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Testing the interplay between physiological and life-history traits: an experimental study in *Drosophila*

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ABSTRACT

Question: Developmental time and metabolic rate are correlated at higher levels of organization. Does this allometric relationship scale down to the intraspecific level?

Methods: We exposed flies of three species of *Drosophila* collected at contrasting altitudes and similar latitudes to varying experimental thermal regimes. Metabolic rate was measured as CO_2 production using a 'closed system'. Developmental time was estimated as the time elapsed from the first instar larval stage until adult emergence.

Results: We observed a positive correlation between metabolic rate and developmental time among species. However, such allometry was not detected within species. Interestingly, both variables presented different patterns of interactions with sex and thermal treatment.

Conclusions: It may not be possible to extrapolate allometric rules of macroecology to within-species variation. Indeed, the factors that affect variation in physiological variables at the intraspecific and interspecific levels should be different.

Keywords: developmental time, *Drosophila*, metabolic rate, thermal adaptation.

INTRODUCTION

Life-history traits such as survival, growth rate, fecundity, and age at maturity are considered indirect measures of an organism's fitness in nature (Stearns, 1992). The expression of these phenotypic traits, as for all traits, is mediated by environmental influences; nevertheless, life-history traits have a particularly strong influence on an organism's fitness. In addition, physiological variation within the life history of an individual may influence fitness. Moreover, the connections between genotype and phenotype have traditionally been the subject of physiology and developmental biology. However, such studies do not inform us about how the phenotype is designed for reproduction and survival, an issue traditionally addressed by evolutionary biologists (Stearns, 1992).

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Even though physiology (and ultimately molecular developmental biology) is able to provide the mechanistic explanations for phenotypic variation, little is known about the proximal causes underlying variation in fitness and fitness-related traits. One possible reason for the small number of studies linking life-history traits and physiology is that rigorous life-history studies are generally done on a mass scale, screening large numbers of individuals and populations; whereas the focus of classical comparative physiological studies is most often higher taxonomic levels. Notwithstanding the importance of comparative physiological studies, little attention has been paid to variation in physiological traits within populations and among populations (for instance, those inhabiting different habitats), and the effect of environmental variables on the physiological response of populations (Spicer and Gaston, 1999; Lardies *et al.*, 2004). However, there are exceptions in the literature that address the link between life history and physiological variables (e.g. Townsend and Calow, 1981; Stearns, 1992; Roff, 2001; Zera and Harshman, 2001; Lardies and Bozinovic, 2006). Our thesis is that the integration of physiology and evolution will improve our ability to identify the proximal factors underlying life-history variation.

Because environmental temperature varies in time and space at different time-scales, ectothermic organisms are continuously challenged to maintain homeostasis. Thus, thermal physiology may be a significant factor underlying the ecological and evolutionary success of animals (Berrigan, 1997; Forsman, 1999; Rogowirz and Chappell, 2000; Huey and Berrigan, 2001; Tomanek and Somero, 2002; Neargarder *et al.*, 2003; Nespolo *et al.*, 2003). Indeed, individuals within populations living at lower altitudes (lowland) often experience higher mean temperatures throughout the year than those living at higher altitudes. However, differences in mean annual temperature are not the only feature differentiating highland and lowland environments. Indeed, abrupt thermal changes occur between day and night in highland environments.

Studies of thermal adaptation in ectothermic organisms have a long tradition (reviewed in Johnston and Bennett, 1996). However, most have compared the response of organisms exposed to thermal treatments that differ in mean constant temperature, whereas studies addressing the effect of thermal amplitude on life-history and/or life-history-related traits are scarce (Economos and Lints, 1986; Loeschcke *et al.*, 1994, 1997; Borash *et al.*, 1998; Petavy *et al.*, 2001).

Developmental time, the time elapsed from the egg to the adult stage, is particularly important in organisms such as *Drosophila* that depend on ephemeral resources. In particular, developmental time is known to be inversely correlated with the temperature at which larvae are raised (Partridge *et al.*, 1994; Gibert and De Jong, 2001; Petavy *et al.*, 2001; Angiletta *et al.*, 2002).

In previous studies, we have observed extensive variation in developmental time within and among populations of *D. buzzatii* and *D. melanogaster*, and that a significant proportion of among-population variation can be accounted for by differences in altitude among sampling localities in both species (G. Folguera, unpublished results). However, developmental time variation seems to be largely independent of body size variation in both species (Folguera, unpublished results), contradicting the notion that developmental time may be correlated with (or just be a mere consequence of) general body size (Cortese *et al.*, 2002).

The relationships between metabolic rate and body mass, and generation time and body mass, are well established (reviewed in Ginzburg and Colyvan, 2004). Based on these allometries, it can be inferred that generation time and metabolic rate might also be correlated. However, if such a relationship does exist, it would be due to the relationship that both variables have with body mass. In any case, metabolic rate (either absolute or mass-specific) may provide the physiological basis for variation in developmental time, an issue that has not been fully investigated.

The main aim of the present study is to unveil the relationship between developmental time and metabolic rate in three sympatric species of *Drosophila* living in two sites located at the same latitude but contrasting altitudes. Our hypothesis is that developmental time is positively correlated with metabolic rate, as might be expected from the inferred allometric relationship, at both the intraspecific (intra- and inter-population) and interspecific levels. Alternatively, it may be logical to assume a negative relationship between developmental time and metabolic rate after removing the 'confounding' effect of body mass. In addition, our experimental design allows us to compare the degree of phenotypic plasticity in response to different thermal regimes: the patterns of between-population variation in the response to constant and alternating temperature thermal regimes in a native species, *D. buzzatii*, and two invasive species that have recently reached the area, *D. melanogaster* and *D. simulans*.

MATERIALS AND METHODS

This work was performed with flies collected in two sampling sites where *Drosophila buzzatii*, *D. melanogaster*, and *D. simulans* co-exist (2340 m above sea level, 25°07′S, 66°09′W; and 720 m above sea level, 24°40′S and 65°02′W). The sampling localities are located in the Monte phytogeographic district, where at least two species of Cactaceae (*Trichocereus terschekki* and *Opuntia sulphurea*) provide the feeding and breeding resources to *D. buzzatii* (Hasson *et al.*, 1995), and vineyards and orchards the breeding and feeding resources for *D. melanogaster* and *D. simulans*, and in the semi-arid Chaco phytogeographic province, where several species of Cactaceae (Hasson *et al.*, 1995) and natural fruit trees provide the breeding and feeding resources to the flies.

Flies were collected by net sweeping on fermented banana baits. Upon arrival at the laboratory, several isofemale lines were established with females of each species collected in both sampling localities and maintained at low density (to avoid competition) for four generations (at 25°C and a photoperiod of 12 h light/12 h dark). In the fifth generation, equal numbers of individuals of several isofemale lines were pooled to initiate six stocks, one for each population and species.

Experimental flies

Two hundred flies of each pool were released into egg-collecting chambers with Petri dishes containing an egg-laying medium (2% agar in distilled water). Twelve hours later, the Petri dishes were removed and incubation allowed until egg hatching (24 h for *D. melanogaster* and *D. simulans* and 36 h for *D. buzzatii*). Then, batches of 30 first-instar larvae for *D. simulans* and *D. melanogaster*, and batches of 40 first-instar larvae for *D. buzzatii*, were transferred from Petri dishes to vials containing *Drosophila* instant medium. Pilot studies have shown that these larval densities are optimal.

Two different sets of experiments were run. The first, aimed at investigating the relationships between developmental time, metabolic rate, and body mass, involved the initiation of 96 vials (as described above), 16 for each combination of species (3) and locality (2). Vials were then randomly assigned to four thermal treatments, in three of which temperature was kept constant at 17°C, 21°C or 25°C; and the fourth consisted of an alternating regime in which temperature varied from 25°C during the 12-h period of light to 17°C during the night. In all cases, thermal treatments were chosen that were within the

natural thermal range (Loeschke *et al.*, 1994; Bubly and Loeschcke, 2005). Vials were inspected every 12 h for the presence of flies. Once flies were detected in a vial, the time and day of the emergence of the first male and female were recorded and used as a proxy of developmental time. Two flies, one of each sex that emerged in each vial, were randomly chosen and scored for metabolic rate and body mass (as described below).

For the second experiment, the aim of which was to investigate sources of variation in developmental time, 40 vials for each combination of species and sampling locality were initiated, giving a total of 240 vials. These vials were randomly assigned to the same thermal treatments described above. Newly emerged adults were recovered every 6 h and developmental time was estimated as the time elapsed since larval seeding to adult emergence.

Metabolic rate

Metabolic rate was measured as CO_2 production using a 'closed system' (Vleck, 1987) consisting of 2-cm^3 glass syringes fitted with three-way valves (see Chappell, 1983; Ashby, 1997; Chown *et al.*, 1997). All measurements were done during the day when flies were active. Measurements were performed on individual adult flies within 24 h of emergence.

Each fly was put in a glass syringe that was sealed and placed in a temperature-controlled incubator at 25°C during the measurement interval (2 h). Three blank syringes served as controls for each batch of measurements. Before injecting air from the glass syringe to a Tygon® tube (20 cm long) connected to a CO_2 analyser, the air was passed through small granules of Baralyme® and Drierite® to absorb CO_2 and water, respectively. At the end of the measurement interval, CO_2 concentrations were determined using a respirometry system (Sable Systems, Henderson, NV). A computer running the program DATACAN recorded the output of the CO_2 analyser.

Rates of CO_2 production (ml $CO_2 \cdot h^{-1}$) were calculated for each syringe using the following equation:

$$vCO_2\,(\mathrm{ml}\cdot\mathrm{h}^{-1}) = (v(F_mCO_2 - F_CCO_2))/t$$

where vCO_2 is the volume of CO_2 considered in the statistical analyses, vF_mCO_2 is the volume of CO_2 in the syringe with the fly, and vF_CCO_2 is the volume of CO_2 in the syringe submitted to a temperature regime but without a fly. In all cases, incubation time (t) was 2 h. Before the assay of CO_2 production, all flies were weighed using an analytical balance.

Data analysis

Data analysis was carried out using standard statistical methods described in Sokal and Rohlf (1997). First, we conducted a correlation analysis between metabolic rate, body mass, and developmental time. Correlation coefficients between pairs of variables were obtained at both the intra- and interspecific levels. These correlations were calculated by pooling males and females, and for each sex separately.

For the analysis of metabolic rate data, our experimental design included five predictor variables: four categorical variables (locality, species, thermal treatment, and sex) and one continuous variable (body mass), which was considered a covariate in the general design.

Developmental time was analysed using a four-way analysis of variance (ANOVA) with thermal treatment, population, species, and sex as the main sources of variation. Before

statistical analyses, developmental time, metabolic rate, and body mass were log transformed. *Post hoc* pairwise comparisons between species and thermal treatments were performed using Tukey's HSD test. All statistical analyses were performed with Statistica for Windows (v. 6.0).

RESULTS

Correlation analysis

Metabolic rate and developmental time were both positively and significantly correlated with body mass in comparisons involving the three species (Table 1). As expected, *D. buzzatii*, the largest species, had a significantly higher metabolic rate and a longer mean developmental time than both *D. melanogaster* and *D. simulans* (Table 2).

The correlation coefficients between developmental time and body mass within each species (pooled across sexes) were not significant, while those between metabolic rate and body mass were positive and significant in *D. simulans* and *D. melanogaster* but not so in *D. buzzatii* (Table 1). Analogous tests performed in males and females separately were largely non-significant, with the exception of *D. simulans* females (Table 1).

Metabolic rate

Metabolic rate exhibited a wide range of variation between the sexes, treatments, sampling localities, and species. Analysis of covariance (ANCOVA) revealed that differences between

Table 1. Correlation coefficients (r) between body mass (BM) and developmental time (DT), and between body mass and metabolic rate (MR), in interspecific and intraspecific comparisons

	DT-BM		MR-BM	
	r	P	r	P
Interspecific	0.234	0.003	0.273	<0.0001
Females	0.298	0.005	0.226	0.015
Males	0.320	0.006	0.220	0.061
Intraspecific				
D. melanogaster	–0.034	0.808	0.403	0.003
Females	0.054	0.780	–0.038	0.844
Males	0.324	0.131	0.329	0.124
D. simulans	–0.060	0.652	0.579	<0.0001
Females	0.147	0.407	0.658	<0.0001
Males	–0.223	0.283	0.359	0.077
D. buzzatii	–0.066	0.649	0.172	0.233
Females	–0.415	0.844	–0.295	0.889
Males	0.104	0.619	0.198	0.343

Table 2. Results of ANCOVA testing for differences in metabolic rate among species, between sampling sites (populations), among thermal treatments, and between sexes, with body weight as the covariate

Metabolic rate	SS	d.f.	*F*	*P*
Mass (mg) (covariate)	1.3×10^{-5}	1	5.048	0.026
Population (P)	1.6×10^{-5}	1	5.843	0.017
Species (Sp)	3.2×10^{-5}	2	6.064	0.003
Thermal treatment (T)	1.0×10^{-5}	3	1.234	0.299
Sex (S)	2.3×10^{-5}	1	8.725	0.004
P*Sp	2.0×10^{-5}	2	0.306	0.736
P*T	1.2×10^{-5}	3	1.517	0.213
Sp*T	6.8×10^{-5}	6	4.231	6.0×10^{-4}
P*S	1.9×10^{-6}	1	0.058	0.810
Sp*S	1.0×10^{-6}	2	0.178	0.836
T*S	7.1×10^{-7}	3	0.041	0.988
P*Sp*T	1.8×10^{-5}	6	1.113	0.357
P*Sp*S	1.9×10^{-6}	2	0.203	0.816
P*T*S	3.0×10^{-6}	3	0.410	0.745
Sp*T*S	1.0×10^{-5}	6	0.654	0.686
P*Sp*T*S	5.0×10^{-5}	6	0.286	0.942
Error	3.7×10^{-4}	139		

localities, species, and sexes were significant (Table 2). These effects were independent of variation in body mass, since this variable was considered a covariate in the ANCOVA. In all thermal treatments, mean metabolic rate in females was greater than that in males and lowland flies exhibited a higher metabolic rate than highland flies. The only significant interaction was species × thermal treatment (Table 2). *Post hoc* comparisons showed that mean metabolic rate at 17°C in *D. buzzatii* and *D. melanogaster* was significantly higher than in *D. simulans*, whereas at 25°C mean metabolic rate in *D. melanogaster* was significantly higher than in the other two species (Figure 1). In addition, metabolic rates were not significantly different between constant and alternating temperature regimes (sharing the same mean temperature, 21°C) in the three species.

Developmental time

Regarding developmental time, we also observed a wide range of variation between localities and among species in all thermal treatments (Table 3). In *D. buzzatii*, minimum and maximum values of mean developmental time were recorded in lowland females at 25°C (298.2 h) and 17°C (513.8 h), respectively. In *D. melanogaster*, developmental time varied from 212.6 h in males of the highland site to 411.1 h in males of the lowland site at 25°C and 17°C, respectively. Finally, in *D. simulans*, developmental time varied from 227.5 h to 435.2 h, in highland females at 25°C and lowland males at 17°C, respectively.

In the ANOVA performed using the complete developmental time data set, all main sources of variation – population, species, thermal treatment, and sex – were significant (Table 3). Mean developmental time was longer in flies reared at 17°C than at higher mean temperatures and females developed faster than males, in agreement with the expected

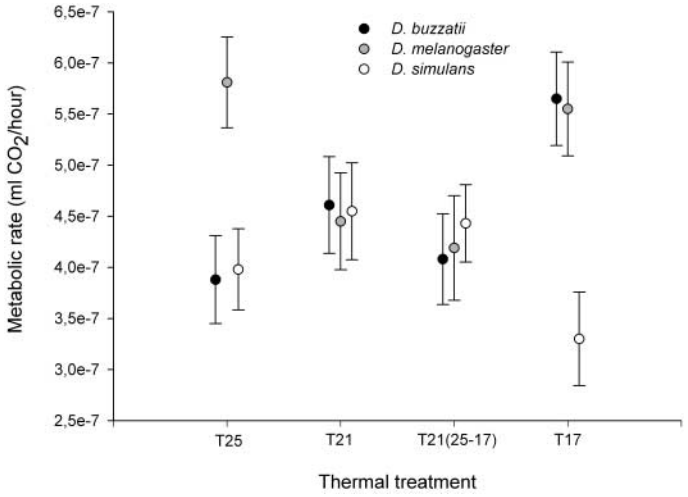

Fig. 1. Mean metabolic rate (averaged over sexes and localities) in *D. buzzatii*, *D. melanogaster*, and *D. simulans* exposed to different thermal treatments.

Table 3. Results of ANOVA testing for differences in mean developmental time among species, between sampling sites (populations), among thermal treatments, and between sexes

Developmental time	SS	d.f.	*F*	*P*
Population (P)	23 946	1	38.44	1.4×10^{-9}
Species (Sp)	688 430	2	552.54	2×10^{-3}
Thermal treatment (T)	1 510 184	3	808.06	3.0×10^{-3}
Sex (S)	8827	1	14.17	1.9×10^{-4}
P*Sp	41 080	2	32.97	5.7×10^{-14}
P*T	12 578	3	6.73	1.9×10^{-4}
Sp*T	58 717	6	15.71	3.3×10^{-16}
P*S	87	1	0.14	0.71
Sp*S	581	2	0.47	0.63
T*S	1250	3	0.67	0.58
P*Sp*T	19 270	6	5.16	4.1×10^{-5}
P*Sp*S	440	2	0.35	0.71
P*T*S	191	3	0.10	0.96
Sp*T*S	2470	6	0.66	0.68
P*Sp*T*S	2101	6	0.56	0.76
Error	246 071	395		

negative relationship between developmental time and temperature (Petavy *et al.*, 2001; Folguera, unpublished data) and the sexual dimorphism often reported in *Drosophila*. The interactions population × species, population × thermal treatment, species × thermal treatment, and the three-way interaction population × species × thermal treatment were also significant. The pattern depicted by the three-way interaction is shown in Figure 2. *Post hoc* comparisons among species revealed that developmental time differences between *D. melanogaster* and

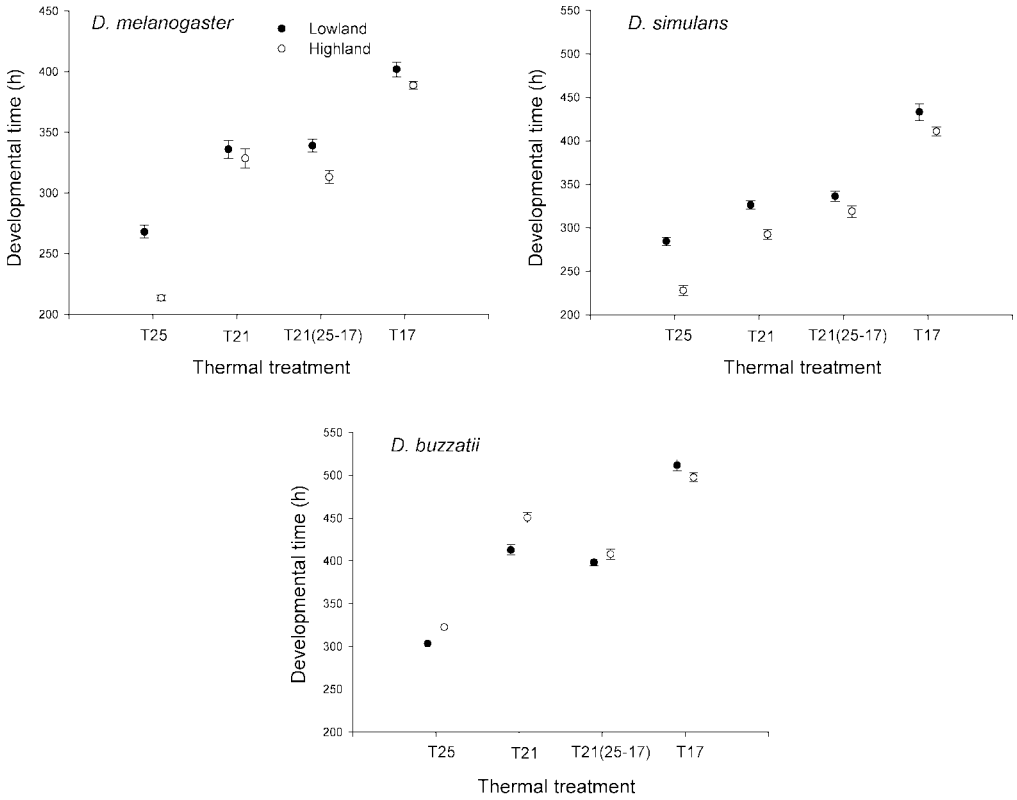

Fig. 2. Developmental time (hours) (averaged over sexes) in *D. melanogaster*, *D. simulans*, and *D. buzzatii* collected in lowland and highland localities exposed to different thermal treatments.

D. simulans were not significant, and that these species developed significantly faster than *D. buzzatii*.

Highland flies developed faster than lowland flies in *D. melanogaster* and *D. simulans*, irrespective of thermal treatments. However, in *D. buzzatii* the response to thermal treatments was different, with lowland flies developing significantly faster at 21°C and 25°C. Further analysis comparing the effect of constant and alternating thermal regimes revealed species-specific responses. On the one hand, differences between thermal regimes were not significant in *D. melanogaster* and *D. simulans*. On the other hand, the time to reach adulthood was significantly shorter in flies raised in the vials exposed to alternating temperature regimes than in flies that emerged in vials incubated at the same mean constant temperature. Interestingly, comparisons between constant versus alternating regimes, within sampling localities in *D. buzzatii*, showed that this interaction is mainly due to the highland site.

DISCUSSION

The first issue we would like to address is the positive correlation between metabolic rate and body mass when the three species were considered as the units in the analysis. Intra-

specific analyses revealed a similar trend in metabolic rate in the three species (although the correlation coefficients were only significant in *D. melanogaster* and *D. simulans*). These results are not trivial since studies in *Drosophila* have yielded contradictory results regarding the relationship between metabolic rate and body mass. In *D. melanogaster*, the most studied species to date, our results are coincident with a recent report showing that metabolic rate and body mass are not correlated in recombinant inbred lines (Van Voorhies *et al.*, 2004) and contrast with the positive relationship reported for flies recently derived from natural populations (Berrigan and Partridge, 1997).

In addition, there are several interesting conclusions that can be drawn from the ANCOVA conducted for metabolic rate, using body mass as the covariate. First, our study shows that metabolic rate is a sexually dimorphic trait (females had a higher metabolic rate than males) – a feature that is common to the three species studied – and, interestingly, independent of body mass (recall that in *Drosophila*, as in other insects, males are generally smaller and lighter than females). Second, differences between lowland and highland localities were also consistent across species, sexes, and thermal treatments. Flies of the three species, derived from collections at the highland site, showed a lower metabolic rate than the lowland flies, suggesting that similar environmental variables correlated with altitude may potentially affect metabolic rate in a consistent manner across species, in agreement with reports showing that variation in metabolic rate correlates with geographic gradients (e.g. Berrigan and Partridge, 1997).

The last issue concerning metabolic rate relates to interspecific variation. The significant species × thermal treatment interaction prevents us from interpreting interspecific differences independently of thermal treatments, since the responses were species-specific. Overall, *D. melanogaster* exhibited the highest metabolic rate, followed by its sibling *D. simulans* and finally by *D. buzzatii*. In addition, it is interesting to note that the specific responses were independent of the sampling locality – that is, differences in metabolic rate between localities were of the same magnitude and direction in the three species and across thermal treatments. Even *D. simulans* and *D. melanogaster*, which are phylogenetically close relatives, showed idiosyncratic responses to thermal treatments.

Especially interesting is the case of *D. buzzatii*. In this species, the metabolic rate of flies raised at 17°C was significantly higher than in treatments with higher mean temperatures, illustrating the so-called 'compensation effect'. Data from comparative studies suggest that compensation is one possible solution to the problem of temperature variation. Moreover, compensation is thought to be adaptive, since it allows higher levels of activity in individuals experiencing colder environments (Berrigan and Partridge, 1997).

Regarding developmental time, patterns of variation differed in many respects from the picture outlined in the preceding paragraphs for metabolic rate. First, the correlation between developmental time and body mass was significant at the interspecific level. At the intraspecific level, however, not only were the correlation coefficients in the three species not significant, but the sign of the coefficient was also negative. As for metabolic rate, all main sources of variation (species, sampling locality, sex, and thermal treatments) contributed significantly to variation in the ANOVA. However, all factors, except sex, were involved in second- and third-order interactions indicating that differences between localities are species-specific as well as the response to thermal treatments. Considering the species × locality interaction, our results show that our *a priori* expectation of a positive relationship between developmental time and altitude was only fulfilled in *D. buzzatii*, a trend that was homogeneous across treatments. However, this pattern, which was

consistent across treatments, was exactly the opposite to that seen in both *D. simulans* and *D. melanogaster*.

In general, the patterns of variation in metabolic rate and developmental time differ in several relevant respects, which suggests that each biological variable responds differentially to either natural environmental variation and/or to experimental conditions. Although flies of the three species face the same macro-environmental conditions in nature, the two biological variables responded in different ways. In this vein, it may be argued that the selective regime imposed by natural conditions promoted similar responses in metabolic rate across species, suggesting that the strength of the constraints governing the evolution of metabolic rate and developmental time may be substantially different.

In summary, our results offer a note of caution in the extrapolation of the so-called allometric rules from the interspecific to the intraspecific level. Interestingly, in our study, fast development appeared to be associated with high mass-specific metabolic rates, particularly in comparisons between the sexes (sexual dimorphism) and among species. Fast developing flies had higher metabolic rates in both *D. melanogaster* and *D. buzzatii*. In turn, *D. simulans* occupied an intermediate position for both variables. In contrast, at the intraspecific level fast development was associated with higher mass-specific metabolic rates only in *D. buzzatii*, since flies collected at lower altitude developed faster and had higher metabolic rate than flies collected in the highland locality.

In conclusion, we did not observe perfect agreement between inter- and intraspecific levels in relation to the predictions of allometries, thus providing experimental support to Kozlowski and Weiner's (1997) assertion that different factors may affect variation in physiological variables at both the intraspecific and interspecific levels.

Finally, recall that our inferences of a relationship between metabolic rate and, in particular, developmental time are based on the measurement of adult flies. Flies of the genus *Drosophila* are holometabolous insects that spend a large part of their lives in early life-cycle stages (as larvae and pupae) confined to a continuously changing environment (which usually consists of pockets of decaying plant tissues). In this sense, we wonder whether the measurement of metabolic rate in adults is representative of earlier stages. Hence, we suggest that the relationship between metabolic rate and developmental rate in early stages of the life cycle may provide new insights into our understanding of the allometric relationship in organisms with complex life histories.

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REFERENCES

Angiletta, M.J., Jr., Niewiarowski, P.H. and Navas, C.A. 2002. The evolution of thermal physiology in ectotherms. *J. Therm. Biol.*, **27**: 249–268.

Ashby, P.D. 1997. Conservation of mass-specific metabolic rate among high- and low-elevation populations of the acridid grass-hopper *Xanthippus corallipes*. *Physiol. Zool.*, **70**: 701–711.

Berrigan, D. 1997. Acclimation of metabolic rate in response to developmental temperature in *Drosophila melanogaster*. *J. Therm. Biol.*, **22**: 213–218.

Berrigan, D. and Partridge, L. 1997. Influence of temperature and activity on the metabolic rate of adult *Drosophila melanogaster*. *Comp. Biochem. Physiol. A*, **118**: 1301–1307.

Borash, D.J., Gibbs, A.G., Joshi, A. and Mueller, L.D. 1998. A genetic polymorphism maintained by natural selection in a temporally varying environment. *Am. Nat.*, **151**: 148–156.

Bubly, O.A. and Loeschcke, V. 2005. Variation of life-history and morphometrical traits in *Drosophila buzzatii* and *D. simulans* collected along an altitudinal gradient from a Canary Island. *Biol. J. Linn. Soc.*, **84**: 119–136.

Chappell, M.A. 1983. Metabolism and thermoregulation in desert and montane grasshoppers. *Oecologia*, **56**: 126–131.

Chown, S.L., van der Merwe, M. and Smith, V.R. 1997. The influence of habitat and altitude on oxygen uptake in sub-Antartic weevils. *Physiol. Zool.*, **70**: 116–124.

Cortese, M.D., Norry, F.M., Piccinali, R. and Hasson, E. 2002. Direct and correlated responses to artificial selection on developmental time and wing length in *Drosophila buzzatii*. *Evolution*, **56**: 2541–2547.

Economos, A.C. and Lints, F.A. 1986. Developmental temperature and life span in *Drosophila melanogaster*. II. Oscillating temperature. *Gerontology*, **32**: 28–36.

Forsman, A. 1999. Variation in thermal sensitivity in performance among colour morphs of a pygmy grasshopper. *J. Evol. Biol.*, **12**: 869–878.

Gibert, P. and De Jong, G. 2001. Temperature dependence of development rate and adult size in *Drosophila* species: biophysical parameters. *J. Evol. Biol.*, **13**: 267–276.

Ginzburg, L. and Colyvan, M. 2004. *Ecological Orbits: How Planets Move and Populations Grow*. Oxford: Oxford University Press.

Hasson, E., Rodriguez, C., Fanara, J.J., Naviera, H., Reig, O.A. and Fontdevila, A. 1995. The evolutionary history of *Drosophila buzzatii*: XXVI. Macrogeographic patterns of inversion polymnorphism in New World populations. *J. Evol. Biol.*, **8**: 369–384.

Huey, R.B. and Berrigan, D. 2001. Temperature, demography and ectotherm performance. *Am. Nat.*, **158**: 204–210.

Johnston, I.A. and Bennett, A.F. 1996. *Animals and Temperature: Phenotypic and Evolutionary Adaptation*. Cambridge: Cambridge University Press.

Kozlowski, J. and Weiner, J. 1997. Interspecific allometries are by-products of body size optimization. *Am. Nat.*, **149**: 352–380.

Lardies, M.A. and Bozinovic, F. 2006. Geographic covariation between metabolic rate and life-history traits. *Evol. Ecol. Res.*, **8**: 1–16.

Lardies, M.A., Catalán, T.P. and Bozinovic, F. 2004. Metabolism and life-history correlates in a lowland and highland population of a terrestrial isopod. *Can. J. Zool.*, **82**: 677–687.

Loeschcke, V., Krebs, R.A. and Barker, J.S.F. 1994. Genetic variation for resistance and acclimation to high temperature stress in *Drosophila buzzatii*. *Biol. J. Linn. Soc.*, **52**: 83–92.

Loeschcke, V., Krebs, R.A., Dahlgaard, J. and Michalak, P. 1997. High-temperature stress and the evolution of thermal resistance in *Drosophila*. In *Environmental Stress, Adaptation and Evolution* (R. Bijlsma and V. Loeschcke, eds.), pp. 175–189. Basel: Birkhäuser-Verlag.

Neargarder, G., Dahlhoff, E.P. and Rank, N.E. 2003. Variation in thermal tolerance is linked to phosphoglucose isomerase genotype in a montane leaf beetle. *Funct. Ecol.*, **17**: 213–221.

Nespolo, R.F., Bacigalupe, L.D. and Bozinovic, F. 2003. Heritability of energetics in a wild mammal, the leaf-eared mouse (*Phyllotis darwini*). *Evolution*, **57**: 1679–1688.

Partridge, L., Barrie, B., Fowler, K. and French, V. 1994. Thermal evolution of pre-adult life history traits in *Drosophila melanogaster*. *J. Evol. Biol.*, **7**: 645–663.

Petavy, G., David, J.R., Gibert, P. and Moreteau, B. 2001. Viability and rate of development at different temperatures in *Drosophila*: a comparison of constant and alternating thermal regimes. *J. Therm. Biol.*, **26**: 29–39.

Roff, D.A. 2001. *Life History Evolution*. Sunderland, MA: Sinauer Associates.

Rogowirz, G.L. and Chappell, M.A. 2000. Energy metabolism of eucalyptus-boring beetles at rest and during locomotion: gender makes a difference. *J. Exp. Biol.*, **203**: 1131–1139.

Sokal, R. and Rohlf, F. 1997. *The Principles and Practice of Statistics in Biological Research*. New York: W.H. Freeman.

Spicer, J.I. and Gaston, K.J. 1999. *Physiological Diversity and its Ecological Implications*. Oxford: Blackwell Science.

Stearns, S.C. 1992. *The Evolution of Life Histories*. Oxford: Oxford University Press.

Tomanek, L. and Somero, G.N. 2002. Interspecific and acclimation-induced variation in levels of heat shock protein 70 (hsp70) and 90 (hsp90) and heat shock transcription factor-1 (HSF1) in congeneric marine snails (genus *Tegula*): implications for regulation of hsp gene expression. *J. Exp. Biol.*, **205**: 677–685.

Townsend, C.R. and Calow, P. 1981. *Physiological Ecology: An Evolutionary Approach to Resource Use*. Oxford: Blackwell Scientific.

Van Voorhies, W.A., Khazaeli, A.A. and Curtsinger, J.W. 2004. Testing the 'rate of living' model: further evidence that longevity and metabolic rate are not inversely correlated in *Drosophila melanogaster*. *J. Insect Physiol.*, **50**: 445–453.

Vleck, D. 1987. Measurement of O_2 consumption, CO_2 production, and water vapor production in a closed system. *J. Appl. Physiol.*, **62**: 2103–2106.

Zera, A.J. and Harshman, L.G. 2001. The physiology of life-history trade-offs in animals. *Annu. Rev. Ecol. Syst.*, **32**: 95–126.