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Non-adaptive strategies explain variation in rate of development under different thermal conditions

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ABSTRACT

Objective: To test for an effect of isomorphy on development rate under different experimental thermal environments.

Methods: In three different thermal environments, we studied the following characters as response variables during development: metabolic rate, body mass, and body length. We used as our study model the terrestrial isopod *Armadillidium vulgare* during the first 14 weeks of development.

Conclusions: Our results suggest that although metabolic rate and body mass may depend on the internal state of individuals, body length may present an isomorphic response. This would be evidence that a non-adaptive interpretation could explain developmental patterns and thus highlight the importance of avoiding the frequent use of unsupported adaptive explanations in evolutionary ecology.

Keywords: adaptation, *Armadillidium vulgare*, development, isomorphy.

INTRODUCTION

Theoretically, among ectotherms, the proportion of total developmental time spent in a particular developmental stage does not change with temperature. This hypothesis predicts similar proportional responses among different life stages (Gillooly *et al.*, 2002; Jarosik *et al.*, 2002, 2004), and is called 'developmental isomorphy'. Some authors have suggested that isomorphy may constrain the evolution of the life-history strategies of ectotherms (Jarosik *et al.*, 2004), and that there is a canalized response for each stage. Such an approach highlights another feature that is relevant to an understanding of other aspects related to the strategies of an organism's growth. It refers to patterns of development rate through (and not yet among) life cycles under different environmental conditions (Fig. 1). In this sense, the analysis of patterns of development rate would provide useful information about developmental strategies. In addition, the use of different phenotypic variables might be useful for providing comparative information in the study of differences in development rate.

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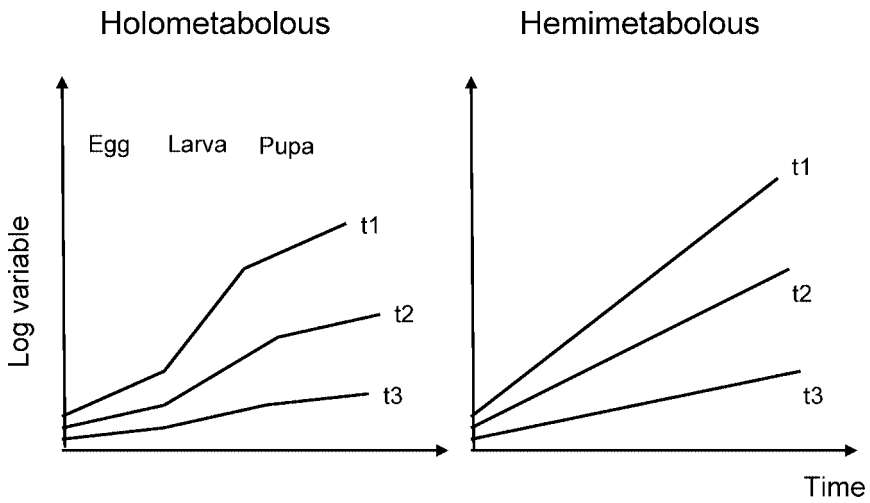


Fig. 1. Two models of the isomorphy theory for holometabolous and hemimetabolous organisms maintained under different thermal conditions.

Accordingly, a comparison among ontogenetic traits is viewed as trait co-variation among individuals between developmental stages or along a growth course (Schlichting and Pigliucci, 1998), allowing the identification of factors and constraints that affect development (Garland and Carter, 1994). Moreover, a comparison of ontogenetic trajectories might also help the integration of fitness variation at different periods in the life cycle in an overall view of fitness (Chippindale *et al.*, 1997). In this context and based on the ideas of Gotthard and Nylin (1995) and Hassall *et al.* (2005), we ask the following question: Is the variation in development rate under different thermal scenarios the result of non-adaptive phenotypic responses, or adaptive strategies selected to increase fitness under different conditions? We test for the presence of isomorphy on development rate under different thermal conditions using the following traits as response variables during ontogeny: metabolic rate (V_{CO2}), body mass, and body length. We used an animal model, the terrestrial isopod *Armadillidium vulgare*. This woodlouse appears to be a good model, since it copes with extreme abiotic habitat conditions and exhibits phenotypic plasticity in development rates at different temperatures (Helden and Hassall, 1998).

MATERIALS AND METHODS

Experimental populations and variables studied

The study was performed with woodlice collected at the Estación de Investigaciones Ecológicas Mediterráneas in San Carlos de Apoquindo (33°23′S, 70°31′W) (Jaksic, 2001). After collection, organisms were sorted by sex. Pregnant females were identified in the laboratory and placed under standard conditions of light (light/dark = 12 h : 12 h) and temperature (22.5°C) in culture boxes ($2.2 \times 2.2 \times 2.4$ cm) with a layer of damp sand 1–1.5 cm thick. Forty-seven females with a mean body mass of ~40 mg were selected to start the experiments. Later, their progeny were randomly assigned to each of the three thermal treatments (15°C, 22.5°C, and 30°C). Nearly 500 individuals were assigned to each thermal treatment. Experimental temperatures were chosen because they are within the

thermal range of this species' tolerance (Miller and Cameron, 1987; Helden and Hassall, 1998). Each week, 12 organisms from each thermal treatment were chosen at random and measured. This protocol was repeated during the first 14 weeks of development. First, each woodlouse was weighted in an analytical balance (AT-21 Comparator; ± 0.001 mg; Mettler, Toledo). Later, V_{CO2} was measured based on CO_2 production using a 'closed system' (Vleck, 1987) consisting of 2-ml glass syringes fitted with three-way valves (see Lighton, 2008). In short, each woodlouse was placed inside glass syringes that were sealed and placed in a temperature-controlled incubator, at 15°C, 22.5°C, and 30°C for 4 h (measurement interval). Three blank syringes served as controls for each batch of measurements. Before injecting air from the glass syringe into a Tygon® tube (20 cm long) connected to a CO_2 analyser, the air passed through small granules of Drierite® to absorb water. At the end of the measurement interval, CO_2 concentrations were determined using a respirometry system (Sable Systems, Henderson, NV). A computer equipped with the program EXPEDATA recorded the output of the CO_2 analyser (for methodological details, see Folguera *et al.*, 2007). We also measured the body length of each animal as the distance (mm) between the cephalothorax to the limit of the pleotelson. Once measurements were registered, organisms were discarded.

Statistical analyses: regression analyses and parallelisms in single characters

Regression analyses of V_{CO2}, body mass, and body length on weeks of development were used to examine patterns of variation in each variable over time. In all cases, males and females were analysed together because previous analyses have revealed that the sex ratio of *A. vulgare* does not depart significantly from the expected 1 : 1. Comparison of rates of each variable through development was performed by slope comparison of regression analyses. Studies that have investigated aptitude–treatment interactions have adopted the homogeneity of regressions test as the standard for assessing differences in regression slopes across treatments. In this case, we test the hypothesis that the slopes are equal through development of organisms at each temperature (Fig. 2); we considered the different

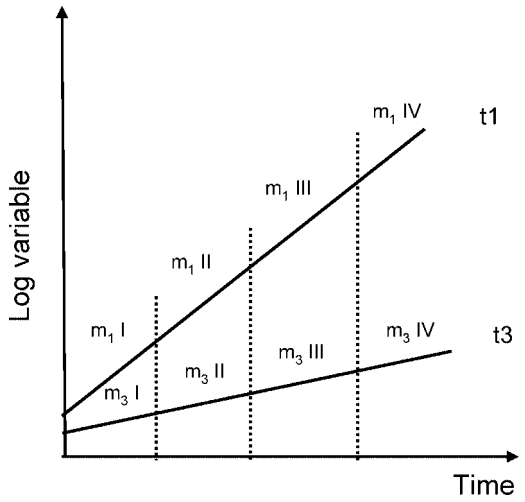
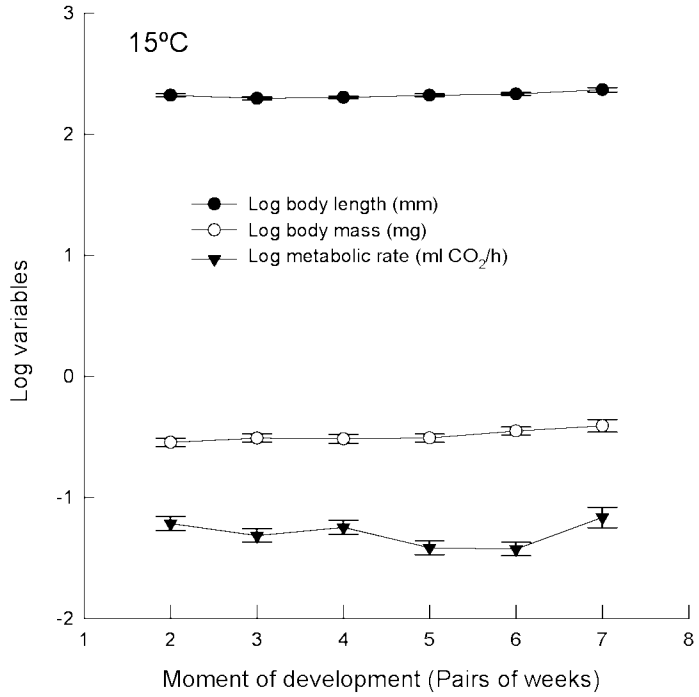
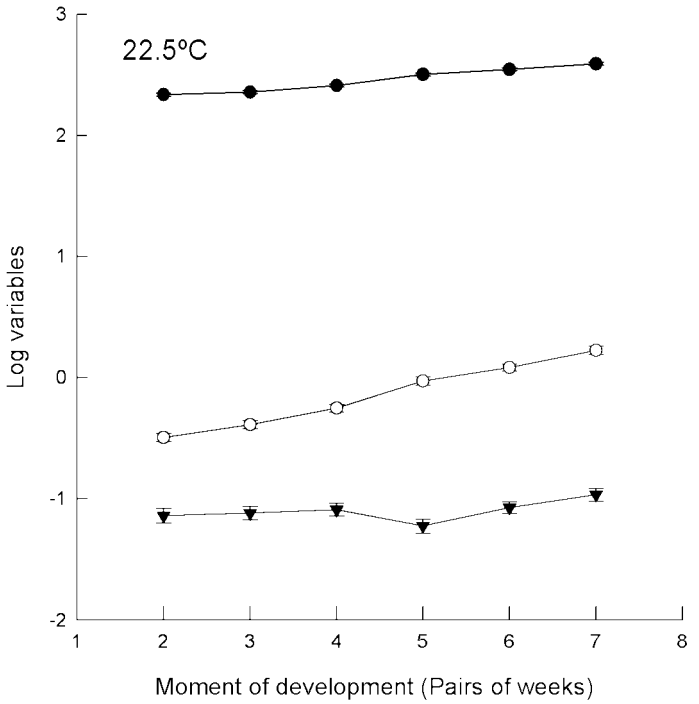


Fig. 2. Hypothetical growth trajectories under two different thermal regimes (t1 and t3). Comparisons in this study were performed within each growth trajectory, i.e. among slopes of development rate and among different periods of time for organisms maintained under each thermal regime.


15ºC
Log body length (mm)
Log body mass (mg)
Log metabolic rate (ml CO_2/h)
Log variables
Moment of development (Pairs of weeks)


22.5ºC
Log variables
Moment of development (Pairs of weeks)

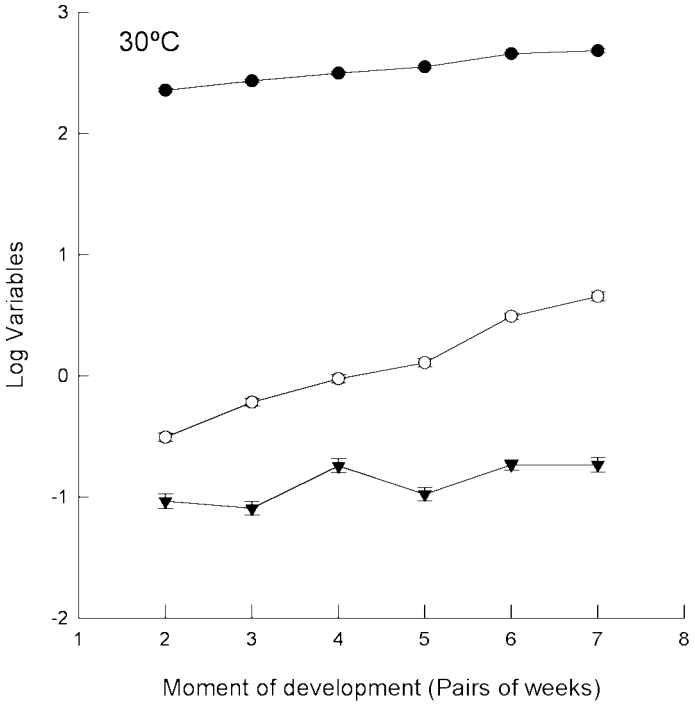


Fig. 3. Ontogenetic trajectories of body length, body mass, and metabolic rate over 14 weeks under three thermal regimes (15°C, 22.5°C, and 30°C). Error bars represent the standard deviation among organisms every 2 weeks.

responses for organisms reared at each of three temperatures. Data for pairs of weeks were grouped, giving a total of six groups (2–3, 4–5, 6–7, 8–9, 10–11, and 12–14 weeks). Before statistical analysis, all data were log-transformed. All analyses were conducted using the statistical package Statistica® for Windows (Statsoft, 2001).

RESULTS

We observed that body length exhibited a wide range of variation through development. Regressions on weeks of development yielded significant results among temperatures (Fig. 3). Body length increased in organisms maintained at 15°C (slope (b) = 0.34), 22.5°C (b = 0.84), and 30°C (b = 0.85). In addition, analysis of isomorphy revealed the absence of an interaction between 'period of development' and slope, suggesting similar magnitudes in developmental rate under each thermal regime (Table 1). In addition, body mass followed the same general pattern as body length. For body mass, values of slopes exhibited comparable magnitudes at 15°C (b = 0.39), 22.5°C (b = 0.86), and 30°C (b = 0.87). However, the analysis of isomorphy in body mass exhibited some differences, in contrast to isomorphy in body length. Woodlice maintained at 22.5°C and 30°C, but not 15°C (Table 1), showed a significant interaction between 'period of development' and slope, suggesting fluctuating developmental rates at both 22.5°C and 30°C. Finally, metabolic rate versus time did not exhibit significant values in organisms developed at 15°C or at 22.5°C, but a positive

Table 1. Results of analyses of variance for body mass, body length, and metabolic rate tested for differences among 'period of development' (PD) and slope (S) in *Armadillidium vulgare*

	15°C			22.5°C			30°C		
	d.f.	*F*	*P*	d.f.	*F*	*P*	d.f.	*F*	*P*
Body mass (mg)									
PD	4	1.887	0.119	5	11.445	0.0001	5	8.90	0.0001
S	1	0.031	0.862	1	2.326	0.129	1	14.12	0.0001
PD × S	4	1.609	0.178	5	2.446	0.037	5	2.32	0.046
Error	96			143			143		
Body length (mm)									
PD	4	1.82	0.132	5	7.46	0.0001	5	11.06	0.0001
S	1	2.33	0.130	1	0.22	0.637	1	13.12	0.0001
PD × S	4	0.94	0.443	5	1.04	0.399	5	0.91	0.357
Error	87			129			133		
Metabolic rate (ml $CO_2 \cdot h^{-1}$)									
PD	4	0.497	0.738	5	3.043	0.012	5	1.697	0.139
S	1	0.259	0.612	1	6.243	0.014	1	1.227	0.270
PD × S	4	1.122	0.351	5	5.399	0.001	5	3.681	0.004
Error	96			140			141		

Note: The PD × S interaction is an estimate of the differences among slopes of rate of development at different periods of time for organisms maintained under each thermal regime.

relationship was observed in individuals maintained at 30°C ($b = 0.37$). Interestingly, this physiological character was similar to body mass, with a significant interaction at 22.5°C and 30°C.

DISCUSSION

In this paper, we tested the isomorphic response hypothesis during development under different thermal regimes. Interestingly, our results showed a diversity of responses to thermal regimes and among characters. We observed that V_{CO2} and body mass at 22.5°C and 30°C suggested the absence of an effect of isomorphy on development, which was not the case for body length. This is an interesting difference between two characters that are usually considered to respond similarly in an ecological scenario.

Van der Have and de Jong (1996) proposed that a basic process regulates the timing of major developmental events. They suggested that the rate of differentiation is determined primarily by cell division – that is, an increase in cell number during development. Specifically, the duration of cell division should be the key process regulating the rate of differentiation. In addition, similar rates of development in the case of body length could be viewed as an indicator of structural size. In this sense, this approach could interpret similar rates of development as a constant number of cells in organisms through growth under all thermal conditions. In contrast, body mass reflects environmental and physiological conditions (Dobson, 1992; Green, 2001). Therefore, greater variability through development is

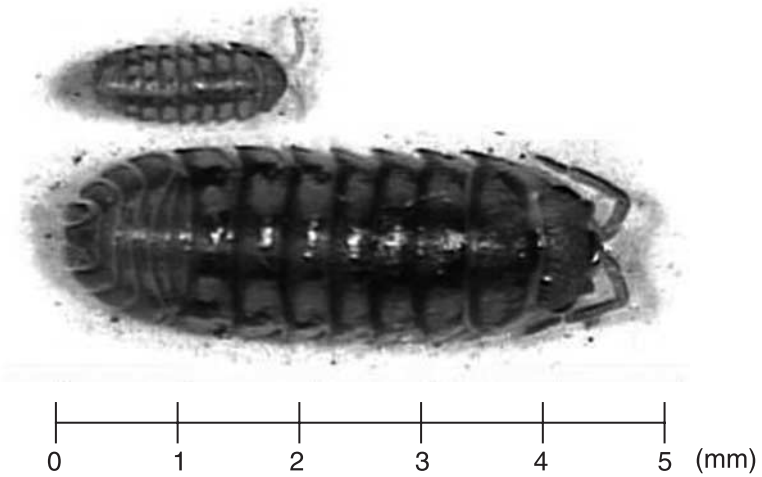


Fig. 4. Two woodlice siblings at 10 weeks of development, one maintained at 15°C (top) and the other at 30°C (bottom).

expected in body mass rather than in body length, thus explaining the observed fluctuating results for body mass. This pattern would explain the V_{CO2} results at 22.5°C and 30°C. In the case of animals maintained at 15°C, results should not be considered due to the lower rates of development observed (Fig. 4).

We now ask whether differences through development should be interpreted as non-adaptive phenotypic responses or as adaptive strategies selected to increase fitness under different environmental conditions. In the adaptive scenario, strategies to cope with environmental changes are diverse not only among organisms of different populations and species, but also during the life cycle of the same organism (Marquet *et al.*, 1989; Pörtner *et al.*, 2006). In this context, the state-dependent life-history decision (Houston and McNamara, 1992) predicts that the responses of individuals to environmental thermal variation may depend on the state of the organism (McNamara and Houston, 1996; Rombough, 2003), including its environment as well as different aspects of its own design (Houston and McNamara, 1992). Thus, the different periods of development of organisms may require different 'decisions'. According to Houston and McNamara (1992, p. 243), this would be 'a genetically determined rule which prescribes the action that should be taken in each possible state' in relation to life history. Therefore, different stages may have different 'decisions' in relation to life history. In this way, the 'decisions' are considered 'a genetically determined rule which prescribes the action that should be taken in each possible state' (ibid.). Interestingly, life-history state-dependent theory might be a conceptual model to characterize different 'mechanisms of growth' (Gotthard *et al.*, 2000; Gotthard, 2001). However, our results – for example, the isomorphy pattern for body length (explained by a constant increase in the number of cells) and the variability in body mass and V_{CO2} – support a non-adaptive explanation for developmental patterns. These examples may be useful to show the importance of avoiding the frequent use of unsupported adaptive explanations in evolutionary ecology.

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