Limited mass-independent individual variation in resting metabolic rate in a wild population of snow voles *(Chionomys nivalis)*

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Abstract

Resting metabolic rate (RMR) is a potentially important axis of physiological adaptation to the thermal environment. However, our understanding of the causes and consequences of individual variation in RMR in the wild is hampered by a lack of data, as well as analytical challenges. RMR measurements in the wild are generally characterized by large measurement errors and a strong dependency on mass. The latter is problematic when assessing the ability of RMR to evolve independently of mass. Mixed models provide a powerful and flexible tool to tackle these challenges, but they have rarely been used to estimate repeatability of mass-independent RMR from field data. We used respirometry to obtain repeated measurements of RMR in a long-term study population of snow voles (*Chionomys nivalis*) inhabiting an environment subject to large circadian and seasonal fluctuations in temperature. Using both uni- and bivariate mixed models, we quantify individual repeatability in RMR and decompose repeatability into mass-dependent and mass-independent components, while accounting for measurement error. RMR varies among individuals, *i.e.* is repeatable (R=0.46), and strongly co-varies with BM. Indeed, much of the repeatability of RMR is attributable to individual variation in BM, and the repeatability of mass-independent RMR is reduced by 41% to R=0.27. These empirical results suggest that the evolutionary potential of RMR independent of mass may be severely constrained. This study illustrates how to leverage bivariate mixed models to model field data for metabolic traits, correct for measurement error, and decompose the relative importance of mass-dependent and mass-independent physiological variation.

Keywords

adaptation, constraints, measurement error, metabolism, mixed model, repeatability, rodent

Introduction

Given its key role in maintaining homeostasis, metabolism is a major dimension of adaptation, especially to climatic variation (Lovegrove, 2003). The number of studies focusing on the causes and consequences of physiological variation, mostly by measuring metabolic rate (White *et al.*, 2013), has therefore increased profoundly during the last decade. However, despite technological advances, obtaining respirometric measurements in the wild remains challenging (Nespolo & Franco, 2007), and hence estimates of individual variation of metabolic rates and other physiological traits under natural conditions continue to be scarce. As a consequence, the causes and consequences of individual variation in physiological traits in a natural setting remain poorly understood.

In addition to any technical complications, the interpretation of metabolic measurements is often hampered by their strong dependency on body mass (e.g. Kleiber 1932; Darveau et al. 2002; White & Seymour 2003). Metabolic rates typically scale allometrically with body mass, and the corresponding scaling exponents and the underlying explanations have been the subject of debate for over a century (White & Seymour, 2003). The relationship depends on taxonomic group (White & Seymour, 2003; Glazier, 2005, 2008), but also on factors such as physiological state and activity level, suggesting that an universal scaling law does not exist (Hayssen & Lacy, 1985; Glazier, 2008). The strong correlation between metabolic rate and body mass hampers the ability to identify both the selective agents and the target of selection.

The interdependency of body mass and metabolism raises the question regarding the extent to which metabolism has the potential to evolve independently from body mass (Rønning *et al.*, 2007; Nilsson *et al.*, 2009). As the raw material on which natural selection is acting are consistent between-individual differences, a necessary pre-condition for metabolism to evolve independently of body mass is the existence of individual variation in metabolism that is independent of body mass (White *et al.*, 2013). The constancy of individual phenotypes over time is typically quantified by the repeatability, defined as the proportion of phenotypic variation that is attributable to between-individual differences (Dohm, 2002), and this captures both genetic variation and permanent environmental effects. Hence the repeatability can be considered as an estimate for the upper limit of heritability (Falconer & Mackay, 1996); but see Dohm (2002) for exceptions. The non-repeatable fraction of the phenotypic variation includes phenotypic flexibility and measurement error (Nakagawa & Schielzeth, 2010). Hence, repeatability estimates have long been a useful concept in evolutionary biology, and studies on the repeatability of metabolic rates have increased in recent years (White *et al.*, 2013), especially when formal quantitative genetic analyses are not possible (Wilson, 2018).

Here we aim to estimate evolutionary potential of a metabolic trait, the resting metabolic rate (RMR), in a wild population of snow voles (*Chionomys nivalis*, Martins 1842) in the Swiss Alps.

Snow voles live in a high-altitude rocky environment characterized by large daily and seasonal fluctuations in temperature. This may have led to fine-tuned physiological adaptations, making RMR a trait of selective importance in this species. We used short- and long-term repeated measurements of RMR made during two consecutive years to decompose variation into within- and among-individual variation while explicitly accounting for any covariation between RMR and body mass, with the ultimate aim to estimate the mass-independent repeatability of RMR.

Although statistical methods to decompose phenotypic variation (e.g. ANOVA, linear mixed effect models) are well established, how to derive a meaningful estimate of repeatability from such a decomposition is non-trivial and depends on the question at hand (Wilson, 2018). Although nuisance parameters that contribute to the residual variance should be controlled for and excluded from the calculation of repeatability (Nakagawa & Schielzeth, 2010), the question what constitutes a nuisance parameter is not always easily answered. Furthermore, measurement error can lead to substantial downward bias in repeatability estimates. Whenever possible, measurement error variance should therefore be explicitly estimated and excluded from the total phenotypic variance. This is particularly important when measuring metabolic rates, as oxygen consumption is usually measured by flow-through respirometry, a technique that includes considerable error variance (Nespolo & Franco, 2007). Repeated measurements taken over different time scales (i.e., short-term and long-term repeated measurements) can be used to separate transient (e.g. measurement error) from permanent environmental effects and hence to obtain unbiased repeatability estimates (Ponzi *et al.*, 2018). Finally, in physiological research, it is common to rescale metabolic rates to obtain mass-specific metabolic rates. However, this can lead to misleading conclusions (Wilson, 2018).

Here, we avoid these problems by building upon Wilson (2018) and Ponzi *et al.*, (2018). We explicitly model the variance-covariance structure of body mass and RMR by 1) fitting mass as an additional covariate, and 2) as an additional dependent variable in a bivariate model, to estimate the individual repeatability of (body mass-independent) RMR, as well as the importance of body mass, sex, age and time of day in shaping variation in RMR. Furthermore, we explicitly quantify measurement error variance. By decomposing phenotypic variation in metabolic rate into repeatable and non-repeatable variation, we provide insights into the adaptive potential of metabolism in a wild rodent population, and we contribute to the understanding of physiological adaptations to seasonal climatological changes in wild animal populations in general.

Material and methods

Study species, study area and trapping

The snow vole is a medium-sized (up to ~ 13 cm) rodent of the family Cricetidae, with adult males being approximately 10% heavier than adult females (Metcheva *et al.*, 2008). It is widely distributed

across the mountainous regions of Europe and almost exclusively inhabits screes up to 4000 meters above sea level (Luque-Larena *et al.*, 2002). Snow voles do not hibernate and instead are active underneath the snow layer during winter (Janeau & Aulagnier, 1997).

A snow vole population located near the Churer Joch at 2030 meter above sea level in the Swiss Alps (46°47' N, 9°34' E) has been intensively monitored since 2006 (Bonnet *et al.*, 2017). The site covers approximately 5 ha and is characterized by isolated screes interrupted by grass, providing typical snow vole habitat (Janeau & Aulagnier, 1997). The average monthly temperature ranges from -4 to 12 °C, and during approximately six months of the year (from November to April) the site is covered by snow.

Snow voles are live-trapped between mid-June and early October in a standardized manner. To this end, the study area is subdivided into 559 cells of 10 × 10 m. Four trapping nights are necessary to trap once in each grid cell. A live-trap (Longworth model, Penlon Ltd, Oxford, UK) supplied with hay and grains and baited with peanut butter and a piece of apple is set within each of the grid cells. Individuals caught for the first time are equipped with a PIT tag (ISO transponder, Tierchip Dasmann, Tecklenburg, Germany) for identification at recapture. For all captured individuals, sex, age (juvenile or adult), body mass, body length, tail length, abundance of external parasites (mites) as well as time and place of capture were recorded. Snow voles were classified as 'juvenile' when captured in the same year as they were born, and as 'adult' after they had survived their first winter. Distinguishing between juveniles and adults is possible based on body mass and length (adults are never below 30 g and 97 mm), fur colour and quality (juveniles have softer, thinner hairs). Note that although early-born juveniles are largely indistinguishable from adults towards the end of the season, the regular monitoring of the population before and during the reproductive period means that these individuals will have been caught at least once earlier in the season when they could still be identified as juvenile.

Metabolic measurements

In addition to the standard monitoring protocol, additional trapping sessions took place during two weeks in September and October 2014 and 2015. Depending on the part of the study area, we placed 57–63 traps in locations in which trapping success is relatively high. Whereas normally trapping takes place overnight, these trapping sessions took place between 5:00 (approximately two hours before sunrise) and 16:00. Traps were checked every two to three hours, up to three times per session. Newly tagged individuals, as well as pregnant or lactating females, were released upon capture to minimize stress and because these factors may influence metabolic rates. Captured individuals were transported in plastic respiration chambers (18.4 × 13 × 8.9 cm) to the respirometer, located approximately 1 km away from the field site. Body mass was measured twice, directly before and after respirometry using an electronic scale with a precision of 1 g. To minimize stress before respirometric measurements, the

remaining phenotypic data (i.e., sex, age, body length, tail length, and external parasites) were recorded only just before release at the point of capture. The complete procedure from the time when the voles were removed from the traps and released took on average 3 h 30 min.

RMR is defined as the metabolic rate of an animal that is resting in a thermoneutral environment but that is not in a post-absorptive state (IUPS Thermal Commission 2001). Here we aimed at measuring RMR as oxygen consumption ($mL \cdot min^{-1}$) using an open-flow respirometric system (Sable Systems International, Las Vegas, USA). Air was pumped into each chamber at 500-530 $mL \cdot min^{-1}$ by means of an eight-channel mass flow meter system (Flowbar-8 Mass Flow Meter/Pump FB-8-1, Sable Systems International, Las Vegas, USA). Air was sampled from one chamber at a time (Multiplexer Intelligent RM-8-2, Sable Systems International, Las Vegas, USA), dried (magnesium perchlorate, Sigma-Aldrich, USA), and analysed every second by a barometric pressure-compensated fuel cell O_2 analyser and a dual wavelength infrared bench CO_2 analyser (Foxbox, Sable Systems International, Las Vegas, USA). The data was recorded using ExpeData software v1.01 (Sable Systems International, Las Vegas, USA). The respiration chambers, always including one empty chamber used as a control, were placed within one of two temperature-controlled incubators (Exo-Terra) set at 20 °C. This is the upper limit of the thermoneutral zone for snow voles, which ranges from 15 to 20 °C (Bienkowski & Marszalek, 1974; Metcheva *et al.*, 2008). As digestive processes influence metabolic rates (White & Seymour, 2003), animals were kept without food or water. In addition, animals were kept in the dark to reduce stress. During a measurement session, each chamber was measured for 5 minutes before switching to the next chamber. To get short-term repeated measurements, and to maximise the probability an animal reached a resting state, each chamber (including the control chamber) was measured three times. See the Discussion for a critical discussion of the accuracy and precision of the measurements obtained using this setup.

We took the body mass before the beginning of a measurement session to represent an individual's mass during the first measurement, and the mass immediately after measurements had ended as the mass during the third measurement. The body mass during the second measurement was interpolated by taking the mean of the mass at the beginning and at the end.

Calculation of oxygen Consumption

All analyses were performed using R v 3.5.3 (R Core Team 2019). To correct for drift in the fractional oxygen and carbon dioxide content of the incurrent air over the course of a measurement session, both were interpolated from measurements from the control chamber at the start and finish of the session. Given that the mass flow meter was upstream from the metabolic chamber and so CO_2 was not removed from the excurrent air stream, we used the following equation to calculate consumed oxygen (VO_2) (Lighton, 2008):

$$VO_2 = \frac{FR_i[(F_iO_2 - F_eO_2) - F_eO_2(F_eCO_2 - F_iCO_2)]}{1 - F_eO_2}. \quad \text{eqn 1}$$

FR_i is the total incurrent (*i*) flow rate, and F_iO_2, F_iCO_2, F_eO_2 and F_eCO_2 are the fractional concentrations of the incurrent (*i*) and excurrent (*e*) gas species (O_2 and CO_2), respectively.

Large fluctuations in consumed oxygen are indicative of either the animal being in a non-resting state or of measurement error. To select in an efficient and objective manner only those measurements where an animal was likely to be resting, we first calculated the mean oxygen consumption and its standard deviation for all possible 60 s intervals within each measurement period. We subsequently excluded those intervals with a standard deviation greater than 0.015 $mL \cdot min^{-1}$. Of the remaining intervals, we used the interval with the lowest mean oxygen consumption. The threshold of 0.015 $mL \cdot min^{-1}$ corresponds to three times the typical standard deviation of the measurements within a 60 s interval of the baseline (i.e., control chamber; ~ 0.005 $mL \cdot min^{-1}$).

Over a total of 14 trapping days, 23 measurement sessions and 99 captures, we were able to obtain 172 measurements for a total of 59 individuals (Fig. 1). Twenty-eight individuals were captured multiple times, five of which were captured in both years. RMR could not be obtained for 10 individuals (15 captures) because they were never in a resting state. In addition, RMR of one animal, which probably was in poor condition, was more than three standard deviations below the mean RMR, and therefore excluded from further analyses.

Respiratory quotient

We calculated the respiratory quotient (RQ), the ratio of produced CO_2 (VCO_2) over consumed O_2 (VO_2), to assess the quality of our RMR measurements. RQ is an indicator of the fuel source used in metabolism (Richardson, 1929), and at least in theory an RQ close to 0.7 is suggestive of mainly fat is being metabolized, whereas it approximates 1 in the case of purely carbohydrate-based metabolism. It has been suggested that the range of RQ values observed can be used to assess the quality of RMR measurements (Compher *et al.*, 2006). However, under atypical metabolic and respiratory conditions the RQ can substantially deviate from the range 0.7-1. For example, RQ's below 0.7 have been interpreted as the result of gluconeogenesis (Mori, 1979) or incomplete fat oxidation (Walsberg & Wolf, 1995). To calculate RQ, we first calculated the mean VCO_2 across all time points for which an RMR measurement was available using

$$VCO_2 = \frac{FR_i[(F_eCO_2 - F_iCO_2) - F_eCO_2(F_iO_2 - F_eO_2)]}{1 - F_eCO_2}. \quad \text{eqn 2}$$

We then calculated the RQ as VCO_2/VO_2. RQ values ranged from 0.61 to 0.86, with 78% of the RQ values being above 0.7 and all values being smaller than 1 (Fig. S1).

Variance decomposition

To identify the biologically relevant sources of variation in RMR, we fitted a series of linear mixed effect models using (restricted) maximum likelihood. The fixed part of the model included hour of day (mean-standardised) and hour of day^2 (squared after mean-standardisation) as covariates, as well as age (juvenile or adult), sex and year as factors. As expected, effects of outdoor temperature and (realised) incubator temperature (ºC), and of incubator and outdoor relative humidity (%), were small and non-significant (incubator temperature: b=0.017±0.022, χ^2_1=0.700, *P*=0.403; outdoor temperature: b=-0.002±0.011, χ^2_1=0.064, *P*=0.800; incubator humidity: b=-0.0041±0.0028, χ^2_1=2.037, *P*=0.154; outdoor humidity: b=-0.0006±0.0019, χ^2_1=0.108, *P*=0.743). Hence, these covariates were excluded from any further analyses.

Individual ID was fitted as a random effect to estimate the variance attributable to systematic differences in RMR among individuals. Furthermore, we fitted respirometer chamber number (15 levels, as except for the control chamber, different chambers were used in both years) and trapping date, to account for systematic differences among chambers and trapping days, respectively. Finally, we fitted the identity × trapping day interaction, again as a random effect.

The simultaneous inclusion of ID and the ID × trapping session interaction as random effects allows us to separate the variance among individuals across two time-scales. The random ID effect will estimate the degree of individual consistency across longer periods of time (from days up to a year; V_{ID}). The ID × trapping session interaction on the other hand will capture the individual consistency of RMR within a single measurement session (i.e., across the five-minute measurement periods within a single capture; $V_{ID\times trap\ session}$) *over and above* the consistency across sessions as captured by the random ID effect. A useful consequence of this random effects specification is that, assuming an individual's true RMR remains constant over the course of a measurement session, the residual variance (i.e., the variance in RMR left unexplained by the model; V_R) is equal to the within-individual and -measurement session variance, and thereby provides an estimate of the measurement error variance.

From the above it follows that the proportion of the variance attributable to individual differences in RMR that is free from the variance introduced by measurement error, *i.e.* the repeatability, can be calculated as

$$R = \frac{V_{ID}}{V_{ID} + V_{trap\ session} + V_{ID\times trap\ session}} \qquad \text{eqn 3}$$

Importantly, the repeatability calculated is conditioned on the fixed effects included in the model. Furthermore, note that we did not include the variance accounted for by chamber in the denominator, as this is not a natural source of variation in RMR.

The proportion of the variance in RMR explained by the fixed effects, as well as the proportion of variance explained by the fixed and random effects combined, were calculated following Nakagawa & Schielzeth (2013).

To quantify the degree to which individual variation in BM shapes the long-term repeatability of RMR, we subsequently repeated the analyses above, but this time including BM as a covariate. This provides an estimate of the degree to which, for example, differences in RMR among juveniles and adults, and among males and females, are mediated by differences in BM. Furthermore, it allows us to obtain an estimate of how much of the short- and long-term repeatability of RMR is independent of individual variation in BM.

For completeness, and in preparation for the bivariate model outlined below, we also fitted a model with the same fixed and random effects structure for BM itself. Note that similar to the RMR model, the residual variance estimate provided by the model represents the variance in BM within a single measurement session (animals were weighed at the beginning and the end). This variance is composed of measurement error and any short-term (most likely negative) changes in BM. The variance captured by ID × trap session represents (biologically more interesting) within-individual changes in BM across longer periods of time. As was the case for RMR, the residual variance was excluded from the denominator when calculating the repeatability.

As an alternative to the univariate model including BM as a covariate, we fitted a bivariate model with BM as an additional dependent variable. To aid convergence, we did not fit a random respirometer chamber effect and trap session effect for BM (which were effectively zero), but otherwise the fixed and random effect structure was the same as for the univariate RMR and BM models. Importantly, this bivariate model does not only provide an estimate for all fixed and random effects, but it also estimates the among- and within-individual covariance between RMR and BM ($COV_{ID_{BM,RMR}}$ and $COV_{ID\times trap\ session_{BM,RMR}}$, respectively). Unlike the univariate model including BM, this bivariate model hence allows us to estimate the effect of BM on RMR on both a between- and a within-individual level, where the inter-individual slope of RMR regressed on BM is given by

$$b_{between} = \frac{COV_{ID_{BM,RMR}}}{V_{ID_{BM}}} \qquad \text{eqn 4}$$

and the intra-individual slope by

$$b_{within} = \frac{COV_{ID\times trap\ session_{BM,RMR}}}{V_{ID\times trap\ session_{BM}}} \quad \text{eqn 5}$$

Similarly, the among and within-individual correlations between RMR and BM are provided by

$$r_{between} = \frac{COV_{ID_{BM,RMR}}}{\sqrt{V_{ID_{BM}} V_{ID_{RMR}}}} \quad \text{eqn 6}$$

and

$$r_{within} = \frac{COV_{ID\times trap\ session_{BM,RMR}}}{\sqrt{V_{ID\times trap\ session_{BM}} V_{ID\times trap\ session_{RMR}}}} \quad \text{eqn 7}$$

These correlation coefficients squared (i.e., $r^2_{between}=R_{between}$ and $r^2_{within}=R_{within}$) provide us with the proportion of variance that is shared between BM and RMR on the between- and within-individual level.

To aid interpretation, we use non-transformed RMR and BM in all analyses outlined above. However, with an eye to further comparative analyses, we also estimated the intraspecific mass-scaling exponent (Kleiber, 1932) by refitting the models above with $\log_{10}$-transformed RMR and BM. Albeit on a log-scale, these models provided very similar results and other than the mass-scaling exponent itself these results are not presented here.

Mixed models were fitted using the R packages lme4 (Bates *et al.*, 2015) and ASReml-R v4 (Butler *et al.*, 2018). Statistical significance of fixed and random effects is based on likelihood ratio tests, where the degrees of freedom is equal to the number of parameters estimated by the effect in question (fixed effects), or a mixture of 0 and 1 degrees of freedom (random effects; following Self & Liang 1987).

Results

Mixed models excluding BM as a covariate revealed that RMR was higher in adults than in juveniles, and was higher at the beginning and the end of the day (Table 1). All other fixed effects were poorly estimated and/or statistically non-significant. In total, this model explained 85% of the variation in RMR, and 46% of the variation could be attributed to the fixed effects. Accounting for measurement error variance and variance among respirometer chambers, as well as the variance explained by the fixed effects, the repeatability of RMR (SE) was substantial at 0.46 (0.18).

As expected, the inclusion of BM as a covariate revealed a strong and positive relationship between BM and RMR. Furthermore, this removed the effect of age (i.e., older individuals have a higher RMR because they are heavier), but the quadratic effect of time of day remained. Inclusion of BM increased the proportion of the variance in RMR explained by the fixed effects to 53%, but as the variance explained by ID went down, the total variance explained remained very similar (84%). After accounting for variation in BM, the repeatability of RMR (SE) was 0.27 (0.2).

In accordance with previous analyses of BM in this population (Bonnet & Postma, 2016; Bonnet *et al.*, 2017), we found that adults are heavier than juveniles, males are heavier than females, and BM declines during the day (Table 1). Furthermore, variation in body mass was repeatable ($R \pm SE = 0.45 \pm 0.14$).

The bivariate model provided very similar estimates for the fixed effects as those obtained by the two univariate models (not presented), as well for the random effects (Table 2). Most importantly, there was a significant and strong association between RMR and BM on the among-individual level only. In other words, a difference in BM of 1 gram *between* two individuals translate into a difference in RMR of 0.056 $mL \cdot min^{-1}$. On the other hand, an individual gaining a gram only increases its RMR by a (non-significant) 0.011 $mL \cdot min^{-1}$.

Using log_{10}-transformed RMR and BM in the univariate model provided an intraspecific mass-scaling exponent of 0.506 ± 0.095. However, this estimate ignores the origin of the variation in BM and assumes that an increase in BM has the same effect on RMR if it is the result of age, sex, within- or among-individual effects. Importantly, the bivariate model with log_{10}-transformed RMR and BM instead provides us with an estimate of the mass-scaling exponent on both an among and a within-individual level, while conditioning on age and sex effects on BM. When we do this, we find an among-individual mass-scaling exponent of 0.90 ± 0.25.

Discussion

Metabolism is a major dimension of adaptation, especially to climatic variation (Lovegrove, 2003). Here we used respirometry to obtain repeated measurements of resting metabolic rate (RMR) in a wild population of snow voles in the Swiss Alps. Using a mixed model framework, we quantified the individual repeatability of RMR and the part of repeatability that is independent of body mass (BM). We found RMR to vary consistently among individuals, *i.e.* to be repeatable, and a strong positive relationship between RMR and BM. Indeed, much of the repeatability of RMR is attributable to individual variation in BM, and the inclusion of BM reduced the repeatability of RMR from 46% to 27%. In addition, we assessed the importance of sex, age, and time of day. We found a significant positive quadratic relationship between RMR and the time of the day, with RMR being higher at the

beginning and the end of the day. The effect of age on RMR was mainly explained by juveniles being lighter than adults. In contrast, despite a sex difference in BM, there was no clear sex difference in RMR. Below we will first critically evaluate the quality of our RMR measurements, before discussing the analytical framework as adopted here, and finally the implications of our findings for the current and future adaptation of the species.

Quality of the metabolic measurements

Respirometric measurements in the field are challenging, and before interpreting our results biologically, we must discuss what it is that was measured, and whether it is an accurate and precise measurement of RMR. Whereas in other snow vole populations the TNZ has been estimated to range from approximately 15 to 20 °C (Bienkowski & Marszalek, 1974; Metcheva *et al.*, 2008), our measurements were taken at a temperature of around 20 °C (median: 19.7 °C; range: 17.9 °C to 22.9 °C). Although given the small range of temperatures statistical power is relatively low, we found no relationship between RMR and incubator temperature, which is in line with measurements having been obtained within the snow vole TNZ.

A second potential issue is the latency time that is intrinsic to open respirometry: if an animal changes its metabolic rate, it may take several minutes for the gas mix in the chamber to reach its new equilibrium (Lighton, 2008). Therefore, RMR measurements must be taken when the animals are resting and have been resting already for a few minutes. As snow voles show a daily activity rhythm with a peak during the night (24:00–06:00) and a resting period during the day (Bienkowski & Marszalek, 1974), our metabolic measurements (which took place between 08:00 and 18:00) were made during a period when they are more likely to be resting. However, by definition all animals were active at the moment they were trapped, and although some of our animals appeared to be resting for the full duration of the measurement, large fluctuation in oxygen consumption suggested others were occasionally active. To this end, we calculated RMR only for those 1-minute intervals where VO_2 showed a standard deviation smaller than 0.015 $mL \cdot min^{-1}$, which corresponds to a situation where the animal was resting and the chamber was approximately at equilibrium. Nevertheless, any bouts of activity that happened less than a few minutes before the plateaus for which we calculated RMR would have affected the gas content in the chamber and would have biased our estimation of absolute RMR values upward. However, assuming that individuals do not inherently differ in their propensity to be active before the time of measurement, this will generate random noise that is captured by the measurement error term in our models, and it will not affect RMR measurements expressed relative to the population mean.

Finally, although most RQ values fell within the expected range of 0.7 to 1, 22% were smaller than 0.7, which is likely to indicate a low quality of at least these measurements.

Overall, taken in isolation each measurement is probably an imprecise estimate of the true RMR of an individual, and because of latency effects the estimates are likely to be slight overestimations. However, our average measurement of RMR (3.3 $mL \cdot g^{-1} \cdot h^{-1}$) is only a bit higher than the RMR reported in another study on snow voles (3.06 $mL \cdot g^{-1} \cdot h^{-1}$ at 20 °C) measured using a Kalabukhov–Skvortzov respirometer under laboratory conditions (Bienkowski & Marszalek, 1974). Moreover, our measurements are likely to all be biased in a similar way, and as demonstrated by our statistical models, they do capture biologically relevant variation among individuals.

Physiological characteristics of snow voles

Snow voles live in a high-altitude rocky environment characterized by large daily and seasonal fluctuations in temperature. They do not hibernate and instead are active underneath the snow layer during winter. Physiological adaptations can thus be expected to be highly distinctive. It has been reported that animals living under cold conditions have relatively lower critical temperatures and show a wider TNZ than those adapted to warmer conditions (Broekman *et al.*, 2006; Zhao *et al.*, 2014). Also, it has been proposed that insulative properties of the body and metabolic thermogenesis are involved in the shift of the TNZ in order to cope with cold stress (Broekman *et al.*, 2006; Zhao *et al.*, 2010b; a, 2014). Consistent with these predictions, the thermal insulation of the snow vole's body was shown to be much better than that of the common vole (*Microtus arvalis*), a typical lowland rodent of the same family (Bienkowski & Marszalek, 1974). Moreover, the RMR of snow voles (3.3 $mL \cdot g^{-1} \cdot h^{-1}$) is much higher than that of common voles (1.97 $mL \cdot g^{-1} \cdot h^{-1}$, Lehto Hürlimann *et al.*, 2019), and the TNZ extends further into lower temperatures (snow vole: 20 °C; common vole: 30 °C) (Bienkowski & Marszalek, 1974). These are physiological characteristics which are thought to improve survival in cold environments (Song & Wang, 2003).

Interdependency of body mass and metabolism

Using an univariate model including $\log_{10}$-transformed BM as a covariate, we found that RMR in snow voles is proportional to $BM^{0.51}$, which is somewhat lower than the allometric relationships found in other small mammals (Heusner 1991; Lovegrove 2000; Glazier 2005), and significantly lower compared to exponents found by Kleiber (1961) and Brown & Sibly (2012). However, there is increasing evidence that the scaling of metabolism with body mass depends not only on taxonomic group (White & Seymour, 2003; Glazier, 2005, 2008), but also on factors such as physiological state and activity level, which has led some to suggest that a universal scaling law does not exist (Hayssen & Lacy, 1985; Glazier, 2008). This realisation has led Glazier (2005) to propose the metabolic-level boundaries hypothesis. According to this model, most mass-scaling exponents should vary between 2/3 and 1: if the energetic demands of maintenance are relatively high, as is the case in endotherms, the metabolic rate is mainly constrained by body surface area and therefore should scale with $BM^{2/3}$. However, if energetic demands are relatively low, as in ectotherms, metabolism should scale with

$BM^{3/4}$ or even steeper, mainly because of volume constraints (Glazier, 2005). Interestingly, the intraspecific mass-scaling exponent for RMR in snow voles was lower than 2/3. This might be due to differences in the capacity of effective endothermy and temperature regulation depending on body size, which cause shallower scaling relationships of small versus large mammals (McNab, 1983; Glazier, 2005). Importantly however, our bivariate model provided a mass-scaling exponent that was 0.90. Mass-scaling exponents close to 1 are predicted when the energy demands of maintenance are relatively low, e.g. in species with relatively low metabolic rates (Glazier, 2005).

This raises the question which scaling exponent is more appropriate? Since scaling exponents are slopes, they depend on the variance in BM. If BM is fitted as a covariate, this treats all sources of variance in BM equally. However, sex or age differences in BM do not necessarily translate into similar changes in RMR. Similarly, within-individual changes in BM may be the result of e.g. hydration status, which is unlikely to affect RMR. Unlike a univariate model including BM, a bivariate model allows us not only to estimate the effect of BM on RMR on both a between- and a within-individual level, but also to treat the sources of variance in RMR and BM separately. Accordingly, we found that within individuals, RMR barely increases with increasing mass (0.011 $mL \cdot min^{-1}$ per gram), while RMR increases steeply with BM between individuals (0.056 $mL \cdot min^{-1}$ per gram). The among- versus within-individual discrepancy in the relationship between BM and RMR may be explained by a difference in the metabolic activity of the tissues that give rise to among- versus within-individual variation. For instance, gut content, hydration status, and short-term variation in fat storage levels may affect an individual's BM, but not its RMR. Variation in BM among individuals on the other hand may be largely attributable to variation in overall size, which is accompanied by variation in the size of metabolically active tissues, and in the surface-area-to-volume ratio. However, further research is required to fully understand the mechanisms underlying the among- versus within-individual variation in the relationship between BM and RMR.

All things considered, we argue that a bivariate model framework is the most appropriate way to estimate metabolic scaling relationships that are independent of additional extrinsic (e.g. temperature) and intrinsic (e.g. age, sex) effects. We furthermore demonstrate that this can lead to strikingly different estimates of mass-scaling exponents, providing a first step towards a better understanding of the role of body mass in shaping metabolic rates. The application of the multivariate mixed model framework outlined here to other species and populations will provide more insight into the generality of these findings, and intra- and interspecific variation in the dependency of metabolic rate on body size in general.

Repeatability of RMR

Repeatability is a useful concept in evolutionary biology, as it provides an estimate of the upper limit of heritability (Falconer & Mackay 1996; but see Dohm 2002). Accounting for measurement error

variance, but not for BM, we found a substantial amount of individual variation in RMR measurements. However, RMR also strongly co-varied with BM, and consequently, much of the repeatable variation in RMR is attributable to individual variation in BM. Particularly, when accounting for BM, the repeatability of RMR is reduced substantially. Similarly, in *Gammarus fossarum*, the repeatability of individual metabolic reaction norms was approximately three times lower when metabolic rate was corrected for body mass (Réveillon *et al.*, 2019). This suggests a limited amount of additive genetic variation in RMR that is independent of BM. In other systems, empirical evidence suggests that the mass-independent heritability of RMR (or BMR) is close to zero (Dohm, Hayes & Garland 2001; Nespolo, Bacigalupe & Bozinovic 2003; Nespolo et al. 2005; Rønning et al. 2007; but see e.g. Sadowska et al. 2005; Nilsson et al. 2009). As a consequence, the potential of RMR to evolve in response to selection is severely constrained by its dependency on BM.

Previous studies on wild mammals generally found higher mass-corrected repeatability estimates of RMR, ranging between 47% (Zub *et al.*, 2012) and 83% (Fournier & Thomas, 1999). Among others, the discrepancy between previous reports and our estimation of mass-corrected repeatability of RMR may have arisen from differences in the statistical method used to calculate repeatability (i.e., linear mixed effect models versus product–moment correlation or intraclass correlation coefficients), the way of accounting for BM, but is likely to be also due to differences in the time interval between repeated measurements. Typically, the repeatability of metabolic rates tends to be high when calculated over relatively short time periods, but it decreases as the time interval between measurements increases (Chappell *et al.*, 1995; Norin & Malte, 2011; White *et al.*, 2013). This may not be surprising, given that the environmental conditions are likely to change over time (Vézina & Williams, 2005). Generally, for selection to be effective, the repeatability has to persist over a period that is long enough to enable the trait to influence individual fitness (Rønning *et al.*, 2005).

Implications for natural selection

The raw material on which natural selection is acting are consistent between-individual differences (White *et al.*, 2013). The fact that RMR is repeatable indicates that it may be subject to selection, provided that the repeatable component of variation in RMR causes fitness variation (Falconer & Mackay, 1996). For instance, variation in metabolism has been proposed to underlie variation in over-winter survival probability in several non-hibernating rodents (e.g. Jackson, Trayhurn & Speakman 2001; Boratyński & Koteja 2009; Zub et al. 2014). Likewise, metabolic rate can be expected to influence fecundity (Gittleman & Thompson, 1988; Nilsson, 2002; Blackmer *et al.*, 2005), as reproductive effort has significant energetic costs (Nilsson & Svensson, 1996). According to the increased energy-intake hypothesis of Nilsson (2002), animals with increased metabolic rate achieve better reproductive performance because they are able to take in more energy, which can be allocated

to reproduction. Typically, however, the strong dependency of RMR on BM hampers the ability to identify both the selective agents and the targets of selection. Although both low body mass and metabolic rate may be associated with lower total energy requirements (Iverson & Turner, 1974; Dohm *et al.*, 2001), the increased surface to volume ratio that comes with small size will result in proportionally higher heat loss (Merritt, 1995). At the same time, individuals with high body mass and metabolic rate may be more robust to low temperatures and starvation due to an increased thermogenic capacity, but also require higher absolute food intake (Jackson *et al.*, 2001). Given this complex interplay between body mass and metabolic rate, Speakman & McQueenie (1996) have suggested that the relative costs and benefits of metabolic rate are environment-dependent, resulting in a variety of fitness optima, potentially contributing to the high diversity of metabolic rates among individuals.

Conclusions

Here we show that a bivariate mixed model approach can provide a powerful analytic framework to decompose variation in RMR and to quantify the dependency of RMR on BM. Moreover, repeated measurements taken over different time scales (i.e., short-term and long-term repeated measurements) can be used to explicitly account for measurement error and hence to obtain unbiased estimates of repeatability (Ponzi *et al.*, 2018). This is particularly important when measuring metabolic rates, as oxygen consumption is usually measured by flow-through respirometry, a technique that includes considerable error variance (Nespolo & Franco, 2007). Applying this to RMR data collected in a wild rodent population inhabiting an environment subject to large fluctuations in temperature during the day and across the year, we show that the evolutionary potential of this key physiological trait is severely constrained by its dependency on body size.

Data Accessibility

The data reported in this paper will be archived at Dryad

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Tables

Table 1. Estimates of fixed (A) and random (B) effects on resting metabolic rate (RMR), without and with body mass as covariate (– BM and + BM, respectively), and on body mass (BM). SE: standard error. Estimates in **bold** are significantly different from zero at α=0.05 based on conditional Wald F tests (fixed effects) and likelihood ratio tests (random effects).

A)

	RMR (– BM)		RMR (+ BM)		BM	
Fixed effect	Estimate	SE	Estimate	SE	Estimate	SE
Intercept	1.612	0.057	0.873	0.176	28.12	0.67
Body mass (g)	-	-	**0.026**	**0.006**	-	-
Sex (male)	0.066	0.057	0.007	0.051	**2.23**	**0.92**
Age (adult)	**0.446**	**0.048**	0.113	0.087	**12.78**	**0.75**
Time of day (hours)	**-0.043**	**0.011**	**-0.034**	**0.011**	**-0.46**	**0.05**
Time of day^2 (hours^2)	**0.009**	**0.003**	**0.009**	**0.003**	-0.01	0.02
Year (2015)	-0.061	0.075	-0.021	0.071	-1.46	0.81

B)

	RMR (– BM)		RMR (+BM)		BM	
Random effect	Variance	%*	Variance	%*	Variance	%*
ID	**0.0198**	**31.8**	0.0091	17.7	**5.201**	**44.2**
Trap session	**0.0125**	**20.1**	**0.0116**	**22.6**	0.044	0.4
ID × Trap session	**0.0110**	**17.7**	**0.0130**	**25.2**	**6.348**	**54.0**
Respirometer chamber	0.0018	2.9	0.0003	0.6	0.000	0.0
Residual	0.0171	27.5	0.0175	33.9	0.171	1.5

*Conditional on the fixed effects listed in (A)

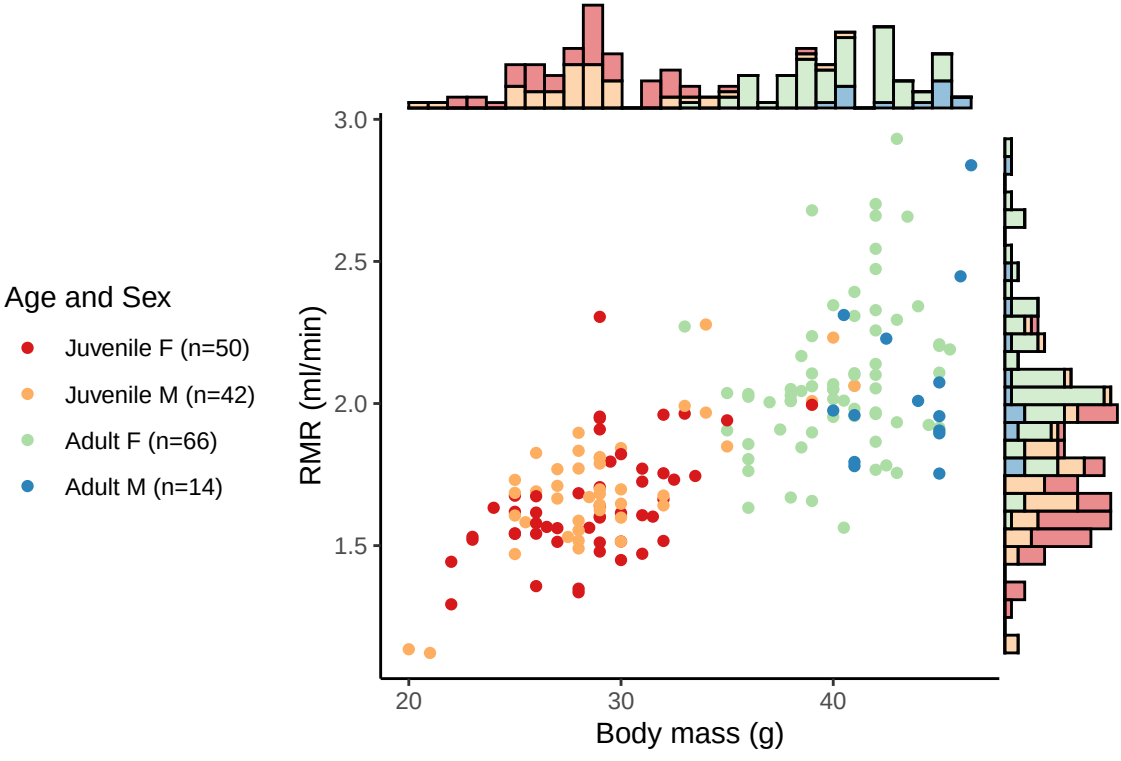
Table 2. Results from a bivariate model, simultaneously decomposing the variance in RMR and BM, as well as estimating the covariances among both traits. Correlation coefficients and slopes are accompanied by their approximate standard errors, and estimates in **bold** are significantly different from 0 at $\boldsymbol{\alpha}$=0.05 based on likelihood ratio tests. $b_{RMR,BM}$ is the slope of a regression of RMR against BM.

Random effect	V_{RMR}	V_{BM}	$COV_{RMR, BM}$	$r_{RMR,BM}$	$b_{RMR,BM}$
ID	**0.0227**	**5.3989**	**0.3006**	**0.859 ± 0.189**	**0.056 ± 0.018**
Trap session*	**0.0104**	-	-	-	-
ID × Trap session	**0.0119**	**6.3715**	0.0684	0.249 ± 0.247	0.011 ± 0.011
Respirometer chamber *	0.0000	-	-	-	-
Residual	0.0170	0.1705	-0.0059	-0.109 ± 0.118	-0.034 ± 0.037

*The random effects of Trap session and Respirometer chamber were not included for BM.

Figure legends

Fig. 1. Joint distribution of body mass (in grams) and Resting Metabolic Rate (RMR, in milliliters of O_2 per minute) measurements used in all models. Colors indicate the sex of individuals and their age at the time of measurement. Numbers in legend indicate the number of measurements per Age-Sex category.


Age and Sex
Juvenile F (n=50)
Juvenile M (n=42)
Adult F (n=66)
Adult M (n=14)
RMR (ml/min)
3.0
2.5
2.0
1.5
20
30
40
Body mass (g)