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Is there a trade-off between energetics and spleen mass? A quantitative genetic study in the leaf-eared mouse

Leonardo D. Bacigalupe,[1]* Roberto F. Nespolo,[2] Diego M. Bustamante[1] and Francisco Bozinovic[1]

[1]*Center for Advanced Studies in Ecology & Biodiversity and Departamento de Ecología, Facultad de Ciencias Biológicas, Pontificia Universidad Católica de Chile, Santiago 6513677 and* [2]*Instituto de Ecología y Evolución, Universidad Austral de Chile, Casilla 567, Valdivia, Chile*

ABSTRACT

Question: Is there a microevolutionary trade-off between energetics and spleen mass?

Hypothesis: A negative genetic correlation between energetics and spleen mass would indicate a trade-off.

Methods: We carried out a quantitative genetic study in cold-acclimated individuals of the leaf-eared mouse, *Phyllotis darwini*.

Conclusions: Our results (1) do not support a microevolutionary trade-off between spleen mass and energetic traits, and (2) do not provide evidence that spleen mass can respond to current natural selection

Keywords: energetics, heritability, spleen, trade-off.

INTRODUCTION

During the last decade, the immune system has been the focus of intense research within the life-history theory framework, given that it competes for the limiting resources of an organism (Lochmiller and Deerenberg, 2000). In this context, much evidence has been accumulated regarding trade-offs with life-history traits (e.g. Nordling *et al.*, 1998; Ilmonen *et al.*, 2000; Soler *et al.*, 2003; Ardia *et al.*, 2003) as well as between other traits (e.g. Kraaijeveld *et al.*, 2001; Mallon *et al.*, 2003).

There has also been increased interest in understanding the immune response of endotherms under their most important energetic cost: the maintenance of a constant and high body temperature (Dabbert *et al.*, 1997; Svensson *et al.*, 1998; Ots *et al.*, 2001; Cichon *et al.*, 2002; Raberg *et al.*, 2002; Martin *et al.*, 2003). This is not only because thermoregulation requires a constant input of energy, but also because it is the principal demand that must be met by any mammal or bird

* Address all correspondence to Leonardo D. Bacigalupe, Department of Animal and Plant Sciences, University of Sheffield, Sheffield S10 2TN, UK. e-mail: l.bacigalupe@sheffield.ac.uk

(McNab, 2002). However, most studies of this trade-off have utilized a strictly physiological approach. In other words, the trade-off between the immune response and thermoregulation is caused by differential allocation of limited energy resources within a single individual. Alternatively, as far as we know, there is little evidence about such a physiological trade-off underlying a microevolutionary one (Stearns *et al.*, 1991; Stearns, 1992). A microevolutionary trade-off occurs when a population exhibits a negative genetic correlation between traits. Therefore, if natural selection induces a change in one trait that increases fitness, there would be a correlated response in another trait that decreases fitness (Stearns, 1992). In this study, we tested for a genetic correlation between non-shivering thermogenesis, maximum metabolic rate, sustained metabolic rate and spleen mass (as an index of immune machinery, see below) in cold-acclimated individuals of the leaf-eared mouse, *Phyllotis darwini*. We selected these physiological traits because they reflect maximum thermogenic capacities and, therefore, are paramount to maintaining body temperature in cold seasonal environments when evasive strategies (e.g. hibernation, torpor) are not present. Maximum metabolic rate is the maximum capacity for heat production induced by cold exposure (Rosenmann and Morrison, 1974); non-shivering thermogenesis is the maximum capacity for heat production in the brown adipose tissue (Jansky, 1973); and sustained metabolic rate is the maximum rate of energy expenditure fuelled by concurrent energy intake over long periods of cold exposure (Bacigalupe and Bozinovic, 2002). In addition, we measured basal metabolic rate, which is the minimum energy requirement of an endotherm and one of the most studied traits in comparative physiology (McNab, 2002).

It is important to note that our approximation has two important limitations. First, we have only studied one specific part of the immune system machinery (i.e. the spleen). Second, by using spleen mass as an index of immune response, we assume that spleen mass indicates its strength as an immune organ. The mammal spleen is involved in different immune activities: (1) maturation of lymphocytes; (2) identification and filtration of antigens, immune complexes and parasitized blood cells; (3) antibody synthesis; and (4) development of macrophages (John, 1994; Roitt *et al.*, 1998). Several studies have shown that spleen mass is larger in immunized animals (e.g. Ksiazek *et al.*, 2003) or is positively associated with antibody production (e.g. Cichon *et al.*, 2002).

In addition, some comparative studies have shown a positive relationship between spleen size and the extent of parasitism (e.g. Moller *et al.*, 1998; Morand and Poulin, 2000; Nunn, 2002). It is unclear whether larger spleens reflect greater evolutionary investment in the immunological response, or simply reflect a proximate response to current levels of parasites among other possible causes (e.g. season, sex) (Smith and Hunt, 2004).

We believe that our experimental design allowed us to overcome at least some of these possible confounding effects. First, the spleen was measured in animals born in the laboratory; therefore, they were not exposed to their natural parasites (with the exception of those parasites that could be passed through the mother). Second, the chance of being parasitized in the acclimation chamber was nil. Third, all animals experienced exactly the same conditions of temperature and photoperiod and were measured around the same age. Fourth, estimates of spleen variance and covariances with energetic traits were corrected by sex and carcass mass (i.e. mass of the remaining animal without organs). Given these controls, together with the empirical intraspecific evidence (Cichon *et al.*, 2002; Ksiazek *et al.*, 2003), we consider that the remaining 'residual' variation in spleen size should be representative of immunocompetence.

MATERIALS AND METHODS

The leaf-eared mouse *P. darwini* (Rodentia: Sigmodontidae) inhabits semi-arid and Mediterranean scrublands in central Chile, from 25° to 38°S and from sea level to 2000 m above sea level. Seventy-seven adult females and 26 adult males were captured during the austral autumn (April 2001) using 200 Sherman live traps in central Chile (33°31′S, 70°50′W, 500 m above sea level). All individuals were transported to the laboratory on the same day of capture. A detailed description of lab maintenance, breeding procedures, physiological measurements and heritability estimation are available in Bacigalupe *et al.* (2004) and Nespolo *et al.* (2003).

Spleen morphometrics

Following the physiological measurements, mice were sacrificed and dissected. The spleen and remaining carcass were dried to constant mass in an oven at 60°C for at least 7 days, and then weighed in an analytical electronic balance (Chyo JK-180) to within ± 0.0001 g.

RESULTS

Descriptive data and heritability estimates for energetic traits can be found elsewhere (Nespolo *et al.*, 2003; Bacigalupe *et al.*, 2004). Mean spleen mass was 0.011 ± 0.004 g (range 0.004 to 0.031). Estimates of additive genetic variance (V_A), post-natal common environmental variance (V_C) and specific environmental variance (V_E) for spleen mass are presented in Table 1. The contribution of V_A was not significant in a likelihood ratio test (CE versus ACE, $\chi^2 = 0$, $P > 0.05$), while the contribution of V_C was significant (AE versus ACE, $\chi^2 = 3.09$, $P < 0.05$). Results for bivariate analyses are shown in Table 2. In brief, no genetic covariance was statistically different from zero.

DISCUSSION

A microevolutionary trade-off is defined in terms of a population's evolutionary response to natural or sexual selection (Stearns, 1992). Such a response is contingent upon the genetic architecture underlying the traits: a negative genetic correlation among traits results in a trade-off. In such a case, if selection changes one trait that increases fitness, there will be a correlated evolutionary response in another trait that decreases it (Roff, 1997). Our results show that all genetic covariances between energetic traits and spleen mass were indistinguishable from zero. Given that it is not feasible that all loci for a trait are completely fixed [i.e. $V_A \equiv 0$ (Stearns, 1992)], our estimates of additive genetic variance and covariances were possibly too low to be detected by our analysis. This could represent a problem with the power of our design. Indeed, our breeding design does not allow us to detect narrow-sense heritability (h^2) below about 0.2 (Lynch and Walsh, 1998). In this sense, we are cautious in stating that no microevolutionary trade-off exists between spleen mass and energetic traits in this species. However, we can assert that the magnitude of the correlated evolutionary response between spleen mass and energetic traits must be small.

In addition, our V_A estimates of spleen mass were not different from zero. In contrast with our results, other studies have found evidence of underlying V_A for spleen mass in mammals (see Rocha *et al.*, 2004, and references therein) and birds (Rance *et al.*, 2002). Our results do not

Table 1. Estimates of variance components for ACE, AE and CE models

	ACE	CE	AE
V_A	—	—	0.0543
h^2	—	—	0.41 (0.30)
V_C	**0.0273**	0.0273	—
c^2	**0.21 (0.13)**	0.21 (0.13)	—
V_E	0.1034	0.1034	0.0791
e^2	0.79 (0.13)	0.79 (0.13)	0.59 (0.21)
V_P	0.1307	0.1307	0.1334
−2LL	−275.379	−275.379	−272.288

Note: V_A is additive genetic variance; h^2 is narrow-sense heritability (V_A/V_P); V_C is post-natal common environmental variance; c^2 is the post-natal component (V_C/V_P); V_E is the specific environmental variance; and e^2 is the residual component (V_E/V_P). Significant estimators are denoted in **bold** face, after a likelihood-ratio test between the complete model ACE and the reduced models AE and CE (see Methods for details). Parentheses denote standard errors and the symbol '–' indicates that estimates were smaller than 0.00001.

agree with the assumed pattern of h^2 among trait categories (Roff, 1997). In particular, morphological traits, such as spleen mass, are expected to show high values of h^2 because direct natural selection on these traits is usually weak, and thus V_A should not have been eroded (Roff, 1997). Because h^2 is the ratio between V_A and V_P, high values of V_E and V_C (both of which are included in V_P) could be the reason behind the low h^2 estimates. As can be seen in Table 1, spleen mass presented high levels of both V_E (79%) and V_C (21%). Mothers are known to transfer different immune defence agents to their offspring, providing them with their first protection against local parasites (e.g. Rossiter, 1996; Gasparini *et al.*, 2001, 2002). However, the evolutionary consequences of such maternal transference have only just begun to be understood (Cheverud and Moore, 1994; Mousseau and Fox, 1998; Wolf *et al.*, 1998). In our breeding design, such maternal influences are included in V_C, which also includes dominance variance and other litter variance (Lynch and Walsh, 1998), thus clouding its evolutionary relevance.

As previously stated, the general approach to studying the trade-off between immune response and thermoregulation has been from a physiological perspective, meaning that an observed trade-off would be caused by differential allocation of limited resources to both systems. Therefore, maintenance of body temperature, which is the principal activity that must be met by any endotherm, would constrain the energy available to mount an immune response. Evidence for such a trade-off is not clear. Studies in birds have suggested that such a trade-off may indeed exist (Dabbert *et al.*, 1997; Svensson *et al.*, 1998), and that immune activity increases energy expenditure (Ots *et al.*, 2001; Martin *et al.*, 2003), which could be considered indirect evidence of the trade-off. In addition, studies with mammals have suggested that mounting an immune response is costly (Cichon *et al.*, 2002; Derting and Crompton, 2003), although the results are not always straightforward (Ksiazek *et al.*, 2003) In short, evidence is accumulating to suggest that physiological trade-offs exist. However, evidence that a physiological trade-off underlies a microevolutionary one is still lacking, and unfortunately our results are not straightforward either.

Table 2. Estimates of additive genetic (cov_A) and post-natal common environmental (cov_C) covariances between spleen mass and energetic traits

	MMR_{MAX}	MMR_{MEAN}	MMR_{ST}	BMR	NST_{MAX}	NST_C	SusMR
cov_A	0.893 (1.0)	0.271 (0.87)	0.611 (0.96)	−0.78 (−0.99)	−0.01 (−0.01)	1.341 (1.00)	−0.375 (−1.0)
cov_C	−0.005 (−0.97)	−0.251 (−0.88)	0.165 (1.0)	−0.133 (−1.0)	−0.908 (−0.17)	−1.440 (−0.25)	0.776 (0.56)
−2 LL ACE	2313.830	1815.576	2130.776	1202.530	1987.546	1978.445	1346.444
−2 LL CE	2313.832	1815.644	2130.870	1202.598	1987.546	1978.568	1346.616
−2 LL AE	2313.928	1815.669	2130.787	1203.109	1988.134	1979.295	1348.565

Note: MMR_{MAX}, MMR_{MEAN} and MMR_{ST} are the maximum single-sample, average and steady-state (12 min) metabolic rates, respectively. BMR is the lowest steady-state metabolic rate over 3 min. NST_{MAX} is the highest steady-state metabolic rate over 3 min after norepinephrine injection, and $NST_C = NST_{MAX} - BMR$. Correlations are denoted in parentheses. There were no significant estimators after a likelihood-ratio test between the complete model ACE and the reduced models AE and CE.

In general, our results (1) show no evidence of a microevolutionary trade-off between spleen mass and energetic traits, and (2) show no evidence that spleen mass is heritable and thus could respond to current natural selection.

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