drosophila melanogaster*. Solid bars represent the differences between the least-squared means (scaled in standard deviation units) for treatment at 25°C minus that at 18°C (for sire, dam, and developmental temperature treatments). Unfilled bars represent the effect of sex (female minus male). Significance levels (from Tables 2, 4, and 6) are indicated adjacent to each bar (* = 0.05, ** = 0.01, *** = 0.001).

flies evolving at high temperature (Anderson 1966; Cavicchi et al. 1989; Partridge et al. 1995). Similarly, body size is positively related to latitude (hence inversely related to mean ambient temperature) in several species (David and Bocquet 1975; Pegueroles et al. 1995).

Wing size was more plastic with respect to developmental temperature than was dry mass (Fig. 1). Consequently, wing loading (mass per unit area of wing) may be reduced at low developmental temperatures. However, measurements of mass and wing size on the same individuals are required to test this

expectation. Wing loading is lower during cool seasons in *D. melanogaster* collected from nature (Stalker 1980) or in flies raised at low temperature (Starmer and Wolf 1989). Several possible functional consequences are discussed in Starmer and Wolf (1989; see also Curtsinger and Laurie-Ahlberg 1981).

Cross-generational effects on body size in flies are generally thought to be small (Riska et al. 1989), but temperature-induced, cross-generational effects have not previously been examined to our knowledge. In our studies, dry body mass—but not wing size—was sensitive to dam temperature (Fig. 1). Flies whose mother had been raised at low temperature were slightly heavier, but had wings that were similar in length, than flies whose mother had been raised at low temperature (Fig. 1). (Note: a recent study confirms our finding that maternal temperature has no significant effect on wing length in *D. melanogaster* [A. A. Hoffmann, pers. comm., 1994].) In contrast, body mass and wing size were insensitive to sire temperature (Fig. 1). Whether the effect of dam temperature on body mass is adaptive requires investigation. The effect could reflect (in part) the larger eggs of low-temperature females (see below).

Abdominal Melanization

Results

On average, 42.6 ± 1.27% of the posterior three tergites of females were melanized in lateral projection view. The model accounted for 77% of the variation in melanization. Developmental temperature had a major impact: flies that developed at 18°C had 20% more of their projected surface area covered by melanin than did flies that developed at 25°C (Tables 1–2, $P < 0.0001$, Fig. 1). None of the remaining main factors had a significant effect (all $P > 0.5$, Table 2, Fig. 1). Of the two-way interactions, only the dam by developmental temperature was significant ($P = 0.0009$, Table 2): the effect of developmental temperature was accentuated if the dam developed at 18°C rather than at 25°C.

Discussion

Increased abdominal melanization in response to low developmental temperatures (Table 1) has been well documented in *Drosophila* (David et al. 1985) and in other insects (Watt 1990). Melanization also increases (genetically) with latitude in *D. melanogaster* and *D. simulans* (David et al. 1985; Capy et al. 1988). Thus, as was the case with body size (above), developmental and apparent evolutionary responses of melanization to temperature are parallel. The lack of significant between-generational effects (Table 2) may reflect limited statistical power, as we scored only six flies per group. David et al. (1985) found a maternal effect for trident pigmentation in crosses of different geographical strains of *D. melanogaster.*

Increased melanization at lower temperatures is generally thought to be adaptive for thermoregulation (David et al. 1983, 1985; Capy et al. 1988): darker objects absorb more visible radiation and thus should heat more quickly and reach higher equilibrium body temperatures in a cold environment (Stevenson 1985). These thermoregulatory consequences are well established for ectotherms that are large in size relative to *Drosophila* (Stevenson 1985; Watt 1990). Nevertheless, the heat balance of tiny ectotherms, which have minute boundary layers, is dominated by convection and not by radiation: thus any radiant heat that is absorbed will quickly be lost by convection (Stevenson 1985). Consequently, melanization should not influence body temperature of *Drosophila*-sized organisms (Stevenson 1985), although empirical studies have not addressed this prediction.

The adaptive significance of the effect of developmental temperature on melanization of *Drosophila*-sized organisms thus requires re-evaluation. Color is a complex trait (Kingsolver and Wiernasz 1990), and we suggest several explanations and encourage their investigation. First, increased melanization might reflect a non-adaptive linkage with other genes that are adaptive at low temperature. In fact, some alleles affecting abdominal pigmentation in *D. melanogaster* also affect sternopleural bristle number (Robertson et al. 1977). Second, because melanin influences cuticular strength (Roseland et al. 1987), more melanized cuticles at low temperature might promote structural rigidity and thus help compensate for larger body size. Third, increased melanization might protect against UV radiation (Porter 1967): flies in cool climates might thermoregulate by spending time in relatively open, sunny microhabitats, where UV radiation is high. Fourth, even though a tiny insect can't bask effectively, it can raise its temperature by sitting within the warm boundary layer of a large, dark object (e.g., rock, flower). Increased melanization might thus enhance concealment (from visually hunting predators) at such times.

TABLE 3. Egg size dimensions (arbitrary units) of females with different parental and developmental temperatures. Values given are means ± standard errors. For the various temperature treatments, H = 25°C and L = 18°C.

T_{dev}	T_{dam}	T_{sire}	N	Width	Length
H	H	H	38	147.63 ± 0.155	397.00 ± 0.357
H	H	L	36	150.36 ± 0.174	398.17 ± 0.425
H	L	H	41	150.24 ± 0.158	391.83 ± 0.363
H	L	L	38	150.21 ± 0.181	390.18 ± 0.302
L	H	H	39	148.87 ± 0.152	393.03 ± 0.393
L	H	L	39	155.85 ± 0.161	402.31 ± 0.322
L	L	H	42	152.33 ± 0.114	400.12 ± 0.295
L	L	L	41	156.07 ± 0.150	405.93 ± 0.388

Egg Size

Results

Egg length and width were moderately correlated ($r = 0.29$), and so we analyzed principal component scores. PC1 (overall size index) and PC2 (shape) accounted for 64% and 36% of the variance. ANOVAs based on PC scores accounted for only 25% (PC1) and 5% (PC2) of the overall variance in egg size; and so we report only the PC1 analysis. PC1 was inversely related to developmental ($P < 0.0001$) and sire ($P < 0.0001$) temperature, but surprisingly not to dam temperature ($P = 0.21$; Tables 3–4, Fig. 1). All interactions were significant. For example, flies laid exceptionally small eggs if they developed at high temperature and that also had a parent that was raised at high temperature. Moreover, this effect was especially marked if the high-temperature parent was a male.

TABLE 4. ANOVA tables for egg size (PC1) derived from principal components analysis.

Source	df	Sum of sq	Mean sq	F value	Pr(F)
T_{sire}	1	22.38	22.38	21.58	< 0.001
T_{dam}	1	1.64	1.64	1.58	0.209
T_{dev}	1	37.47	37.47	36.13	< 0.001
T_{sire}:T_{dam}	1	4.30	4.30	4.15	0.043
T_{sire}:T_{dev}	1	12.59	12.59	12.14	< 0.001
T_{dam}:T_{dev}	1	8.05	8.05	7.76	0.006
Error	307	318.36	1.04		

Discussion

If raised at low developmental temperatures, females of several species of *Drosophila* produce relatively large eggs (Imai 1934; Avelar 1993; Table 3). However, the actual size difference in our samples was small: egg volume (assuming a prolate spheroid shape) was only 7% larger for development at 18°C versus 25°C. Techniques are available to determine whether the slightly larger eggs of flies developing at low temperature is adaptive (e.g., Curtsinger 1976; Sinervo et al. 1992), but such techniques have not yet been applied to *Drosophila.* Larger eggs might alternatively be a nonadaptive result either of longer egg retention by the female at low temperature (Avelar 1993) or merely of the larger body size of females that developed at low temperature (Table 1; Congdon and Gibbons 1987).

Heat Resistance

Results

Mean knock-down temperature was higher in females (by 0.35°C) than in males (P = 0.0002), and was higher (by 0.8°C) in flies developing at high temperature ($P \ll 0.001$, Tables 5–6). Mean knock-down temperature was weakly and positively related to dam temperature (~0.15°C, P = 0.048), but unrelated to sire temperature (P = 0.535). No interaction was significant.

Discussion

A positive effect of developmental temperature on adult heat tolerance, which is usually indexed as the percentage of flies surviving a heat shock, has commonly been found in *Drosophila* (Maynard Smith 1957; Levins 1969; Quintana and Prevosti 1990a,b) and in many other organisms (Prosser 1986). Developmental temperature also positively affects adult knock-down temperature (Fig. 1).

The relationship between adult heat tolerance and developmental temperature is usually interpreted as adaptive (Prosser 1986): a high developmental temperature may be a cue (Levins 1968) that the adult will likely emerge into a warm environment where the risk of heat stress is high and where increased heat tolerance may thus promote fitness. This hypothesis, though appealing, has never directly been tested in nature. Furthermore, recent laboratory evidence contradicts it (Leroi et al. 1994; Zamudio et al. 1995; see also Krebs and Loeschcke 1994; Hoffmann 1995). Consequently, this adaptive hypothesis deserves a direct test.

Possible cross-generational effects of parental temperatures on measures of heat or cold resistance have rarely been studied (Crill 1991; Jenkins and Hoffmann 1994; Watson and Hoffmann 1996). In our present study, dam temperature had a weak effect on knock-down temperature. In a previous, small-scale study of *D. melanogaster* in which both parents were raised together at 18°C or at 25°C, high parental temperature positively affected knock-down temperature (Crill 1991). (However, this analysis was based on measurements of individual flies, not on run means, such that P-values were exaggerated [see Methods and Materials].) Knock-down time (not temperature) of the F_1 offspring of field-collected *D. simulans* from Australia is positively influenced by a maternal effect (Jenkins and Hoffmann 1994). Further, in a live-bearing fish (*Heterandria formosa*), female offspring born from parents raised at 21°C have higher—not lower—heat resistance than did offspring born from parents at 29°C (Forster-Blouin 1989). These studies suggest that cross-generational effects on heat resistance may be more common than appreciated.

The potential adaptive significance of cross-generational

TABLE 5. Physiological measurements of flies with different parental and developmental temperatures. Values given are means ± standard errors. Sample sizes are shown in parentheses. For the various temperature treatments, H = 25°C and L = 18°C.

Sex	T_{dev}	T_{dam}	T_{sire}	μ_{max} (cm/s)	Breadth (°C)	T_{opt} (°C)	T_{kd} (°C)
F	H	H	H	4.42 ± 0.042 (29)	10.65 ± 0.104 (29)	23.97 ± 0.125 (29)	39.31 ± 0.001 (1091)
F	H	H	L	4.43 ± 0.056 (27)	9.83 ± 0.182 (27)	24.26 ± 0.183 (27)	39.43 ± 0.001 (1230)
F	H	L	H	4.83 ± 0.042 (31)	11.07 ± 0.126 (31)	23.55 ± 0.157 (31)	39.21 ± 0.001 (1163)
F	H	L	L	4.34 ± 0.041 (29)	10.33 ± 0.123 (29)	24.31 ± 0.188 (29)	39.16 ± 0.001 (1061)
F	L	H	H	4.17 ± 0.038 (30)	10.09 ± 0.119 (30)	24.33 ± 0.150 (30)	38.53 ± 0.001 (1183)
F	L	H	L	3.94 ± 0.033 (31)	10.50 ± 0.130 (31)	26.45 ± 0.145 (31)	38.34 ± 0.001 (1073)
F	L	L	H	4.17 ± 0.048 (30)	9.79 ± 0.144 (30)	26.00 ± 0.134 (30)	38.32 ± 0.001 (1201)
F	L	L	L	4.06 ± 0.049 (29)	9.23 ± 0.096 (29)	26.03 ± 0.141 (29)	38.20 ± 0.001 (1114)
M	H	H	H	4.78 ± 0.055 (26)	12.72 ± 0.171 (26)	25.96 ± 0.181 (26)	38.90 ± 0.001 (1008)
M	H	H	L	4.54 ± 0.034 (30)	12.01 ± 0.150 (30)	24.00 ± 0.166 (30)	38.91 ± 0.001 (1029)
M	H	L	H	4.86 ± 0.039 (27)	11.02 ± 0.182 (27)	24.07 ± 0.192 (27)	38.80 ± 0.001 (1138)
M	H	L	L	5.10 ± 0.047 (31)	9.85 ± 0.123 (31)	24.35 ± 0.154 (31)	38.62 ± 0.001 (1006)
M	L	H	H	4.85 ± 0.047 (30)	9.24 ± 0.115 (30)	25.83 ± 0.139 (30)	38.20 ± 0.001 (1119)
M	L	H	L	4.47 ± 0.036 (29)	9.09 ± 0.125 (29)	25.52 ± 0.133 (29)	38.12 ± 0.001 (914)
M	L	L	H	4.67 ± 0.036 (30)	9.49 ± 0.095 (30)	25.50 ± 0.119 (30)	38.04 ± 0.001 (1029)
M	L	L	L	4.87 ± 0.062 (31)	9.98 ± 0.121 (31)	25.65 ± 0.143 (31)	38.12 ± 0.002 (895)

TABLE 6. ANOVA tables for physiological performance traits.

Source	df	Sum of sq	Mean sq	F value	Pr(F)
μ_{max}:					
Sex	1	1.552	1.552	18.321	< 0.001
T_{sire}	1	0.123	0.123	1.452	0.229
T_{dam}	1	0.112	0.112	1.321	0.251
T_{dev}	1	0.517	0.517	6.107	0.014
Sex:T_{sire}	1	0.028	0.028	0.327	0.568
Sex:T_{dam}	1	0.016	0.016	0.190	0.663
Sex:T_{dev}	1	0.166	0.166	1.960	0.162
T_{sire}:T_{dam}	1	0.008	0.008	0.091	0.764
T_{sire}:T_{dev}	1	0.000	0.000	0.001	0.971
T_{dam}:T_{dev}	1	0.090	0.090	1.064	0.303
Error	459	38.877	0.085		
Breadth:					
Sex	1	0.037	0.037	0.258	0.611
T_{sire}	1	0.255	0.255	1.801	0.180
T_{dam}	1	0.138	0.138	0.975	0.324
T_{dev}	1	1.586	1.586	11.202	< 0.001
Sex:T_{sire}	1	0.000	0.000	0.004	0.948
Sex:T_{dam}	1	0.049	0.049	0.346	0.557
Sex:T_{dev}	1	0.444	0.444	3.139	0.077
T_{sire}:T_{dam}	1	0.005	0.005	0.034	0.855
T_{sire}:T_{dev}	1	0.211	0.211	1.494	0.222
T_{dam}:T_{dev}	1	0.137	0.137	0.964	0.327
Error	459	64.978	0.142		
T_{kd}:					
Sex	1	1.029	1.029	21.120	< 0.001
T_{sire}	1	0.019	0.019	0.397	0.535
T_{dam}	1	0.215	0.215	4.411	0.048
T_{dev}	1	5.117	5.117	105.068	< 0.001
Sex:T_{sire}	1	0.001	0.001	0.025	0.876
Sex:T_{dam}	1	0.004	0.004	0.092	0.765
Sex:T_{dev}	1	0.102	0.102	2.103	0.162
T_{sire}:T_{dam}	1	0.004	0.004	0.077	0.784
T_{sire}:T_{dev}	1	0.000	0.000	0.014	0.907
T_{dam}:T_{dev}	1	0.006	0.006	0.120	0.733
Error	21	1.023	0.049		

effects of temperature on heat or cold resistance has received little discussion (Jenkins and Hoffmann 1994; Watson and Hoffmann 1996). However, positive cross-generational effects on heat tolerance could be adaptive, at least for organisms with generation times that are short relative to the periodicity of environmental change (Levins 1968): an individual's phenotype could benefit from environmental information accumulated during its parents' lifetimes as well as during its own (Levins 1968; Giesel 1988; Mousseau and Dingle 1991). This adaptive hypothesis has not yet been adequately tested. However, male *D. melanogaster* that were raised at 25°C and whose parents were also raised at 25°C were dominant over males that were raised at 25°C but whose parents were raised at 18°C, when both males were tested in paired encounters at 27°C (Zamudio et al. 1995). To our knowledge this is the only suggestive example of a positive, cross-generational effect of temperature on a component of fitness.

The mechanistic bases for the positive effects of developmental temperature on heat tolerance (Somero 1995; Prosser 1986) and knock-down temperature have not been explored in depth for *Drosophila*. However, developmental temperature influences composition and melting points of epicuticular lipids in *Drosophila* and in orthopterans (Gibbs et al. 1991; Toolson and Kuper-Simbrón 1989; Markow and Toolson 1990). Developmental temperature might also influence membrane viscosity (Prosser 1986) or enzyme activities (Somero 1995). The mechanistic basis for the apparent maternal effect on heat tolerance is unknown.

The greater heat resistance of females (relative to males) is probably not causally related to their larger body size. First, development at low temperature increases adult size but actually reduces heat resistance. Second, when body size in *D. melanogaster* was experimentally manipulated by adjusting larval crowding, heat resistance was unaffected (Oudman et al. 1988). Third, female *Drosophila* spp. do not always have higher heat resistance (Quintana and Prevosti 1990a,b; Loeschcke and Krebs 1994; Cavicchi et al. 1995). Fourth, selection for increased knock-down temperature has not altered body size (Huey and Gilchrist, unpubl. data).

Thermal Dependence of Walking Speed

Results

Flies developing at 25°C had significantly higher maximal speeds (u_{max}, $P = 0.014$) and broader performance curves ($P < 0.001$) than did flies developing at 18°C (Fig. 2, Tables 5–6). Males were faster than females ($P < 0.001$), but the sexes did not differ in performance breadth ($P = 0.61$, Table 6). Neither sire nor dam temperature influenced maximal speed or performance breadth (Table 6). Optimal temperatures were not normally distributed and so were compared using Kruskal-Wallace tests. Females developing at 25°C had a lower (not higher) T_{opt} than females at 18°C ($H = 6.1366$, $P = 0.0132$); males showed a similar but not significant trend ($H = 2.3538$, $P = 0.125$). The combined probability ($P = 0.009$; Rice 1989) suggests that optimal temperature is inversely—not positively—related to developmental temperature. Neither sire nor dam temperature significantly affected T_{opt}, T_{br}, or u_{max}. The more powerful, repeated-measures analysis confirms these general patterns. Sex ($P < 0.001$) and developmental temperature ($P < 0.001$) were the most significant experimental factors other than measurement temperature itself (Table 7).

The greater performance breadth (above) of flies developing at 25°C is primarily a result of these flies walking relatively faster at low temperature (i.e., < 25°) than did flies developing at 18°C. Specifically, flies developing at 25° ran significantly faster at 10°, 15°, and 20°C than did 18°C flies (Fig. 2; $P < 0.02$, statistical analyses not shown). In contrast, flies developing at 18°C and 25°C had similar walking performances at 25°C and 30°C ($P > 0.4$). Surprisingly, flies developing at 18°C had significantly higher speeds at 35°C ($P < 0.002$) than did flies developing at 25°C (Fig. 2; statistical analyses not shown).

Parental temperatures had little influence on maximal speed or performance breadth (both $P > 0.17$, Fig. 2, Table 6). However, dam temperature had a significant effect on the overall performance curve ($P = 0.005$, Table 7), but the effect was small relative to that of developmental temperature (Table 7).

Discussion

Developmental temperature has complex effects on the thermal sensitivity of walking speed. The responses contra-

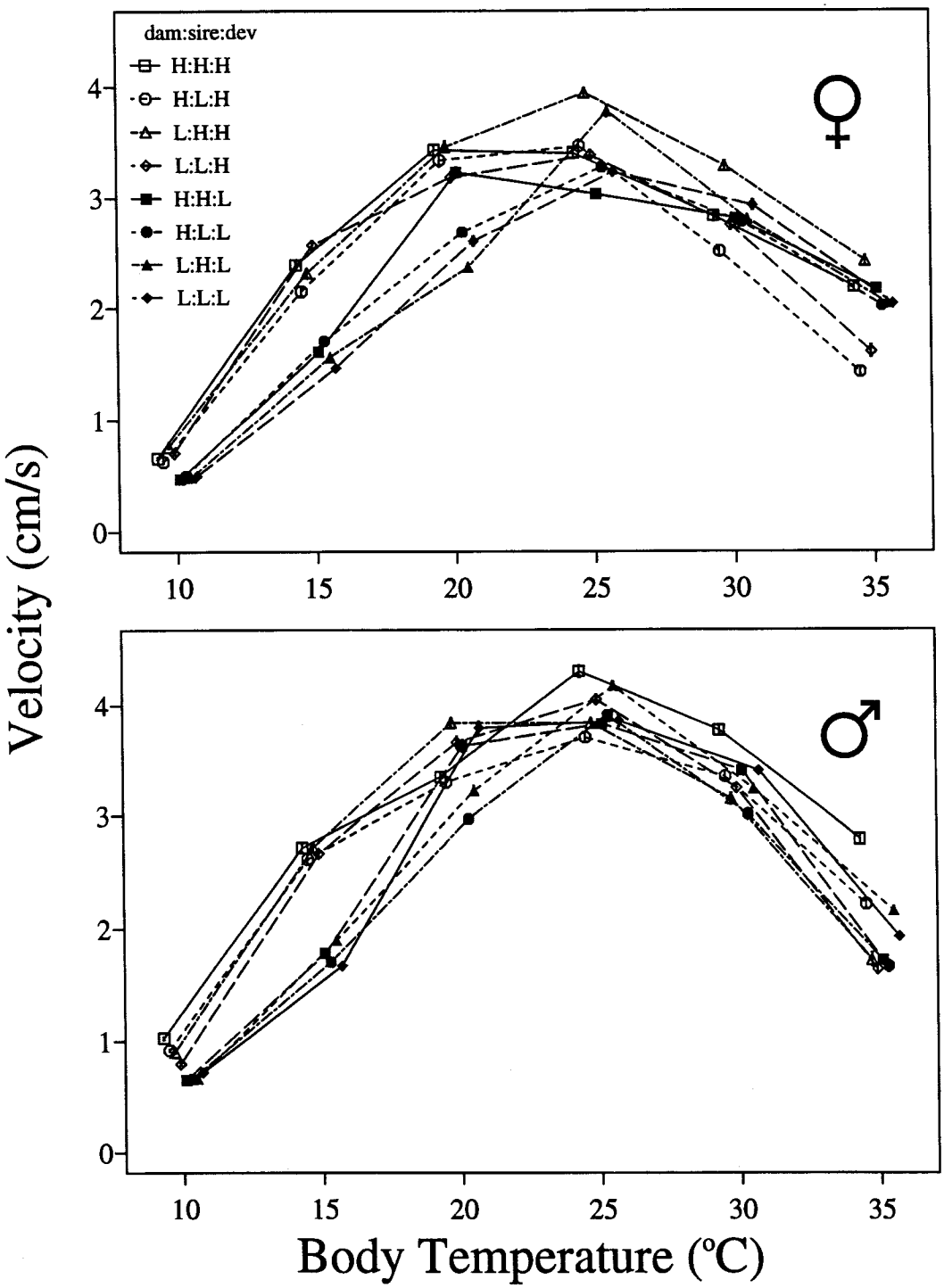

FIG. 2. Effects of temperature on walking speed of *Drosophila melanogaster*. The treatment groups are indicated in the legend, where the sequence is dam, sire, and developmental temperature. Development at 25°C increases speed, especially at low temperatures, and thus thermal performance breadth.

dict a simple hypothesis of "beneficial acclimation" (Leroi et al. 1994; Zamudio et al. 1995), which predicts that flies reared at high temperature should run faster at high test temperatures than flies raised at low temperature (and the reverse pattern at low test temperatures). In contrast, flies raised at 25°C generally ran quickly at low temperatures (< 25°C) relative to flies raised at 18°C (Fig. 2; Table 6). Even so, development at 25°C resulted in a significantly higher maximal speed (u_{max}, Table 6). Cohet (1975) and Cohet and David (1978) suggest that 21° to 25°C are optimal developmental

TABLE 7. Repeated measures ANOVA of locomotor performance curves. "Temp" refers to measurement temperatures. Tests of hypotheses use Type III SS. For the among-subjects analysis, the error term was (replicate*sex*T_{sire}*T_{dam}*T_{dev}) nested within that for individuals.

Source	df	Sum of sq	Mean sq	F value	Pr(F)
Temp	5	2836.371	567.274	300.535	< 0.001
Sex	1	68.216	68.216	36.140	< 0.001
T_{sire}	1	0.749	0.749	0.397	0.529
T_{dam}	1	14.705	14.705	7.791	0.005
T_{dev}	1	54.258	54.258	28.745	< 0.001
Temp*sex	5	24.888	4.978	2.637	0.023
Temp*T_{sire}	5	3.967	0.793	0.420	0.835
Temp*T_{dam}	5	6.827	1.365	0.723	0.606
Temp*T_{dev}	5	54.576	10.915	5.783	< 0.001
Sex*T_{sire}	1	0.546	0.546	0.289	0.591
Sex*T_{dam}	1	0.183	0.183	0.097	0.756
Sex*T_{dev}	1	0.000	0.000	0.000	0.986
T_{sire}*T_{dam}	1	1.365	1.365	0.723	0.396
T_{sire}*T_{dev}	1	0.357	0.357	0.189	0.664
T_{dam}*T_{dev}	1	4.056	4.056	2.149	0.143
Error	459	866.384	1.888		

temperatures for *D. melanogaster,* and a variety of evidence supports this hypothesis (Zamudio et al. 1995; Huey and Berrigan 1996).

Relative Effects on Phenotypes

Our experiments allow us to quantify the relative effects of sex as well as of different developmental, paternal, and maternal temperatures on adult phenotypic traits. Figure 1 summarizes patterns for each trait: it plots the difference between the standardized means for specific temperature treatments (e.g., mean for development at 25°C versus mean for development at 18°C) and for females versus males.

Effects of Sex

Drosophila melanogaster is strongly sexually dimorphic. Not surprisingly, sex affected most physiological and morphological traits examined here. As was expected (Ashburner 1989), females were heavier in dry body mass and had longer wings than did males (Fig. 1). Females also had relatively high knock-down temperature, which is consistent with previous studies of heat resistance in several species (Maynard Smith 1957; Huey et al. 1992; but see Quintana and Prevosti 1990a,b; Loeschcke and Krebs 1994; Cavicchi et al. 1995). As noted above, the greater heat resistance of females is probably not causally related to their larger body size.

Effects of Temperature

Developmental temperature significantly affected all traits examined here. Development at the higher temperature (25°C versus 18°C) produced flies that were lighter in mass, had shorter wings, had less abdominal melanization, had enhanced heat resistance, ran faster over a broader range of temperatures, had a lower optimal temperature for walking, and produced smaller eggs (Fig. 1). Other life history (Giesel et al. 1982; David et al. 1983; but see Huey et al. 1995) and behavioral traits (Cohet 1972, 1974; David and Cohet 1974) are also influenced by developmental temperature.

Paternal or maternal temperature had significant effects on several traits. The magnitude of these effects is usually small relative to those of sex and developmental temperature (Fig. 1). Surprisingly, paternal temperature sometimes had effects that were stronger (or even in a different direction) than those of maternal temperature (Fig. 1).

The mechanistic bases for the maternal and paternal effects are unknown. However, numerous mechanisms (e.g., cytoplasmic factors, egg size and shape) exist for maternal effects (see Kirkpatrick and Lande 1989; Mousseau and Dingle 1991). The possible factors contributing to observed paternal effects are less clear. However, the complete male sperm (potentially with temperature inducible factors) is incorporated into the egg during fertilization in *D. melanogaster* (Karr 1991), and male accessory gland secretions (Chapman et al. 1995) might be involved as well.

CONCLUDING REMARKS

We used a factorial experimental design to explore the sensitivity of various phenotypic traits of *D. melanogaster* to different maternal, paternal, and developmental temperatures as well as to sex. The experimental temperatures used are not extreme and are probably within the range experienced by *Drosophila* developing in nature (Parsons 1978; McKenzie and McKechnie 1979; Jones et al. 1987; Feder 1996). Our results show that diverse aspects of the adult phenotype are sensitive to within-generation effects of temperature, and sometimes even to between-generational effects (Fig. 1). However, different traits may not respond in parallel: for example, dry body mass and wing size show different sensitivities to dam temperature (Table 2). These patterns reinforce an important generalization: the phenotype is the result of complex dynamics involving an organism's developmental and even parental environments as well as its genotype (Levins 1969; Gupta and Lewontin 1982; Giesel 1988; Mousseau and Dingle 1991).

The documentation of cross-generational effects has implications for evolutionary studies. First, models attempting to predict short-term responses to selection may need to consider the possible impact of parental-effect reaction norms (Kirkpatrick and Lande 1989) in addition to developmental-effect reaction norms. Indeed, some "paradoxical" responses to artificial selection result from cross-generational effects (Falconer 1965; Janssen et al. 1988; Watson and Hoffmann 1996).

Second, the significance of cross-generational effects implies that comparative studies (e.g., of different populations or species) should raise the study organisms through at least two generations in a common-garden environment. The short acclimation periods traditionally used by comparative physiologists may thus be inadequate (Garland and Adolph 1991). Physiologists studying vertebrates shouldn't dismiss this as "a problem only for flies." Cross-generational environmental effects have also been documented in mammals (e.g., Falconer 1965).

Third, several recent studies have attempted to measure the "field heritabilities" of phenotypic traits by collecting and scoring females directly from nature, crossing them in the laboratory, scoring their offspring phenotypes, and then

estimating heritabilities from dam-offspring correlations (Coyne and Beecham 1987; Hoffmann 1991; Jenkins and Hoffmann 1994). These protocols explicitly assume (Riska et al. 1989) that parental effects are insignificant. However, this assumption is not always warranted (e.g., Fig. 1; Jenkins and Hoffmann 1994; Watson and Hoffmann 1996), suggesting that field heritabilities should be estimated only for traits in which cross-generational effects are known to be unimportant.

Finally, our review of the literature demonstrates two conspicuous gaps concerning developmental and cross-generational effects of temperature. First, the actual mechanistic bases of these effects is poorly known in *Drosophila*. Second, direct tests of the adaptive significance (or lack thereof) of within- and especially of between-generational reaction norms for temperature are essentially nonexistent. Indeed, the few empirical studies completed to date actually contradict a simple "beneficial acclimation" hypothesis (Krebs and Loeschcke 1994; Leroi et al. 1994; Hoffmann 1995; Huey et al. 1995; Zamudio et al. 1995). The exploration of these issues in evolutionary physiology warrants attention.

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