Testing the metabolic cold adaptation hypothesis: an intraspecific latitudinal comparison in the common woodlouse

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ABSTRACT

The metabolic cold adaptation (MCA) hypothesis predicts an increase in the metabolic rate of ectotherms from cold environments compared with their more temperate counterparts. This adaptive hypothesis is one of the most controversial in physiological ecology, for which the evidence comes principally from the meta-analysis of data sets of arthropods. Important for the study of metabolic cold adaptation are comparisons at a geographic level, especially on a latitudinal scale, because mean annual temperature decreases towards high latitudes. Furthermore, few studies have conducted intraspecific comparisons of metabolic rates along a latitudinal gradient. We tested the MCA hypothesis in the common woodlouse, *Porcellio laevis*, using different populations along a distributional range with a wide range of mean ambient temperatures (5°, 12°, 18° and 25°C) in Chile. Our results demonstrated that metabolic rate increased towards low latitudes – that is, woodlice from the warmer (i.e. northern) part of the distribution range had markedly higher metabolic rates than those from the cooler (i.e. southern) region, for almost all experimental temperatures. Thus, our results provide direct evidence of intraspecific latitudinal differences in metabolism, rejecting the MCA hypothesis, which is more difficult to resolve with interspecific level comparisons.

Keywords: latitudinal cline, local adaptation, metabolism, *Porcellio laevis*, temperature.

INTRODUCTION

Much controversy and speculation has surrounded the metabolic cold adaptation (MCA) hypothesis and the latitudinal compensation hypothesis (e.g. Krogh, 1916; Chown and Gaston, 1999; Steffensen, 2002; Hodkinson, 2003). The MCA hypothesis refers to the observation that at the same ambient temperature the metabolic rate of ectotherms from cold climates is higher than that of their counterparts from warm climates (i.e. temperate or tropical). The increase in metabolic rate is considered to be an adaptation to compensate for the short period of favourable environmental conditions for development, growth and

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reproduction (Scholander *et al.*, 1953; Clarke, 1993). The MCA hypothesis has also been used to explain metabolic differences in altitude, latitude and habitat conditions, since it is thought to be a general evolutionary adaptation among ectotherms to compensate for a low ambient temperature (for a review, see Chown and Gaston, 1999).

Several interspecific studies support the MCA hypothesis for a suite of both terrestrial and marine species (e.g. Young, 1979; Torres and Somero, 1988; Chown *et al.*, 1997; Chown and Gaston, 1999; Addo-Bediako *et al.*, 2002). However, several authors have also failed to find an increase in oxygen consumption at lower ambient temperatures (Lee and Baust, 1982a,b; Kevan and Danks, 1986; Clarke, 1991; Nylund 1991; Steffensen, 2002). Classically, studies testing the MCA hypothesis have emphasized the analysis of species as a unit. Despite its importance, little attention has been paid to metabolic cold adaptation between populations of the same species inhabiting different habitats within a region (but see Sømme *et al.*, 1989; Nylund, 1991). It is broadly accepted by evolutionary ecologists that to evaluate evolutionary changes it is necessary to compare populations within species (intraspecific, population level) as well as individuals within breeding populations. One of the major problems in understanding ecological physiology is that studies comparing individuals within populations are scarce. Nevertheless, such studies are crucial for understanding how differences in physiological variables evolve and can be affected by ecological and geographical factors. Consequently, studying populations from the same region, but from different habitats, should provide the most effective tool for understanding physiological tolerances, and their consequences for the MCA hypothesis. In addition, there are several problems attached to comparisons of metabolic rate for organisms from different areas. Factors such as acclimation, activity level, sex, reproductive status, food intake and experimental technique may strongly influence the results if they are not taken into account (Clarke, 1991; Chown and Gaston, 1999).

In broadly distributed ectotherm species, intraspecific clinal patterns of variation are frequently attributed to the action of natural selection, and hence are presumed to reflect genetic adaptation within populations to local conditions (Alatalo and Gustafson, 1988; Mousseau and Roff, 1989; Mousseau *et al.*, 2000). Environmental gradients are common in nature and are considered to have major effects on intraspecific variation patterns (Mizera and Meszéna, 2003). Important for the study of the MCA hypothesis are comparisons at a geographic level, especially on a latitudinal scale, since mean annual temperature decreases towards high latitudes. In this study, we tested the MCA hypothesis at an intraspecific level, along a temperature gradient. For this purpose, we utilized the common woodlice *Porcellio laevis* (Isopoda: Oniscidea) as a study model. This isopod presents a range of geographic distribution with a gradient across 13° of latitude. We tested the MCA hypothesis in a laboratory experiment in which woodlice from different localities were measured at different air temperatures, paralleling conditions in the wild.

MATERIALS AND METHODS

Animal capture and study sites

Porcellio laevis is widely distributed in Chile, as well as throughout several other biogeographic areas around the world (Leistikow and Wägele, 1999). In this study, woodlice were collected during the austral summer of 2002 from four coastal localities in northern and central Chile: Iquique (20°10′S), Antofagasta (23°38′S), La Serena (29°55′S) and

San Carlos de Apoquindo in Santiago (33°23′S). Climatological data for the study sites are presented in Table 1. Woodlice from all study sites were collected by hand, from under stones, pieces of wood and soil litter. Specimens were placed in plastic containers with vegetable soil and carrot slices, then transported to the laboratory where they were sexed. To remove possible confounding effects of sex, we used only males.

Maintenance, acclimation and metabolic rate

All live specimens were maintained in plastic containers. The base of the containers was covered with a layer of plaster of Paris to ensure uniformity of the acclimation conditions before recording metabolic rate. Periodically, we added water to the base to provide moisture. Food was supplied weekly in the form of mashed rabbit food pellets, and the photoperiod was maintained at 12 : 12 dark : light. After an initial 4-week period of acclimation to laboratory conditions, and before each measurement of metabolic rate, the woodlice were maintained in environmental chambers for 3 weeks at 5 ± 1°C, 12 ± 1°C, 18 ± 1°C and 25 ± 1°C. After each thermal acclimation, metabolic rate was measured (at the same temperature as acclimation). We chose these acclimation temperatures since they are close to the average extremes of the natural temperature range in the habitats where the woodlice were collected (see Table 1).

Rate of oxygen consumption or metabolic rate was determined using a 'closed system' (Vleck, 1987), consisting of disposable 10-ml syringes fitted with three-way valves (see also Chappell, 1983; Ashby, 1997; Chown $et\ al.$, 1997; Nespolo $et\ al.$, 2003). All measurements were made during the daytime when the woodlice were inactive (Warburg, 1987). The woodlice were weighed to the nearest milligram using an analytical balance to determine body mass and then placed individually within syringes. The syringes were then sealed from the atmosphere and placed in a temperature-controlled incubator for the duration of the measurement interval ($c.$ 3–7 h, depending on the experimental temperature). The concentration of oxygen inside the syringe never decreased more than 10% of the initial value (usually less than 5%) during the measurements. Three blank syringes served as controls for each series of measurements. After injecting air from the syringe into a Tygon tube (1.5 m long) connected to an oxygen analyser, the air passed through small granules of Baralyme and Drierite to absorb CO_2 and H_2O, respectively. These absorbent granules were also contained in each syringe, in a compartment isolated from the woodlice. At the end of the measurement interval, the concentration of oxygen was determined using a Fox Field Oxygen Analysis System (Sable System International) supplied with barometric pressure compensation. Rates of oxygen consumption (in $\mu l\ O_2 \cdot h^{-1}$) were calculated for each syringe using the following equation from Vleck (1987):

$$\dot{V}O_2 = V(F_1O_2 - F_EO_2)/(1 - F_EO_2) \times t$$

where V is the initial volume of dry CO_2-free air in the syringe at standard temperature and pressure; F_1O_2 and F_EO_2 are the oxygen fractions inside the syringe at the beginning and end of the incubation period, respectively; and t is the duration of the incubation period in hours. This system was not intended to measure the instantaneous rate of $\dot{V}O_2$, or to resolve discontinuous gas exchange (e.g. Chappell and Rogowitz, 2000), since each measurement is an average of resting $\dot{V}O_2$ over several hours. Nevertheless, technical errors associated with this measurement method are small (see Anderson $et\ al.$, 1989), and its simplicity allows for the simultaneous measurement of a large number of individuals (Nespolo $et\ al.$, 2003).

Table 1. Climatological data for the four study sites

	Iquique (20°10′S)	Antofagasta (23°38′S)	La Serena (29°55′S)	Santiago (33°23′S)	Years of observation	Source
Temperature (°C)	18.7 (22.2–15.1)	17.0 (20.1–13.3)	14.8 (18.9–11.2)	13.9 (22.1–7.7)	35	di Castri and Hajek (1976); FAO (1985)
Relative humidity (%)	80	72	80	72	35	di Castri and Hajek (1976); FAO (1985)
Precipitation (mm)	2.1	2.2	127.4	356.2	65	di Castri and Hajek (1976); FAO (1985)
Total radiation (cal · cm^{-2}· day^{-1})	424	423	340	310	20	FAO (1985)
Climate	Desert	Desert	Semi-arid	Mediterranean		di Castri and Hajek (1976)

Note: The temperature is presented as the annual average with the annual average minimum and maximum indicated in parentheses.

Statistical analyses

Before all statistical analyses, we tested the data for assumptions of homogeneity of variances and normality using Levene and Kolmogrov-Smirnov tests, respectively. Where necessary to meet the assumptions, the variables were $\log_{10}$-transformed. For analysis of covariance, we checked for parallelism using a test for interaction with the covariate (i.e. body mass). If necessary, a separate slopes model was carried out. We designated $\alpha = 5\%$ as the probability level for falsely accepting a null hypothesis (Type I error). All statistical analyses were performed using Statistica 6.0 (StatSoft, 2001) for Windows.

Since a positive relationship between metabolic rate and ambient temperature is well known for ectotherms (McNab, 2002), within populations we carried out planned comparisons to test the following alternative hypothesis: $\mu5°C < \mu12°C < \mu18°C < \mu25°C$. Planned comparisons were also used between populations to test for differences within each acclimation temperature along the latitudinal gradient: San Carlos *vs* La Serena (C1), La Serena *vs* Antofagasta (C2) and Antofagasta *vs* Iquique (C3).

RESULTS

General trends in metabolic rate within and between populations ranging from approximately 20 to 33°S of the distribution are depicted in Fig. 1A. As expected, within all populations metabolic rate increased as a result of thermal acclimation. Nevertheless, at 25°C the metabolic rate of individuals from Antofagasta (north) was 322% higher than that of individuals from Santiago (southern population). An Arrhenius plot of log metabolism versus 1/temperature (K) for different populations is presented in Fig. 1B. After analysing this plot, it is apparent that there were no abrupt changes in slope among the populations studied.

The estimated thermal sensitivities (Q_{10}) are presented in Table 2. Interestingly, similar values were seen for all populations, ranging from 2.034 at 5–12°C to 3.358 at 18–25°C in woodlice from La Serena. Only two outliers were observed at a temperature range of 12–18°C, one individual from Antofagasta (~23°S) and one from La Serena (~29°S).

The results of planned comparisons of metabolic rate within each population are presented in Table 3, while planned comparisons between populations are presented in Table 4. Finally, a brief summary of interpopulation comparisons is shown in Table 5. We conclude that, in general, our results show that metabolic rate increased towards low latitudes for almost all temperature trials. At three of the four temperatures tested, the MCA hypothesis was rejected.

DISCUSSION

The empirical study of intraspecific variation is important for understanding the ecology and evolution of organisms, and for gaining insight into possible sources of variation. Our results do not support the metabolic cold adaptation hypothesis; instead, they reveal a gradual increment in metabolic rate towards populations at low latitudes, which is to be expected for ectotherms. This pattern is consistent for almost all experimental temperatures, with the exception of populations from Iquique and Antofagasta at 18 and 25°C; nevertheless, this result does not alter the general pattern observed, since these localities present
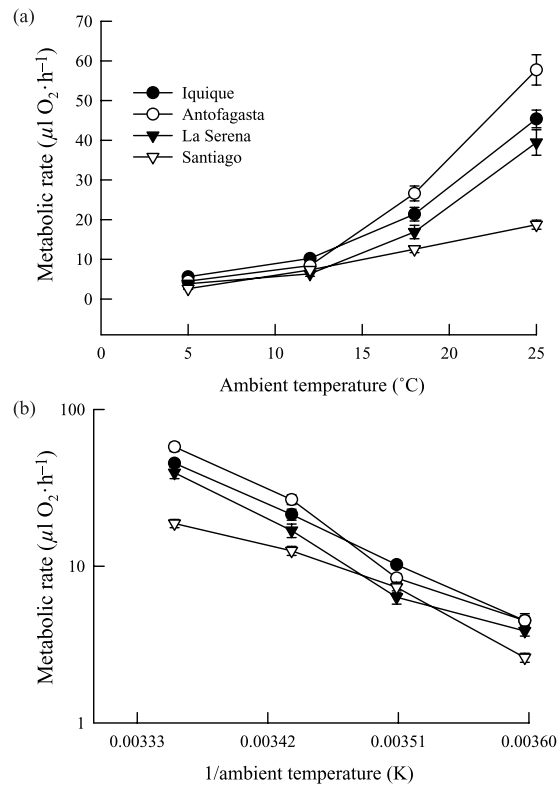
Fig. 1. (a) Metabolic rates of different populations of *Porcellio laevis* at different experimental temperatures. (b) Arrhenius plot of log metabolism versus 1/temperature (K) for different populations of *P. laevis* along a latitudinal gradient. Results are shown as the mean ± 1 standard deviation. The northernmost (warmest) population is Iquique (20°10′S), and the southernmost (coldest) population is Santiago (33°23′S).

very similar climates (see Table 1). In addition, in spite of the acclimation period, differences in metabolic rate between populations were observed, indicating a possible local adaptation and a genetic-heritable basis for this trait. Maternal and acclimation effects could also be operating. Unfortunately, we are unable to control for all the possible environmental signals that affect the variability in metabolic rate among populations.

We consider metabolic cold adaptation to be a component of the general term 'cold adaptation', which encompasses all those aspects of the physiology of an organism that allow it to inhabit polar regions (Clarke, 1991). Empirical support for the MCA hypothesis is controversial and puzzling and comes principally from meta-analyses of data sets of insects (see Addo-Bediako *et al.*, 2002). Despite the importance of meta-analyses, this approach does not account for possible confounding factors, such as life stage, activity, seasonality, feeding, anoxia, experimental temperature, acclimation, techniques, and interspecific comparisons without correction for phylogeny, among others. While meta-analysis is a valuable tool, according to Hodkinson (2003) it should not be used to extrapolate beyond the measured data range. Following Chown *et al.* (2003), large-scale studies are useful for understanding physiological variability, the factors affecting it and the

Table 2. Thermal sensitivity of the terrestrial isopod *Porcellio laevis*, from populations at different latitudes, measured at four different experimental temperatures

Ambient temperature (°C)	Temperature interval (°C)	Iquique (20°10′S)		Antofagasta (23°38′S)		La Serena (29°55′S)		Santiago (33°23′S)	
		Q_{10}	n	Q_{10}	n	Q_{10}	n	Q_{10}	n
5			15		14		8		20
12	5–12	2.353	15	2.449	18	2.034	8	2.573	19
18	12–18	3.411	17	4.812	15	5.106	17	2.445	28
25	18–25	2.932	18	3.022	23	3.358	10	1.773	21

Note: Q_{10} = thermal sensitivity; n = number of individuals.

Table 3. Planned comparisons within each population

Population			SS	d.f.	MS	F	P
Iquique	5°C < 12°C	Temperature	0.493	1	0.493	709.016	<0.001
		Error	0.041	60	0.001		
	12°C < 18°C	Temperature	0.797	1	0.797	1145.104	<0.001
		Error	0.041	60	0.001		
	18°C < 25°C	Temperature	0.938	1	0.938	1348.367	<0.001
		Error	0.041	60	0.001		
Antofagasta	5°C < 12°C	Temperature	0.509	1	0.509	474.821	<0.001
		Error	0.066	62	0.001		
	12°C < 18°C	Temperature	1.950	1	1.950	1816.488	<0.001
		Error	0.066	62	0.001		
	18°C < 25°C	Temperature	0.781	1	0.781	727.301	<0.001
		Error	0.066	62	0.001		
La Serena	5°C < 12°C	Temperature	0.168	1	0.168	146.506	<0.001
		Error	0.043	38	0.001		
	12°C < 18°C	Temperature	0.852	1	0.852	742.131	<0.001
		Error	0.043	38	0.001		
	18°C < 25°C	Temperature	0.867114	1	0.867	755.092	<0.001
		Error	0.043637	38	0.001		
Santiago	5°C < 12°C	Temperature	1.887522	1	1.887	2204.475	<0.001
		Error	0.070210	82	0.001		
	12°C < 18°C	Temperature	0.597499	1	0.597	697.831	<0.001
		Error	0.070210	82	0.001		
	18°C < 25°C	Temperature	0.358943	1	0.358	419.217	<0.001
		Error	0.070210	82	0.001		

implication thereof for explaining macroecological patterns. We wish to stress that the issue is not which approach is better, but that they reveal rather different things, and what is revealed by one often cannot be revealed by the other.

Table 4. Comparisons along the latitudinal gradient

Experimental temperature		SS	d.f.	MS	*F*	*P*
5°C	Santiago–La Serena	0.158	1	0.158	169.702	<0.001
		0.217	233	0.001		
	La Serena–Antofagasta	0.011	1	0.011	12.729	<0.001
		0.217	233	0.001		
	Antofagasta–Iquique	0.039	1	0.039	41.845	<0.001
		0.217	233	0.001		
12°C	Santiago–La Serena	0.018	1	0.018	19.937	<0.001
		0.217	233	0.001		
	La Serena–Antofagasta	0.072	1	0.072	78.112	<0.001
		0.217	233	0.001		
	Antofagasta–Iquique	0.052	1	0.052	56.325	<0.001
		0.217	233	0.001		
18°C	Santiago–La Serena	0.117	1	0.117	126.013	<0.001
		0.217	233	0.001		
	La Serena–Antofagasta	0.297	1	0.297	319.542	<0.001
		0.217	233	0.001		
	Antofagasta–Iquique	0.070	1	0.070	75.327	<0.001
		0.217	233	0.001		
25°C	Santiago–La Serena	0.696	1	0.696	747.325	<0.001
		0.217	233	0.001		
	La Serena–Antofagasta	0.146	1	0.146	156.669	<0.001
		0.217	233	0.001		
	Antofagasta–Iquique	0.072	1	0.072	77.453	<0.001
		0.217	233	0.001		

Table 5. Summary of interpopulation comparisons within each acclimation temperature trial (H$_0$: MCA = Santiago > La Serena > Antofagasta > Iquique)

Temperature	Results	MCA hypothesis
5°C	Santiago < La Serena < Antofagasta < Iquique	Rejected
12°C	Santiago > La Serena < Antofagasta < Iquique	Uncertain
18°C	Santiago < La Serena < Antofagasta > Iquique	Rejected
25°C	Santiago < La Serena < Antofagasta > Iquique	Rejected

At the intraspecific level, decreases in metabolic rate have been reported for high latitude populations of vertebrates and invertebrate ectotherms (Chown and Gaston, 1999; Nielsen *et al.*, 1999; Angilletta, 2001; Peck, 2002). Here we demonstrate the opposite of the pattern predicted by the MCA hypothesis, along 13° latitude, making appropriate controls for confounding factors, such as seasonality, life stage, activity, diet, sex, experimental tech-

nique and temperature acclimation period. That is, metabolic rate increased towards low latitude within our range of experimental temperatures. Therefore, our results do not support the idea that an elevated metabolic rate is advantageous to woodlice occupying cooler habitats (see also Sømme *et al.*, 1989). Furthermore, at 5°C (i.e. the lowest experimental temperature) the predictions of the MCA hypothesis should be most apparent; however, at this temperature metabolic rate gradually increased towards low latitude populations. Our results confirm the obvious pattern found for all ectothermic organisms, in the sense that metabolic rate is strongly dependent on both temperature and body size (Clarke and Johnston, 1999).

The highest thermal sensitivities for the metabolic rate–ambient temperature curves were found in the mid-latitude populations (i.e. La Serena and Antofagasta), which does not support the idea that metabolic rate increases with a decrease in temperature (i.e. an increase in latitude). This contrasts with other studies of sub-Arctic invertebrates, which have shown that northern species are more sensitive than southern species (e.g. Nielsen *et al.*, 1999). The relationship between thermal sensitivity and latitude is important. It means that woodlice in Antofagasta and La Serena respond relatively more to increased temperature than other populations of the same species originating from lower and higher latitudes. We do not know the reason for this. One plausible explanation is that the higher thermal sensitivity in the temperature intervals of 12–18°C for mid-latitude populations is near their average environmental temperature in their habitat (see Table 1); consequently, these individuals are able to respond quickly to rising temperatures and are physiologically more active within this temperature interval. On the other hand, metabolic cold adaptation should be best expressed through the activation energy of metabolism, calculated from the gradient of the Arrhenius plot (see Fig. 1B) of log metabolism against 1/temperature (K) (Lee and Baust, 1982a). The more cold-adapted a species is, the lower the activation energy, and thermal sensitivities in excess of 3 will likely be observed throughout the lower end of the temperature range (Hodkinson, 2003). This predicted pattern is entirely contrary to our findings, where the population at the highest latitude (i.e. Santiago) had the lowest thermal sensitivities for almost all ambient temperatures. Consequently, we are able to reject the MCA hypothesis because it predicts a higher metabolic rate, and more thermal sensitivity, in populations inhabiting colder habitats.

Higher metabolic rates have been interpreted as advantageous in ectotherms, especially in insects inhabiting high latitude/altitude environments, because it enables them to metabolize foodstuffs more rapidly, to develop faster, and thus to complete their life cycle in a period of time compatible with a reduced growing season (Hadley and Massion, 1985; Chown and Gaston, 1999; Gotthard *et al.*, 2000). Nevertheless, for iteroparous organisms with a life cycle longer than one year and which are active throughout the year – as many terrestrial isopods are (Warburg, 1987) – higher metabolic rates are not an advantage, since individuals can grow until the next reproductive season and then are able to increase their reproductive success (i.e. by producing more offspring of better quality). In this sense, we agree with Clarke (1993), in that metabolism represents a cost to the organism, not a benefit, which therefore makes it an improbable selective benefit for maintaining or elevating respiration. This is because an elevated metabolic rate imposes a cost on growth and development, which is necessary for the completion of life cycles in relatively short and cold growing seasons in high latitude/altitude zones (Addo-Bediako *et al.*, 2002).

Based on our review of the literature and our own results, we believe that the MCA hypothesis is more applicable to semelparous, ectothermic organisms, with annual cycles,

which need to grow and develop faster to reach reproductive size in one season (Chown *et al.*, 1997; Chown and Gaston, 1999; Gotthard *et al.*, 2000; Addo-Bediako *et al.*, 2002). In this sense, we believe that metabolic cold adaptation is more likely to be found in species that show latitudinal compensation in growth (Levinton and Monahan, 1983), or the inverse Bergmann's Rule (Masaki, 1978). Given that resting metabolism is by far the largest component of the energy budget of animals, the energy lost to metabolism comprises over 80% of annual energy expenditure (e.g. Anderson and Karasov, 1988; Green and Ydenberg, 1994; Beaupre, 1996). Therefore, the MCA hypothesis is probably linked to the latitudinal compensation hypothesis, or the inverse Bergmann's Rule. That is, we suggest for future studies that likely candidates for metabolic cold adaptation are any organisms whose life-cycle is linked to season length and demonstrates a relationship between development time, body size and metabolism. In this case, we could establish a link between physiological and life-history differences (see Rickelfs and Wilkelski, 2002) in natural populations of a given species living along a thermal (e.g. latitudinal) gradient (Lardies, 2003). This link is logical since in any study of growth, body size and age of first reproduction, temperature may have to be considered as a primary influence on life history and physiological traits.

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