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Influence of Temperature and Activity on the Metabolic Rate of Adult *Drosophila melanogaster*

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ABSTRACT. We measured metabolic rates of adult male *Drosophila melanogaster* allowed to evolve in the laboratory at 18 and 25°C and compared these with measurements of metabolic rates of flies collected along a latitudinal gradient in Australia. Metabolic rates of flies that had evolved in the laboratory at low temperature were 5–7% higher than those of flies allowed to evolve at high temperature. Metabolic rates of field collected increased with latitude when measured at 18°C but not at higher temperature (25°C) and were about 9% greater in high latitude (~41'00) flies than low latitude (16'53) flies. Metabolic rate was strongly influenced by measurement temperature; estimated Q_{10}s ranged from 1.79 to 2.5 for measurements made at 18 and 25°C. Metabolic rate scaled isometrically with body mass; the estimated slope of a ln-ln regression of metabolic rate and body mass was 1.03 ± 0.1. We used our measures of metabolic rate and activity to estimate the minimum cost of transport (MCOT) while walking. The estimates of MCOT have high standard errors (lab, 34.30 ± 14.2 ml O_2/g/km; and field, 38.0 ± 17.0 ml O_2/g/km); however, they differ by only 3–9% from predicted values based on allometric relationships reported in the literature. COMP BIOCHEM PHYSIOL 118A;4:1301–1307, 1997. © 1997 Elsevier Science Inc.

KEY WORDS. Metabolic rate, cost of locomotion, MCOT, life history, temperature, compensation, latitudinal gradient, *Drosophila melanogaster*

INTRODUCTION

Temperature is a major influence on the life histories of ectotherms (2–4,6,34,40–45). For example, most ectotherms mature later at a larger size when reared at low temperature. Temperature has evolutionary and environmental effects on life history traits, and these evolutionary effects may be similar to the environmental influence of temperature or they may be in the opposite direction. In *Drosophila*, the evolutionary influence of temperature can result in earlier maturation at a larger size (2,3,11,26). Adult life history traits also evolve in response to temperature. Replicated lines of *D. melanogaster* held at different temperatures in the laboratory show evidence for genetic changes in survivorship and fecundity (41,42). These flies survived longer and had higher fecundity at the temperatures at which they had evolved than at the alternative temperature. Here, we explore the relationship between metabolic rate and life history variation by measuring metabolic rates of these replicated lines of flies that have been evolving under different fixed temperature regimes.

Metabolic rates of ectotherms are strongly temperature dependent (22). Furthermore, the metabolic response to temperature is known to differ among species and among populations within a species (46,49). Data from comparative studies suggest that one major solution to the problem of temperature variation involves compensation. With compensation, metabolic rates of organisms from relatively cold environments are higher than those of organisms from relatively warm environments (43,44). Compensation is thought to be adaptive because it allows higher levels of activity for an individual experiencing a particular temperature; however, there is little *direct* evidence that compensation is adaptive (12). There is evidence for genetically based compensatory changes in metabolic rate in adult *Drosophila* from an unreplicated laboratory natural selection experiment (27). Thus, we predicted that our laboratory populations would show compensation.

Furthermore, diverse ecotherms collected from higher latitudes have higher metabolic rates than con-specifics collected from lower latitudes [e.g., (9,35,43,49).] Higher latitude and cold-adapted *D. melanogaster* mature earlier and at a larger size than lower latitude and warm-adapted flies (28), and latitudinal clines in size are known in several ectotherms [(10,34); *Drosophila* literature reviewed in (28).] Parallel changes in life history traits in flies from laboratory natural selection experiments at different temperatures and

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in populations collected along latitudinal gradients have been used to argue that average temperature plays an important selective role in the field, and a similar argument could be made for physiological rather than a life history traits. To determine if metabolic rate evolves in parallel in response to temperature in the laboratory and field, we also report measures of metabolic rate from males collected along a latitudinal gradient in Australia. Positive correlations between latitude and metabolic rate are predicted if compensation is present.

Our third goal is to estimate the cost of pedestrian locomotion in *D. melanogaster*. Activity is likely to influence metabolic rate, but very few if any past studies of metabolic rate in *Drosophila* quantified this influence [e.g., (19,20, 27,36); and many others.] Additionally, estimates of the cost of pedestrian locomotion should be useful for understanding variation in male mating and territorial behavior [e.g., (23,50)].

To achieve these three goals, we measured metabolic rate and walking speed in adult male *D. melanogaster* from replicated lines of flies evolving at 16.5–18°C and at 25°C for at least 100 generations and in adult males from six populations of flies collected along a latitudinal gradient in Australia. We chose to measure males because they do not develop eggs. Variation among females in the degree of development of the ovarioles could have a major influence on measurements of metabolic rate and the cost of locomotion. We also report estimates of the minimum cost of transport for walking *Drosophila*.

MATERIALS AND METHODS

Fly Stocks

The stocks of *D. melanogaster* used here have been described in some detail elsewhere (25,28). The flies used in the laboratory natural selection experiment were originally collected near Brighton, England in June 1984. After 1 year, this stock was subdivided into three replicate populations at 16.5 and 25°C, a 12L:12D photoperiod and supplied with culture bottles of Lewis medium. In January 1994, the 16.5°C flies were moved to new incubators set at 18°C. In May 1994, the flies were transported from the United Kingdom to Seattle, Washington and transferred to a slightly different media (corn meal, molasses, yeast, agar, tegosept). We waited at least three generations before beginning any of the measurements described here. Based on development time data (25,28), both high temperature and low temperature flies had experienced more than 100 generations of selection.

Field populations from different latitudes in Australia were collected in February 1993 and reared at 16.5/18°C in the laboratory in population cages for 10 generations before these experiments (28). We examined six populations, MO, IN, CH, CI, FT and RN. MO and IN were collected near Cairns, Queensland at latitude 16′53 from a compost heap and a banana farm dump about 10-km apart, CH and CI were collected near Coffs Harbor, New South Wales at latitude 30′19 from banana baits placed about 10-km apart. FT and RN were collected in Tasmania at latitudes 41′11 and 42′53 from tomatoes and crushed apples, respectively. Baits were used when flies were not present in existing waste fruit and, the type of bait used depended on the availability of ripe produce. These field sites were selected haphazardly to span a wide range of latitudes. Measurements of life history traits of flies from additional collections are described in James *et al.* (28,29).

Respirometry

We estimated the metabolic rate of individual adult male *D. melanogaster* using flow-through respirometry (6,33). In brief, we used a Sable Systems TR-3 respirometry apparatus (Sable Systems Inc., Las Vegas, NV) to measure CO_2 production. This consisted of a Licor 6262 infrared CO_2 detector (Licor, Inc., Lincoln, NB) together with a computer controlled baselining device, flow controller and A/D converter board attached to a computer for data recording. We placed individual flies in a small glass tube in the CO_2-free airstream of this system, and after a 4- to 6-min equilibration period, we recorded the CO_2 output for 2–4 min. An infrared motion detector was placed around the tube, sending a voltage to the computer when the fly walked past the sensor. The temperature of the cuvette was monitored to detect heating (none was found) due to the sensor. From the measured CO_2 concentration and flow rate (80 ml/min), we calculated CO_2 outputs (33). After a set of measurements, we weighed each fly to the nearest 0.01 mg.

CO_2 output was converted to O_2 consumption based on a respiratory quotient (RQ) of 1.0. Measurements of other strains of adult *D. melanogaster* have resulted in RQs not significantly different from 1 (e.g., Lighton and Berrigan, unpublished data, found RQ = 0.91, SE = 0.07, n = 12). Metabolic rates are reported as ml O_2/hr or ml O_2/g/hr. Note that these data can be converted to units of J/s using the relationship MR = (15.97 + 5.164RQ) Vco_2 (33). Finally, the minimum cost of transport data are reported as ml O_2/g/km for ease of comparison with past studies.

Walking speed estimates were obtained by multiplying the number of times the infrared detector was tripped times the length of the cuvette holding the fly (5.5 cm) and dividing by the total time of the recording. The flies tended to walk back and forth fairly regularly, suggesting that this procedure is reasonable [see also (7)]. This procedure does not control for differences in behavior that do not trip the motion detector. Flies did not attempt to fly in the cuvette but did groom themselves and occasionally twitch their wings.

Common Garden Experiments

We measured the metabolic rates of individual adult males from the six laboratory and six field populations at 18 and

25°C. The laboratory populations had been evolving at 16.5/18°C and 25°C for over a 100 generations and the field collected flies had been held at 16.5/18°C for 10 generations before the start of the experiments. The flies were then reared for two generations at the measurement temperature to control for parental effects on metabolic rate and activity (26). Eggs were collected from each line by placing adults into yeasted bottles of medium. After 24 hr at 15°C or 48 hr at 18°C, the adults were removed and these bottles were cultured at 18 or 25°C. Three- to 5-day-old adults from these bottles were transferred to fresh bottles and allowed to lay eggs over 8-hr periods at 25°C and 24 hr at 18°C. To control for density effects (8), we sliced out pieces of media containing 50–60 eggs and placed them in individual 75 × 25 mm diameter vials containing Lewis medium seeded with a drop of live yeast suspension.

Adult males from the vials described above were used for measurements of metabolic rates. Flies were measured at their respective rearing temperatures (e.g., males reared at 18°C were measured at 18°C). We were able to measure the metabolic rate of 36 individual males in a day. For the temperature stocks, we measured metabolic rates for 4 days at both temperatures. This resulted in 24 individuals from each of the replicate selection lines at both measurement temperatures. A similar protocol was followed for the measurements on the Australian flies except that we only performed 2 days of measurements at each of the two measurement temperatures, resulting in data for 12 individuals from each of the six stocks at both temperatures.

Analysis

All the statistical analyses described here were performed with JMP (SAS Institute Inc., Cary, NC, U.S.A.). In some cases, data were ln transformed before analysis. To explore correlations between metabolic rate, speed and mass and to summarize the data, we first performed a principal components analysis on the three dependent variables at two measurement. We then analyzed mass-specific metabolic rates using ANCOVA with speed as the covariate. Preliminary analysis indicated that metabolic rate scaled isometrically with body mass. For the selection lines, the estimated slope of the relationship between ln metabolic rate (ml O^2/hr) and ln mass (g) is 1.03 ± 0.10 (means ± SE). For the field populations, the estimated slope was 0.96 ± 0.07. Neither of these estimates is significantly different from 1.0. Therefore, the analysis of mass-specific metabolic rate is not be misleading as a consequence of the allometry of metabolic rate (39). Additional issues involving the analysis of physiological variables correlated with mass are discussed in Packer and Boardman (39) and Hayes (21). In the analysis of data from the laboratory selection populations replicate line was treated as a nested random effect. This conservative approach uses variation between lines to test for an effect of selection temperature on metabolic rate. Finally, we estimated the minimum cost of locomotion as the inter-individual correlation between speed and metabolic rate. This procedure assumes no difference in the relationship between speed and metabolic rate within and between individuals (5,7).

RESULTS

Overall, larger flies had higher metabolic rates and walked more slowly (Table 1). The first component of a principal component analysis (PCA) on the laboratory and field population data explained 50–65% of the variation in metabolic rate, mass and walking speed and loads positively on mass and metabolic rate; bigger flies have higher metabolic rates. The second principal component explained an addi-

TABLE 1. Standardized principal components analysis summarizing the relationships between natural log transformed mass (g), metabolic rate (ml O^2/g/hr) and speed (m/sec) in adult male *Drosophila melanogaster* from laboratory and field populations of flies studied at two temperatures

	Laboratory selection lines		Field collections	
	PC1	PC2	PC1	PC2
Measured at 18°C				
Eigen value	1.47	1.18	1.76	1.00
% variation	49.0	39.4	58.6	33.3
Eigenvectors				
ln mass	0.65	−0.47	0.68	−0.25
ln metabolic rate	0.74	0.20	0.70	0.00
ln speed	0.18	0.86	0.18	0.97
Measured at 25°C				
Eigen value	1.51	1.01	2.00	0.84
% variation	50.3	33.7	66.6	27.8
Eigenvectors				
ln mass	0.70	−0.14	0.64	−0.37
ln metabolic rate	0.71	0.07	0.66	−0.17
ln speed	0.05	0.98	0.39	0.91

TABLE 2. ANCOVA testing the effects of selection temperature, measurement temperature, replicate line and speed (m/sec) on the metabolic rate (ml O_2/g/hr) of adult male *Drosophila*

Source	*df*	SS	*F* Ratio	*P*
Measurement temperature (MT)	1	976.4	787.5	≤0.001
Selection temperature (ST)	1	10.1	5.1	≤0.084
MT · ST	1	1.7	1.3	≤0.312
Line*	4	8.1	1.6	≤0.327
Line · MT*	4	5.02	1.3	<0.271
Speed (m/sec)	1	5.7	5.8	≤0.016
Error	275	266.1		

Prior analysis indicated that the interaction terms between speed and measurement temperature were not significant; therefore, ANCOVA is the appropriate test. Estimated slopes are given in the text. Replicate line is a random effect nested in selection temperature.
*Nested in selection temperature; SS = sum of squares.

tional 30–40% of the variation and loads positively on speed and negatively on mass; bigger flies walk less or they walk more slowly. At 25°C flies from the laboratory natural selection experiment ranged in mass from 5.5×10^{-4} to 1.23×10^{-3} g (mean, 1.0×10^{-3}) and had metabolic rates ranging from 3.6 to 9.8×10^{-3} ml O_2/hr (mean, 6.2×10^{-3} ml O_2/hr) while walking from 7.1×10^{-3} to 9.1×10^{-4} m/sec (mean, 3.4×10^{-3} m/sec).

Selection Lines

Measurement temperature and speed had highly significant effects on metabolic rate of adult male *D. melanogaster* allowed to evolve at different temperatures in the laboratory (Table 2). Metabolic rate increased with temperature (Fig. 1), averaging about 4.2 (ml O_2/g hr) at 18°C and about 8.0 (ml O_2/g/C hr) at 25°C. This results in a Q_{10} of 2.5 for flies from either selection regime.

Selection temperature had a small effect on metabolic rate; flies that evolved at low temperature had mass-specific metabolic rates 5–7% higher than flies that had evolved at higher temperature (Fig. 1). This effect was only marginally significant ($P = 0.084$); however, we treated replicate line as a random effect nested in selection regime. This conservative approach tests for the effect of selection temperature using the number of replicate lines to determine the degrees of freedom available. If we reanalyze the data without this restrictive assumption, the effect of selection temperature ($F_{1,275} = 10.4$, $P = 0.0014$) is highly significant.

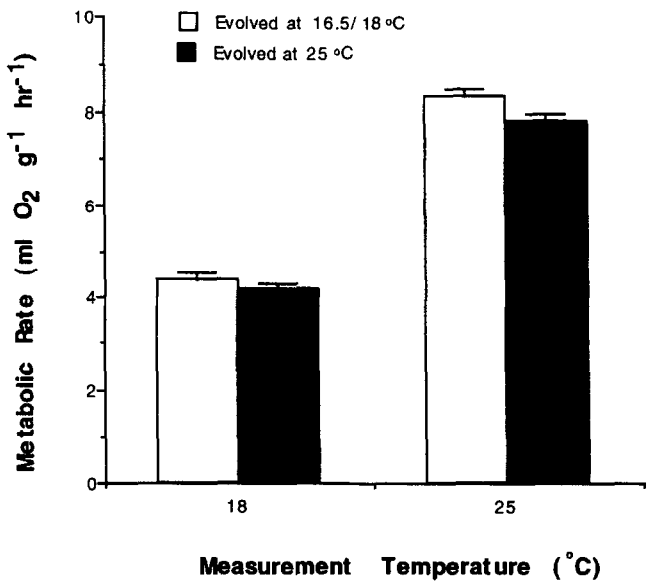

FIG. 1. Influence of measurement and selection temperature on metabolic rate of adult male *Drosophila melanogaster*.

Metabolic rate increased with speed (Table 2). The slope of the regression from this analysis of covariance is an estimate of the minimum cost of transport (MCOT). The estimated slope of the line is 34.3 ± 14.2 ml O_2/g/km. This slope is obtained from the analysis of covariance in Table 2 ($r^2 = 0.81$, $n = 288$).

Australian Populations

We found highly significant effects of measurement temperature and speed on the metabolic rate of adult male *D. melanogaster* collected from different latitudes in Australia (Table 3). Metabolic rate increased with temperature (Fig. 2) with Q_{10} values ranging from 1.79 to 2.36 (2.06 ± 0.08, $n = 6$). Sensitivity of metabolic rate to temperature was not correlated with latitude, a regression of Q_{10} on latitude was not significant ($P = 0.13$).

Metabolic rate increased with speed (Table 3). The slope of the regression from this analysis of covariance is an estimate of the MCOT. The estimated slope of the line is 38.0 ± 17.4 ml O_2/g/km. This slope is obtained from the analysis in Table 3 ($r^2 = 0.81$, $n = 141$).

Metabolic rate and latitude are positively and significantly correlated in flies measured at 18°C ($P = 0.022$) but not when measured at 25°C ($P = 0.867$). To test for this relationship between metabolic rate and latitude, we used least-square mean metabolic rates for each field collection from the ANCOVA (Table 3) in a linear regression with latitude. Inspection of Fig. 2 should make it clear that the influence of collection site on metabolic rate is small. The bars in Fig. 2 are arranged in order of latitude. Metabolic rate increases about 9% from the collections at the two low latitude sites (IN and MO) collected at about 17′ south to

TABLE 3. ANCOVA testing the effects of measurement temperature, geographic origin and speed (m/sec) on the metabolic rate (ml O^2/g/hr) of adult male *Drosophila*

Source	*df*	SS	*F* Ratio	*P*
Measurement temperature (MT)	1	214.3	351.54	≤0.001
Line	5	3.1	1.03	≤0.401
MT · Line	5	3.8	1.25	≤0.289
Speed (m/sec)	1	2.9	4.75	≤0.031
Error	131	79.8		

Prior analysis indicated that the interaction terms between speed and measurement temperature were not significant. Estimated slopes are given in the text.
SS = sum of squares.

the collections from the two high latitude sites (FT and RN) collected at about 42′ south.

DISCUSSION

In this study we report measurements of metabolic rate on adult male *D. melanogaster* from several geographic collections and from a laboratory natural selection experiment, and we use these data to estimate the cost of pedestrian locomotion in *Drosophila*. Flies evolving in the laboratory for more than 100 generations at 18°C have higher mass-specific metabolic rates than flies evolving at 25°C. Furthermore, flies from field collections along a latitudinal gradient also show evidence for compensation. Higher latitude flies have higher metabolic rates than lower latitude flies at both 18 and 25°C. The correlation between metabolic rate and latitude was significant only at 18°C.

For both laboratory and field populations, the magnitude of the compensatory increases in metabolic in low temperature laboratory selection lines and in high latitude field collections were less than 10% and near the limits of detection of our statistical methods. We found no evidence for differences between populations in walking speed (analysis not shown). Future studies of adult metabolic rates in these lines and other *Drosophila* could use measurements of groups of adults to increase the power of the analysis.

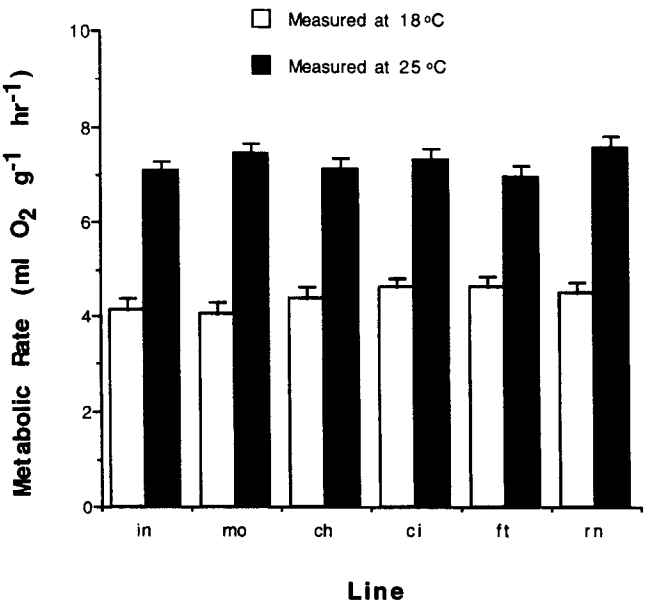

FIG. 2. Influence of collection site and measurement temperature on metabolic rate of adult male *Drosophila melanogaster*. The lines are arranged in order from low to high latitude.

D. melanogaster are a cosmopolitan species and highly mobile (14). High levels of migration could reduce the power of selection for compensatory increases in metabolic rate in the field. This could explain the fact that we saw only small differences in metabolic rates between high and low latitude populations. However, these field-collected flies do show evidence for clinal variation in body size and development time (28,29). Flies from the laboratory natural selection experiment also showed small differences in metabolic rate between high and low temperature lines. Laboratory natural selection experiments impose weak selection, and it might be possible to obtain larger differences in metabolic rate between lines using artificial selection. These lines could be useful for measurements of the costs and the benefits of compensation.

Compensation in metabolic rate has been observed in diverse organisms [reviews in (12,43,46)]. Past studies of compensation typically involve collections of organisms in the field, acclimation for some time in the laboratory, followed by measurement at one or more temperatures. This design ignores the presence of cross-generational effects on physiological rates even though these effects have been well documented (26). In this study, we controlled for cross-generational effects by rearing flies in common for two generations immediately before measurement. This may be in part responsible for the relatively small increases in metabolic rate in the cold adapted and higher latitude flies.

Hunter (27) also reported evidence for compensation in *D. melanogaster*, with adult female flies from a 15°C treatment having metabolic rates about 15% higher than flies from a 25°C treatment when both are reared at 20°C. Males in her 4-year-long laboratory natural selection experiment did not show signs of compensation. Hunter's study confounds cross-generational and evolutionary changes in metabolic rate and involved unreplicated lines. Additionally, Hunter's selection regime used different densities at high and low temperatures with the cold flies reared at higher

density than the warm flies. This is particularly important because larval and adult *D. melanogaster* show diverse evolutionary and phenotypic responses to density (37). Nevertheless, the results of Hunter's experiments and the present study provide evidence that *D. melanogaster* show compensatory increases in metabolic rate as a consequence of decreased temperature in the laboratory.

The cost of walking in *D. melanogaster* males is 34–38 ml O_2/g/km. This is indistinguishable from the predicted cost based on allometric relationships. For example, Lighton *et al.* (32) report an allometric equation based on data for a variety of insects: MCOT = 4.904 $W^{-0.272}$ with W = mass in grams and MCOT in ml O_2/g/km. The average mass of the laboratory and field flies in this study were 8.83 and 7.91 $\times 10^{-4}$, respectively. This results in predicted MCOTs of 33.2 and 34.2 ml O_2/g/km, very similar to the observed values. A similar allometric relationship was obtained by Full (18).

MCOTs are traditionally estimated by measuring the metabolic rate of individual animals forced or allowed to walk at different speeds. In this study we made single measurements of speed and metabolic rate in different individuals. Thus, we are assuming that the inter- and intra-individual relationships between speed and metabolic rate are indistinguishable. Two studies of metabolic costs of locomotion in Diptera have compared the inter- and intra-individual relationship between metabolic rate and speed and in both cases failed to reject the above assumption (5,7).

Studies of insect locomotion have focused primarily on flight (1,16,18; references therein). Metabolic rate during pedestrian locomotion is much lower than that during flight (1). Dickinson and Lighton (15) report metabolic rates 7.4 times greater in flight than at rest (140.2 vs 18.9 W/kg) in *Drosophila hydei*, and Laurie-Ahlberg *et al.* (31) estimated metabolic rates of about 50 ml O_2/g/hr for *D. melanogaster*. These two studies indicate that metabolic rate during flight is five to seven times greater than the resting metabolic rate. Based on the results of the present study, walking is much less costly. The predicted metabolic rate of a 1.0-mg fly walking at average speed (3.4×10^{-1} m/sec) is only 5–10% greater than its routine metabolic rate.

Despite the large difference in the cost of walking vs flying, the cost of walking could still comprise a significant portion of a fly's total energy budget. This is particularly likely in species that spend little time aloft and where males and/or females search for food, explore potential oviposition substrates or engage in elaborate mating rituals on foot. We know of no studies reporting activity budgets of *Drosophila* in the laboratory or field.

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