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Individual differences in the behavioural responses of meadow voles to an unfamiliar environment are not correlated with variation in resting metabolic rate

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Abstract

Resting metabolic rate (RMR) is highly variable between individuals within a single species and the relationship between body mass and RMR does not wholly explain this variability. One factor that could account for a portion of the residual variation is animal personality or consistent individual differences (CIDs) in behaviour, but no study has examined this relationship in a free-living population of mammals. In this paper, we test for a relationship between RMR and CIDs in activity in live-trapped meadow voles *Microtus pennsylvanicus* after controlling for the effect of body mass. We quantified the activity levels of voles both in an unfamiliar environment and for the first 2 min in the metabolic apparatus, and then measured RMR using open-flow respirometry. As expected, there was a linear relationship between RMR and body mass, and we found strong evidence for repeatable differences in activity levels between individuals. However, contrary to the hypothesis, we did not identify a significant correlation between CIDs in behaviour and RMR after controlling for body mass. Our results suggest that, at least within species, higher activity levels may not require a greater investment in energetically demanding tissues or increased capacity for processing of energy. Alternatively, if a relationship exists, our inability to detect it may reflect a weak behavioural signal in noisy RMR data that are influenced by many factors. Our results could also reflect an artefact of individual responses to stress or a sampling bias towards more exploratory individuals in animals captured by live-trapping.

Introduction

Basal metabolic rate or resting metabolic rate (RMR), the minimum amount of energy required to sustain life, is highly variable when compared across species and between individuals of a single species (Kleiber, 1961; we modify Speakman, Krol & Johnson's (2004) RMR_t and use 'RMR' to include resting, thermo-neutral animals that may not be post-absorptive). Given the importance of energy balance to survival and fitness, understanding the causes of variation in RMR has implications for understanding many aspects of animal ecology, physiology and behaviour (e.g. McNab, 2008). Although there is a strong relationship between body mass and RMR, body mass only accounts for a percentage of variation in RMR within species (Careau *et al.*, 2008). To account for this residual variation, the effects of many factors on within-species variation in mass-corrected RMR have been tested, including diet (Cruz-Neto & Bozinovic, 2004), geographic region (Hammond *et al.*, 1999; Tieleman *et al.*, 2003), life-history traits (Derting & McClure, 1989; Earle & Lavigne, 1990; Hayes, Garland & Dohm, 1992) and organ weight (Konarzewski & Diamond, 1995; Nespolo *et al.*, 2002; Brzek *et al.*, 2007; Russell & Chappell, 2007). While some of these factors have been shown to influence within-species variation in RMR, other traits have not, and residual variation still remains (Careau *et al.*, 2008).

The influence of consistent individual differences (CIDs) in behaviour on RMR has not been well studied, but may explain some of the residual variability in RMR (Careau *et al.*, 2008; Biro & Stamps, 2010). CIDs in behaviour refer to traits consistently exhibited by an individual in response to different situations that are distinguishable from the behavioural tendencies of conspecifics. CIDs in activity, exploratory behaviour and aggressiveness are heritable and have physiological consequences that can indirectly influence life-history traits and fitness in wild populations (Koolhaas *et al.*, 1999; Réale *et al.*, 2000; Biro & Stamps, 2008).

CIDs in various behaviours can also be correlated, and the relationship between these traits, often defined as animal personality, is typically used to categorize individuals along a continuum ranging from proactive to reactive (Sih, Bell & Johnson, 2004; Sih *et al.*, 2007; Careau *et al.*, 2008). Proactive individuals tend to be more active and aggressive than their conspecifics, and show rapid, superficial exploration of a novel environment (Koolhaas *et al.*, 1999; Careau

et al., 2008). They are predicted to exhibit higher RMRs than reactive individuals because a more energetically expensive lifestyle may require a greater investment in metabolically active tissue and organ systems (Careau *et al*., 2008, 2009; Biro & Stamps, 2010). This hypothesis is supported by the fact that, between species of rodents, those that exhibit rapid but superficial exploration in an open-field test (i.e. proactive species) have higher RMRs than species that explore the novel environment thoroughly (i.e. reactive species; Careau *et al*., 2009). Our objective was to test Careau *et al*.'s (2008) hypothesis that, within species, CIDs in behaviour are correlated with RMR after accounting for the effect of body mass. We predicted that individuals displaying high activity levels in an unfamiliar environment would have higher RMRs than less active individuals.

Methods

Study areas

We captured meadow voles *Microtus pennsylvanicus* at two study sites in southern Manitoba, Canada, during the summers of 2008 and 2009. Both sites were wooded areas with a dense understorey located in a forest-marshland. The first was an 8.4 ha woodlot located within the town of St Léon, Manitoba (49°21′N 98°35′W). The second was located at the University of Manitoba Delta Marsh Field Station (50°11′N 98°23′W), <100 km north of St Léon.

Animal care

All procedures were approved by the University of Winnipeg Animal Care Committee. We used Sherman live traps placed at 10 m intervals to capture voles during July and August, 2008 at St Léon, and the end of July, 2009 at Delta Marsh. We baited each trap with a mixture of peanut butter and rolled oats, and provided cotton wool as nesting material. Traps were set between 22:00 and 23:00 h and checked between 06:00 and 07:00 h the following morning (2008), or left open continuously and checked every 6 h (2009). We recorded body mass at capture and sex for each individual at our field laboratory, located <100 m from the woodlot, and classified voles as adults (>33 g), sub-adults (22–33 g) and juveniles (<22 g) based on their body mass at capture (Boonstra & Rodd, 1983).

Voles were individually housed under semi-natural conditions (natural photoperiod, 25 °C) in standard rodent cages ($40 \times 26 \times 18$ cm) supplied with grass, dried leaves and cotton wool, and a small amount (*c*. 1.0–2.5 g) of the bait mixture. Water was always available but food was removed at least 1 h before metabolic testing. We did not fast animals for longer than 1 h because food deprivation can elevate small rodent activity levels, making it difficult to obtain RMRs (Speakman *et al*., 2004). All voles were tested within 3 days of capture to limit the influence of captivity on our results.

Behavioural testing

We observed activity levels in response to an unfamiliar environment and during respirometry measurements using simple modifications to standard respirometry protocols following Careau *et al*. (2008). In 2008, voles were placed in a transparent plastic container (5.7 cm diameter, 17 cm high, constructed from a 591 mL beverage bottle) large enough for the animal to turn a complete circle and move freely. In 2009, voles were placed in an unfamiliar arena ($60 \times 90 \times 45$ cm) also made of transparent plastic. Each vole's behaviour was recorded for 2 min using a digital video camera. We used these recordings to quantify the proportion of the trial each individual spent investigating the chamber (i.e. searching), grooming and motionless (i.e. frozen). Fewer than half of the individuals ($n = 16/35$) exhibited grooming behaviour during the observation period; hence, we pooled grooming and searching into one category (i.e. activity). To test for short-term repeatability of behaviour within individuals, we also recorded the activity of most study subjects during the first 2 min of their metabolic trials. We were unable to record the behaviour of five animals during the 2008 metabolic trials because the field of view of the camera only allowed observation of two chambers at once, and we occasionally recorded RMR from three individuals simultaneously.

In 2009, we quantified responses to a novel environment using a hole-board test, modified from that described by Martin & Réale (2008). The hole-board arena was built using a transparent plastic box ($60 \times 90 \times 45$ cm) with an enclosed entrance chamber ($15 \times 15 \times 15$ cm) on one long wall and four blind holes (3 cm diameter, 2 cm deep) in the floor. Two of the holes were located near a corner, 5.5 cm from the wall, and two were nearer the centre of the arena, 15 cm from the wall. Animals were placed in the entrance chamber for 2 min before the door to the arena was opened. Voles that had not left the entrance chamber after the door had been open for 3 min were gently pushed into the arena. Once the vole entered the arena, the door to the entrance chamber was closed and the behaviour of each vole was recorded for 10 min using a video camera mounted above the arena. These recordings were later used to quantify nine behaviour variables (Table 1; Martin & Réale, 2008). At the end of each trial, the vole was returned to its home cage, the number of faecal pellets and urine spots in the arena were counted and the arena was cleaned using a mild bleach solution, rinsed with clean water and dried with a paper towel.

RMR

We measured metabolic rate (MR) as oxygen consumption (VO_2) and carbon dioxide production (VCO_2) using open-flow respirometry. We conducted metabolic trials in a dark, temperature-controlled cabinet equipped with an infrared video camera. We maintained the ambient temperature at 30–31.5 °C, which is within the thermoneutral zone for meadow voles (Kurta & Ferkin, 1991). Voles typically

Table 1 Behavioural variables measured during the 10-min hole-board test

Behaviour	Definition
Latency to exit	Number of seconds to exit the entrance chamber and enter the main arena, with a maximum of 180 s. Animals were gently pushed into the arena if they failed to enter on their own after 180 s.
Locomotion	Walking or running around the arena
Scanning	Small movements of the head while otherwise sitting or standing still
Sniffing/chewing	Interactions with the apparatus (walls, floor, door to the entrance chamber, edge of blind holes) either in the form of sniffing or chewing
Rearing	Standing on the hind legs with forepaws on a wall of the arena
Grooming	Washing, scratching or biting of the body
Motionless	Sitting or standing motionless with no apparent movements of the head, includes resting or sleeping
Head-dipping corners	Nose and eyes inserted into one of the two blind holes located near the corners of the arena (5.5 cm from side wall)
Head-dipping centre	Nose and eyes inserted into one of the two blind holes located near the centre of the arena (15 cm from side wall)
# Pellets/urine	Sum of the number of faecal pellets and urine spots deposited in the arena

exhibit nocturnal and diurnal of activity bouts; hence, RMR can be recorded during the resting phase independent of the time of day.

Within an hour of the behavioural test, each vole was sealed in a 255 mL (7.8 cm diameter, 17.5 cm high) cylindrical Plexiglas metabolic chamber. Outside air was dried using Drierite (W.A. Hammond Drierite Co. Ltd, Xenia, OH, USA), and pumped continuously through up to four separate channels (an outside-air reference channel and up to three animal channels) using a diaphragm pump (SGO, Schego, Offenbach, Germany). We used mass flow controllers (GFC17, Aalborg Instruments Inc., Orangeburg, NY, USA) to maintain airflow between 250 and 500 mL min^{-1} depending on the individual's MR. We sub-sampled dried excurrent air from chamber airstreams at a flow rate of 80–150 mL min^{-1} using the flow control system of the gas analyser (Foxbox, Sable Systems International, Las Vegas, NV, USA) and used a computer-controlled respirometry multiplexer (Intelligent Multiplexer V3, Foxbox Sable Systems International) to switch between the reference and the animal channels. We recorded metabolic measurements (including mass flow rates) and corrected gas measurements for drift based on our reference measurements, as well as the lag time between O_2 and CO_2 signals, using ExpeData 1.0.24 (Sable Systems International). In 2008, we recorded from each animal for 9.5 min of every half-hour because we switched channels between up to three animals at a time. In 2009, we recorded data from one animal per session; hence, we were able to record for 13 of every 15-min sampling period, relative to a 2-min sample from the reference channel. We calculated MR for each vole based on $\%O_2$ consumed and $\%CO_2$ produced during the lowest and the most stable 30-s period within the final 90 s of each sampling period (Withers, 2001). VO_2 and VCO_2 were calculated using equations 10.6 and 10.7 from Lighton (2008). We ensured accurate values of RMR by discarding readings taken during the first hour of each trial, and we only analysed the most stable MR values recorded after this point (Lighton, 2008). In addition, the entire metabolic trial was video-recorded for 30/35 individuals to confirm that animals were resting during recording of the MR value we designated as their RMR. We weighed each vole to the nearest ± 0.1 g immediately before and after the metabolic trial, and assumed a linear decrease in body mass during the trial. We used this relationship to determine the mass of each animal at the time during its trial when RMR was recorded. All individuals were released at the site of capture following their metabolic trials after being marked on the ventrum with a non-toxic, indelible felt marker. These marks remained visible throughout the study period, but recaptures were rare and recaptured individuals were not re-tested.

Statistical analysis

We calculated the short-term repeatability of activity levels during the behavioural test and the first 2 min in the respirometry chamber using the coefficient of intraclass correlation (ρ; Hayes & Jenkins, 1997). We used principal components analysis (PCA) to create composite behavioural variables for each vole tested using the hole-board test and selected the number of principal components to retain based on the Kaiser–Guttman criterion (eigen values >1; Kaiser, 1991). Our study population included both males and females from three age classes captured at two locations, and we tested the effect of sex, age and capture site on activity levels and RMR using ANCOVA with body mass as a covariate. We found no influence of sex or age on activity levels or RMR (Table 2); hence, we tested for the influence of our activity measures on RMR using ANCOVA, including capture site but not sex or age in the models, with body mass as a covariate. In addition to testing the influence of activity as a continuous variable, we also categorized individuals as either proactive or reactive based on their activity levels compared with the average duration of activity for all individuals during behavioural testing. We tested for differences between the RMR for individuals of the two behavioural categories using ANCOVA including capture site in the model and body mass as a covariate. We also tested for relationships between mass- and capture site-independent residuals of RMR and our two behavioural categories, as well as our activity measures, using ordinary least squares regression. Similarly, we tested for the relationship between the retained principal components scores for each vole and mass-independent residuals of RMR using

least squares regression. The relationship between log body mass and log RMR was tested using a two-factor ANOVA with capture site and an interaction term (log body mass × capture site) included in the model. We used SPSS 12 (SPSS Inc, Chicago, IL, USA) to calculate repeatability, G*POWER 3.0.8 (Faul *et al.*, 2007) for power analyses, and SYSTAT 11 (Systat Software Inc, Chicago, IL, USA) for all other statistical analyses. Values are expressed as the mean ± SEM. The significance of all tests was assessed at a critical α value of 0.05.

Results

We quantified behaviour and RMR of 36 meadow voles during the study. One pregnant female was excluded from subsequent analyses. Excluding this individual, voles weighed 21.4 ± 1.0 g at the time of capture, with no difference between males and females ($t = 1.9$, d.f. = 33, $P = 0.07$; Table 3). The mean RMR was 71.9 ± 3.2 mL O_2 h^{-1}, which is lower than the values previously published for this species (80.4 ± 3.4 mL O_2 h^{-1} for adult males with an average mass of 44.7 ± 2.0 g; Kurta & Ferkin, 1991). As expected, body mass and RMR were strongly related (Fig. 1). There was no effect of sex or age, but animals captured in 2009 had lower RMRs (Table 2). The relationship between the logarithms of RMR and body mass was also significant after controlling for capture location ($F_{1,33} = 16.1$, $r^2 = 0.33$, $P < 0.001$, RMR = 0.368 × mass−0.480). There was no effect of the interaction between the logarithm of body mass and capture site on log RMR ($F_{1,33} = 1.2$, $P = 0.3$), indicating that the slope of this relationship was not significantly different between the two capture sites.

Table 2 Summary of ANCOVA results for metabolic and behavioural data collected from 35 meadow voles *Microtus pennsylvanicus* captured in southern Manitoba during the summers of 2008 and 2009

Trait	Variable	$F_{1,33}$	P
RMR	Sex	1.2	0.28
	Age	0.33	0.72
	Capture site	**13.1**	**0.001**
	Body mass	**8.1**	**0.01**
Activity	Sex	0.006	0.94
	Age	0.60	0.56
	Capture site	0.19	0.67
	Body mass	0.006	0.94
Searching	Sex	0.55	0.46
	Age	0.85	0.44
	Capture site	0.12	0.73
	Body mass	0.41	0.53

Significant effects are highlighted in bold text. Refer to the 'Methods' section for details of data collection methods and statistical tests.
RMR, resting metabolic rate.

Behavioural testing

Activity levels were repeatable within individuals. Voles that spent more time active during the pre-trial behavioural test were more likely to be active during the metabolic trial (Fig. 2; interclass correlation coefficient, $\rho = 0.67$, $F_{1,29} = 5.1$, $P < 0.001$). This relationship was significant for animals tested in both years (2008: $\rho = 0.68$, $F = 5.3$, $P = 0.001$; 2009: $\rho = 0.59$, $F = 4.7$, $P = 0.003$). We found no significant effect of sex, age, body mass or site on time spent active during the pre-trial behavioural test (Table 2). Similarly, there was no effect of sex, age, body mass or site on the time spent searching (Table 2). There was also no effect of sex, age or site on the likelihood of an individual being categorized as proactive or reactive (Table 3; sex: $\chi^2 = 0.2$, $P = 0.63$; age: $\chi^2 = 0.7$, $P = 0.69$ site: $\chi^2 = 0.04$, $P = 0.84$).

Behaviour in the hole-board test was best described by three variables in the PCA, which explained 79% of the total variance (Table 4). The first component showed that animals spending a larger proportion of the test motionless were less likely to visit the two holes nearer the centre of the arena. Based on the second component, voles that showed a high level of grooming behaviour were less likely to rear during the test. Finally, the third component opposed passive exploration (i.e. scanning) and visits to the holes

Table 3 Mean (± standard error) body mass and RMR, and proportion of proactive male and female meadow voles *Microtus pennsylvanicus* from three age classes trapped at two capture locations in southern Manitoba

Site	Age	Sex	n	Body mass (g)	RMR (mL O_2 h^{-1})	Proportion proactive
St Léon	Juvenile	Male	5	19.3 ± 1.3	70.4 ± 5.8	3/5
		Female	5	17.6 ± 1.2	71.2 ± 4.8	3/5
	Sub-adult	Male	3	26.7 ± 2.8	98.0 ± 8.2	1/3
		Female	5	28.4 ± 1.5	87.4 ± 5.0	2/5
	Adult	Male	1	33.75	118.0	1/1
		Female	1	35.6	96.7	0/1
Delta Marsh	Juvenile	Male	11	16.6 ± 0.8	57.5 ± 2.4	6/11
		Female	0	–	–	–
	Sub-adult	Male	4	22.8 ± 0.5	57.5 ± 4.4	2/4
		Female	0	–	–	–

Note that no adults were captured at the Delta Marsh field site.
RMR, resting metabolic rate.

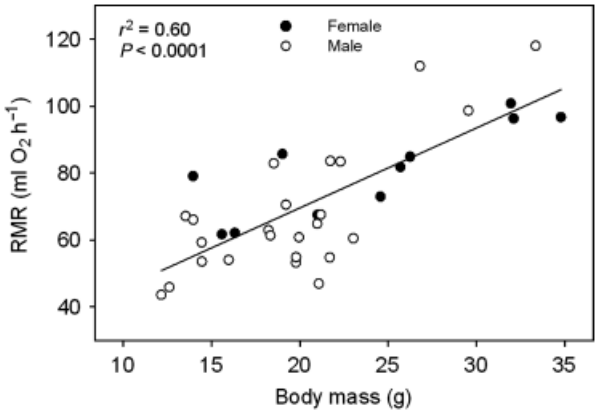

Figure 1 Resting metabolic rate (RMR) as a function of body mass for 35 wild-caught meadow voles *Microtus pennsylvanicus*. Males (open circle) and females (closed circle) are plotted separately, but the regression line shown is calculated for the sexes combined. Although there is a highly significant effect of body mass on RMR, a considerable proportion of the variation remains unexplained (see 'Results').

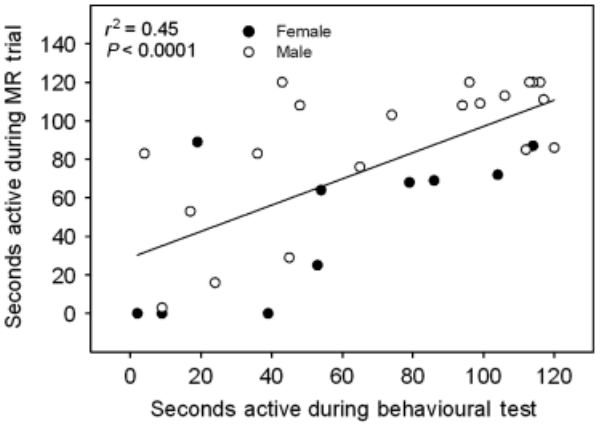

Figure 2 Confirmation of the repeatability of activity levels for 30 voles *Microtus pennsylvanicus* measured during a 2-min behavioural test and during the first 2 min of the metabolic trial. Voles that spent more time active during the first test were also highly active in the metabolic chamber. A regression line (for males and females combined) is included as a reference, but was not used to determine the repeatability of activity levels (see 'Results').

closer to the corners of the arena. There was no effect of age on PCA scores (PC1: $F_{1,14} = 0.9$, $P = 0.36$; PC2: $F_{1,14} = 0.1$, $P = 0.81$; PC3: $F_{1,14} = 1.9$, $P = 0.19$).

Activity and RMR

The amount of time an individual spent active during the pre-trial behavioural test had no influence on RMR ($F_{1,33} = 0.17$, $P = 0.68$), although the effect of body mass on RMR was significant ($F_{1,33} = 30.1$, $P < 0.001$), as was the site of capture ($F_{1,33} = 13.4$, $P = 0.001$). Similarly, there was no effect of the time spent searching on RMR ($F_{1,33} = 0.13$, $P = 0.71$), while the effects of body mass ($F_{1,33} = 30.1$, $P < 0.001$) and capture site ($F_{1,33} = 12.9$, $P = 0.001$) were significant. We also calculated mass- and site-independent residuals of RMR and tested for a relationship between these residuals and our measures of activity. There was no relationship between residuals and pre-trial activity ($F_{1,33} = 0.04$, $P = 0.84$, $r^2 = 0.001$) or searching time ($F_{1,33} = 0.06$, $P = 0.80$, $r^2 = 0.002$). Based on a prospective power analysis, the sample size required to detect a medium to large effect size for this kind of analysis was between 55 and 25 animals (i.e. $f^2 = 0.15$ and 0.35), very close to our sample size. Thus, our results indicate that, if an effect exists, it is relatively small.

Similarly, when we divided individuals into proactive and reactive categories, we found no effect on RMR. After controlling for the significant effect of body mass ($F_{1,33} = 29.5$, $P < 0.001$) and capture site ($F_{1,33} = 13.5$, $P = 0.001$), we found no significant effect of personality on RMR ($F_{1,33} = 0.05$, $P = 0.82$). When we compared mass- and site-independent RMR residuals between proactive and reactive individuals, again, there was no significant relationship ($F_{1,33} = 0.04$, $P = 0.84$). Furthermore, when we considered the behaviour of animals observed in the more rigorous hole-board test, we found no effect of the principal components scores on mass-independent RMR residuals (PC1: $F_{1,13} = 0.04$, $P = 0.84$, $r^2 = 0.003$; PC2: $F_{1,13} = 0.06$, $P = 0.80$, $r^2 = 0.005$; PC3: $F_{1,13} = 0.47$, $P = 0.50$, $r^2 = 0.03$).

Table 4 Summary of principal components analysis of the hole-board test data for 15 voles *Microtus pennsylvanicus* captured at Delta Marsh Field Station in July, 2009

Variables	Component 1	Component 2	Component 3
Latency to exit	−0.31	−0.17	0.31
Locomotion	0.37	−0.32	−0.06
Scanning	0.26	−0.10	**0.60**
Sniffing/chewing	0.32	0.23	0.35
Rearing	0.25	**−0.52**	−0.33
Grooming	0.29	**0.48**	0.05
Motionless	**−0.44**	0.06	−0.01
Head-dipping corners	0.18	0.31	**−0.56**
Head-dipping centre	**0.41**	−0.26	0.03
# Pellets/urine	0.24	0.36	0.01
Standard deviance	4.97	1.73	1.21
% of total variance	0.50	0.17	0.12
Cumulative proportion of total variance	0.50	0.67	0.79

Only the principal components retained based on the Kaiser–Guttman criterion (Kaiser, 1991) are shown. Bolded values represent behaviours that contributed significantly to a particular component (eigen vectors >0.4; Martin & Réale, 2008).

Discussion

We found strong evidence of repeatable differences in behavioural activity between individual meadow voles. Voles that were more active during behavioural testing also

exhibited higher levels of activity during the recording period at the beginning of the metabolic trial. However, although we identified individual differences in activity that were repeatable over time and consistent in different environments, contrary to our prediction, we found no evidence of a correlation between CIDs in behaviour and RMR. We expected more active individuals to exhibit higher RMRs than less active animals for two reasons. First, a more energetically expensive lifestyle characterized by higher levels of aggression and activity might require larger than average organ systems and metabolically active tissues to support this behaviour (Careau *et al*., 2009, 2008; Biro & Stamps, 2010). Within a species, organ size is positively correlated with RMR in some studies (Konarzewski & Diamond, 1995; Nespolo *et al*., 2002; Brzek *et al*., 2007; Russell & Chappell, 2007; but see: Koteja, 1996; Corp, Gorman & Speakman, 1997; Speakman *et al*., 2004), which could result in increased RMR for proactive animals. Second, this pattern has been observed in a comparison across species. Rodent species that spend more time exploring in a novel environment have higher RMRs than less exploratory species (Careau *et al*., 2009).

Although individual differences in physiology and behaviour are viewed as the raw material necessary for larger-scale evolutionary change, the presence of a significant relationship between RMR and CIDs in activity at the interspecific, but not the intraspecific level, is not entirely surprising. For example, although life-history traits and RMR appear to be correlated between species (e.g. McNab, 1980, 2008; Kalcounis-Rüppel, 2007; but see Harvey, Pagel & Rees, 1991), the same relationship has been difficult to demonstrate within species (Derting & McClure, 1989; Earle & Lavigne, 1990; Hayes *et al*., 1992; Speakman *et al*., 2004). Stearns (1983) predicted that the number of life-history traits covarying with body size would decline with the taxonomic level; hence, there would be fewer, different or weaker relationships at the intraspecific level compared with between species. As a result, factors other than CIDs in behaviour could exert a stronger influence on the investment in metabolically active tissues and RMR within species. One possibility is that both proactive and reactive voles face similar selection pressure to minimize energy expenditure while at rest (i.e. direct selection on RMR) and/or maximize it during intense activities like escaping a predator (i.e. indirect selection influencing RMR). Thus, at least within our study species, it is possible that CIDs in activity do not require a greater investment in energetically demanding tissues or increased capacity for energy processing.

Various factors may have contributed to our inability to detect a weak relationship between RMR and CIDs in behaviour at the intraspecific level. For example, proactive animals are easier to trap because they actively search out new environments; hence, live-trapped samples are biased towards more active individuals (Biro & Dingemanse, 2009). Live-trapping of meadow voles is also known to be stressful (Fletcher & Boonstra, 2006). Thus, some voles that spent less time active during behavioural testing may in fact have been highly stressed, resulting in artificially elevated RMR values due to an increase in breathing and heart rate (Careau *et al*., 2008). Our behavioural measures may also have been influenced by a confound between the testing environment and the capture site. However, we found no evidence of an activity difference between the two sites/testing environments and voles that showed high levels of activity in either type of test were also highly active in the metabolic chamber. Moreover, we did identify an effect of capture site on RMR, which implies we had sufficient power to detect a difference in the traits of voles between sites. Although we did not identify an effect of age on RMR or behaviour in our study population, age can also have a significant effect on RMR (Biro & Stamps, 2010; Larivée *et al*., 2010). In contrast, CIDs in behaviour are thought to remain stable with age (Carere *et al*., 2005; Ray & Hansen, 2005). The metabolic cost of reproduction, particularly in females, could also influence RMR (Trebatická *et al*., 2007; Biro & Stamps, 2010). However, reproductive status, including different stages of pregnancy, has a surprisingly small effect on the RMR of individual females, with most of the variation explained by changes in body mass associated with pregnancy and lactation (Trebatická *et al*., 2007). As we saw no effect of sex and age on the body mass/RMR relationship, this may not have been a significant source of variation in RMR. Lastly, although our sample size is comparable to or larger than typical for many ecophysiological studies of mammals, and our statistical power was within the normal range for studies in animal ecology, this range is considered lower than ideal (e.g. Nakagawa, 2004) and may be insufficient to detect a weak effect of CIDs in behaviour in noisy RMR data.

We designed our study based on some of the recommendations made by Careau *et al*. (2008) when proposing their hypothesis, but our data do not support the hypothesis that CIDs in behaviour are correlated with RMR within species. We recommend that future studies assess multiple CIDs in behaviour and control for the possible influences of sex and reproductive status (Biro & Stamps, 2010). Although this was not possible for our study, we suggest repeating measurements of MR and behaviour following the release and re-recapture of individuals to examine repeatability over longer timescales, and to control for the effects of age (Biro & Stamps, 2010). We also recommend using larger sample sizes than typical for ecophysiological studies (e.g. $n = 300+$ animals; Faul *et al*., 2007) to improve statistical power in the face of small effect sizes. Taken together, these steps may improve the ability to detect a weak but potentially important intraspecific relationship between CIDs in behaviour and RMR.

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Don Thomas, a co-author on the article that inspired this study.

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