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# Body size as a latent variable in a structural equation model: thermal acclimation and energetics of the leaf-eared mouse

Roberto F. Nespolo*, Matías Arim and Francisco Bozinovic

*Centro de estudios avanzados en Ecología y Biodiversidad, Departamento de Ecología, Facultad Ciencias Biológicas, Pontificia Universidad Católica de Chile, PO Box 6513677, Santiago, Chile*

*Author for correspondence at present address: Instituto de Ecología y Evolución, Universidad Austral de Chile, Casilla 567, Valdivia, Chile (e-mail: robertonespolo@uach.cl)

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**Summary**

**Body size is one of the most important determinants of energy metabolism in mammals. However, the usual physiological variables measured to characterize energy metabolism and heat dissipation in endotherms are strongly affected by thermal acclimation, and are also correlated among themselves. In addition to choosing the appropriate measurement of body size, these problems create additional complications when analyzing the relationships among physiological variables such as basal metabolism, non-shivering thermogenesis, thermoregulatory maximum metabolic rate and minimum thermal conductance, body size dependence, and the effect of thermal acclimation on them.**

**We measured these variables in *Phyllotis darwini*, a murid rodent from central Chile, under conditions of warm and cold acclimation. In addition to standard statistical analyses to determine the effect of thermal acclimation on each variable and the body-mass-controlled correlation among them, we performed a Structural Equation Modeling analysis to evaluate the effects of three different measurements of body size (body mass, $m_b$; body length, $L_b$ and foot length, $L_f$) on energy metabolism and thermal conductance. We found that thermal acclimation changed the correlation among physiological variables. Only cold-acclimated animals supported our *a priori* path models, and $m_b$ appeared to be the best descriptor of body size (compared with $L_b$ and $L_f$) when dealing with energy metabolism and thermal conductance. However, while $m_b$ appeared to be the strongest determinant of energy metabolism, there was an important and significant contribution of $L_b$ (but not $L_f$) to thermal conductance. This study demonstrates how additional information can be drawn from physiological ecology and general organismal studies by applying Structural Equation Modeling when multiple variables are measured in the same individuals.**

Key words: basal metabolic rate, maximum metabolic rate, thermal acclimation, structural equation modeling, body size, leaf-eared mouse, *Phyllotis darwini*.

## Introduction

The balance between acquisition and expenditure of energy is critical to the survival and reproductive success of organisms. This balance depends on the interplay between energy intake and processing, thermoregulation, metabolic expenditures and production (Karasov, 1986). Nevertheless, as suggested by Hammond and Diamond (1997), the energetics of an organism may also change as a result of its own physiological features and constraints. In endotherms, for example, the elevated costs of thermoregulation require them to sustain expensive metabolic machinery, reflected by a high cost of maintenance. Metabolic rate, measured as oxygen consumption ($\dot{V}_{O_2}$), is a standard measure of these costs of living under various conditions. Metabolic rate measured during resting, post-absorptive adult and non-reproductive animals is known as basal metabolic rate (BMR) (McNab, 1988). This variable measures the minimum cost of maintenance in euthermy, and has become one of the most ubiquitous variables studied by physiological ecologists (see McNab, 2002). $\dot{V}_{O_2}$ determined for an endotherm faced with conditions of extreme cold, usually accomplished by forced convection (Hayes and O'Connor, 1999) or a Helium–Oxygen atmosphere (Rosenmann and Morrison, 1974; Chappell and Bachman, 1995), defines the thermoregulatory maximum metabolic rate (MMR). This variable has been shown to change seasonally, as an adaptive feature in animals facing cold and fluctuating environments (Wickler, 1980; Hayes, 1989; Holloway and Geiser, 2001), as well as among species inhabiting contrasting climates (Rosenmann and Morrison, 1974; Bozinovic and Rosenmann, 1989). In eutherian mammals, a third respirometric variable, which is related to maximum capacities, is non-shivering thermogenesis (NST). This variable is usually measured as the $\dot{V}_{O_2}$ response to norepinephrine injection, within thermoneutrality (Jansky, 1973; Wunder and Gettinger, 1996), and is related to the

formation of brown adipose tissue, a special thermogenic tissue identified only in eutherians, that grows following chronic cold exposure (Himms-Hagen, 1990), and provides the explanation for most of the seasonal variation seen in MMR (Bockler and Heldmaier, 1983; Wunder and Gettinger, 1996).

The roles of BMR, NST and MMR (known collectively as 'energy metabolism') in maintaining the thermal homeostasis of endotherms depends on the efficiency of heat conservation in the body, which is, in turn, a function of body mass ($m_b$) and thermal conductance (C). Thermal conductance, among other things, is a function of the insulating properties of fur, the thermal gradient, evaporative water loss, the composition of the atmosphere surrounding the body, and environmental factors such as radiant temperature and wind velocity (McNab, 1980; Wooden and Walsberg, 2002). A reliable estimation of 'wet' thermal conductance (i.e. including evaporative water loss; McNab, 1980) is obtained from the equation $C=V_{O_2}/(T_b-T_a)$, where $T_b$ is body temperature and $T_a$ is ambient temperature (McNab, 1980). This simplified calculation of C is useful as long as $\dot{V}_{O_2}$ is recorded below thermoneutrality (Anderson et al., 1997). It is known that C, as well as energy metabolism, can change seasonally and in response to thermal acclimation (Maddocks and Geiser, 2000; Merritt et al., 2001). The mechanisms and physiological processes responsible for these changes and their ecological significance are well understood (for reviews, see Jansky, 1973; Heldmaier et al., 1985; Wunder and Gettinger, 1996; McNab, 2002).

Body mass, as a proxy for body size, is the main determinant of energy metabolism and thermal conductance in animals (Schmidt-Nielsen, 1995; Schleucher and Withers, 2001; McNab, 2002). This dependence is not simple, and complicates statistical analyses, especially when defining how to deal with body mass when analyzing mass-independent physiological data (Hayes, 1996, 2001; Christians, 1999). Several analytical methods exist for solving these problems, most of them related to common statistical procedures such as analysis of covariance (ANCOVA), multiple regression and residual analysis (Christians, 1999). Body size, however, is an abstraction, whereas $m_b$ is a correlated variable that can be measured. It has been shown that other correlates of body size could yield different strengths of association with physiological variables (Gosler, 2000; Tracy and Walsberg, 2002), and hence different results from the analyses (Gosler, 2000; Milner et al., 2000; Tracy and Walsberg, 2002). The problem is that most statistical methods are good at treating each physiological variable separately, but performing multi-variable analyses is not easy, especially when physiological variables differ in their dependence on $m_b$.

Structural equation modeling (SEM) is a powerful set of procedures which, combined with an adequate design, could solve these problems. With SEM it is possible to test complete path diagrams of causation and correlation among variables, and to include latent variables (variables that cannot be measured without error) or theoretical constructions associated with observed variables (Everitt, 1984; Cox and Wermuth, 1996). In addition, comparisons among standardized path coefficients are straightforward because they are weighted indices that represent the proportional contribution of each causation path to an observed, manifest variable (i.e. they can be compared because they are scale-independent). SEM is widely used as a research tool in psychology, sociology and medicine (e.g. Koch et al., 2001; MacLullich et al., 2002), and has been applied by evolutionary biologists (Crespi and Bookstein, 1989), geneticists (Dohm, 2002) community (Wootton, 1993) and ecosystem ecologists (Ferguson, 2002) and, to a lesser extent, by physiological ecologists (Hayes and Schonkwiler, 1996).

This study had two aims: (1) to determine the mass-controlled effects of thermal acclimation on energy metabolism (BMR, NST and MMR) and thermal conductance (C) in a murid rodent *Phyllotis darwini*, and (2) to explore and compare the effect of different measurements of body size ($m_b$, $L_t$ and $L_f$) on energy metabolism and C, and their reliability as good approximations for body size using SEM. We hypothetized that cold thermal acclimation has a profound effect over physiological variables, modifying the effect of body size on them. Accordingly, we predict that SEM models will adjust differently in warm- and cold-acclimated individuals.

## Materials and methods

### *Animals and thermal acclimation*

Adult *Phyllotis darwini* Waterhouse 1837, 15 males ($m_b$=64.6±9.1 g, mean ± S.D.) and 25 females ($m_b$=57.7±11 g), were captured using 200 Sherman live traps between July and August 1999 (middle to late winter) in central Chile (33°29′S; 70°56′W, 500 m above mean sea level. Animals were maintained in the laboratory at ambient temperature (26±2°C, mean ± S.E.M.) and natural photoperiod. Individuals were maintained with commercial rabbit and rat food pellets, and water *ad libitum*. Each of 15 males were randomly assigned to three non-pregnant females, one at a time, for 10 days each. Gravid females were isolated and weighed weekly using a Sartorious (Gottingen, Germany) electronic balance (accurate to ±0.1 g). Young were isolated after weaning (at day 15) and maintained under the conditions described above until adulthood (approximately 150 days). Physiological measurements were performed on adult offspring on two occasions (in 1 month): warm acclimation (30°C) followed by cold (12°C) acclimation. We obtained one large ($N$=108) dataset for each acclimation, which included 7 manifest variables (i.e. measured variables: $m_b$ values recorded before BMR/NST, MMR and C measurements, plus the three respirometric variables, plus C), and one smaller set of data ($N$=73) with two additional manifest variables: foot and body length ($L_f$ and $L_b$, respectively), measured only in cold-acclimated animals.

### *Basal metabolic rate and non-shivering thermogenesis*

Upon completing each thermal acclimation, and prior to measurements of BMR and NST, animals were fasted for 6 h

(Nespolo et al., 2002). BMR and NST were measured according to the following protocol. Oxygen consumption ($V_{O_2}$) was measured in a computerized (Datacan V) open-flow respirometry system (Sable Systems, Henderson, Nevada, USA). Animals were kept in steel metabolic chambers of 1000 ml volume, at $T_a$ of 30.0±0.5°C, which is within the thermoneutral zone for this species (Bozinovic and Rosenmann, 1988). The metabolic chamber received dried air at a rate of 505±3 ml min$^{-1}$ from mass flow controllers (Sierra Instruments, Monterey, CA, USA), which was enough to ensure adequate mixing in the chamber. Air passed through $CO_2$-absorbent granules of Baralyme® and Drierite® before and after passing through the chamber, and was monitored every 5 s by an Applied Electrochemistry $O_2$-analyzer, model S-3A/I (Ametek, Pittsburgh, PA, USA). Oxygen consumption values were calculated using equation 4a of Withers (1977). All metabolic trials were completed between 08:00 and 16:00 h. Body mass was measured prior to the metabolic measurements using an electronic balance (to ±0.1 g), and rectal body temperature ($T_b$) was recorded at the end of each measurement with a Cu/copper-constant thermocouple (Cole–Parmer, Illinois, USA).

The experimental protocol was: (1) $\dot{V}_{O_2}$ recorded for a 1.5 h period at rest, (2) intramuscular injection of norepinephrine (NE), followed by (3) a final 30 min period of $\dot{V}_{O_2}$ recording. Recording ended when $\dot{V}_{O_2}$ reached a sustained maximum value during a 10 min period. Doses of NE were calculated according to Wunder and Gettinger's equation (Wunder and Gettinger, 1996: p. 133). Basal metabolic rate was estimated as the lowest mean value recorded over a 3 min interval after the first period of $\dot{V}_{O_2}$ recording. We previously measured BMR to determine the optimal time of recording needed to reach minimum metabolism and we found that this species reaches a steady state after 15–20 min, with no changes of $\dot{V}_{O_2}$ >15% in the following 3 h (see also Nespolo et al., 2002). To calculate NST from the recording, we used the highest sample above BMR, following the standard procedure (i.e. maximum $\dot{V}_{O_2}$ after NE injection minus BMR; e.g. Klaus et al., 1988; Wunder and Gettinger, 1996).

### *Minimum thermal conductance*

In addition to NST and BMR measurements, we measured $\dot{V}_{O_2}$ using the same procedure as above but at a $T_a$ of 6±2°C, in order to determine 'wet' minimum thermal conductance (C). To monitor $T_a$ inside the chamber, we used a copper-constant thermocouple set on its geometric center and approximately 4 cm above the animal. Temperature was recorded every 4 s with a Data Logger (Digi-Sense, Illinois, USA). To avoid heat loss due to contact of the animal with the steel floor of the chamber, or with urine and feces, we covered the chamber floor with 0.5 cm of sawdust, which was renewed before each new measurement. Rectal $T_b$ was measured within 30 s of the last recording. The lowest value of $\dot{V}_{O_2}$ within the last 10 min period of $\dot{V}_{O_2}$ measurements was taken to determine C. For each individual we calculated minimum C using the equation $C=V_{O_2}/(T_b–T_a)$ (McNab, 1980).

### *Maximum metabolic rate*

We measured MMR in a He–$O_2$ atmosphere according to the procedure of Rosenmann and Morrison (1974), using an open circuit respirometer, as described by Chappel 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), which was maintained at 1002±10 ml min$^{-1}$. This flow rate prevented the partial oxygen pressure from falling below 20 kPa, a value far above those considered hypoxic (Rosenmann and Morrison, 1975). As in the case of BMR measurements, 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®. Chamber temperature (–5.0±0.5°C) and $T_b$ were measured. The highest steady-state 3 min period of recordings were taken as MMR.

### *Statistics and structural equation modeling*

All statistical analyses were performed with Statistica version 6.0 (StatSoft, Tulsa, OK, USA). Differences in measured variables between acclimation groups (BMR, NST, MMR and C) were tested by repeated-measures (RM) ANCOVA with $m_b$ as a changing covariate (StatSoft). Partial product–moment correlations were used to test associations between variables within each acclimation temperature, controlled by $m_b$.

The Structural Equation Modeling (SEPATH) module of Statistica (StatSoft) was used to compute standardized path coefficients among measured variables, and to test the overall path diagram as a likely cause of observed data. We used maximum likelihood (ML) to estimate parameters, with their respective standard errors. Since this procedure is based on asymptotic statistics (large sample sizes), we performed Monte Carlo simulations to assess the behavior of our sample statistic and the iteration procedure at different sample sizes. Our three measurements of body size were assumed as measured consequences of a latent variable, BODY SIZE, that we included in the model (see Appendix). Computationally, SEM makes a linear combination of these variables to build the latent variable (Shipley, 2000). We hypothesized that energy metabolism and thermal conductance were primarily a function of the amount of tissue in the body, which is better described by $m_b$, and not by linear measurements ($L_b$ and $L_f$). For this reason, we included the paths from body mass to physiological variables. The first model (the 'indirect' model, Fig. 1) consisted of this causal structure (i.e. $m_{bBN}$→BMR, $m_{bBN}$→NST; $m_{bM}$→MMR; $m_{bC}$→C) (see Fig. 1), which supposes that the effects of body size on physiological variables are expressed indirectly through body mass. The second model considered an additional path structure from 'BODY SIZE' to each metabolic variable (the 'direct–indirect' model, Fig. 2). Hence, in this model the effect of body size over physiological variables was decomposed into indirect effects *via* $m_b$, as in the first model, and direct effects from 'BODY SIZE' to physiological variables (Fig. 2). In the context of the

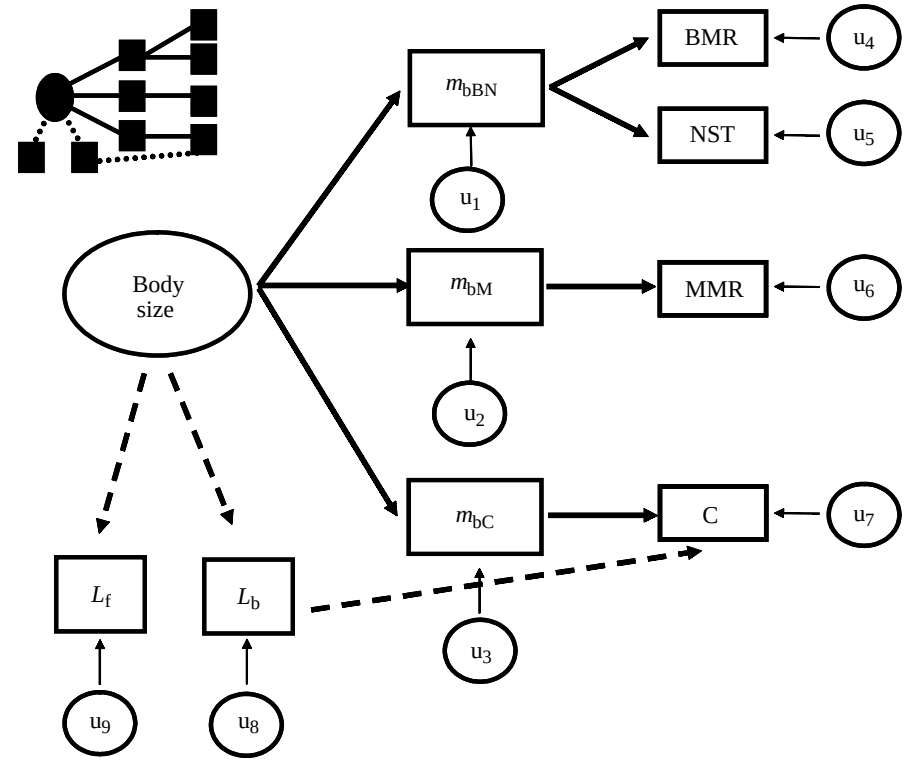



Fig. 1. Path diagram of the 'indirect' model tested with measured data. BMR, basal metabolic rate; NST, non-shivering thermogenesis; MMR, maximum metabolic rate; C, thermal conductance; $m_{bBN}$, body mass recorded at the moment of BMR and NST measurement; $m_{bM}$, body mass recorded at the moment of MMR measurement; $m_{bC}$, body mass recorded at the moment of C measurement; u, residual error. The broken line shows the additional paths from variables other than $m_b$, which were considered as indicators of body size: $L_f$ (foot length) and $L_b$ (body length). These variables were measured only for cold-acclimated individuals (the 'small' dataset). Latent variables are in circles and manifest variables in rectangles. The small diagram at the upper left identifies the path model in Table 3 (see Results).

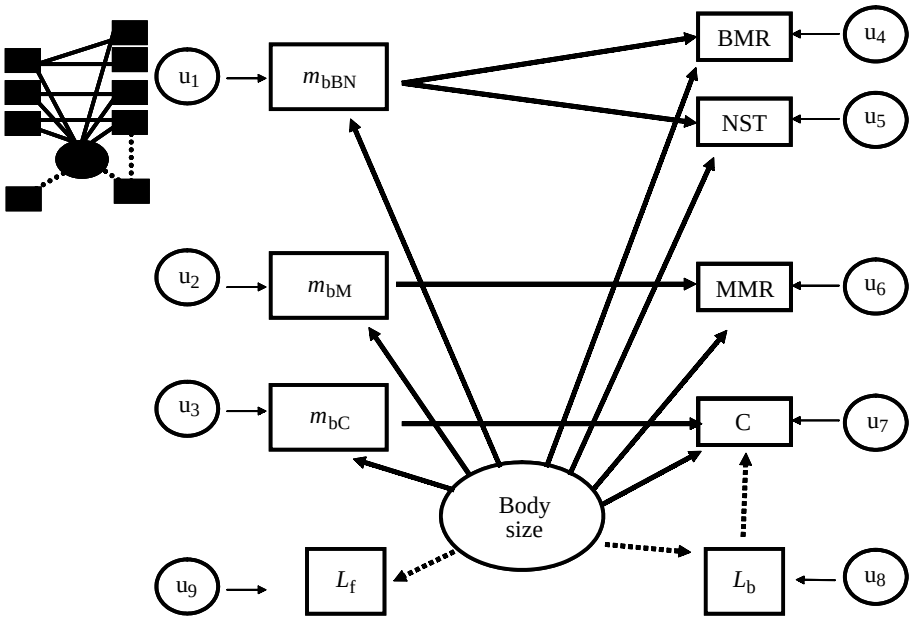



Fig. 2. Path diagram of the 'direct–indirect' model tested with observed data. Symbols and broken lines as in Fig. 1.

physiological variables studied here, if the indirect models were accepted, we would conclude that $m_b$ is the variable that best explains body size. In contrast, the acceptance of the direct–indirect model would mean that both $L_f$ and $L_b$ are important variables explaining body size, in addition to $m_b$.

## Results

Body mass differed significantly between acclimations (warm $m_b$: 57.3±12.5; cold $m_b$: 65.6±11.3, $t_{95}$=7.85, $P$<0.001). Thermal acclimation had a significant effect on all measured variables (BMR: $F_{1,106}$=5.46, $P$=0.021; NST: $F_{1,106}$=51.01, $P$<0.0001; MMR: $F_{1,106}$=115.6, $P$<0.0001; C: $F_{1,106}$=24.72, $P$<0.0001, RM-ANCOVA). Whereas metabolic variables were higher in cold-acclimated individuals, thermal conductance presented an opposite trend (Fig. 3). Correlations between all physiological variables and $m_b$ were significant for both acclimations (Fig. 4). Partial correlations (controlled by $m_b$) showed that in warm-acclimated animals, BMR was significantly associated with MMR and with NST (Table 1). In cold-acclimated animals, there was only one significant partial correlation: BMR with NST (Table 1). Morphological variables measured in cold-acclimated animals were all highly intercorrelated (Table 2).

The adjustments of path models are presented in Table 3. Based on nominal $\chi^2$ $P$-values, only three of eight path models presented non-significant differences from the expected covariance structure (i.e. are supported by the data). However, from the Monte Carlo simulated distribution, two additional models are supported by the data (Table 3). In general, for the large dataset (i.e. using only $m_b$ as a measure of body size; see Materials and methods), cold-acclimated individuals presented better adjustment than warm-acclimated ones (Table 3; models 1 and 2), and for the small dataset (i.e. body mass plus $L_f$ and $L_b$, models 3–6, only in cold-acclimated animals; see

Materials and methods), the single path $L_f \rightarrow C$ improved the adjustment significantly (Table 4). This effect is also observed in the path diagram that includes only indirect effects of body size to physiological variables (model 3 *versus* 4, Table 4), and in the path diagram including direct and indirect effects of body size to physiological variables (model 5 *versus* 6, Table 4). Comparisons between nested models (indirect model *versus* direct–indirect model, see Table 3) for warm- and cold-acclimated individuals were both significant (warm-acclimated animals: $\chi^2_{[4]}=15.44$; $P=0.004$; cold-acclimated animals: $\chi^2_{[4]}=9.99$; $P=0.041$).

Considering only the best-adjusted models (i.e. non-significant from Monte Carlo *P*-values, Table 3), path diagrams that included direct and indirect effects of body size over physiological variables were less explanatory than those that included only indirect paths through $m_b$ (Figs 5–9). This is supported by the fact that in all cases all direct paths from body size are non-significant, and several paths $m_b \rightarrow$'physiological variable' have values above 1.0 (Figs 6, 7, 9), which suggest a poor adjustment. This allows us to discard all direct–indirect models (models 2, 5, 6 in Table 3, and Figs 6, 7 and 9), and leaves us only with model 1 for cold-acclimated animals (Fig. 5) and model 4 (Fig. 8).

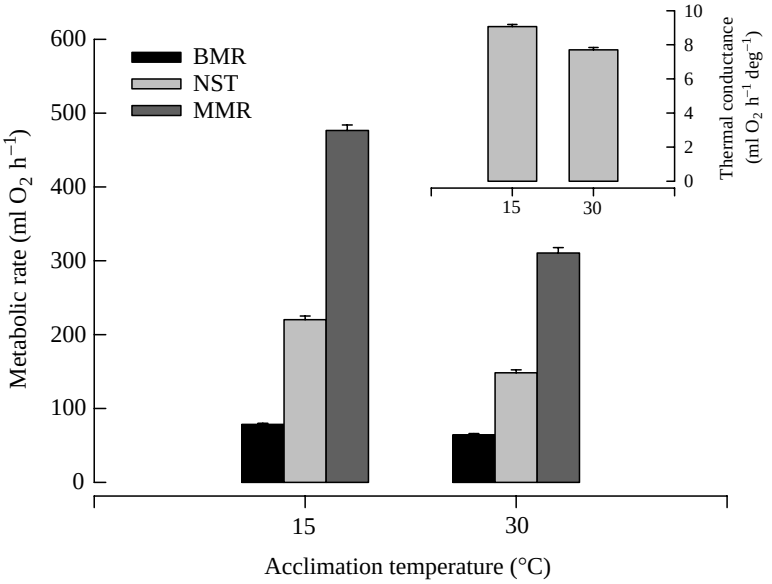



Fig. 3. Adjusted means (± 1 S.E.M., $N=108$) of basal metabolic rate (BMR), non-shivering thermogenesis (NST), maximum metabolic rate (MMR) and thermal conductance in cold- (15°C) and warm- (30°C) acclimated individuals. Significant differences between acclimation temperatures were found in all cases (ANCOVA, $P<0.01$; see Results).

Path coefficients of accepted models (Figs 5 and 8) are similar: large values in paths from body size to body mass and smaller paths relating body mass to physiological variables. A difference between both models is that in the more complex one (Fig. 8) the path relating $m_{bC}$ to C is considerably larger, and error paths for $m_{bC}$ and C are smaller (see Figs 5 and 8).

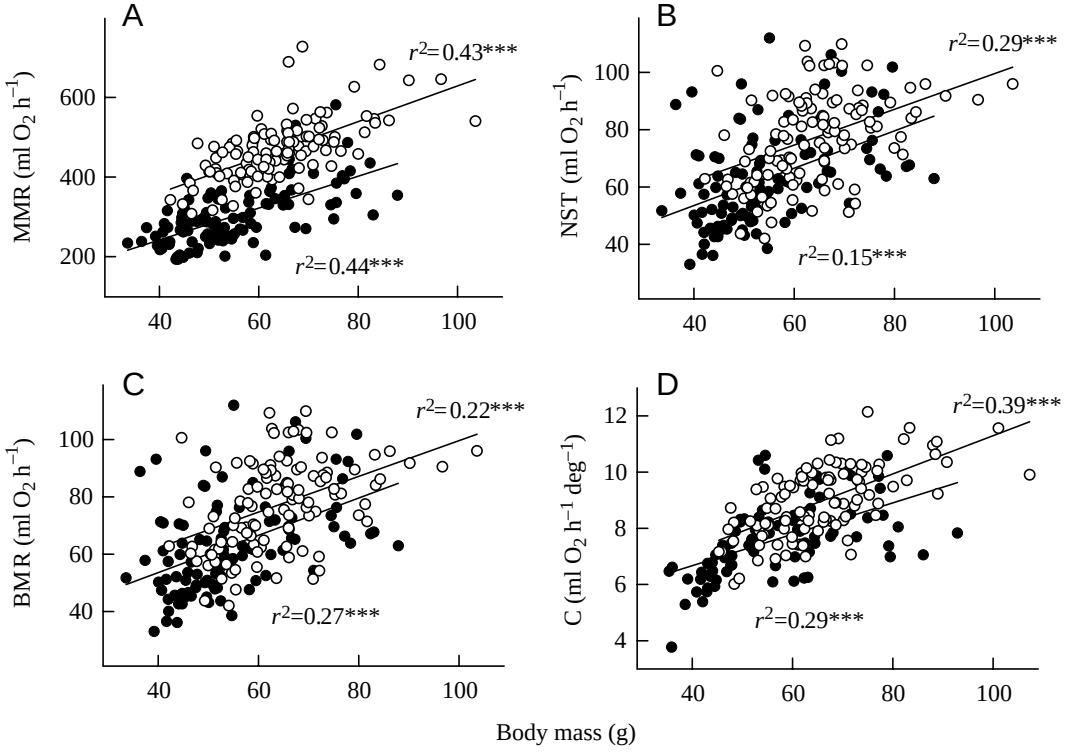



Fig. 4. Correlations between body mass and oxygen consumption parameters. (A) MMR, maximum metabolic rate; (B) NST, non-shivering thermogenesis; (C) BMR, basal metabolic rate; (D) C, thermal conductance. Squared correlation coefficients are shown for cold- (open symbols, top) and warm- (closed symbols, bottom) acclimated individuals in each case. ***, $P<0.001$.

Table 1. *Partial correlations among energetic variables (controlled for by body mass)*

| | MMR | BMR | NST | C |
|---|---|---|---|---|
| MMR | 1.0 | **0.247 (0.023)** | 0.198 (0.07) | 0.127 (0.25) |
| BMR | –0.06 (0.58) | 1.0 | **0.436 (0.001)** | 0.049 (0.66) |
| NST | 0.044 (0.66) | **0.257 (0.009)** | 1.0 | 0.105 (0.34) |
| C | –0.096 (0.34) | 0.065 (0.52) | 0.023 (0.82) | 1.0 |

MMR, maximum metabolic rate; BMR, basal metabolic rate; NST, non-shivering thermogenesis; C, thermal conductance.
Values for warm- and cold-acclimated individuals are shown above and below the diagonal, respectively.
*P*-values are in parentheses; significant comparisons at $P<0.05$ are in bold type.

## Discussion

### *Absolute values of physiological variables and correlations among them*

Since this is not the first determination of physiological/energetic variables in *P. darwini*, it represents a good opportunity to find out the extent by which ignoring the effects of thermal acclimation on energy metabolism may have biased previous works. Reported values of BMR, MMR and C in *P. darwini* (Bozinovic and Rosenmann, 1988, 1989) fall exactly in between our warm and cold measurements (in mass-specific units, see Table 5). These same measurements made in another species of *Phyllotis* (*P. xanthopygus*), which inhabits high altitudes, revealed very similar warm and cold values of BMR and NST, but MMR values were more extreme (Nespolo et al., 1999; Table 5). Predictions from allometric equations for rodents (Bozinovic, 1992) for BMR are 1.31 ml $O_2$ $g^{-1}$ $h^{-1}$ and for MMR, 7.34 ml $O_2$ $g^{-1}$ $h^{-1}$. These values are closer to the cold-acclimated values in our study, but only the MMR values fall inside the confidence intervals of our data (Table 5). Similarly, the expected allometric values for NST are 2.90 ml $O_2$ $g^{-1}$ $h^{-1}$ and 5.56 ml $O_2$ $g^{-1}$ $h^{-1}$ for warm- and cold-acclimated animals, respectively (Wunder and Gettinger, 1996). These values are above our measurements and fall outside our 95% confidence intervals for both acclimations (Table 5).

Table 2. *Correlation matrix for morphological variables in cold-acclimated animals*

| | Body mass | Foot length | Body length |
|---|---|---|---|
| Body mass | 1.0 | **0.62 (0.0001)** | **0.78 (0.0001)** |
| Foot length | | 1.0 | **0.55 (0.0001)** |
| Body length | | | 1.0 |

*P*-values are in parentheses; significant comparisons at $P<0.05$ are in bold type.

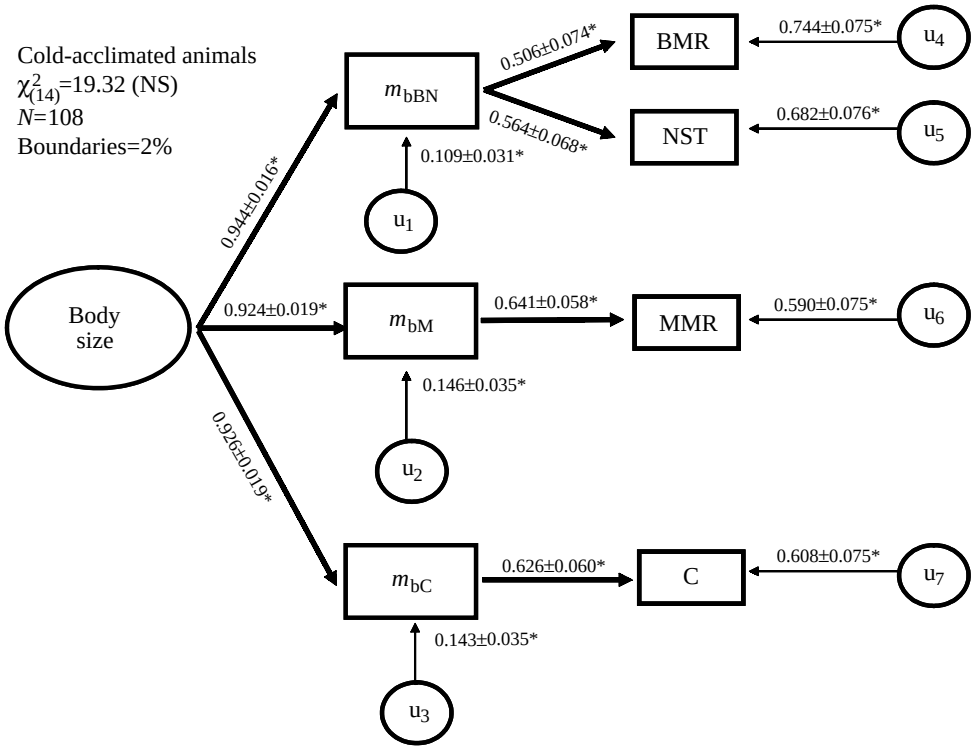
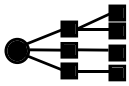
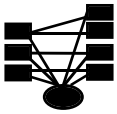
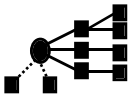
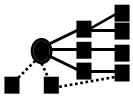
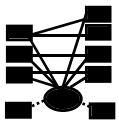
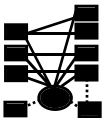



Fig. 5. Path coefficients adjusted for model 1, cold-acclimated animals (Table 3). Values are means ± 1 S.E.M. Asterisks denote significant coefficients ($P<0.05$). Latent variables are in circles; manifest variables are in rectangles. Adjustment statistics are from Table 3 (only non-significant models are presented). Abbreviations as in Fig. 1.

Table 3. *Path models adjusted for warm and cold acclimated individuals*

| Model | Acclimation | $\chi^2$ | d.f. | *N* | Boundary rate (%) | Nominal *P*-value | Monte Carlo *P*-value |
|---|---|---|---|---|---|---|---|
| (1)  | Warm | 30.05 | 14 | 108 | 2 | **0.0075** | **<0.01** |
| | Cold | 19.32 | 14 | 108 | 2 | 0.153 | >0.05 |
| (2)  | Warm | 14.61 | 10 | 108 | 0.1 | 0.147 | >0.05 |
| | Cold | 9.33 | 10 | 108 | 0.1 | 0.501 | >0.05 |
| (3)  | Cold | 50.12 | 27 | 71 | 0.1 | **0.0044** | **<0.01** |
| (4)  | Cold | 42.98 | 26 | 71 | 0.1 | **0.0194** | >0.05 |
| (5)  | Cold | 44.77 | 23 | 71 | 0.1 | **0.0042** | **<0.01** |
| (6)  | Cold | 37.61 | 22 | 71 | 0.1 | **0.020** | >0.05 |

The arrows and names were omitted for simplicity. Manifest (measured) variables are in rectangles and latent (non-measured) variables, i.e. body size, are in circles. Configurations are according to the path models presented in Figs 1 (models 1,3,4) and 2 (models 2,5,6).

Boundary rate is the proportion of parameter estimations that fell outside the parameter space in a Monte Carlo simulation with 100 replications, using the observed sample size *N* for each case.

The nominal *P*-value corresponds to the probability level according to the theoretical $\chi^2$ distribution with the observed degrees of freedom (d.f.).

The Monte Carlo *P*-value is the observed probability from the $\chi^2$ simulated distribution.

Significant comparisons at $P<0.05$ are in bold type.

We found significant partial correlations between BMR and NST for both acclimations, and between BMR and MMR in warm-acclimated animals only. Previous reports of (residual) intraspecific correlations between basal and maximum metabolic capacities show that, in general, they are small but significant (Hayes and Garland, 1995). Since in endotherms BMR measures maintenance costs (i.e. operation of metabolically active organs), the coupling of BMR to maximum capacities (in this case either MMR or NST) reflects the increase in systemic performance when maximum capacities are higher. In cold-acclimated animals, however, the association BMR to MMR was absent (see also Bozinovic et al., 1990), which suggests that cold induced a disproportionate increment of maximum performance, in agreement with systemic adjustments.

Table 4. *$\chi^2$ ratio tests for nested models showed in Table 3*

| | Model | | | | | |
|---|---|---|---|---|---|---|
| | 4 | | 5 | | 6 | |
| Model | $\chi^2$ | *P* | $\chi^2$ | *P* | $\chi^2$ | *P* |
| 3 | **$7.14_{[4]}$** | **0.0075** | $5.35_{[4]}$ | 0.253 | **$12.51_{[5]}$** | **0.028** |
| 4 | | | $1.79_{[3]}$ | 0.617 | $5.37_{[4]}$ | 0.251 |
| 5 | | | | | **$7.16_{[1]}$** | **0.0075** |

Values are $\chi^2$, with degrees of freedom in brackets, and the corresponding *P*-value. Significant comparisons at $P<0.05$ are in bold type.

### *Thermal acclimation*

Thermal acclimation had an effect over all energetic variables, which is well known for MMR and NST in mammals (Lynch, 1973; Wickler, 1980; Hayes and Chappell, 1986; Merritt et al., 2001). Nevertheless, it is rather paradoxical that C increased following cold acclimation since published studies seem to suggest either no change under these conditions, or a decrease in C (Maddocks and Geiser,

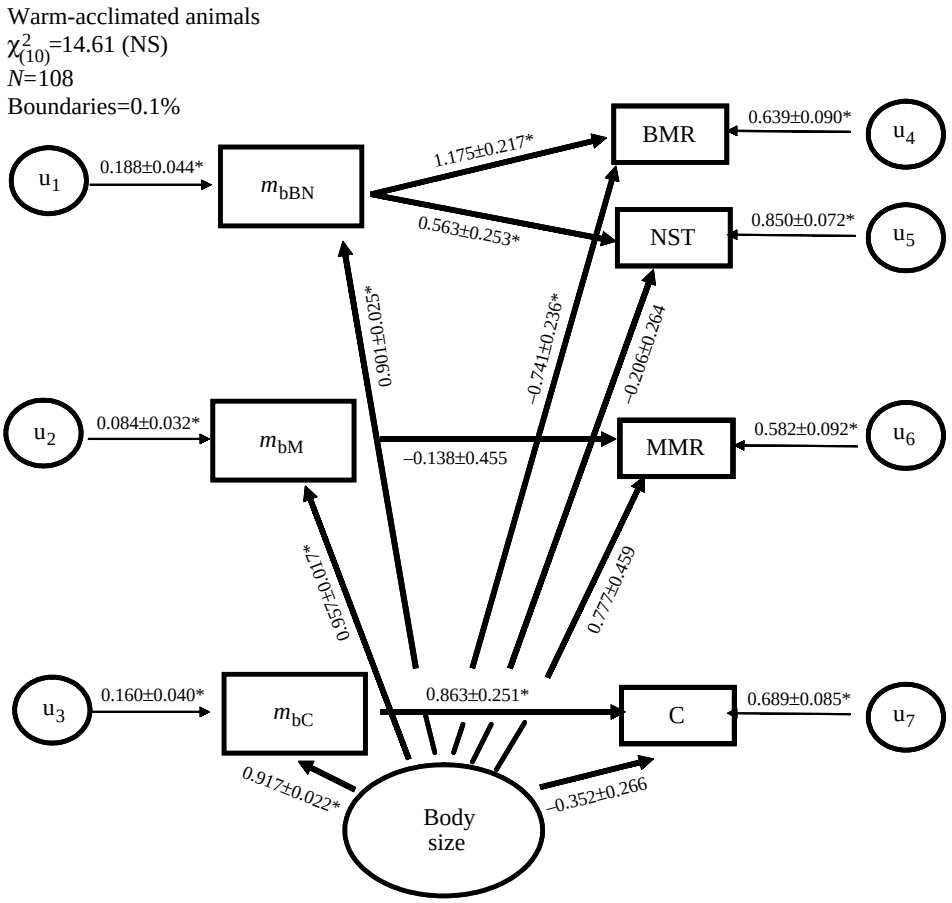



Fig. 6. Path coefficients adjusted for model 2 (indirect effects of latent body size on mass), warm-acclimated individuals (see Table 3) Values are means ± 1 S.E.M. Asterisks denote significant coefficients ($P$<0.05). Latent variables are in circles; manifest variables are in rectangles. Adjustment statistics are from Table 3 (only non-significant models are presented). Abbreviations as in Fig. 1.

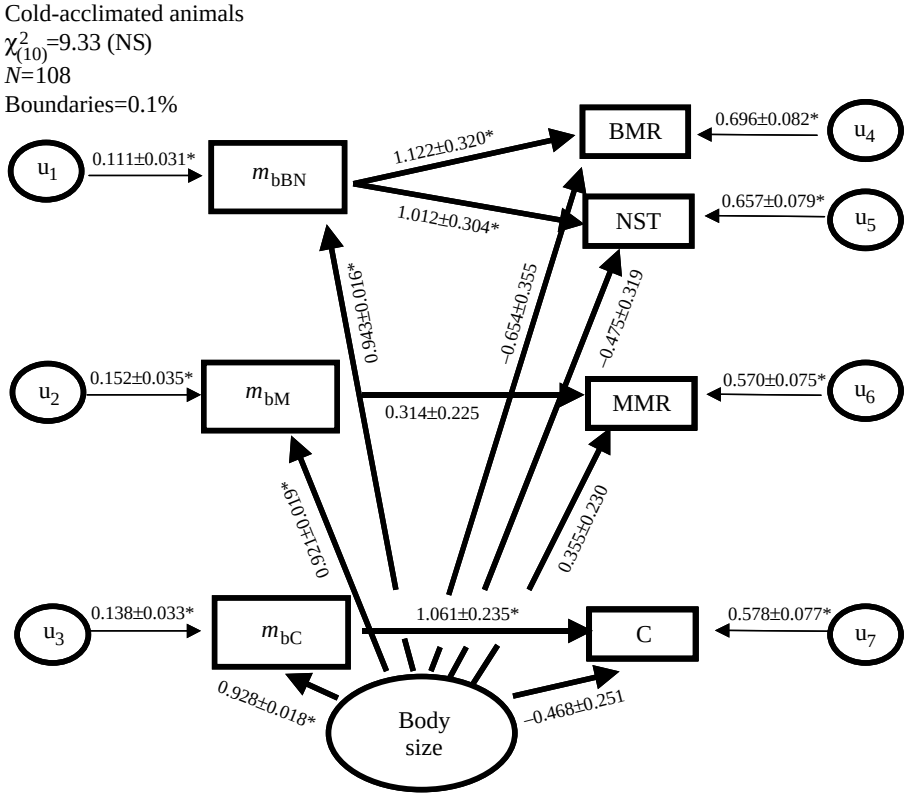



Fig. 7. Path coefficients adjusted for model 2 (indirect effects of latent body size on mass), cold-acclimated individuals (see Table 3). Values are means ± 1 S.E.M. Asterisks denote significant coefficients ($P$<0.05). Latent variables are in circles; manifest variables are in rectangles. Adjustment statistics are from Table 3 (only non-significant models are presented). Abbreviations as in Fig. 1.

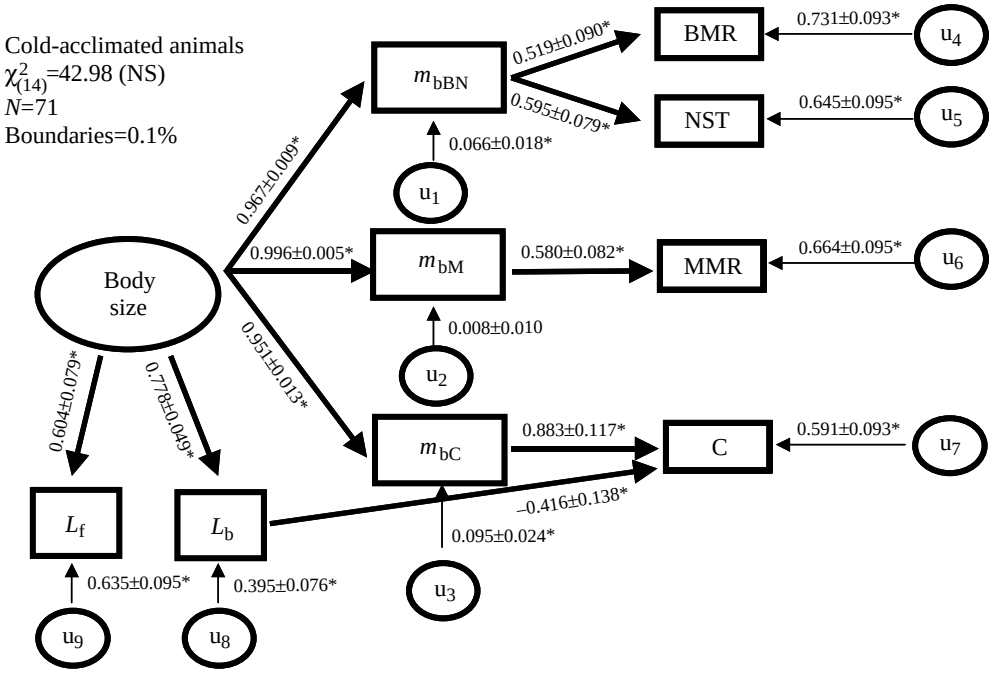



Fig. 8. Path coefficients adjusted for model 1 (direct effects of latent body size on mass), cold-acclimated individuals including foot length ($L_f$) and body length ($L_b$) as proxies for body size (see Table 3). Values are means ± 1 S.E.M. Asterisks denote significant coefficients ($P$<0.05). Latent variables are in circles; manifest variables are in rectangles. Adjustment statistics are from Table 3 (only non-significant models are presented). Abbreviations as in Fig. 1.

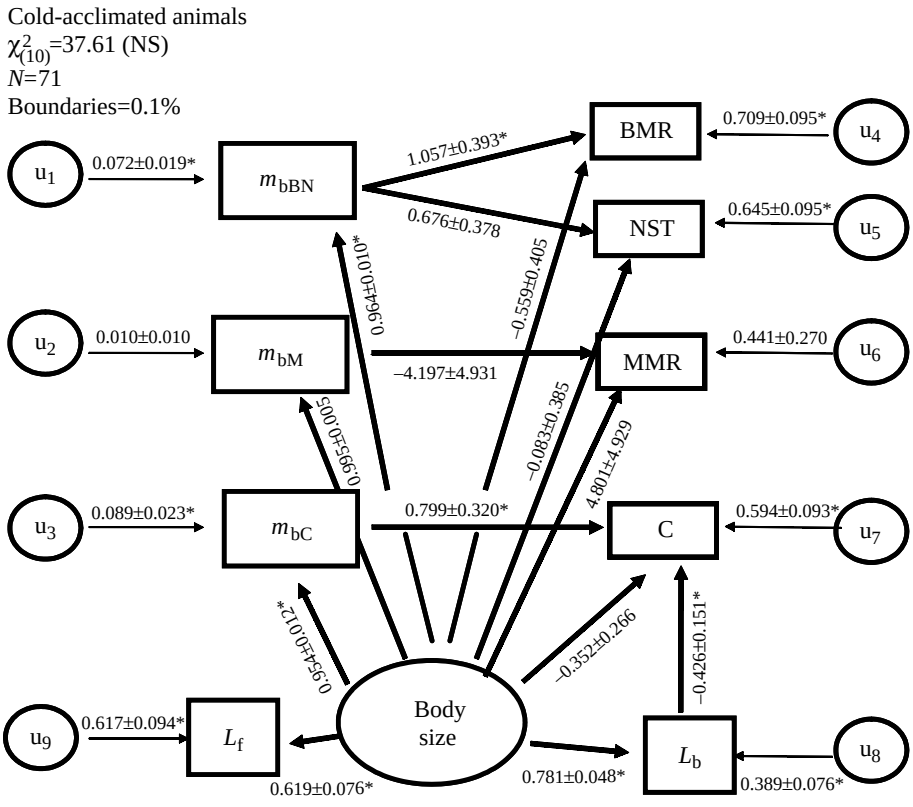



Fig. 9. Path coefficients adjusted for model 2 (indirect effects of latent body size on mass), cold-acclimated individuals including foot length ($L_f$) and body length ($L_b$) as proxies for body size (see Table 3). Values are means ± 1 S.E.M. Asterisks denote significant coefficients ($P$<0.05). Latent variables are in circles; manifest variables are in rectangles. Adjustment statistics are from Table 3 (only non-significant models are presented). Abbreviations as in Fig. 1.

2000; Sharbaugh, 2001). This means that insulation is reduced in cold-acclimated individuals, which suggests a poor performance under cold conditions. However, our measure of C ('wet' thermal conductance; McNab, 1980) is computed from $\dot{V}_{O_2}$. This measurement has several useful properties; for example in small bodies it has been shown to be a better predictor of heat loss than dry C (i.e. heat loss rate measured in dead animals or carcasses) (Klaassen et al., 2002). Actually,

Table 5. *Values of energy metabolism (see Table 1) and thermal conductance reanalyzed as mass-specific values for comparision with previous studies (see Discussion)*

| | Acclimation | | | |
|---|---|---|---|---|
| | Warm | Cold | $F_{1,107}$ | $P$ |
| BMR (ml $O_2$ $g^{-1}$ $h^{-1}$) | 1.17 (1.11-1.22) | 1.22 (1.16-1.28) | 2.28 | 0.134 |
| NST (ml $O_2$ $g^{-1}$ $h^{-1}$) | 2.68 (2.53-2.83 | 3.39 (3.25-3.54) | 57.59 | **<0.0001** |
| MMR (ml $O_2$ $g^{-1}$ $h^{-1}$) | 5.56 (5.37-5.76) | 7.38 (7.18-7.58) | 194.18 | **<0.0001** |
| C (ml $O_2$ $g^{-1}$ $h^{-1}$ $deg^{-1}$) | 0.141 (0.137-0.146) | 0.141 (0.137-0.145) | 0.326 | 0.570 |

BMR, basal metabolic rate; NST, non-shivering thermogenesis; MMR, maximum metabolic rate; C, thermal conductance.
Values are means ± 95% confidence intervals.
Significant differences between acclimations are in bold type ($P$<0.05).

carcasses increase the surface-area-to-volume ratio (Klaassen et al., 2002) and dead animals lack piloerection and other physiological mechanisms that reduce heat loss in live animals (Turnpenny et al., 2000). So, wet C accounts for all the ways that heat loss occurs in live animals, including those that stem from increases in metabolic rate (higher respiratory gas exchange), and enhanced peripheral circulation due to increase in interscapular brown adipose tissue (Lynch, 1973). It is known that after cold acclimation, murids can increase blood flow to brown adipose tissue by a factor of ten (Puchalski et al., 1987). This change is enough to increase heat loss in peripheral areas of the thorax, which is demonstrated by infrared thermography (Jackson et al., 2001). Since our cold-acclimated animals showed an increase in C (which reflect the rate of heat loss), all other things being equal, it would be reasonable to observe a small increase in C, which was the case (less than 10%; see Results).

There still remains the question of why other studies have not detected such an effect of thermal acclimation on C. We believe this is because our analysis is more powerful, due to the control for body mass. In fact, when expressed as mass-specific units, both BMR and C show non-significant effects of thermal acclimation (Table 5). This illustrates the confounding effect of $m_b$ when analyzing mass-specific data. When this ratio is used, the residual variance is increased because $m_b$ is measured with an error that is not controlled, because it is included as a denominator of another variable ($V_{O_2}$) which, in turn, is also measured with an error. It follows that using this procedure, the residual variance of the linear model must be enlarged (in the case of an ANOVA), with the consequent loss of power in the overall analysis (Packard and Boardman, 1999; Hayes, 2001). The use of mass-specific units has probably obscured several subtle effects of acclimation and many other factors on the physiological variables published so far (e.g. Holloway and Geiser, 2001; Tracy and Walsberg, 2002). In agreement with previous authors (Hayes, 1996, 2001; Packard and Boardman, 1999; Christians, 1999; Lleonart et al., 2000), this evidence suggests that mass-specific variables in the literature should be treated with caution.

### *Structural Equation Modeling and body mass as a proxy of body size*

Our three measurements of body size were highly intercorrelated, reflecting the fact that all were reliable estimators of body size. Also, path coefficients relating latent body mass with $m_b$, $L_f$ and $L_b$ were large and significant in all cases. It is interesting, however, that while $m_b$ appears to be the strongest determinant of energy metabolism, there is an important and significant contribution of $L_b$ (and not $L_f$) to C. This is not surprising since C is a function of surface-area-to-volume, and $L_b$ is the total length of the body, and hence is geometrically related to surface area.

In spite of the generalized use of SEM in biology, in addition to the study of Hayes and Shonkwiler (1996), we were unable to find other published works that have addressed the problem of causation of physiological variables in small endotherms. Nevertheless, SEM has been recognized as a powerful tool for correlational experiments by several organismal biologists. For example, Pigliucci and Schlichting (1998) applied path analysis to investigate the differences among populations and the plasticity of plant architecture in fruits of *Arabidopsis*; Kause et al. (1999) used the full capabilities of SEM to test the effects of leaf quality on larval traits in six sawfly species; Miles et al. (2000) used SEM to predict survivorship from life history variables in a lizard; and Ferguson (2002) used SEM to discriminate between direct and indirect effects of demographic and environmental variables on age at maturity in a moose. These examples, together with our results, illustrate how SEM could be a powerful tool to distinguish between contrasting causal models in organismal biology.

SEM proved to be useful for our data analysis in the sense that we could test specific models and subtle effects, such as the inclusion of specific paths, to explore the change in the overall goodness of fit. This enabled us to conclude that our models were good explanations of the relationship between body size and energetic variables. However, this was only true for cold-acclimated animals; warm-acclimated animals did not adjust to our models, possibly because NST and MMR only have physiological significance in cold-acclimated

individuals. This raises the importance of thermal acclimation when measuring respirometric variables in endotherms. Likewise, the partial correlation that was detected among physiological variables was not detected by the SEM analysis (when we included such paths, the iteration did not converge because of the appearance of singular matrix). This occurs because SEM models can often be structurally identified, but numerically underidentified (Shipley, 2000, p. 167). These are limitations of SEM that cannot be ignored, and make it especially important to complement the SEM procedure with standard statistical analyses.

An interesting outcome from the SEM analysis is that the single path $L_b \rightarrow$C significantly improved the goodness of fit. Our best models were those that related body size with physiological variables indirectly, through $m_b$, and not by direct paths. Moreover, by examining the accepted path models, and comparing error paths, we could infer that the strength of association between body size and morphological traits is considerably larger than the (indirect) association between body size and physiological variables. Similarly, it is clear that C has a higher (indirect) dependence from body size than energy metabolism. None of these conclusions would have been possible using only standard statistical procedures.

## List of symbols and abbreviations

| | |
|---|---|
| $L_b$ | body length |
| $L_f$ | foot length |
| $m_b$ | body mass |
| $m_{bBN}$ | body mass at the moment of BMR/NST determination |
| $m_{bM}$ | body mass when MMR was measured |
| $m_{bC}$ | when C was recorded |
| $\dot{V}_{O_2}$ | oxygen consumption |
| $T_a$ | ambient temperature |
| $T_b$ | body temperature |
| BMR | basal metabolic rate |
| C | thermal conductance |
| ML | maximum likelihood |
| MMR | maximum metabolic rate |
| NE | norepinephrine |
| NST | non-shivering thermogenesis |

## Appendix

### *Structural Equation Modeling procedure, Monte Carlo simulations, minimum sample size estimation and hypothesis testing between models*

The minimum number of individuals needed for our analyses was assessed by simulating different sample sizes in 100 replications, and testing the number of boundary cases in each simulation (an estimation outside the parameter space; e.g. an estimated correlation coefficient outside the range |0–1|). Usually, sample sizes above $N$=60 gave less than 5% of boundary cases in the iteration for all models, which we considered a reasonable rate. The significance of the overall path model was assessed using the chi-squared ($\chi^2$) statistic computed from the departure of observed data from the expected covariance matrix (Shipley, 2000). The $P$-value of this statistic was obtained (1) from the theoretical $\chi^2$ distribution at the corresponding degrees of freedom, and (2) from the simulated distribution obtained from the Monte Carlo experiment (Manly, 1998). The last procedure is considered more powerful since the sampling distribution does not always approach the theoretical one (i.e. $\chi^2$) with finite data (Manly, 1998). The null hypothesis of a perfect fit means that the data support the proposed model (Shipley, 2000). By contrast, a significant $\chi^2$ ($P$<0.05) means that the model is not supported by the data and needs to be modified. Structural equation modeling has two basic assumptions: multivariate normality and linearity among variables. Our data satisfied both assumptions and no transformation was necessary. Although physiological variables scale non-linearly with $m_b$ (Schmidt-Nielsen, 1985), the best approximation when data have no more than one order of magnitude is a linear approximation (see Results). We created a latent exogenous variable, 'BODY SIZE', which was considered the cause of the three following measurements of body mass that we used: (1) body mass at the moment of BMR/NST determination ($m_{bBN}$), when MMR was measured ($m_{bM}$), and when C was recorded ($m_{bC}$). Usually, $m_{bBN}$ was lower than $m_{bM}$ and $m_{bC}$ because animals were fasted before the measurement of BMR/NST. We considered body size as the cause of the manifest variables $m_b$, $L_f$ and $L_b$ (the latter two only for the small dataset). However, not all of these variables were considered a direct cause of energy metabolism and thermal conductance.

Nested models (i.e. models differing in path number but with same number of variables) were compared with $\chi^2$ ratio tests since the difference in the maximum likelihood $\chi^2$ values between nested models is, itself, asymptotically distributed as a $\chi^2$ distribution (Shipley, 2000). The degrees of freedom for the resultant $\chi^2$ distribution are equal to the number of parameters that have been freed in the nested model, which is the same as the change in the degrees of freedom between the nested models (Shipley, 2000). This test allowed us to evaluate whether different models that included the same variables were statistically different, using the same set of data. To select the best models, we proceeded as follows: first, we looked at the statistics of the adjustment of the different models and selected (and presented) those that yielded a non-significant $\chi^2$. Second, we performed $\chi^2$ ratio tests to analyze the specific contribution of single paths to the overall model, between nested models. Third, path coefficients of selected models (i.e. non-significant from the Monte Carlo $P$-value) were examined, and those that presented more significant paths were judged to be the best. Although the effects of thermal acclimation were evaluated individually for each physiological variable, the condition of measurement (cold- or warm-acclimated) is explicitly stated in each path diagram.

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## References

**Anderson, M. D., Williams, J. B. and Richardson, P. R. K.** (1997). Laboratory metabolism and evaporative water loss of the aardwolf, *Proteles cristatus*. *Physiol. Biochem. Zool.* **70**, 464-469.

**Bockler, H. and Heldmaier, G.** (1983). Interaction of shivering and non-shivering thermogenesis during cold exposure in seasonally acclimatized djungarian hamster (*Phodopus sungorus*). *J. Therm. Biol.* **8**, 97-98.

**Bozinovic, F., Novoa, F. and Veloso, C.** (1990). Seasonal changes in energy expenditure and digestive tract of *Abrothrix andinus* (Cricetidae) in the Andes Range. *Physiol. Zool*. **63**, 1216-1231.

**Bozinovic, F.** (1992). Scaling of basal and maximum metabolic rate in rodents and the aerobic capacity model for the evolution of endothermy. *Physiol. Zool.* **65**, 921-931.

**Bozinovic, F. and Rosenmann, M.** (1988). Comparative energetics of South American cricetid rodents. *Comp. Biochem. Physiol.* **91A**, 195-202.

**Bozinovic, F. and Rosenmann, M.** (1989). Maximum metabolic rate of rodents: physiological and ecological consequences on distributional limits. *Funct. Ecol.* **3**, 173-181.

**Chappell, M. A. and Bachman, G. C.** (1995). Aerobic performance in Belding's ground squirrels (*Spermophilus beldingi*): variance, ontogeny, and the aerobic capacity model of endothermy. *Physiol. Zool.* **68**, 421-442.

**Christians, J. K.** (1999). Controlling for body mass effects: is part-whole correlation important? *Physiol. Biochem. Zool.* **72**, 250-253.

**Cox, D. R. and Wermuth, N.** (1996). *Multivariate Dependencies – Model Analysis and Interpretation. Monograph on Statistics and Applied Probability*. Second Edition. New York: Chapman and Hall.

**Crespi, B. J. and Bookstein, F. L.** (1989). A path-analytic model for the measurement of selection on morphology. *Evolution* **43**, 18-28.

**Dohm, M. R.** (2002). Repeatability estimates do not always set an upper limit to heritability. *Funct. Ecol.* **16**, 273-280.

**Everitt, B. S.** (1984). *An Introduction to Latent Variables Models. Monograph on Statistics and Applied Probability*. Second Edition. New York: Chapman and Hall.

**Ferguson, S.** (2002). The effects of productivity and seasonality on life history: comparing age at maturity among moose (*Alces alces*) populations. *Glob. Ecol. Biogeog.* **11**, 303-312.

**Gosler, A. G.** (2000). Assessing the heritability of body condition in birds: a challenge exemplified by the great tit *Parus major* L. (Aves). *Biol. J. Linn. Soc.* **71**, 103-117.

**Hammond, K. A. and Diamond, J.** (1997). Maximum sutained energy budgets in humans and animals. *Nature* **386**, 457-462.

**Hayes, J.** (1989). Altitudinal and seasonal effects on aerobic metabolism of deer mice. *J. Comp. Physiol. B* **159**, 453-459.

**Hayes, J. P.** (1996). Analyzing mass-independent data. *Physiol. Zool.* **69**, 974-980.

**Hayes, J. P.** (2001). Mass specific and whole-animal metabolism are not the same concept. *Physiol. Biochem. Zool.* **74**, 147-150.

**Hayes, J. P. and Chappell, M. A.** (1986). Effects of cold acclimation on maximum oxygen consumption during cold exposure and treadmill exercise in deer mice, *Peromyscus maniculatus*. *Physiol. Zool.* **59**, 473-481.

**Hayes, J. and Garland, T.** (1995). The evolution of endothermy: testing the aerobic capacity model. *Evolution* **49**, 836-847.

**Hayes, J. P. and O'Connor, C. S. O.** (1999). Natural selection on thermogenic capacity of high-altitude deer mice. *Evolution* **53**, 1280-1287.

**Hayes, J. P. and Shonkwiler, J. S.** (1996). Altitudinal effects on water fluxes of deer mice: a physiological application of structural equation modeling with latent variables. *Physiol. Zool.* **69**, 509-531.

**Heldmaier, G., Bockler, H., Buchberger, A., Lynch, G. R., Puchalski, W., Steinlechner, S. and Wiesinger, H.** (1985). Seasonal acclimation and thermogenesis. In *Circulation, Respiration and Metaboism* (ed. R. Gilles), pp. 490-501. Heidelberg: Springer Verlag.

**Himms-Hagen, J.** (1990). Brown adipose tissue thermogenesis: interdisciplinary studies. *FASEB J.* **4**, 2890-2891.

**Holloway, J. C. and Geiser, F.** (2001). Seasonal changes in the thermoenergetics of the sugar glider, *Petaurus breviceps*. *J. Comp. Physiol. B* **171**, 643-650.

**Jackson, D. M., Hambly, C., Trayhurn, C. and Speakman, J. R.** (2001). Can non-shivering thermogenesis in brown adipose tissue following NA injection be quantified by changes in overlying surface temperatures using infrared thermography? *Comp. Biochem. Physiol.* **26A**, 85-93.

**Jansky, L.** (1973). Non-shivering thermogenesis and its thermoregulatory significance. *Biol. Rev.* **48**, 85-132.

**Karasov, W. H., Phan, D., Diamond, J. M. and Carpenter, F. L.** (1986). Food passage and intestinal nutrient absortion in hummingbirds. *The Auk* **103**, 453-464.

**Kause, A., Haukioja, E. and Hanhimaki, S.** (1999). Phenotypic plasticity in foraging behavior of sawfly larvae. *Ecology* **80**, 1230-1241.

**Klaasen, M., Agrell, J. and Lindstrom, A.** (2002). Metabolic rate and thermal conductance of lemmings from high-arctic Canada and Siberia. *J. Comp. Physiol. B* **172**, 371-378.

**Klaus, S., Heldmaier, G. and Ricquier, D.** (1988). Seasonal acclimation of bank voles and wood mice: nonshivering thermogenesis and thermogenic properties of brown adipose tissue mitochondria. *J. Comp. Physiol. B* **158**, 157-164.

**Koch, R., Julius, U., Jaross, W. and Schroder, H.** (2001). Estimation of the heritability of latent variables which are included in a structural model for metabolic syndrome. *Hum. Heredity* **52**, 171-176.

**Lleonart, J., Salat, J. and Torres, G. J.** (2000). Removing allometric effects of body size in morphological analysis. *J. Theor. Biol.* **205**, 85-93.

**Lynch, G. R.** (1973). Seasonal changes in thermogenesis, organ weights, and body composition in the white-footed mouse, *Peromyscus leucopus*. *Oecologia (Berlin)* **13**, 363-376.

**MacLullich, A., Ferguson, K., Deary, I. and Seckl, J.** (2002). Intracranial capacity and brain volumes are associated with cognition in healthy elderly men. *Neurology* **59**, 169-174.

**Maddocks, T. A. and Geiser, F.** (2000). Seasonal variations in thermal energetics of Australian silver eyes (*Zosterops lateralis*). *J. Zool. Lond.* **252**, 327-333.

**Manly, B. F.** (1998). *Randomization, Bootstrap and Monte Carlo Methods in Biology*. First edition. New York: Chapman and Hall.

**McNab, B. K.** (1980). On estimating thermal conductance in endotherms. *Physiol. Zool.* **53**, 145-156.

**McNab, B. K.** (1988). Complications inherent in scaling the basal rate of metabolism in mammals. *Quart. Rev. Biol.* **63**, 25-54.

**McNab, B. K.** (2002). *The Physiological Ecology of Vertebrates. A View From Energetics*. First edition, pp. 576. Cornell: Comstock.

**Merritt, J. F., Zegers, D. A. and Rose, L. R.** (2001). Seasonal thermogenesis of southern flying squirrels (*Glaucomys volans*). *J. Mammal.* **82**, 51-64.

**Miles, D. B., Sinervo, B. and Frankino, W. A.** (2000). Reproductive burden, locomotor performance, and the cost of reproduction in free ranging lizards. *Evolution* **54**, 1386-1395.

**Milner, J. M., Pemberton, J. M., Brotherstone, S. D. and Albon, S. D.** (2000). Estimating variance components and heritabilities in the wild: a case study using the 'animal model' approach. *J. Evol. Biol.* **13**, 804-813.

**Nespolo, R. F., Bacigalupe, L. D. and Bozinovic, F.** (2002). The influence of heat increment of feeding on basal metabolic rate in *Phyllotis darwini* (Muridae). *Comp. Biochem. Physiol. A* **134**, 139-345.

**Nespolo, R. F., Opazo, J. C., Rosenmann, M. and Bozinovic, F.** (1999). Thermal acclimation, maximum metabolic rate and nonshivering thermogenesis in *Phyllotis xanthopygus* (Rodentia) inhabiting the Andean range. *J. Mammal.* **80**, 742-748.

**Packard, G. C. and Boardman, T. J.** (1999). The use of percentages and size-specific indices to normalize physiological data for variation in body size: wasted time, wasted effort? *Comp. Biochem. Physiol. A* **122**, 37-44.

**Pigliucci, M. and Schlichting, C. D.** (1998). Reaction norms of Arabidopsis. V. Flowering time controls phenotypic architecture in response to nutrient stress. *J. Evol. Biol.* **11**, 285-301.

**Puchalski, W., Bockler, H., Heldmaier, G. and Langefeld, M.** (1987). Organ blood flow and brown adipose tissue oxygen consumption during noradrenaline-induced nonshivering thermogenesis in the djungarian hamster. *J. Exp. Zool.* **242**, 263-271.

**Rosenmann, M. and Morrison, P.** (1974). Maximum oxygen consumption and heat loss facilitation in small homeotherms by He-$O_2$. *Am. J. Physiol.* **226**, 490-495.

**Schleucher, E. and Withers, P. C.** (2001). Re-evaluation of the allometry of wet thermal conductance for birds. *Comp. Biochem. Physiol. A* **129**, 821-827.

**Schmidt-Nielsen, K.** (1985). *Scaling: Why is Animal Size so Important?* First edition. Cambridge: Cambridge University Press.

**Schmidt-Nielsen, K.** (1995). *Animal Physiology*. Fourth edition. New York: Cambridge University Press.

**Sharbaugh, S. M.** (2001). Seasonal acclimatization to extreme climatic

conditions by black-capped chickadees (*Poecile atricapilla*) in interior Alaska. *Physiol. Biochem. Zool.* **74**, 568-575.

**Shipley, B.** (2000). *Cause and Correlation in Biology*. First edition. Cambridge: Cambridge University Press.

**Tracy, C. and Walsberg, G. E.** (2002). Developmental and acclimatory contributions to water loss in a desert rodent: investigating the time course of adaptive change. *J. Comp. Physiol. B* **171**, 669-679.

**Turnpenny, J. R., Wathes, C. M., Clark, J. A. and McArthur, A. J.** (2000). Thermal balance of livestock 2. Applications of a parsimonious model. *Agr. Forest Meteorol.* **101**, 29-52.

**Wickler, S. J.** (1980). Maximal thermogenic capacity and body temperatures of white-footed mice (*Peromyscus*) in summer and winter. *Physiol. Zool.* **53**, 338-346.

**Withers, P. C.** (1977). Measurements of metabolic rate, $V_{CO_2}$, and evaporative water loss with a flow through mask. *J. Appl. Physiol.* **42**, 120-123.

**Wooden, K. and Walsberg, G. E.** (2002). Effect of wind and solar radiation on metabolic heat production in a small desert rodent, *Spermophilus tereticaudus*. *J. Exp. Biol.* **203**, 879-888.

**Wootton, J. T.** (1993). Indirect effects and habitat use in an intertidal community: interaction chains and interaction modifications. *Am. Nat.* **141**, 71-89.

**Wunder, B. A. and Gettinger, R. D.** (1996). Effects of body mass and temperature acclimation on the nonshivering thermogenic response of small mammals. In *Adaptations to the Cold: Tenth International Hibernation Symposium* (ed. F. Geiser, A. J. Hulbert and S. C. Nicol), pp. 131-139. Armidale: University of New England Press.