Long-term repeatability of body mass and body temperature (but not basal metabolism) in the free-ranging leaf-eared mouse

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ABSTRACT

Question: The value of a trait must be repeatable (i.e. consistent in each individual over time) for natural selection to have the power to mould it. How repeatable are physiological (energetic) traits in the field during a prolonged period?

Methods: During each of 17 months, I measured body mass, body temperature, and basal metabolic rate in adult males and in non-reproductive females of the leaf-eared mouse, *Phyllotis darwini* (Muridae) in the Mediterranean habitats of central Chile. I analysed the trait values obtained in the earliest and latest appearances of each individual in the data set.

Results: The absolute value of the difference between the two measurements (earliest and latest) of a trait did not depend on the time interval between them. Thus, one could assess repeatability by comparing the measurements of all individuals regardless of the size of that time interval. Both body mass and body temperature were repeatable. However, neither whole basal metabolic rate nor mass-independent basal metabolism was repeatable in this mouse.

Conclusions: Both body mass and body temperature may be able to respond to current selective forces, but basal metabolism may not be. That is, most species of endotherms do show repeatability in basal metabolism, but it is not safe to assume that all do.

Keywords: energetics, heritable variation, long-term field physiology, repeatability, small mammals.

INTRODUCTION

Energy metabolism is associated with the rate at which animals acquire and process energy to fuel their existence. Since metabolic rates and thermoregulatory capacities set the pace of life, the measurement and analysis of their variability has been, and continues to be, of vital importance to several contemporary evolutionary and ecological theories (Kooijman, 2000; Brown *et al.*, 2004). McNab (1992), Hulbert and Else (2004), and Speakman *et al.* (2004) provide an

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overview of the development of various metabolic measurements and describe how one such measurement came to be known as the basal metabolic rate (BMR). Originally defined as a way to index the minimum rate of energy use necessary to maintain homeostasis, basal metabolic rate is the most widely measured energetic parameters for endothermic vertebrates.

Identification of the selective factors that have led to diversification in metabolic rates has been a major goal of ecological and evolutionary physiology (Garland and Carter, 1994; McNab, 2002). A common approach is to correlate variation in metabolic rate with variation in various biotic or abiotic factors. In this vein, correlations are generally taken as evidence of physiological flexibility and metabolic adaptation in the genetic and evolutionary sense, although a variety of caveats apply. Indeed, a trait can be the target of natural selection only if it is reliable over time – that is, the trait must be repeatable (Hayes and O'Connor, 1999). In fact, evolutionary geneticists have demonstrated that repeatability is related to heritability in the sense that the former sets the upper limit of the latter (Falconer and Mackay, 1997). Hence, the demonstration of significant repeatability in a trait necessarily precludes any attempt to demonstrate its selective significance. Recently, Nespolo and Franco (in press) performed a broad meta-analysis to determine whether metabolic rate is significantly repeatable. Their results show that metabolic rate is significantly repeatable and the magnitude of its effect is not statically affected by factors such as species identity, metabolic measurements, type of estimator, time between measurements, and number of individuals tested. In addition, these authors reported that the magnitude of whole-animal metabolic rate fluctuates from 0.6 to 0.8. Interestingly, Russell and Chappell (2007) recently reported a lack of repeatability of basal metabolic rate in deer mice (*Peromyscus maniculatus*) and concluded that there is little potential for direct selection to drive BMR evolution in this species. It is important to note that practically all studies of repeatability of metabolic rates have been laboratory oriented (see Bech *et al.*, 1999, for an exception) and conducted over comparatively short time intervals. However, Szafranska *et al.* (in press) recently conducted the first long-term study of repeatability in free-living weasels (*Mustela nivalis*). These authors reported a significant repeatability of body mass and resting metabolic rate under natural conditions, supporting potentially heritable variation in these traits.

On the other hand, Goldstein and Pinshow (2006) reported that quantifications of organismal response to changing environments may provide a bridge between mechanistic physiology, ecology, and evolutionary biology. Although uncontrolled biotic and abiotic factors in natural environments may complicate our capacity to define accurate and causal relationships among variables, field approaches to physiological problems provide opportunities not available under laboratory conditions. While comparative physiology documents the range of physiological variation across a range of organisms, measures of integrated physiological traits in wild-caught animals could provide insight into physiological traits under high environmental variability that is not possible in a laboratory setting. Thus, in the present study I investigated the long-term (17 months) repeatability of body mass, basal metabolic rate, and body temperature of marked individuals in a free-ranging population of the leaf-eared mouse, *Phyllotis darwini* (see Methods). Body mass, basal metabolic rate, and body temperature were measured on freshly caught animals from the field since they experience natural seasonal variations in climatic factors, habitat productivity, and diet quality (F. Bozinovic, unpublished data).

MATERIAL AND METHODS

I used adult males and non-reproductive females of the leaf-eared mouse, *Phyllotis darwini* (Muridae). This species inhabits semi-arid and Mediterranean scrublands in central Chile, from 25° to 38°S and from sea level to 2000 m above sea level (Steppan, 1998). The vegetation at the study site is sclerophyllous, described physiognomically as evergreen scrub, and more specifically known as 'matorral'. The climate is Mediterranean, with an annual mean precipitation of 376 mm, 65% of which falls in winter (i.e. from June to August). Precipitation is minimal from December to March, accounting for only 3% of the yearly total. Temperatures are highest from December to March, corresponding to austral summer, and lowest from June to August, during austral winter. A combination of high temperatures and low precipitation from December to March characterizes the typical summer drought of this Mediterranean climate (Jaksic, 2001; www.bio.puc.cl/sca/).

All individuals were ear tagged and captured monthly, from July 2005 to November 2006, in Quebrada de la Plata, central Chile (33°31′S, 70°50′W, 500 m above sea level), using 100 clean Sherman live traps. I recaptured a total of 22 rodents. Animals were transported to the laboratory within the same day of capture and released 24 h later, after the measurement of body mass, basal metabolic rate and body temperature. Following capture, all animals were maintained in the laboratory for less than 24 h at ambient temperature and photoperiod and fed fresh apples. Basal metabolic rate was measured in the laboratory by recording oxygen consumption in a computerized (Datacan V) open-flow respirometry system (Sable Systems, Henderson, NV). Post-absorptive animals were maintained in plexiglass metabolic chambers (1000 ml), at an aural temperature of $30.0 \pm 0.5°C$ (Nespolo *et al.*, 2003b), which is within the thermoneutral zone of this species (Bozinovic and Rosenmann, 1988). The metabolic chamber received dried air at a rate of 800 ml·min^{-1}, from mass flow controllers (Sierra Instruments, Monterey, CA), which is enough to ensure adequate mixing in the chamber. Air passed through CO_2-absorbent granules of Baralyme and H_2O-absorbent granules of Drierite both before and after passing through the chamber, and was sampled every 5 s by an Applied Electrochemistry oxygen analyser (model S-3A/I, Ametek, Pittsburgh, PA). Oxygen consumption ($\dot{V}O_2$) was calculated using equation (4a) of Withers (1977). Since *P. darwini* is nocturnal, all metabolic trials were completed between 09.00 and 17.00 h, when individuals were resting. Body mass was measured before metabolic measurements using an electronic balance (± 0.1 g), and colonic body temperature was recorded at the end of each measurement using a Digi-Sense copper-constant thermocouple. Basal metabolic rate was estimated as the lowest steady-state period of 3 min duration, recorded during $\dot{V}O_2$ measurements (~2 h).

The statistical analyses were performed using the Statistica® (StatSoft, 2001) software package for Windows. Pearson's correlation coefficient (r_p) was used to assess the repeatability of measurement of body mass, basal metabolic rate, and body temperature using the two measurements separated by the greatest number of days (i.e. the two measurements of the same individual furthest apart in time). In some cases, I measured one individual during nine consecutive months; in other cases, and more commonly, during just two or three consecutive months.

To eliminate the effect of body mass on basal metabolic rate (Kleiber, 1961), I conducted the same analysis but using the residuals from the linear regression between log body mass and log basal metabolic rate. To test for the effect of time on the studied variables, I conducted a correlation of the absolute difference between the two measurements of body mass, basal

metabolic rate, and body temperature separated by the greatest number of days versus time (Szafranska et al., in press). Before the analyses, I tested assumptions of normality and constant variance.

RESULTS AND DISCUSSION

There were no significant correlations in the absolute value of the differences between the two measurements of body mass, basal metabolic rate, and body temperature separated by the greatest number of days (body mass: $r_p = 0.042$, $P = 0.853$; residuals of basal metabolic rate: $r_p = 0.106$, $P = 0.640$; body temperature: $r_p = 0.032$, $P = 0.888$). These statistical patterns indicate that changes in all measured variables are independent of time.

I observed a significant correlation between the two measurements separated by the greatest number of days for body mass ($r_p = 0.610$, $P = 0.0026$) and body temperature ($r_p = 0.432$, $P = 0.045$) (Fig. 1a, c). No significant repeatability was observed between basal metabolic rate at trial 1 and the two values separated by the greatest number of days ($r_p = -0.05$, $P = 0.810$; Fig. 1b). When the effect of body mass was removed from a linear regression between log body mass versus log basal metabolic rate, the residuals of basal metabolic rate were also not significantly repeatable ($r_p = -0.0991$, $P = 0.686$; Fig. 2).

Intraspecific analysis focuses on the analysis of the proximate factors responsible for physiological variability, as well as their underlying mechanisms and functional significance. In this sense, despite the considerable amount of research on this topic, many questions about how organisms adapt physiologically to different environments and the selective significance thereof remain unanswered. To answer such questions, further study of the repeatability of physiological traits is required. In fact, the use of repeatability and its magnitude allows one to determine a trait's capacity to respond to current natural selection. Thus, repeatability appears to be an important parameter in evolutionary physiological ecology (Garland and Carter, 1994). In this sense, physiological ecologists can study not only how animals work, but also how physiological systems might evolve (Rezende et al. 2005; Bozinovic and Gallardo, 2006).

On the other hand, since natural selection reduces variation, trait heritability (h^2) represents the extent to which a trait has been under selection in the past. Bacigalupe et al. (2004) and Nespolo et al. (2003a, 2005) examined the magnitude of h^2 for physiological as well as life-history traits in *P. darwini* under laboratory conditions. They reported non-significant h^2 values for basal metabolic rate, suggesting pure environmental determination of this trait (see Lacy and Lynch, 1979; Dohm et al., 2001). However, Sadowska et al. (2005) reported significant h^2 values for basal metabolic rate in the bank vole.

In this study, I used a 'natural experiment', in which animals must deal with natural seasonal changes in biotic and abiotic environmental conditions. I found long-term consistency in two phenotypic trials, body mass and body temperature, of the leaf-eared mouse in the field. This observation suggests a significant level of heritable variation. In other words, the significant repeatability of both body mass and body temperature suggests that direct selection could produce micro-evolutionary changes in both variables. These data are consistent with previous reports of h^2 values in this species (Bacigalupe et al., 2004; Nespolo et al., 2003a, 2005).

Furthermore, I observed no significant long-term repeatability of basal metabolic rate. This observation is consistent with the non-significant h^2 values reported previously for the same species and in the same habitat (Bacigalupe et al., 2004; Nespolo et al., 2003a, 2005), and with a
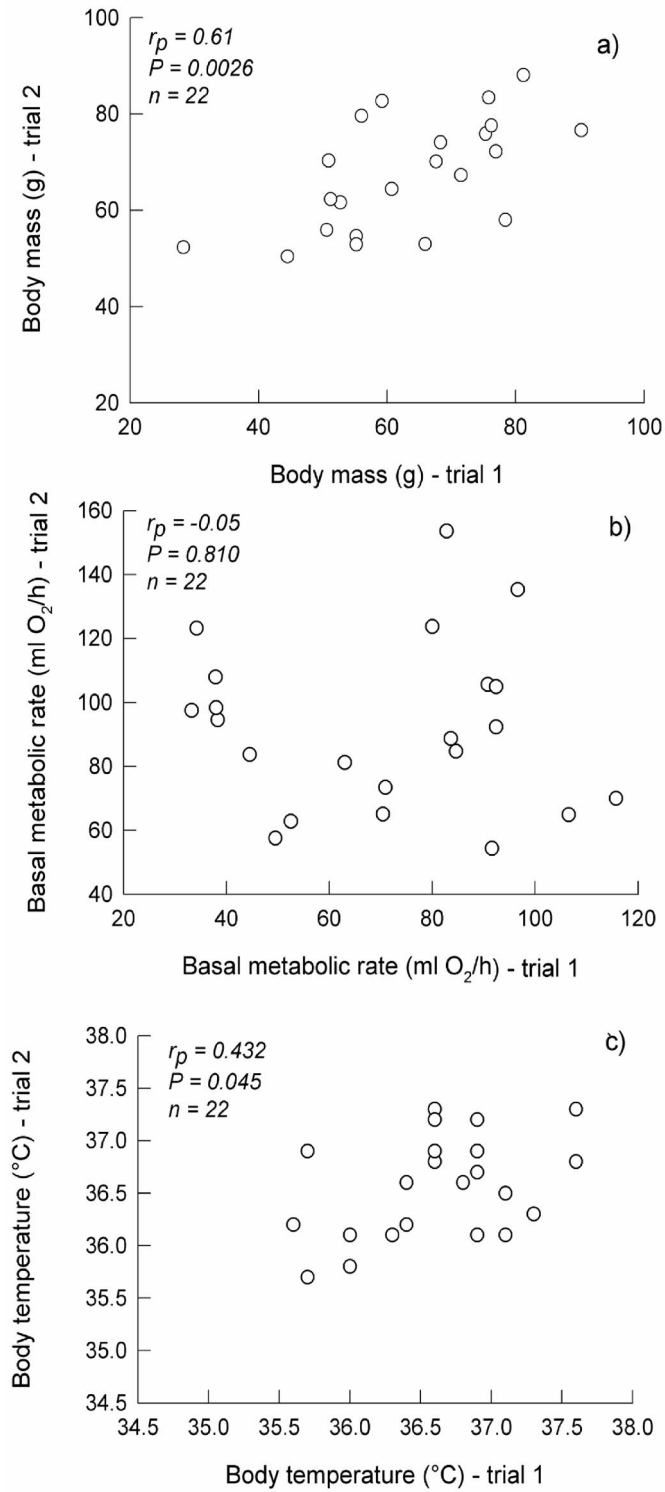
Fig. 1. Correlations between the two measurements separated by the greatest number of days for: (a) body mass, (b) basal metabolic rate, and (c) body temperature. Each data point represents a different individual.
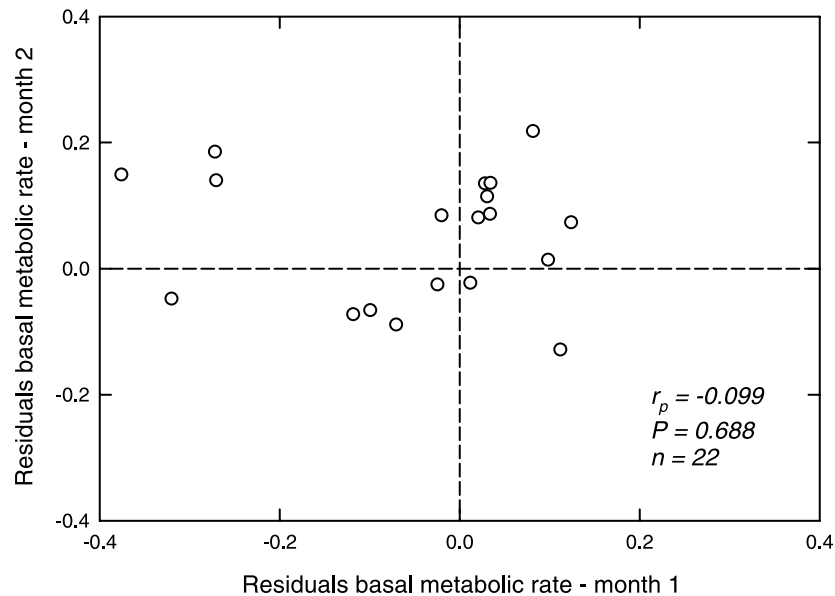
Fig. 2. Correlation between the residuals from a linear regression between log body mass and log basal metabolic rate. Residuals of basal metabolism were not significantly repeatable. Each data point represents a different individual.

previous report of deer mice inhabiting the white mountains of eastern California (Russell and Chappell, 2007). Lack of repeatability makes it unlikely that direct selection could generate micro-evolutionary changes in this metabolic trait. However, as mentioned earlier, Szafranska *et al.* (in press) documented significant long-term repeatability of body mass and basal metabolic rate for *Mustela nivalis*. As expected, this result allows the opposite conclusion – basal metabolic rate is likely to respond to selection.

Based only on these two long-term studies, it would appear that most species of endotherms do show repeatability of basal metabolic rate, but it is not safe to assume that all do. What appears to be important is that long-term studies of repeatability of physiological traits in the field may provide specific insights into the evolution of metabolic capacities.

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