J Comp Physiol B (2010) 180:293–299
DOI 10.1007/s00360-009-0410-0

ORIGINAL PAPER

Phenotypic integration of morphology and energetic performance under routine capacities: a study in the leaf-eared mouse *Phyllotis darwini*

Leonardo D. Bacigalupe · Diego M. Bustamante · Francisco Bozinovic · Roberto F. Nespolo

Received: 15 July 2009 / Revised: 2 September 2009 / Accepted: 10 September 2009 / Published online: 10 October 2009

Abstract A major goal of evolutionary physiology is to understand the intrinsic and the extrinsic factors that impose limitations on an animal's energy budget. Although natural selection acts upon organismal traits such as performance (e.g., burst, sustained metabolic rates), from a mechanistic perspective, organismal performance results from the integrated functioning of different levels of biological organization. Hence, a better understanding of whole-animal performance must necessarily incorporate an explicit analysis of the integration between those different levels. Although this topic has been under intense scrutiny, overall there have been very few consistent patterns. Here, we explore the phenotypic integration between organ masses and the overall energy budget under routine capacities by statistically decomposing the covariance matrix (using path analysis and canonical correlation analysis) between organ masses and thermoregulatory burst and sustained metabolisms in cold acclimated individuals of *Phyllotis darwini*. Our results suggest that (a) central organs associated with the processing of food (cecum and liver), residuals (kidneys) and pumping of O_2 (heart) are tightly integrated to sustained expenditure and between themselves; (b) with the exception of the heart, central energy supplying organs are weakly related to burst expenditures; (c) sustained and burst metabolisms refer to complete different strategies and (d) basal metabolic rate is not related to any of the physiological or morphological traits considered in this study. Overall, our results support the hypothesis of an economic phenotype: animals maintain their excess capacities to face those critical extreme events, but their physiology and internal morphology are tightly integrated to function under routine needs.

Keywords Sustained metabolic rate · Basal metabolic rate · Maximum metabolic rate · Organ masses · Path analysis · Canonical correlation

Introduction

It is well known the negative relationship that exists between the rate of expenditure and the duration of activity (Peterson et al. 1990; Speakman 2000). This means that burst metabolic rates of activity or thermoregulation performed during short periods of time (i.e., minutes, hours) cannot be sustained indefinitely because organisms are not in energy balance during the exertion (Hammond and Diamond 1997). In fact, an important part of the expenditure is fueled by body reserves, which are depleted while activity is maintained. On the other hand, during longer periods of activity (i.e., days or weeks), energy expenditure must be fueled by concurrent energy intake, which is known as the sustained metabolic rate (SusMR) if body mass is not decreasing (Hammond and Diamond 1997). Understanding which factors impose ceilings on burst or SusMR is critical because ceilings on energy expenditure represent the upper limit below which all energy-consuming activities performed by an individual must engage (Bacigalupe and Bozinovic 2002). For example, in small

Communicated by I. D. Hume.

L. D. Bacigalupe (✉) · R. F. Nespolo
Facultad de Ciencias, Instituto de Ecología y Evolución, Universidad Austral de Chile, Casilla 567, Valdivia, Chile
e-mail: lbacigal@gmail.com

D. M. Bustamante · F. Bozinovic
Center for Advanced Studies in Ecology and Biodiversity, Pontificia Universidad Católica de Chile, Santiago CP 6513677, Chile

mammals, it is well known that asymptotic ceilings on SusMR could limit individual reproductive effort (since offspring number and quality depends on milk production; Knight et al. 1986; Rogowitz and McClure 1995; Rogowitz 1996, 1998), activity (e.g., foraging, escape from predators), thermoregulatory capabilities and survival to long-term cold exposures (Konarzweski and Diamond 1994; McDevitt and Speakman 1994).

Although natural selection acts upon organismal traits such as performance (e.g., burst, SusMR), from a mechanistic perspective, it is clear that overall organismal performance results from the integrated functioning of different levels of biological organization. Hence, a better understanding of whole-animal performance must necessarily incorporate an explicit analysis of the integration between those different levels of organization (e.g., Chappell et al. 1999, 2007; Hammond et al. 2000, 2001). Although this topic has been under intense scrutiny in the past decades, overall there have been very few consistent patterns (e.g., Chappell et al. 2007). A potential reason for this may be that the research in this area has been largely biased to an experimentalist tradition. Either in field or in laboratory animals, physiological capacities have been submitted to a manipulative treatment (e.g., thermal and dietary acclimation, lactation challenges, artificial selection, see Speakman and Krol 2005) that ultimately leads to a mechanistic view of the physiological process being studied. On the other hand, one of the simplest approaches to explore the underlying (i.e., biological) associations among variables is to measure the different traits of interest (e.g., biochemical, morphological and physiological) in the same sampling units (i.e., individuals) and to explore the resulting variance–covariance structure (Quinn and Keough 2002). Here, we addressed this issue in two forms: first, by statistically decomposing the covariance matrix (in the form of a canonical correlation) between organ masses and burst and sustained metabolisms; second, by summarizing the main patterns of phenotypic integration between physiology and morphology using a structural equation modeling approach. Our main goal was to explore the phenotypic integration between organ masses and the overall energy budget under routine capacities. That is, our experimental protocol did not push the animals to their maximum physiological limit, as we were interested in understanding how physiology and morphology are interrelated in the daily experience of our study model in its habitat (see below).

We characterized endothermal energy expenditure by measuring a suite of standard energetic variables derived from thermoregulatory physiology, which represent the ceiling of burst (thermoregulatory maximum metabolic rate, MMR), and sustained capacity for aerobic work (SusMR) and the minimum cost of existence (basal metabolic rate, BMR). Furthermore, we included total turnover time (TT), a digesta-processing variable that is known to change in response to ambient temperature in our study model (Naya et al. 2005). We chose *Phyllotis darwini* (Rodentia: Sigmodontidae) given that it is a strictly nocturnal and non-hibernating species, which faces seasonal and daily exposures to temperatures below thermoneutrality (Rezende and Bozinovic 2001; Bacigalupe et al. 2004) and thus, mechanisms that control its thermoregulatory homeostasis are of paramount importance (Nespolo et al. 2003). In addition, it is well known that acclimatization to cold in small mammals involves the hypertrophy of central energy supplying organs (i.e., liver, kidneys, heart, and gut) associated with the increase in food consumption (Bacigalupe and Bozinovic 2002 and references therein). Accordingly, we included the masses of such organs as an underlying factor that could have a mechanistic link with whole-animal performance traits.

Materials and methods

Animals

One hundred and eighty-three newborn individuals of *P. darwini* were obtained from a captive population we had in the lab. Until weaning (day 15), all siblings were maintained with their mothers at 26 ± 2°C, photoperiod 16L:8D and water ad libitum. After weaning, siblings were kept together under the previously described laboratory conditions until day 30 (about 1 month). After this time, animals were isolated individually and acclimated to 12 ± 2°C for at least 60 days in an environmental chamber prior to metabolic and morphological measurements were performed. Animals were provided with food (rat food pellets) and water ad libitum.

Basal metabolic rate

Upon completion of thermal acclimation, and prior to measurements of BMR, animals were fasted for 12 h. Oxygen consumption (VO_2) was measured during 1.5 h in a computerized (Datacan V) open flow respirometry system (Sable Systems, Henderson, NV). Animals were kept in plexiglass metabolic chambers of 1,000 ml, at 30.0 ± 0.5°C (T_a), which is within the thermoneutral zone for this species (non-acclimated individuals; Bozinovic et al. 1988). The metabolic chamber received dried air at a rate of 505 ml min^{-1}, from mass flow controllers (Sierra Instruments, Monterey, CA), which was enough to ensure adequate mixing in the chamber (Nespolo et al. 2003). Air passed through CO_2-absorbent granules of Baralyme and Drierite both before and after passing through the chamber,

and was sampled every 5 s by an Applied Electrochemistry O_2-analyzer, model S-3A/I (Ametek, Pittsburgh, PA). Oxygen consumption values were calculated using Eq. 4a of Withers (1977). Since *P. darwini* is nocturnal, all metabolic trials were completed between 0800 and 1600 hours, when individuals were resting. Body mass was measured prior to metabolic measurements using an electronic balance (±0.1 g), and rectal body temperature (T_b) was recorded at the end of each measurement, using a Digi-Sense copper-constant thermocouple. BMR was estimated as the lowest steady-state period of 3 min. Measurements of BMR in this species indicate that VO_2 reached a steady state after 15–20 min, without changes above 15% of VO_2 in the following 4 h (Nespolo et al. 2003).

Maximum metabolic rate

We measured thermoregulatory MMR in a He–O_2 atmosphere, according to the procedure outlined by Rosenmann and Morrison (1974), but using an open circuit respirometer, as described by Chappell and Bachman (1995). In brief, a mixture of He (80%) and O_2 (20%) was passed through a volumetric flowmeter before entering the chamber (i.e., a positive pressure system). Flow was maintained at 1,002 ± 3 ml min^{-1} to prevent partial oxygen pressure from falling below 150 Torr, a value far above those considered hypoxic (Rosenmann and Morrison 1975). The mixture passed through CO_2-absorbent granules of Baralyme and Drierite before and after passing through the chamber, which was tightly sealed with teflon and vaseline. We continuously measured chamber temperature (0.0 ± 0.5°C), and T_b was measured at the end of each trial. MMR records lasted approximately 20 min. We computed MMR as the mean of maximum steady-state VO_2 that was sustained for at least 12 continuous minutes of recording. Following MMR trials, all rodents showed signs of hypothermia, indicating that MMR was attained (Chappell and Bachman 1995).

SusMR and TT

Sustained expenditure was determined as energy intake (food intake in g day^{-1} × energy content of food in kJ g^{-1}) × digestive efficiency (Hammond and Diamond 1997; Speakman 2000). As a measure of digestive efficiency, we used apparent dry matter digestibility, calculated as food intake minus fecal output, divided by food intake. Digestibility is apparent because fecal contributions of endogenous protein and gut microflora were not considered. Food intake and fecal output were controlled for 3 days by placing each animal individually in a cage with an elevated wire bottom. Feces and remaining food dropped to the cage bottom and were dried at 60°C for at least 96 h to constant mass, and then separated from each other. The difference between dry food offered and remnant is food intake. Food dry matter content was ≈90%, and energy content was 18.4 kJ g^{-1} (Veloso and Bozinovic 2000). Total TT was calculated as the ratio between the total amount of digesta (i.e., material rinsed out of each organ and integrated over the entire gut) and food intake over 24 h (Naya et al. 2005).

Organ morphometrics

Following physiological measurements, mice were killed and the gut (small intestine, cecum, and large intestine), heart, kidneys, and liver were removed and weighed (i.e., fresh masses) in an analytical electronic balance (Chyo JK-180, 0.0001 g). Any residual digesta contained in the small intestine, cecum, and large intestine was removed before weighing as well as any adherent fat. All organs and the remaining carcass (i.e., dry body mass) were dried to constant mass in an oven at 60°C for at least 7 days, and then weighed in an analytical electronic balance.

Statistics I: structural equation modeling

We investigated the main patterns of phenotypic integration between physiology and morphology using path analysis (Shipley 2000). In order to reduce collinearity among individual points (i.e., each offspring), analyses were based on mother offspring means and included mothers with a single offspring ($N = 5$). Variables were $\log_{10}$ transformed when necessary to meet normality assumptions. Because of scaling effects, we used residuals between body mass and performance traits and between dry body mass and dry organ masses in subsequent analyses. Given the many possible paths (i.e., 36) that might exist among our 10 variables and our relatively low sample size (i.e., 54 mothers) (Shipley 2000), we built an appropriate path model based on the statistical significance of pairwise Pearson correlations. BMR was the only variable left out of the evaluated model, as it did not correlate with any of the morphological or physiological variables (Fig. 1). The fit of the model to the observed variance–covariance matrix was assessed from its departure from the expected variance–covariance matrix by means of a Chi-squared (χ^2) statistic (Shipley 2000). Path coefficients and standard errors were computed using maximum likelihood. All analyses were implemented in R (2009) using package sem (Fox 2009).

Statistics II: canonical correlation analysis

We performed a canonical correlation analysis to explore the correlation between physiological and morphological

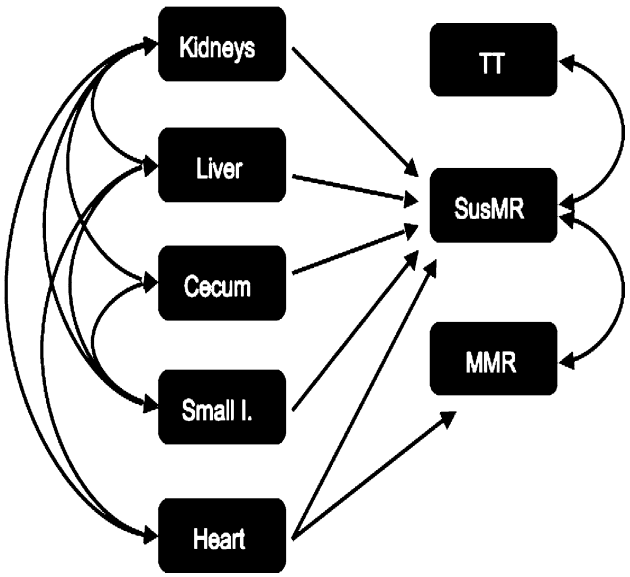

Fig. 1 Path diagram suggested by correlational analysis (see text for details). *SusMR* sustained metabolic rate, *MMR* maximum metabolic rate, *TT* turnover time

Table 1 Standardized parameter estimates and standard errors for the paths depicted in Fig. 1

Path	Estimate	SE	z	P
Cecum → SusMR	0.333	0.112	2.978	0.003
Kidneys → SusMR	−0.236	0.112	−2.102	0.036
Liver → SusMR	0.277	0.109	2.545	0.011
Heart → SusMR	0.206	0.109	1.896	0.058
Small I. → SusMR	0.125	0.112	1.112	0.266
Heart → MMR	−0.266	0.132	−2.008	0.045
Kidneys ↔ Liver	0.422	0.143	2.949	0.003
Kidneys ↔ Heart	0.346	0.139	2.500	0.012
Kidneys ↔ Small I.	0.303	0.128	2.360	0.018
Kidneys ↔ Cecum	0.212	0.124	1.711	0.087
Heart ↔ Liver	0.298	0.137	2.179	0.029
Small I. ↔ Liver	0.285	0.122	2.347	0.019
Small I. ↔ Cecum	0.473	0.146	3.242	0.001
SusMR ↔ MMR	−0.268	0.104	−2.588	0.010
SusMR ↔ TT	−0.517	0.140	−3.700	<0.001

SusMR sustained metabolic rate, *MMR* maximum metabolic rate, *TT* turnover time. → represents a unidirectional path, ↔ represents a bidirectional path (i.e., a correlation)

variables. In brief, canonical analysis extracts linear combinations of variables (roots) from the two sets of variables so that the first component for one set has the maximum correlation with the first component from the second set (Quinn and Keough 2002). Several roots are extracted from the dataset in such a way that the first root is unrelated to the second root, the second is unrelated to the third and so on. The statistical significance of each root was evaluated with χ^2 tests of successive roots removed from the canonical correlation. The sign and magnitude of the correlation of each variable with the canonical root (i.e., canonical loadings) give a measure of the contribution of that variable to the overall canonical correlation. Canonical correlation analysis was implemented using package yacca (Butts 2009).

Results

The general path model (Fig. 1) was not different from the expected variance–covariance matrix ($\chi^2_{13} = 10.766$, $P = 0.630$). The standardized parameter estimates and their standard errors are presented in Table 1. With the exception of the path from small intestine to SusMR and the correlation between cecum and kidneys, all paths were statistically significant. The canonical correlation between physiological and morphological traits was significant ($r = 0.64$, $\chi^2_{20} = 32.67$, $P = 0.037$), with 100% of variance extracted in the physiological dataset and 84% in the morphological one. Redundancy, a measure of how redundant is one set of variables given the other set, was low (i.e., both sets of variables were informative to the overall canonical correlation): 11.3% for the physiological variables and 13.7% for the morphological ones. Only the first root was significant according to the χ^2 tests of successive roots removed (Root 1: $\chi^2_{20} = 32.67$, $P = 0.037$; Root 2: $\chi^2_{12} = 8.97$, $P = 0.706$; Root 3: $\chi^2_6 = 3.39$, $P = 0.759$; Root 4: $\chi^2_2 = 1.31$, $P = 0.519$). The canonical loadings suggest that the most determinant variables for the canonical correlation in the physiological set were SusMR and TT (Table 2). Among the morphological traits, the most important contributors to the canonical correlation were the liver, heart, small intestine, and cecum. Between types of traits, canonical loadings with the same sign suggest direct associations while different signs suggest opposite associations. An inspection of Table 2 suggests that liver, cecum, heart and small intestine are positively associated with SusMR and TT. Overall, both path and canonical correlation analyses provided similar results regarding the integration of morphological and physiological variables.

Discussion

The main goal of this study was to explore the phenotypic integration between whole-animal performance traits and the main central energy supplying organs under routine capacities. In order to achieve that, we explored the covariance matrix between organ masses and burst and sustained metabolisms in cold acclimated individuals of

Table 2 Canonical weights for the significant roots (see text for details)

Physiology	Root 1	Morphology	Root 1
BMR (ml O_2 h^{-1})	0.049	Kidneys (g)	−0.084
MMR (ml O_2 h^{-1})	0.100	Liver (g)	−0.522
SusMR (kJ day^{-1})	−0.645	Heart (g)	−0.338
TT (h)	−0.349	Small intestine (g)	−0.567
		Cecum (g)	−0.775

BMR basal metabolic rate, *SusMR* sustained metabolic rate, *MMR* maximum metabolic rate, *TT* turnover time

P. darwini. Overall, our results suggest that (a) central organs associated with the processing of food (cecum and liver), residuals (kidneys) and pumping of O_2 (heart) are tightly integrated to sustained expenditure and between themselves; (b) with the exception of the heart, central energy supplying organs are weakly related to burst expenditures; (c) sustained and burst metabolisms refer to complete different strategies and (d) BMR is not related to any of the physiological or morphological traits considered in this study.

From a mechanistic perspective, it is clear that organismal performance has to be the result of the integrated phenotypic functioning of different levels of biological organization (Weibel 2002). This unavoidable fact implies that any underlying structure/organ to performance can set the limit to it, even if performance is below its intrinsic physiological maximum (e.g., SusMR at 12°C is the *ceiling* food consumption at that temperature). Regarding both sustained and burst expenditures, during the 1980s and 1990s, experimental as well as correlational evidences suggested that both rates were centrally limited (i.e., regardless of the particular mode of energy expenditure, shared features of energy acquisition and utilization limit energy expenditure to a common value) (Drent and Daan 1980; Kirkwood 1983; Weiner 1989; Peterson et al. 1990). In addition, since 1960s, many studies have observed that digestive features (i.e., mainly gut morphology) do change in a seasonal basis, in parallel to temperature, diet quality, alimentary, and reproductive cycles (see Naya et al. 2007, 2008; Naya and Bacigalupe 2008). Together, all these evidences indicate that if there is a digestive limit to expenditure it is not rigid but rather flexible (Karasov and McWilliams 2005). Probably, the observed (body mass independent) association between resting and both sustained and burst expenditures have been the main idea behind the proposal that energy budgets are centrally limited. This suggestion stems from the fact that animals with higher expenditures should support their demand by increasing food consumption, which in turn produces an increase in the masses of the energy supplying organs. Given the high-specific metabolism of these organs and their direct contribution to the resting metabolic rate, then resting and both sustained and burst expenditures should increase jointly (Bacigalupe and Bozinovic 2002). Although the logic of this reasoning has been undermined (Bacigalupe and Bozinovic 2002), here we not only found that the contribution of BMR to the overall canonical correlation was extremely low, but also that BMR did not significantly correlate with SusMR, MMR or the central organs. Even though it is apparent that high-energy budgets depend on expensive metabolic machinery (i.e., central organs), the association between that machinery and resting metabolism is far from evident (for a review, see Bacigalupe and Bozinovic 2002; Chappell et al. 2007). A potential explanation for the lack of association between BMR and organs size in our study is that the processes associated to BMR (e.g., maintenance of cell polarity and protein synthesis, Weibel 2002) are simply not reflected in the weight of the organs. On the other hand, sustained expenditure showed a strong integration with the central energy supplying organs (i.e., liver, kidneys, heart, and cecum) involved in acquisition, processing and allocation of energy, resources and waste products associated with the increase in food consumption (Bacigalupe and Bozinovic 2002). This suggests that the masses of such organs can certainly be considered an underlying factor that has a mechanistic link with SusMR.

Our results suggest that sustained and burst expenditures clearly refer to different organismal processes, potentially involving different underlying mechanisms, as evidenced by the negative correlation in the path analysis and the opposite signs they showed in the first root of the canonical correlation. Sustained expenditure must be fueled by concurrent food consumption, and thus, it involves processing (i.e., digestion, absorption and transport) greater amounts of nutrients, O_2 and residuals. Probably at SusMR, O_2 is consumed in all cells involved in the different aspects of those processes. However, at burst expenditures (MMR), a great percentage of O_2 will be mainly consumed at the brown adipose tissue and muscular system to generate heat. In addition, we found that organs associated with the processing and transport of food (cecum and liver), residuals (kidneys) and O_2 (heart) were, as expected, positively integrated between them (Bacigalupe and Bozinovic 2002). However, unexpectedly, kidneys were negatively associated to SusMR and the small intestine presented a weak, but positive, association with SusMR. In addition, we were surprised to find a negative relationship between burst expenditure and the heart, since this is the central organ directly associated with the transport of O_2 to the tissues. Although we have no explanations for those unanticipated patterns, it seems difficult to make strong inferences regarding these correlations between performance traits and potential underlying organs. Because of practical

reasons, dry mass has been broadly used as a *proxy* for organ activity in most physiological-ecology type of studies. Therefore, the basic assumption has always been that bigger organ masses do imply more organ activity. However, it is likely that many other variables, not necessarily associated to organ weight, do play a crucial role as underlying factors for aerobic performance (e.g., enzyme activity, capillary density, mitochondrial density and efficiency, see Weibel 2000). Moreover, we would expect those more precise measurements of organ activity would provide not only a better adjustment to performance traits, but also a more realistic one. Although it may be possible that a stronger and clear integration between morphology and physiology would have been seen if animals were at their maximum physiological limit, our interest was to understand the physiology of *P. darwini* in relation to what it experiences in its habitat: 12°C (i.e., the acclimation temperature) is the annual mean temperature at the study site. Finally, it is known that *P. darwini* is able to meet moderate changes in ambient temperature modifying not only its food intake but also the processing capacities of digesta (e.g., TT, Naya et al. 2005). Therefore, the observed negative correlation between SusMR and TT (i.e., TT = 1/retention time) is in concordance with those findings: a higher SusMR is achieved by increasing the time food is withheld in the gut.

Some studies suggest that physiological capacities should be shaped by routine needs, while burst capacities should be shaped by those unusual events that are critical to survival (e.g., predation, thermoregulation; Hertz et al. 1988). Overall, our results support the hypothesis of an *economic phenotype*: animals maintain their excess capacities to face those critical extreme events, but their physiology and internal morphology are tightly integrated to function under routine needs.

Acknowledgments We would like to thank comments by two reviewers that greatly improved a previous version of the manuscript. This work was funded by grants Fondo Nacional de Desarrollo de Áreas Prioritarias 1501-0001 (Program 1) to FB and Fondo Nacional de Ciencia y Tecnología 2000002 to RFN. All experiments were conducted according to current Chilean law and under Servicio Agrícola Ganadero permit 698.

References

Bacigalupe LD, Bozinovic F (2002) Design, limitation and sustained metabolic rate: lessons from small mammals. J Exp Biol 205:2963–2970

Bacigalupe LD, Nespolo RF, Opazo JC, Bozinovic F (2004) Phenotypic flexibility in a novel thermal environment: phylogenetic inertia in thermogenic capacity and evolutionary adaptation in organ size. Physiol Biochem Zool 77:805–815

Bozinovic F, Rosenmann M, Veloso C (1988) Termorregulación conductual en *Phyllotis darwini* (Rodentia: Cricetidae): efecto de la temperatura ambiente, uso de nidos y agrupamiento social sobre el gasto de energía. Rev Chil Hist Nat 61:81–86

Butts CT (2009) yacca: yet another canonical correlation analysis package. R package version 1.1

Chappell MA, Bachman GC (1995) Aerobic performance in beldings ground-squirrels (*Spermophilus beldingi*): variance, ontogeny and the aerobic capacity model of endothermy. Physiol Zool 68:421–442

Chappell MA, Bech C, Buttemer WA (1999) The relationship of central and peripheral organ masses to aerobic performance variation in house sparrows. J Exp Biol 202:2269–2279

Chappell MA, Garland T Jr, Robertson GF, Saltzman W (2007) Relationships among running performance, aerobic physiology and organ mass in male Mongolian gerbils. J Exp Biol 210:4179–4197

Drent RH, Daan S (1980) The prudent parent: energetic adjustments in avian breeding. Ardea 68:225–253

Fox J (2009) sem: structural equation models. R package version 0.9-17. http://CRAN.R-project.org/package=sem

Hammond KA, Diamond JM (1997) Maximal sustained energy budgets in humans and animals. Nature 386:457–462

Hammond KA, Chappell MA, Cardullo RA, Lin RS, Johnsen TS (2000) The mechanistic basic of aerobic performance variation in red junglefowl. J Exp Biol 203:2053–2064

Hammond KA, Chappell MA, Cardullo RA, Lin RS, Johnsen TS (2001) Effects of altitude and temperature on organ phenotypic plasticity along an altitudinal gradient. J Exp Biol 204:1991–2000

Hertz PF, Huey RB, Garland T Jr (1988) Time budgets, thermoregulation, and maximal locomotor performance: are reptiles Olympians or Boy Scouts? Am Zool 28:927–938

Karasov WH, McWilliams SR (2005) Digestive constraints in mammalian and avian ecology. In: Starck JM, Wang T (eds) Physiological and ecological adaptations to feeding in vertebrates. Science Publishers, New Hampshire, pp 87–112

Kirkwood JK (1983) A limit to metabolisable energy intake in mammals and birds. Comp Biochem Physiol A 75:1–3

Knight CH, Maltz E, Docherty AH (1986) Milk yield and composition in mice: effects of litter size and lactation number. Comp Biochem Physiol A 84:127–133

Konarzweski M, Diamond JM (1994) Peak sustained metabolic rate and its individual variation in cold-stressed mice. Physiol Zool 67:1186–1212

McDevitt RM, Speakman JR (1994) Central limits to sustainable metabolic rate have no role in cold acclimation of the short-tailed field vole (*Microtus agrestis*). Physiol Zool 67:1117–1139

Naya DE, Bacigalupe LD (2008) Metabolic constraints to resource allocation. In: Rauw W (ed) Resource allocation theory applied to farm animals. CAB International Publishing, UK, pp 87–112

Naya DE, Bacigalupe LD, Bustamante DM, Bozinovic F (2005) Dynamic digestive responses to increased energy demands in the leaf-eared mouse (*Phyllotis darwini*). J Comp Physiol B 175:31–36

Naya DE, Karasov WH, Bozinovic F (2007) Phenotypic plasticity in laboratory mice and rats: a meta-analysis of current ideas on gut size flexibility. Evol Ecol Res 9:1363–1374

Naya DE, Bozinovic F, Karasov WH (2008) Latitudinal trends in digestive flexibility: testing the climatic variability hypothesis with data on the intestinal length in rodents. Am Nat 172:122–134

Nespolo RF, Bacigalupe LD, Bozinovic F (2003) On the heritability of ecophysiological type traits in endotherms: a study in leaf-eared mice (*Phyllotis darwini*). Evolution 57:1679–1688

Peterson CC, Nagy KA, Diamond JM (1990) Sustained metabolic scope. Proc Natl Acad Sci USA 87:2324–2328

Quinn GP, Keough MJ (2002) Experimental design and data analysis for biologists. Cambridge University Press, Cambridge

R Development Core Team (2009) R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0. http://www.R-project.org

Rezende EL, Bozinovic F (2001) Patterns of daily activity in the leaf-eared mouse (*Phyllotis darwini*): effects of food availability. J Arid Environ 47:95–100

Rogowitz GL (1996) Trade-offs in energy allocation during lactation. Am Zool 36:197–204

Rogowitz GL (1998) Limits to milk flow and energy allocation during lactation of the hispid cotton rat (*Sigmodon hispidus*). Physiol Zool 71:312–320

Rogowitz GL, McClure PA (1995) Energy export and offspring growth during lactation in cotton rats (*Sigmodon hispidus*). Funct Ecol 9:143–150

Rosenmann M, Morrison PR (1974) Maximum oxygen consumption and heat loss facilitation in small homeotherms by He–O_2. Am J Physiol 226:490–495

Rosenmann M, Morrison PR (1975) Metabolic response of highland and lowland rodents to simulated high altitudes and cold. Comp Biochem Physiol A 51:523–530

Shipley B (2000) Cause and correlation in biology: a user's guide to path analysis, structural equations and causal inference. Cambridge University Press, Cambridge

Speakman JR (2000) The cost of living: field metabolic rates of small mammals. Adv Ecol Res 30:177–297

Speakman JR, Krol E (2005) Limits to sustained energy intake IX: a review of hypotheses. J Comp Physiol B 175:375–394

Veloso C, Bozinovic F (2000) Effect of food quality on the energetics of reproduction in a precocial rodent, *Octodon degus*. J Mamm 81:971–978

Weibel ER (2000) Symmorphosis. On form and function in shaping life. Harvard University Press, Cambridge

Weibel ER (2002) The pitfalls of power laws. Nature 417:131–132

Weiner J (1989) Metabolic constraints to mammalian energy budgets. Acta Theriol 34:3–35

Withers PC (1977) Measurement of VO_2, VCO_2, and evaporative water loss with a flow-through mask. J Appl Physiol 42:120–123