Resting and Maximal Metabolic Rates in Wild White-Footed mice

(*Peromyscus leucopus*)

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Abstract

Resting metabolic rate (RMR) represents the lowest level of aerobic metabolism in a resting individual. By contrast, maximal metabolic rate (MMR) reflects the upper limit of aerobic metabolism achieved during intensive exercise. As RMR and MMR define the boundaries of the possible levels of metabolism expressed by a normothermic individual, a key question is whether RMR and MMR are correlated. To evaluate the relationship between RMR and MMR, I took repeated paired measurements of RMR and MMR on 165 white-footed mice (*Peromyscus leucopus*) during the summer of 2018. Repeatability (R±se) was significant for both RMR and MMR (R_{RMR}=0.15±0.07 and R_{MMR}=0.27±0.12). At the residual level (within-individual), RMR and MMR were significantly and positively correlated (r_e=0.20, 95% confidence intervals: 0.04, 0.34). Such a positive residual correlation could be result of correlated phenotypic plasticity. By contrast, RMR and MMR were significantly and negatively correlated at the among-individual level (r_{ind}=-0.87, 95% confidence intervals: -0.99, -0.28). The negative among-individual correlation suggests there are trade-offs between the maintenance and active components of the energy budget (allocation model). Future research should investigate the relationship between RMR and other energetically expensive behaviours and activities to understand how energy is allocated among individuals.

Résumé

Le taux métabolique au repos (TMR) représente le plus bas niveau de métabolisme aérobique chez un individu au repos. En revanche, le taux métabolique maximal (TMM) correspond à la limite supérieure de métabolisme aérobique au cours d'un exercice intense. Alors que TMR et TMM définissent les limites des niveaux possibles de métabolisme pouvant être exprimés par un individu, une question clé est de savoir si le TMR et le TMM sont corrélés. Pour évaluer la relation entre le TMR et le TMM, j'ai obtenu des mesures répétées et couplées des deux traits chez 165 souris à pattes blanches (*Peromyscus leucopus*) durant l'été 2018. Les répétabilités (R±se) pour le TMR et le TMM étaient significatives (R_{RMR}=0.15±0.07 et R_{MMR}=0.27±0.12). Au niveau résiduel (intra-individuel), le TMR et le TMM étaient corrélés de manière significative et positive (r_e=0.20, intervalles de confiances à 95%: 0.04, 0.34). Cette corrélation résiduelle positive pourrait être le résultat d'une plasticité phénotypique corrélée. Par contre, le TMR et le TMM étaient corrélés de manière significative et négative au niveau inter-individuel (r_{ind}=-0.87, intervalles de confiance à 95%: -0.99, -0.28). Cette corrélation négative au niveau inter-individuel suggère qu'il y a des compromis entre les composantes de maintenance et de surplus au sein du budget énergétique (modèle d'allocation). Les études futures devraient investiguer la relation entre le TMR et d'autres comportements et activités énergiquement coûteuses pour comprendre comment l'énergie est allouée chez les individus.

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Section 1: Introduction

1.1 Relevance of metabolic rate

Metabolic rate (MR) is one of the most important aspects of an animal's physiology since it describes the rate at which substrates are oxidized to fuel all biological processes within organisms (Brown et al., 2004). Over 100 years of MR measurements – either directly via heat production or indirectly via CO_2 production or O_2 consumption – have shown that MR is extremely variable (Benedict, 1938; Lighton, 2008). Indeed, the MR of an individual over a given time period is greatly influenced by changes in temperature, activity, digestion, growth, and reproduction. Therefore, standardisation has been established in order to obtain comparable measures of MR across species, population, and individuals (Hulbert and Else, 2004; McNab, 1997). Two widely recognized standardized measures are basal and maximal MR which, together, define the boundaries of the possible levels of MR expressed by a normothermic individual.

1.2 Basal and resting metabolic rate

In endotherms, basal metabolic rate (BMR) represents the minimal energetic cost of living. More specifically, it is the lowest rate at which substrates are oxidized for the animal to simply stay alive (Hulbert and Else, 2004). BMR is measured while the animal is alert but resting, fasting (i.e., post-absorptive), not reproducing or growing, within its thermal neutral zone, and is measured during the inactive part of the animal's daily cycle (Speakman, 2013). Despite the high degree of standardisation involved in its measurement, BMR is highly variable among and within species and individuals. Numerous comparative studies have identified intrinsic and extrinsic factors

explaining most of the inter-specific variance in BMR (Hulbert and Else, 2004; McNab, 1988; McNab, 2015; Pettersen et al., 2018; White and Kearney, 2013) showing how BMR changed as species adapted to different environments, diets, and lifestyles. By contrast, explaining inter-individual differences in BMR has proven much more difficult (Speakman et al., 2004). After accounting for body mass, sex, and age, BMR is usually repeatable (Nespolo and Franco, 2007; Ronning, 2005; White et al., 2013). Explaining the sources of individual variation in BMR is a key endeavour in evolutionary physiology (Burton et al., 2011; Speakman et al., 2004; White and Kearney, 2013).

1.3 Maximal metabolic rate

The highest rate of MR that can be supported by an organism's aerobic system is termed maximal metabolic rate (MMR). MMR can be reached during periods of intensive exercise (exercise-induced MMR; hereafter MMR). As physical activity increases, locomotor and cardiac muscles require more ATP to support work. These energy demands are met by the aerobic system, which creates ATP in the presence of O_2 (Bennett and Ruben, 1979). Aerobic metabolism, however, appears limited by the rate of O_2 delivery to the muscles by the cardiovascular system (Bassett and Howley, 2000). Technically, MMR is reached when the O_2 demands of the locomotor and cardiac muscles surpass O_2 consumption of the organism (these additional energy requirements must be met by the anaerobic system, where ATP is produced in the absence of O_2). Thus, MMR – measured as the maximum O_2 consumption of an animal during intense exercise – is the single most important factor determining an organism's aerobic capacity, or its ability to maintain high levels of activity (Swallow et al., 1998). The ability to maintain high levels of activity is important

in populations where individuals compete for resources, territory and mates, and as such MMR is a key metabolic trait. Just as BMR, MMR is also usually a repeatable trait (Careau et al., 2014a; Nespolo and Franco, 2007), implying that individual differences are consistent through time and hence can be subject to natural selection.

1.4 Co-adaptation of MMR and BMR

As BMR and MMR define the metabolic scope within which all aerobic activities can be expressed, a key question is whether BMR and MMR are somewhat linked and if so, if they are positively or negatively corelated. A positive correlation would support the notion that energy is allocated according to the performance model, which stipulates that larger or more developed metabolic machinery permits higher energy output, but also requires more energy input for maintenance. The general idea behind the performance model comes from the aerobic capacity model, which was proposed by Bennett and Ruben (1979) as a viable and popular theory explaining the evolution of endothermy, and consequently the existence of BMR. Bennett and Ruben (1979) envisioned an evolutionary process beginning with selection for individuals that can sustain higher levels of activity, making them be better at gathering food, fleeing predators, territorial defense or invasion, courtship, mating, and provisioning offspring (Bennett and Ruben, 1979). Sustaining these energetically expensive activities at a higher rate necessitates higher aerobic capacity and hence a higher MMR. When higher MMR is positively selected, the aerobic capacity model predicts that BMR increases as a correlated response due to a genetic covariance between the two (Bennett and Ruben, 1979; Hedrick and Hillman, 2016; Sadowska et al., 2005; Wone et al., 2009). The presence of a genetic covariance would indicate that the same physiological

pathways involved in MMR are also implicated in BMR. The mechanism linking BMR and MMR has yet to be determined, but some possibilities have been explored (e.g., density of mitochondria: Raichlen et al., 2010; Weibel, 2005; Weibel et al., 2004; and cellular membrane composition: Hulbert, 2007; Wone et al., 2013). Such mechanistic and genetic correlations (r_G) should result in an observable phenotypic correlation (r_P), unless the environmental or residual correlations (r_e) are of opposite sign (e.g., Sadowska et al., 2005). Therefore, examining covariance between BMR and MMR is relevant to study all levels of variance.

Another possibility is that energy is managed according to the allocation model (also called the compensation model) which would result in a negative correlation between BMR and MMR. The allocation model describes a trade-off where animals have a fixed amount of energy available that can be allocated either to maintenance or to energetically expensive activities (Baktoft et al., 2016; Careau et al., 2008). In other words, individuals who devote more energy to the maintenance of systems contributing to BMR (e.g., immunological defence or DNA repair), would have less energy available to allocate to expensive activities. Presumably, individuals who do not regularly engage in energetically expensive activities do not need a high MMR and, therefore, the allocation model could be applied to predict a negative correlation between BMR and MMR.

1.5 Empirical evidence for the BMR-MMR link

Over the last 35 years, several studies have been carried out to quantify the phenotypic correlation (r_P) and the genetic correlation (r_G) between BMR and MMR, with mixed results. r_G is an estimate of the proportion of variance two traits share as a result of genetic causes (Falconer,

1960). A positive r_G between BMR and MMR has been found in laboratory house mice and in voles (Dohm et al., 2001; Sadowska et al., 2005; Wone et al., 2009), while a non-significant r_G has been found in another population of laboratory mice (Gebczynski and Konarzewski, 2009). Interestingly, divergent artificial selection on BMR produced correlated changes in MMR that suggest the presence of a negative r_G between BMR and MMR (Ksiazek et al., 2004). Many other studies on BMR and MMR have quantified r_P instead of r_G. Significant and positive r_P have been found in deer mice (Hammond et al., 2002), while a non-significant r_P was found in Junglefowl (Hammond, 2000) and wild voles (Boratyński and Koteja, 2009). A recent meta-analysis reported an overall nonsignificant r_P between BMR and MMR in mammals (Auer et al., 2017; also see Koteja, 1987).

While the empirical evidence seems to point towards a lack of evidence for a link between BMR and MMR, many of the studies that have found non-significant r_P had small samples sizes hence low power to detect an association (Auer et al., 2017; Chappell et al., 2007; Song and Wang, 2002). Additionally, most studies on BMR and MMR did not include repeated paired measurements and hence did not allow the partitioning of r_P at the among vs. within-individual levels (but see Careau et al., 2014a). Such partitioning of r_P is relevant since the among-individual correlation (r_{ind}) represents shared covariance between BMR and MMR that was constant over the time period of the measurements; hence, a r_{ind} can arise due to a r_G and/or permanent environmental effects shared between BMR and MMR. Compared to r_P, r_{ind} is one step closer to r_G since it excludes specific sources of covariance due to specific environmental correlations and/or correlated measurement error, which end up in the residual (or within-individual) correlation (r_e). Finally, almost all studies were carried out on animals raised under laboratory

conditions. While the laboratory allows a certain control over environmental variables, there is also a merit to longitudinal studies on animals living in their natural setting. The higher environmental variability that exists in an animal's natural setting leads to higher variability and lower repeatability in metabolic traits (Auer et al., 2016). To better understand the nature of the BMR-MMR link, studies should also be conducted in the conditions where natural selection would have occurred. To date, only one longitudinal study of BMR and exercise-induced MMR exists on wild mammals (Boratyński and Koteja, 2009).

1.6 Challenges of measuring BMR and MMR in wild animals

Studying the BMR-MMR link in wild animals raises several challenges related to the actual measurement of both traits. In some field situations, it is impossible to meet all of the above criteria to measure BMR, yet it is still interesting to measure and compare MR among individuals with a certain degree of standardisation (Speakman et al 2004). In this case, measurements are referred to as resting metabolic rate (RMR), which is more loosely defined as the minimum MR of a resting animal within the thermal neutral zone (Speakman, 2013). Although RMR measurements violate one or more of the criteria for measuring BMR, their analysis allows the evaluation of the effect of some factors on MR, such as growth and reproductive status. Still, given the high similarity between BMR and RMR measurements, they are usually considered to be analogous traits (Biro and Stamps, 2010; Hochachka et al., 2003).

Measuring MMR using a forced-exercise test is challenging because it involves exerting a maximally motivated animal until exhaustion. It is widely known that individuals react differently to the testing procedure; some may readily run to their complete exhaustion when prompted to

do so, while others may “freeze” (Foster et al., 2015). The extent to which motivation varies among and within individuals, however, remains unknown. One solution is to train animals until they appear able to run to their maximum capacity within the testing apparatus, but this option imposes constraints on the sample size (Roberts et al., 1996) and is not always possible when studying short-lived, wild animals. One way to deal with this challenge is to attempt measuring MMR on every capture (without training) and objectively score the individual’s apparent willingness to run (Coleman et al., 1998; Swallow et al., 1998). This way, it is possible to evaluate the extent to which willingness to run differs among and within individuals. Moreover, low-motivation trials can be removed so that the analysis of the BMR-MMR link only includes the trials where the individual was maximally motivated or at the very least was very close to being maximally motivated.

1.7 Objectives

The objectives of this study are to: (1) identify sources of variation in RMR and MMR in wild white-footed mice (*Peromyscus leucopus*); (2) estimate the repeatability of RMR and MMR; (3) quantify the magnitude of among- vs within-individual variation (i.e., repeatability) in the willingness to run during a forced exercise test; and (4) determine if RMR and MMR are correlated. To do so, I took multiple repeated pairs of RMR and MMR measurements and used bivariate mixed models to partition the r_P into r_{ind} and r_e. Such partitioning is important since, phenotypic correlations are shaped by both among-individual and residual correlations (Dingemanse and Dochtermann, 2013), which can differ from one another and hence provide different information on the nature of the RMR-MMR link.

Section 2: Methods

2.1 Species

White-footed mice are small rodents in the Cricetidae family usually found in bushlands and dry forests (M'Closkey and Lajoie, 1975). The home range for white-footed mice averages about 100m^2, but this can vary depending on season and sex (Lackey et al., 1985; Vessey, 1987). Their population densities can fluctuate considerably throughout the season ranging from 5/ha early spring, to more than 100/ha by August (Vessey, 1987). White-footed mice are nocturnal and spend a large portion of the night foraging for nuts, seeds, and insects. *Peromyscus leucopus* are active year-round as they continue to forage for food and may reproduce even through the winter. Overall *Peromyscus leucopus* is a very active species that participates in scramble competition for resources and mating opportunities, and hence aerobic capacity is likely an important trait in determining fitness. Multiple studies report that average litter size ranges from 4.1 to 5.5 pups and mothers will often have multiple litters throughout the year (Fleming and Rauscher, 1978; Hill, 1979; M'Closkey and Lajoie, 1975). The reproductive success and survival of the white-footed mouse has been shown to fluctuate with availability of resources. Reproductive success and survival rates are higher following mast events, when trees synchronously produce a particularly high yield of fruits and nuts (Scarlett, 2004).

2.2 Study site

The study site is located at the Queens University Biological Station (44°34'08"N; 76°19'08"W) where Longworth style "Little Critter" traps (box: 8.57 x 6.35 x 13.97cm; tunnel: 4.45 x 4.76 x

12.7cm) were installed in two permanent sampling grids. The first grid is located on Cow island, a 7-ha island located ~50 meters off the mainland and covered in mature deciduous forest with 115 live traps spaced 30 meters apart. The second grid contains 63 live traps spaced 30 meters apart on a 2.4-ha grid located on the mainland adjacent to Cow Island. Cow island is covered in mature deciduous forest while Blueberry Hill consists of a mix of deciduous forest and juniper bushes. On both sites, permanent sampling locations were equipped with a small enclosure (Layne, 1987) to protect Longworth traps from tampering from raccoons and other animals. Daily temperature data in the Lyndhurst Shawmere region was also collected from the Government of Canada's past weather and climate historical data (Government of Canada, 2018).

2.3 Captures

All captures were authorized by the Ontario Ministry of Natural Resources. All mice were handled according to the following protocols approved by the University of Ottawa Animal Care Committee and the Queen's University Animal Care Committee. From May 1st to October 21st 2018, traps were set at dusk and checked at dawn. All traps were baited with small piece of apples and a few sunflower seeds to encourage mice to enter the traps. Each new individual caught was marked with unique tags in each ear. On each capture, the age (juvenile or adult), sex, reproductive status (active or not), and presence of parasites (yes or no) were recorded. Identification of the animal's age was based on their pelage; a juvenile's pelage is either grey or a mix of grey and brown above the middorsal molt line, while an adult's pelage is entirely brown above the molt line. Reproductive status was recorded as either active or inactive based on observable sexual characteristics. The presence or absence of parasites was recorded based on

the detection of at least one botfly larva, mite, or tick. After manipulations, mice were either released on site or placed back in their traps without food and transported to a laboratory, approximately 600m from the site, for metabolic measurements. Mice were not brought back to the laboratory if they were pregnant, lactating, or had already visited the lab within the previous 5 days.

2.4 Laboratory procedures

In the laboratory, mice first were weighed using an electronic scale (Mettler Toledo, Model ML1602T/00) and then transferred into individual chambers to measure their RMR (see below), usually between 7:30 and 9:30 am (see Fig. 1A). RMR was measured before MMR to avoid the effect of excess post-exercise oxygen consumption that might occur after the MMR trials (Baker and Gleeson, 1998). After 5 hours in the metabolic chambers (usually between 12:00 and 13:30), mice were weighed a second time and transferred back into their traps and allowed to rest and feed on a small piece of apple and sunflower seeds. After a minimum of 40 minutes, mice were transferred one individual at a time into an enclosed treadmill for measuring MMR. These tests usually started around 14:00 and the last test of the day was usually finished by 19:00. Once the tests were completed, mice were released at their capture site.

2.5 Respirometry – resting metabolic rate measurements

RMR was measured using a 8-channel open-flow respirometry system. Each mouse was individually and gently transferred into a cylindrical plexiglass chamber (diameter: 8 cm; length: 15 cm). Inside the chamber, mice rested onto a flat metal mesh with round holes. Each chamber

was placed on a separate activity detector (ADX-C; Sable Systems) that detected and recorded movement within the chamber throughout the trial. All chambers were maintained inside of a custom-made cabinet set at a controlled temperature of 28° C, which is within the thermal neutral zone for *P. leucopus* (Cannon and Nedergaard, 2010).

Ambient air was pumped from the room, scrubbed of water vapour (drierite) and CO_2 (soda lime), and then divided into 10 separate streams (one for each chamber plus two baselines). The air streams to the chambers were directed through a mass-flow meter (FB-8; Sable Systems), which provided a constant flow of ~500 mL·min^{-1} (range: 450-550 mL·min^{-1}). The excurrent air streams from the chambers and the two baselines were directed to a computer-controlled multiplexer (RM-8; Sable Systems), which allowed the simultaneous monitoring of two chambers (or baselines) using a two-channel O_2 analyzer (Oxzilla, Sable Systems). Two ~100 mL·min^{-1} subsamples of baseline air or chamber outflow were dried and pulled through each channel of the O_2 analyzer. Each chamber and baseline was sequentially monitored for 5 min over the course of 5 hours with 30-sec pauses between switches to let the system fully equilibrate between streams. The exact sampling scheme depended on the number of animals to measure; with 8, 6, or 4 animals each chamber was respectively monitored for 5 min every 25, 20, or 15 minutes (not counting pauses).

The software ExpeData (Sable Systems) was used to record trials and extract RMR. To calculate O_2 consumption, I corrected for drift between baseline measurements and applied equation 10.2 from Lighton (2008). Note that CO_2 was not scrubbed from the chamber outflows and a respiratory quotient of 0.8 was assumed to calculate O_2 consumption (Koteja 1996). For each 5-min sampling period, I used the "nadir" function in ExpeData to calculate the average

oxygen consumption for 4 minutes and 30 seconds (recording was paused for 30 seconds during the transition period between chambers). The lowest of these 4.5-min averages for a given individual in a given trial was taken as the RMR measurement (see red dots in Fig. 1A). As expected, there was a strong and positive influence of activity on MR during the RMR trials (Fig. 1B). Most individuals, however, settled down within the chamber and reached low, stable levels of MR towards the end of the trial, when activity levels were low and most of the RMR estimates were extracted (Fig. 1A).

2.6 Respirometry – maximum metabolic rate

MMR was measured using a pull-mode respirometry system and a treadmill encased within a ~2L flow-through respirometry chamber (Modular Enclosed Metabolic Treadmill for Mice; Columbus Instruments). At the bottom end of the treadmill there was a horizontal metal grid, with prongs spaced 0.5 mm apart, that emitted gentle electric currents. The treadmill chamber was connected to a field metabolic system (FMS; Sable Systems), which contains a pump, mass-flow meter, and multiple gas sensors (O_2, CO_2, and water vapour). Air was pumped from outside and directed through the system at a constant rate of ~1.25 $L \cdot min^{-1}$ (range: 1 to 1.5 $L \cdot min^{-1}$).

After an initial baseline measurement was taken, a single mouse was placed on the treadmill and a towel was placed over the chamber to keep the mouse calm and isolated before beginning the forced-exercise trial. Once the readings for CO_2, water vapour, and O_2 were relatively stable for approximately 2 min, the towel was removed, the shock grid was activated, and the treadmill was started at a speed of 20 $m \cdot min^{-1}$. The speed was increased every 2 minutes by 5 $m \cdot min^{-1}$ until it reached a maximum speed of 75 $m \cdot min^{-1}$. This incremental increase is similar

to ramp-up protocols used in other studies (Petrosino et al., 2016). However, the protocol was adjusted slightly since I aimed to incite in the mice exhaustion between 8-17 minutes (Beltz et al., 2016). My ramp-up protocol has higher incremental increases in speed and ultimately reaches a higher maximal speed since these wild mice were capable of sustaining higher speeds than mice in other treadmill protocols (Ferreira et al., 2007). This procedure was followed until the mouse remained on the shocking grid for more than 10 seconds after which the treadmill and shocker grid were turned off. Once MR decreased to a relatively low and stable level, the mouse was removed from the chamber and a second baseline measurement was taken.

For each trial, I scored the individual's willingness to run and signs of exhaustion on a scale of 1 to 4. A score of 1 was assigned when the mouse refused to run completely and showed normal behaviour immediately after trial (e.g., grooming, walking around, grooming, and rearing). A score of 2 was assigned when the mouse ran but not vigorously or consistently. A score of 3 was assigned when the mouse ran vigorously but not consistently or for a very short period of time, while still showing signs of exhaustion after the trial. Finally, a score of 4 was assigned when the mouse ran constantly until complete exhaustion. I considered signs of exhaustion to be: collapsed posture, shaking and/or a lack of movement. For example, a trial with a score of 1 featured a mouse that was upright, not shaking, moving around the chamber and displayed grooming behaviour. Meanwhile a mouse with a score of 4 was often collapsed, shaking, immobile and displayed no grooming behaviour. Note that the trials which had been assigned a score lower than 3 were removed (71 trials) from the RMR-MMR analysis since these individuals were unlikely to have reached MMR. The first forced-exercise tests were conducted

using a larger treadmill without measuring gas concentrations to establish this objective scoring of the willingness to run. After I switched to a smaller treadmill and recorded gas concentrations.

The extraction of MMR differs from RMR since CO_2 and water vapour were not scrubbed from the system. First the channels measuring CO_2, water vapour, and O_2 were all de-spiked smoothed and then z-transformed. A lag correction was applied to align all three gas signals (all three gases are needed to calculate VO_2). A correction for water vapour was applied using the eq. 8.6 and 15.3 from Lighton (2008). Finally, I corrected for any drift that occurred between the two measures of baseline. MMR was extracted as the highest rate of O_2 consumption averaged over 30 seconds. A 30 second interval is a commonly used time interval (Lemaire et al., 2017) for MMR extraction since it balances the trade-off between recording the actual peak in metabolic rate and accounting for imperfect gas mixing in the chamber (Lighton and Halsey, 2011).

2.7 Statistical analysis

All continuous variables were standardised to a mean of 0 and variance of 1. All models were fitted with ASReml-R version 3 (Butler et al., 2018). I first assessed the repeatability of, and the factors that influenced willingness to run during the forced-exercise test using a univariate mixed model using data from all trials (willingness to run scores 1 to 4). The univariate model included body mass, sex, the presence/absence of parasites, reproductive status, date, time of day, test sequence (e.g., test sequence 6 for RMR represents the 6^{th} time RMR was measured for a given individual), and the mean daily temperature fitted as fixed effects. The significance of the fixed effects was assessed with a conditional Wald *F*-statistic and the denominator degrees of freedom (df) were obtained following methods described by Kenward and Roger (1997). Individual identity

was included as a random effect to partition the variance into the among-individual variance (V_{ind}) and residual variance (V_e). Repeatability (R) in the willingness to run was calculated as $R = V_{ind}/(V_{ind} + V_e)$. Approximate standard error for R was calculated using the delta method (pin function in the R package nadiv; Wolak, 2018).

RMR and MMR were analyzed with a bivariate mixed model with several fixed effects fitted to both traits, including body mass, sex, age, presence/absence of parasites, reproductive status, date, time of day, test sequence, and mean daily temperature. An additional fixed effect of activity was fitted to RMR only to account for slight differences in movement during the extraction of RMR, but it was not significant (P=0.50). For MMR, I included additional fixed effects of the willingness to run score (3 or 4) and the FMS unit (one of two different FMS units used throughout the summer; P<0.01). The average temperature of the room during MMR trials was 22.4°C (range: 19.2 to 28.1°C) and was positively correlated with mean daily temperature (r=0.17, P<0.05). Room temperature, however, did not have a significant effect on MMR (P=0.86) and was thus left out of the model.

Individual identity was included as a random effect to partition the phenotypic (co)variance in RMR and MMR at the among-individual and residual levels. First, I used the V_{ind} and V_e estimates from the bivariate mixed model to calculate R in RMR and MMR as described above. The bivariate mixed model included a correlation matrix at both the among-individual and residual levels, which effectively partitioned r_P into r_{ind} and r_e. The 95% confidence intervals (CI) for r_{ind} and r_e were calculated using profile likelihoods with the function proLik() in the R package nadiv (Wolak, 2018). I also calculated the adjusted r_P from the bivariate model using the following formula: $r_p = r_{ind} \sqrt{(R_{RMR} * R_{MMR})} + r_e \sqrt{(1\text{-}R_{RMR} * 1\text{-}R_{MMR})}$ (Dingemanse and Dochtermann, 2013).

I was able to obtain estimations of r_{ind} and r_e since I had repeatedly taken paired measurements of RMR and MMR. The estimate for r_{ind} reveals whether individual mean values of RMR are associated with individual mean values of MMR (conditioning on the fixed effects). The r_{ind} is influenced by permanent effects from the environment on individuals and also genetic differences between individuals (Dingemanse and Dochtermann, 2013). The estimate for r_e indicates the correlation between changes in RMR and MMR, for the average individual whose metabolic traits were measured multiple times throughout the season. This correlation can be indicative of correlated measurement error, correlated plasticity, and/or an unquantified variable that affects both RMR and MMR (Brommer, 2013).

Section 3: Results

3.1 Willingness to run during the forced-exercise test

A total of 310 forced-exercise trials, for 145 mice, were conducted where a score was assigned describing the willingness to run until exhaustion. The residuals of the willingness to run data deviates a little from a perfectly normal distribution. However, generalized linear mixed models are known to be fairly robust towards residuals that are not perfectly normal and therefore remains the most suitable method to analyse this data. Willingness to run was significantly and negatively affected by day of year and test sequence (Table 2). After accounting for these effects, willingness to run was significantly repeatable (R =0.16$\pm$0.07). Both willingness to run and VO_2 were recorded on 253 trials (the 57 initial trials were conducted without gas recordings); as expected, the highest VO_2 expressed during the forced-exercise tests increased with the

willingness to run (Fig. 2). The analysis below excludes 71 trials in which willingness to run was below 3, as these trials clearly did not yield appropriate MMR measurements.

3.2 Descriptive statistics

I measured both RMR and MMR on 155 individuals for a total of 331 RMR measurements and 182 MMR measurements (sample size is lower for MMR because trials in which the mouse refused to run were excluded; see above). My measurements of RMR and MMR were similar to levels of RMR and MMR found in other studies (Fig S1 & S2). On average MMR was 6.6-fold higher than RMR (Table 1).

3.3 Effects of sex, reproduction, parasites, body mass, and test sequence

As expected, body mass had a strong and significant effect on both traits (Fig. 3A & 3D, Table 3). Reproductive status also had a significant effect on RMR and MMR (Fig 3C & 3F, Table 3). Age had a significant effect on RMR alone (Table 3A). By contrast, sex and the presence/absence of parasites had no effect on RMR and MMR (Table 3). RMR and MMR were strongly influenced by mean daily temperature (Fig. 3B & 3E; Table 3). RMR was significantly higher later in the day (Table 3A). Willingness to run had a marginally non-significant effect on MMR (P=0.059; Table 3).

3.4 Repeatability and correlations

Controlling for the fixed effects above, repeatability was significantly different from zero for both RMR (R =0.15$\pm$0.07) and MMR (R =0.27$\pm$0.12) (Fig. 4, Table 4). At the residual level, RMR and MMR were significantly and positively correlated (r_e= 0.20; 95%CI: 0.04, 0.034) (Fig. 5A). By

contrast, at the among-individual level RMR and MMR were significantly and negatively correlated (r_{ind} = -0.87; 95%CI:-0.99,-0.28) (Fig. 5B). Overall, the phenotypic correlation between RMR and MMR was non-significant (Table 5).

Section 4: Discussion

4.1 Overview

The main objectives of this study were to identify sources of variation in – and estimate the repeatability of – RMR and MMR in wild white-footed mice and to assess their relationship at the among-individual and the residual levels. I found that RMR and MMR were significantly and positively correlated at the residual (r_e) level, but significantly and negatively correlated at the among-individual level (r_{ind}). The positive r_e indicates that RMR and MMR were either increasing or decreasing together within a given individual. The negative r_{ind} indicates that, on average, individuals with higher RMR have lower MMR, and vice versa. These contrasting relationships show the importance of properly partitioning co-variance when studying labile traits like RMR and MMR (Hayes, 2010; Nespolo et al., 2005).

4.2 Willingness to Run

Measuring MMR involves the difficult challenge of motivating animals to run to their maximal capacity. To address this issue, I scored each individual's willingness to run during each trial, allowing me to assess the extent to which willingness to run differs among and within individuals. I found that the willingness to run score was repeatable, which is consistent with previous literature that has assessed willingness to run in mice (referred to as trial quality in Swallow et

al., 1998). The significant repeatability implies that some individuals appear consistently more willing to run than other individuals (Coleman et al., 1998; Swallow et al., 1998). Consequently, the elimination of sub-maximal trials likely exert a sampling bias potentially related to stress coping styles, or personality (Biro and Dingemanse, 2009; Careau et al., 2008; Koolhaas et al., 1999). In general, while eliminating the sub-maximal trials may be sufficient for some purposes (e.g., studying performance), it might become problematic when trying to relate RMR and MMR measures to actual measures of behaviour since individuals with a given behavioural phenotype of interest might be more likely to be excluded from the study. In my case, a total of 16 individuals were eliminated from the MMR analysis, which equates to 10% of the individuals who underwent the forced-exercise test at least once during the study. The impact of this bias on my analysis remains unknown, but in my sample, willingness to run was not correlated with body mass nor with RMR. To investigate this further, it would be helpful for future studies to look at whether individuals excluded due to low willingness to run differ in their behaviour and/or other metabolic phenotypes.

To date, very few studies have considered the willingness to run of the individuals in their analysis. It is reasonable to assume that there will always be at least some variation in this trait during forced-exercise tests, especially those involving untrained and wild animals. The studies that do mention assessing willingness to run in some way (i.e., trial quality, stimulation needed to promote running, motivation), explain that they omitted trials where the animals did not reach MMR (Cartee and Farrar, 1987; Wone et al., 2009; Wone et al., 2015), but do not state the number of trials removed and ultimately the number of individuals removed. Since the majority of forced-exercise studies probably have trials that need to be omitted due to unwillingness to

run, I suggest that researchers explicitly state how they quantified trial quality and how many trials and individuals were excluded from the analysis. This way, we can learn more about the factors that determine an animal's willingness to run and control for slight differences in motivation when analysing MMR (sensu Swallow et al., 1998).

Even after deleting submaximal trials (levels 1 & 2; Fig. 2), willingness to run during the forced-exercise test had a marginally non-significant weak effect on MMR. Indeed, trials with a willingness to run score of 3 yielded metabolic rates that were, on average, ~10.9% lower than trials with a score of 4 (Fig. 2). This suggests that the scoring system was able to detect small differences in motivation even among the trials with a high willingness to run score (levels 3 & 4; Fig. 2). There is, of course, a lot of subjectivity in the quantification of willingness to run. Although I cannot confirm that all individuals reached MMR in level 3, most of these trials must have been very close to reaching MMR and some probably did reach MMR. Importantly, I can still use these trials by conditioning my analysis of MMR on the slight differences in perceived motivation (Coleman et al., 1998; Swallow et al., 1998). Therefore, it's not necessarily imperative that all individuals were maximally motivated, as long as the MMR measurement was done at close to maximal motivation and the analysis includes the willingness to run score as a covariate to control for the slight differences in motivation.

The willingness to run was significantly and negatively impacted by test sequence and day of year. The negative effect with test sequence confirms that wild mice did not learn to run better on the treadmill. This is the opposite of what was found in laboratory mice by Swallow et al., (1998) where trial quality increased for subsequent trials. The negative effect of test sequence might instead indicate that individuals have learned how to avoid running until exhaustion during

the trial. I personally noticed that once an animal found a position that allowed them to remain off of the treadmill without experiencing shocks from the grid, it was difficult to prevent them from returning to or staying in that position. Therefore, it is especially important to make sure there are good conditions for the animal to run until maximum capacity during their first trial.

4.3 Fixed Effects for RMR and MMR

Among the intrinsic effects RMR and MMR were significantly affected by body mass and reproductive status. RMR alone was significantly affected by age. The remaining intrinsic effects like sex and the presence/absence of parasites did not have a significant influence on RMR and MMR. Extrinsic effects like temperature and time of day had different effects on RMR and MMR. The positive correlation of body mass with RMR and MMR is unsurprising since it has been well established that metabolic rate scales with body mass at both the interspecific and intraspecific levels (Daan et al., 1989; Feldman and McMahon, 1983; Hochachka et al., 2003; Weibel et al., 2004).

Here I also found that RMR and MMR were negatively affected by the average daily temperature. During days with cooler temperatures, mice had high RMR and would also reach higher MMR. The negative relationship between RMR and temperature makes sense since, at cooler ambient temperatures, animals will increase their metabolic rate to maintain a normothermic body temperature. Even though RMR is measured within the thermoneutral zone, the daily environmental ambient temperature can still affect RMR if the thermogenic machinery increases maintenance costs. Such an effect of the environmental ambient temperature on RMR

has been documented in a number of other mammalian studies (Naya et al., 2018; Rezende et al., 2004; Speakman, 2000).

A significant and negative effect of temperature on MMR has been found in a number of other studies (Maldonado et al., 2016; White et al., 2008). One of the explanations for this relationship is thought to be due to the positive association with activity, which has also been found to be negatively affected by temperature (Chappell et al., 2004; Sears et al., 2009). Sears et al., (2009) found that lab mice (housed in warm nest boxes), made the same number of trips to get food (kept in a separate chamber at various temperatures), but spent less time per trip, when the food chamber was colder. This suggests that the mice were able to limit their exposure to the cold when they had acclimated a higher aerobic capacity to carry out activities outside of their boroughs/nests more quickly (Chappell et al., 2004). Thus, MMR maybe higher at cooler temperatures because cold exposure acts as a form of aerobic training (White et al., 2008).

Controlling for body mass, juvenile mice had higher RMR than adult mice. This finding is consistent with previous literature, which has shown that RMR decreases with age (McCarter and Palmer, 1992; Speakman et al., 2003). The precise explanation for this relationship is unclear, but it has been suggested that the decline of metabolism with age is related to the decreases in ion pumping, and protein synthesis with age. The juvenile mice in our study are also likely still growing, which is energetically expensive, and would therefore increase RMR (Dietz and Drent, 1997; Klaassen and Bech, 1992; Klaassen and Drent, 1991).

Reproductive individuals in my study system had significantly lower RMR. I should point out that this effect is driven almost entirely by reproductive males, since I avoided conducting metabolic tests on reproductively active female. I was able to verify this by testing the model

with and without female reproductive status. Lower metabolic rates in reproductively active individuals, is the opposite of what I expected to find since previous literature has thoroughly outlined how reproduction is energetically expensive (Bergeron et al., 2011; Gittleman and Thompson, 1988; Hayward and Gillooly, 2011; Millar, 1978). The most reasonable explanation for the negative effect is that there is a relationship between reproductive status and the age of the animals. Age was roughly categorised based on pelage, and consequently I do not have an exact record of the age of the adults in my study. Therefore, some of the variance in RMR and MMR that is associated with age differences, which I could not detect, is instead being attributed to reproductive status.

4.4 Repeatability

RMR and MMR have been found overall to be repeatable in mammals and birds (White et al., 2013). My results are consistent with the previous literature, as we found that both RMR and MMR are repeatable in our study. This means that individuals, who were measured multiple times throughout the season, maintained consistent and different levels of RMR and MMR. The repeatability of RMR and MMR, however, was relatively low (R=0.15 for RMR and 0.26 for MMR). A low repeatability indicates that a large proportion of the phenotypic variance in RMR and MMR occurs within individuals, which can be a result of measurement error and/or physiological changes in the animal resulting from micro-environmental effects that are non-permanent and specific to a given measurement. Micro-environmental effects are likely responsible for the finding that repeatability of metabolic rate is significantly lower in animals living in the wild than in those living under laboratory conditions (Auer et al., 2016). The fact that repeatability for

metabolic rate is lower in the field exemplifies the importance of taking as many repeated measures as possible when attempting to correlate RMR and MMR in wild animals. As I mentioned earlier, the laboratory setting lacks the micro-environmental conditions that cause some of the variation in metabolic rate existing in nature. Part of determining how RMR and MMR are linked, is asking if there are consistent among-individual differences in metabolic rate. Therefore, it is important to take repeated measures in wild animals and estimate the relationship at the among-individual level, because simple phenotypic correlations based on single measurements of RMR and MMR will be more representative of residual correlation than among-individual correlation.

4.5 Positive residual correlation

The positive residual correlation means that, for the same individual, with either an increase or a decrease in RMR, there is the same response in the MMR, and *vice versa*. This type of correlation can be an indication of correlated measurement error and/or phenotypic plasticity at the population or individual level. In my study, however, correlated measurement error is unlikely because RMR and MMR were measured with completely separate respirometry systems, making it improbable for any bias in one system to affect the measurement in the other. Correlated plasticity occurs at the population level whenever RMR and MMR are affected by a common factor. In my study, for example, RMR and MMR were both negatively influenced by daily temperature. Such correlated plasticity, however, cannot contribute to r_e because the effect of temperature on RMR and MMR was accounted for in the bivariate mixed model. It is still possible, however, that an unquantified factor (e.g., availability of resources) exerted an

influence on both RMR and MMR; in this case, such correlated plasticity at the population level would contribute to r_e as the effect cannot be accounted for in the analysis (Dingemanse et al., 2012). Correlated plasticity may also occur at the individual level, where individuals typically differ in how their RMR and MMR change in response to a given environmental variable (e.g., temperature) (e.g. Careau et al., 2014b). Modelling individual variation in plasticity requires fitting random regressions in which the slopes of the reaction norms are allowed to vary among individuals with respect to a given variable. In my dataset, allowing individual slopes in RMR and MMR to vary as function of multiple variables (i.e., mean daily temperature, trial sequence, day of year, and time of day) did not significantly improve the fit of the models (results not shown). Therefore, the underlying factors causing the positive residual correlation between RMR and MMR remain unknown.

4.6 Negative among-individual correlation

The negative among-individual correlation reveals that individuals, with on average higher MMR, have on average lower RMR. While this does not contribute any support to the aerobic capacity model, it also does not entirely invalidate the model since I did not estimate the genetic correlation (r_G). The r_{ind} estimate should be closer to r_G than r_P, but could also differ from r_G because of permanent-environment correlations between RMR and MMR.

The negative among-individual correlation between RMR and MMR suggests that energy is managed according to the allocation model, rather than the performance model. According to the allocation model, the individuals in my study with low RMR and high MMR are re-allocating the energy from maintenance to energetically expensive activities that require a high aerobic

capacity (Careau et al., 2008). These mice are likely profiting from the positive aspects of a high MMR including increased mating opportunities, increased foraging ability, and increased ability to defend territory, among other advantages (Bennett and Ruben, 1979; Hedrick and Hillman, 2016). However, these mice would also be experiencing the drawbacks of potentially compromising their physiological maintenance (Nilsson, 2002). As a result, they may be more prone to contracting parasites and diseases and/or sensitive to the effects of having parasites and diseases. Meanwhile, mice with high RMR but low MMR might be less susceptible to parasites and diseases but are not able to access as many resources, acquire many mates or effectively defend their territory.

I found only one other study, by Ksiazek et al., (2004), that reported a significant negative relationship between MMR and BMR. In this study, Ksiazek et al., (2004) used divergent selection on laboratory mice to explore the relationships between MMR, BMR, and the mass of metabolically active organs. Their objective was to determine if there were correlated changes in MMR as a result of divergent selection on BMR to support the aerobic capacity model. Consequently, the interpretation of their results concentrated on explaining how the positive relationship between BMR and the size of the metabolically active organs might support a modified version of the aerobic capacity model rather than explaining the negative relationship between BMR and MMR.

I suggest that future research should focus on exploring the consequences of a negative link between RMR and MMR in terms of behaviour. Biro et al., (2018) suggested that individuals with a higher aerobic scope should display a wider range of behaviours perhaps allowing them to adjust to changing situations as needed. Aerobic scope is relevant to a negative RMR-MMR

correlation since individuals with a high RMR but low MMR will have a much smaller scope than those with low RMR and high MMR. Research on behaviour and aerobic scope is the logical next step, to specifically investigate the behavioural differences among individuals and potentially explain the negative RMR-MMR link. This research could elucidate the behavioural strategies that exist between individuals exhibiting high RMR and low MMR versus those with low RMR and high MMR.

4.7 Conclusion

To summarize, I found that both RMR and MMR were significantly repeatable, indicating that both traits can be subject to natural selection. I also found that willingness to run was repeatable, suggesting that personality-related sampling bias might occur in forced-exercise studies. The extent of the bias will require a more formal evaluation, but in the meantime willingness to run can be included as a covariate in the analysis of MMR under circumstances where individuals cannot be trained to run. I partitioned the phenotypic correlation between RMR and MMR, to reveal a negative among-individual correlation and a positive residual correlation. The positive residual correlation is likely a result of an unaccounted effect influencing both RMR and MMR. The negative among-individual correlation suggests that energy is managed according to the allocation model (i.e., MMR is correlated with energetically costly activities that trade-off with maintenance costs, or RMR) rather than the performance model. It also does not support the aerobic capacity model. Moving forward, I propose that future research investigates the behavioural ramifications of the compensation model exhibited by the negative RMR-MMR link (see Biro et al., 2018).

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Tables and Figures

Table 1. Descriptive statistics, including the number of individuals measured (N_{ID}), total number of observations (n_{OBS}), average number of measurements per individual (mean n_{OBS}), units, and the mean, minimum, and maximum values recorded for body mass, resting metabolic rate (RMR), and maximal metabolic rate (MMR) in white-footed mice (*Peromyscus leucopus*). Paired measurements refer to cases in which both RMR and MMR were measured on the same individual on the same day.

	RMR	MMR	Body Mass	Paired Measurements
N_{ID}	151	117	155	111
n_{OBS}	331	182	340	171
Mean n_{OBS}	2.2	1.6	2.2	1.5
Units	mL $O_2 \cdot min^{-1}$	mL $O_2 \cdot min^{-1}$	g	
Mean	0.83	5.56	20.24	
Min	0.43	3.06	14.91	
Max	1.29	9.64	29.31	

Table 2. Sources of variation in the willingness to run during a forced-exercise test in white-footed mice (*Peromyscus leucopus*). Shown are estimates, standard errors (SE), degrees of freedom of the denominator (df_{den}), Wald-*F* statistic, and *P*-values extracted from a univariate mixed model that included body mass, sex (male/female), parasite (presence/absence), reproductive status (active/not active), time of day, test sequence, day of year, and the mean daily temperature as fixed effects. Also shown are variance estimates (±SE) for individual identity fitted as a random effect (V_{ind}) and residual variance (V_e). All significant sources of variance are bolded (P<0.05).

Fixed effects	Estimate	± SE	df_{den}	*F*	*P*
Intercept	3.1721	± 0.1443			
Body Mass	0.0640	± 0.0751	213.0	0.728	0.395
$Age_{[Juvenile]}$	0.2836	± 0.2093	300.0	1.836	0.176
$Sex_{[M]}$	-0.0915	± 0.1498	136.6	0.373	0.542
$Parasite_{[present]}$	-0.1465	± 0.1543	287.8	0.902	0.343
Reproductive $status_{[yes]}$	-0.0157	± 0.1410	290.1	0.012	0.911
Time of Day	-0.0958	± 0.0667	283.1	2.062	0.152
Test Sequence	**-0.2403**	± **0.0714**	**184.8**	**11.340**	**0.001**
Day of Year	**-0.2228**	± **0.0774**	**269.6**	**8.287**	**0.004**
Mean Daily Temperature	-0.0113	± 0.0629	295.3	0.032	0.857
Random effects	Variance	± SE			
V_{ind}	0.1910	± 0.0916			
V_e	1.0000	± 0.1047			

Table 3. Sources of variation in (A) resting metabolic rate (RMR), and (B) maximal metabolic rate (MMR) in white-footed mice (*Peromyscus leucopus*). Shown are estimates, standard errors (se), degrees of freedom of the denominator (df_{den}), Wald-*F* statistic, and *P*-values extracted from a bivariate mixed model. Fixed effects fitted to both RMR and MMR include body mass, sex (male/female), parasite (presence/absence), reproductive status (active/not active), time of day, test sequence, day of year and the mean daily temperature. Willingness to run (levels 3 and 4) was fitted to MMR only. All significant sources of variance are bolded (*P*<0.05). See Table 4 and 5 for random effects included in the same model.

Source	Estimate	± SE	df_{den}	*F*	*P*
(A) RMR					
Intercept	-2.3182	0.3817			
Body Mass	**0.6470**	**0.0458**	**193.9**	**199.900**	**<0.001**
Age$_{[Juvenile]}$	**0.4135**	**0.1279**	**319.3**	**10.450**	**0.001**
Sex$_{[M]}$	-0.0222	0.1007	168.6	0.049	0.825
Parasite$_{[present]}$	0.0223	0.0912	306.3	0.060	0.807
Reproductive status$_{[yes]}$	**-0.2495**	**0.0988**	**310.3**	**6.381**	**0.012**
Time of Day	**0.0035**	**0.0005**	**319.6**	**40.620**	**<0.001**
Test Sequence	-0.0384	0.0453	128.5	0.721	0.397
Day of Year	-0.0117	0.0540	283.2	0.047	0.828
Mean Daily Temperature	**-0.1713**	**0.0465**	**310.1**	**13.550**	**<0.001**
Activity	0.0264	0.0378	305.9	0.488	0.485
(B) MMR					
Intercept	0.4993	0.6448			
Body Mass	**0.4304**	**0.0712**	**139.6**	**36.540**	**<0.001**
Age$_{[Juvenile]}$	-0.0869	0.1851	166.6	0.220	0.640
Sex$_{[M]}$	0.1131	0.1456	138.4	0.603	0.439
Parasite$_{[present]}$	-0.0105	0.1352	160.7	0.006	0.938
Reproductive status$_{[yes]}$	**-0.2961**	**0.1459**	**151.5**	**4.120**	**0.044**
Time of Day	0.0008	0.0006	159	1.699	0.194
Test Sequence	-0.1008	0.0672	106.1	2.254	0.136
Day of Year	-0.1330	0.0731	168.8	3.308	0.071
Mean Daily Temperature	**-0.2897**	**0.0620**	**166.2**	**21.810**	**<0.001**
Willingness to Run	0.1003	0.0529	163.4	3.594	0.059

Table 4. Among-individual variance (V_{ind}), residual variance (V_e), and repeatability (R) in resting metabolic rate (RMR) and maximal metabolic rate (MMR) in white-footed mice (*Peromyscus leucopus*).

	V_{ind}	±	SE	V_e	±	SE	R	±	SE
RMR	0.0761	±	0.0381	0.4187	±	0.0432	0.1539	±	0.0730
MMR	0.1589	±	0.0753	0.4352	±	0.0734	0.2604	±	0.1156

Table 5. Estimates, standard errors (SE), and 95% confidence intervals for the phenotypic correlation (r_P), residual correlation (r_e), and among-individual correlation (r_{ind}) between resting metabolic rate and maximal metabolic rate in white-footed mice (*Peromyscus leucopus*).

				95% Confidence interval	
	Estimate	±	SE	Lower	Upper
r_P	-0.0151	±	0.1054	-0.2217	0.1916
r_e	0.2045	±	0.1055	0.0458	0.3445
r_{ind}	-0.8677	±	0.3972	-0.9993	-0.2854

Figure captions

Figure 1. A) Oxygen consumption (VO_2; mL $O_2 \cdot min^{-1}$) as a function of time, showing 331 raw respirometry traces (black circles and line) recorded on 151 white-footed mice (*Peromyscus leucopus*) during summer 2018. B) Activity (black circles and line; arbitrary units) as a function of time and C) oxygen consumption (VO_2; mL $O_2 \cdot min^{-1}$) as a function of activity. In all panels, red dots indicate the extracted value for resting metabolic rate (RMR).

Figure 2. Highest oxygen consumption (VO_2; mL $O_2 \cdot min^{-1}$) recorded during a forced-exercise test as a function of the willingness to run in white-footed mice (*Peromyscus leucopus*).

Figure 3. Resting metabolic rate (RMR) and maximal metabolic rate (MMR) as a function of A,D) body mass (g), B,E) mean daily temperature (°C), and C,F) reproductive status (active or not) in white-footed mice (*Peromyscus leucopus*).

Figure 4. Visual representation of the among- and residual variation in A) resting metabolic rate (RMR; mL $O_2 \cdot min^{-1}$) and B) maximal metabolic rate (MMR; mL $O_2 \cdot min^{-1}$) in white-footed mice (*Peromyscus leucopus*) that were measured at least twice. In each panel, individuals are ordered according to their mean metabolic rates.

Figure 5. Correlation between maximal metabolic rate (MMR) and resting metabolic rate (RMR) at the A) residual level (residuals extracted from the bivariate mixed model) and B) among-individual level (individual estimates ± se extracted from the bivariate mixed model). Traits have been standardized to a mean of 0 and a variance of 1 (sd units).

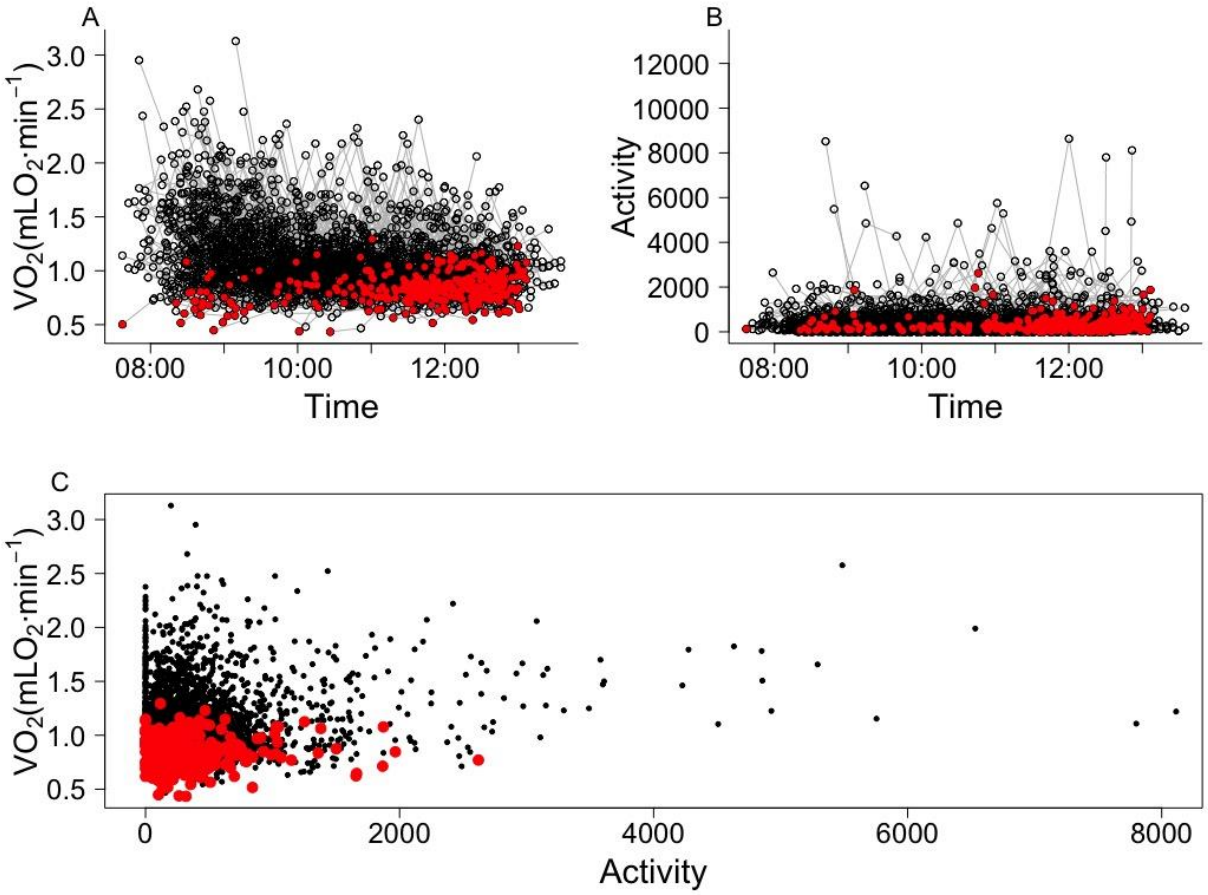
A
B
C
$VO_2(mLO_2 \cdot min^{-1})$
Time
Activity
08:00
10:00
12:00
0.5
1.0
1.5
2.0
2.5
3.0
0
2000
4000
6000
8000
10000
12000

Figure 1

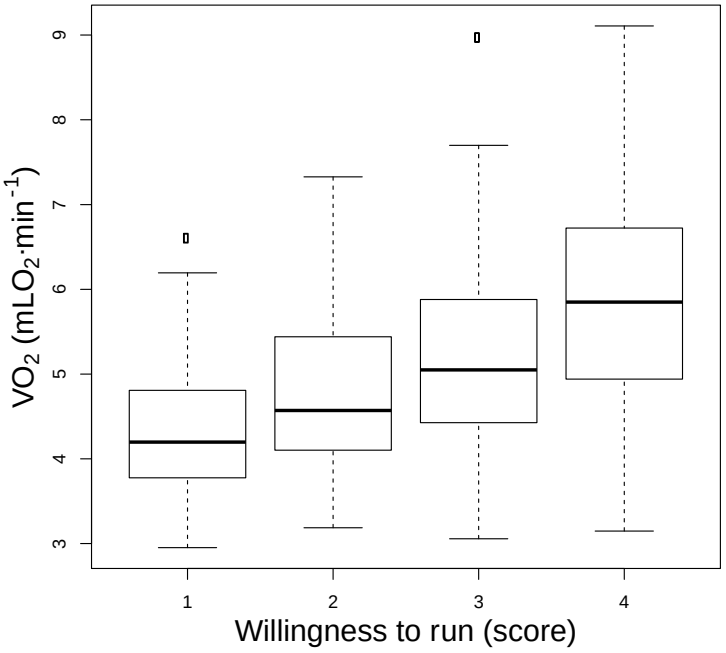

Figure 2

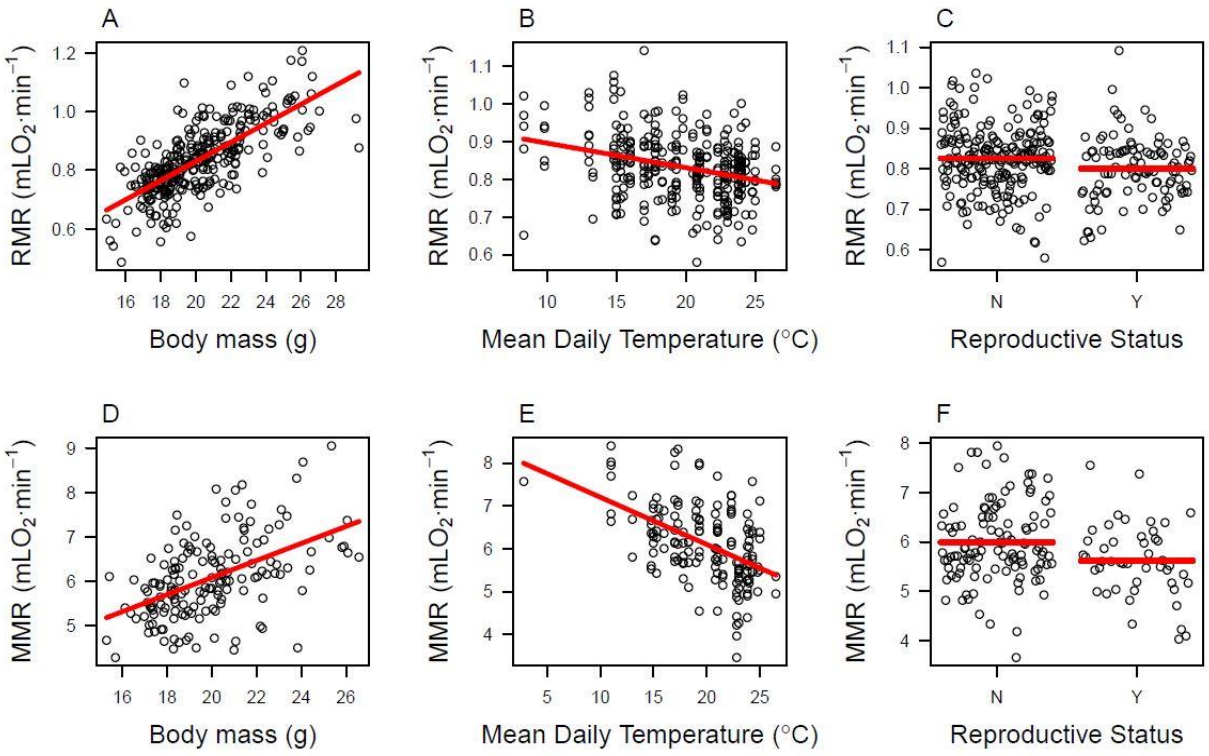
A
B
C
D
E
F
RMR (mLO2·min−1)
MMR (mLO2·min−1)
Body mass (g)
Mean Daily Temperature (°C)
Reproductive Status
N
Y

Figure 3

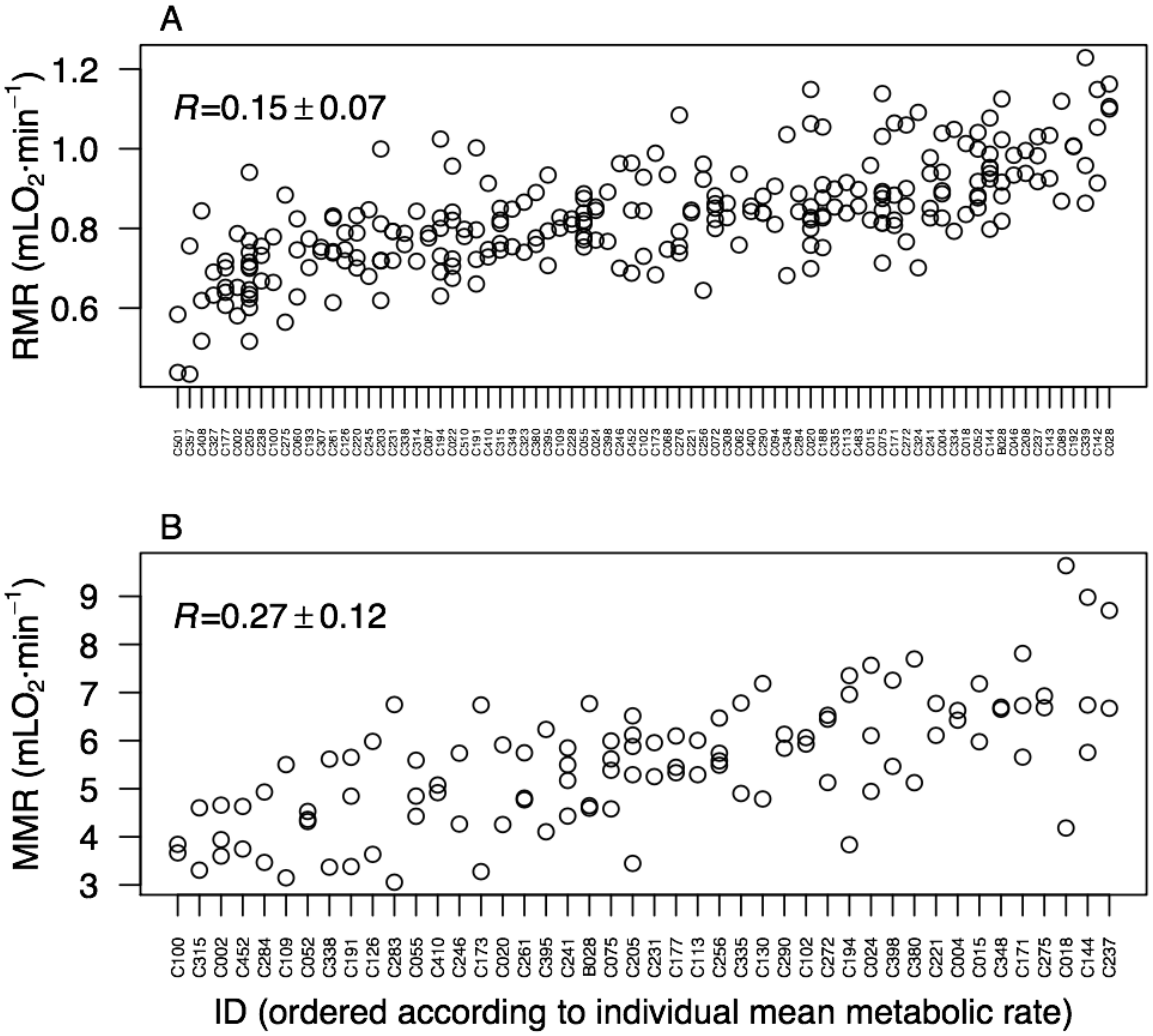
A
R=0.15 ± 0.07
RMR (mLO2·min−1)
1.2
1.0
0.8
0.6
B
R=0.27 ± 0.12
MMR (mLO2·min−1)
9
8
7
6
5
4
3
C100 C315 C002 C452 C284 C109 C052 C338 C191 C126 C283 C055 C410 C246 C173 C020 C261 C395 C241 B028 C075 C205 C231 C177 C113 C256 C335 C130 C290 C102 C272 C194 C024 C398 C380 C221 C004 C015 C348 C171 C275 C018 C144 C237
ID (ordered according to individual mean metabolic rate)

Figure 4

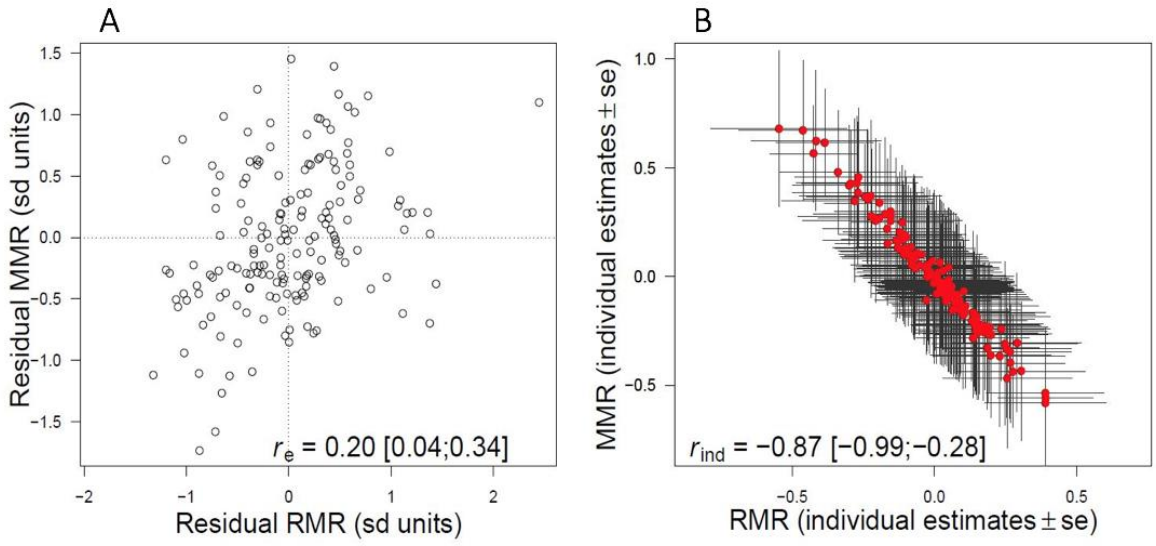
A
B
Residual MMR (sd units)
Residual RMR (sd units)
r_e = 0.20 [0.04;0.34]
MMR (individual estimates ± se)
RMR (individual estimates ± se)
r_{ind} = −0.87 [−0.99;−0.28]

Figure 5

Support Information

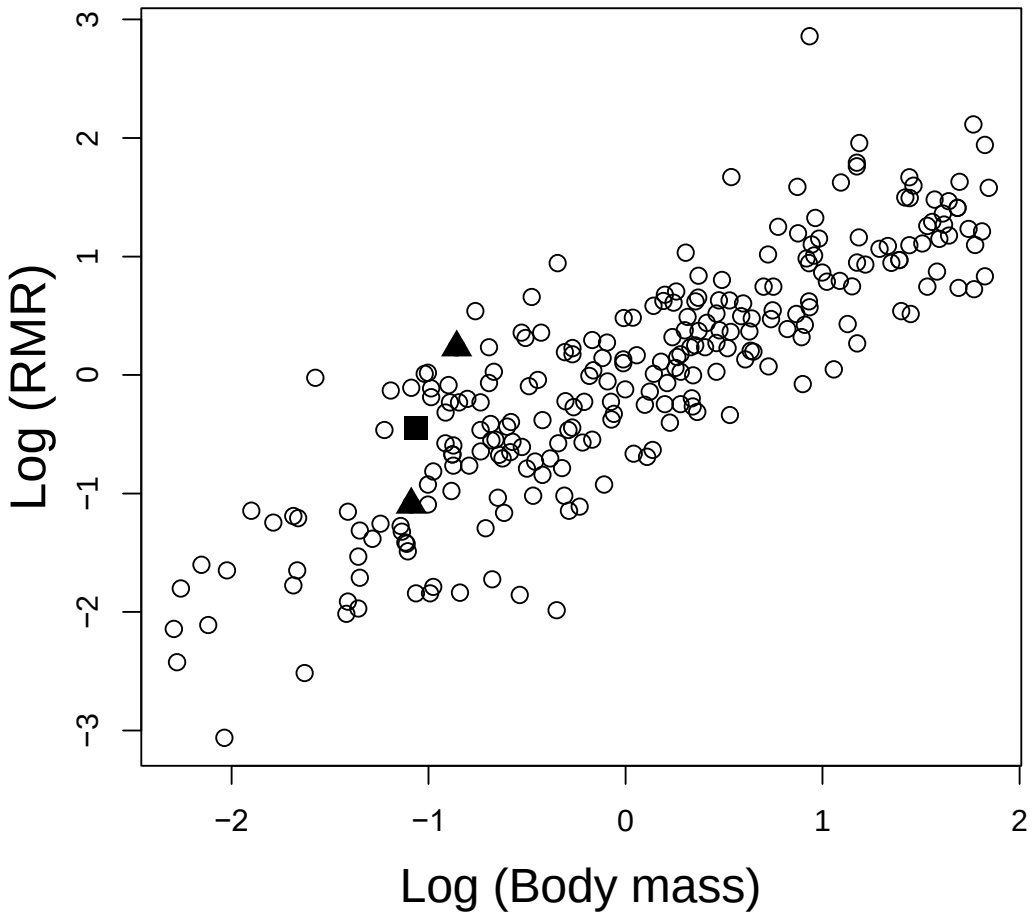

Figure S1. Average resting metabolic rate (RMR; mL $O_2 \cdot min^{-1}$) as a function of body mass (g) in variety of similar sized rodent and other small mammals, taken from (Nespolo et al., 2017). The average RMR measurement for *Peromyscus leucopus* in the current study is represented by the solid square, while the average RMR measurements from two other studies (Deavers and Hudson, 1981; Segrem and Hart, 1967) on *Peromyscus leucopus* are represented by solid triangles.

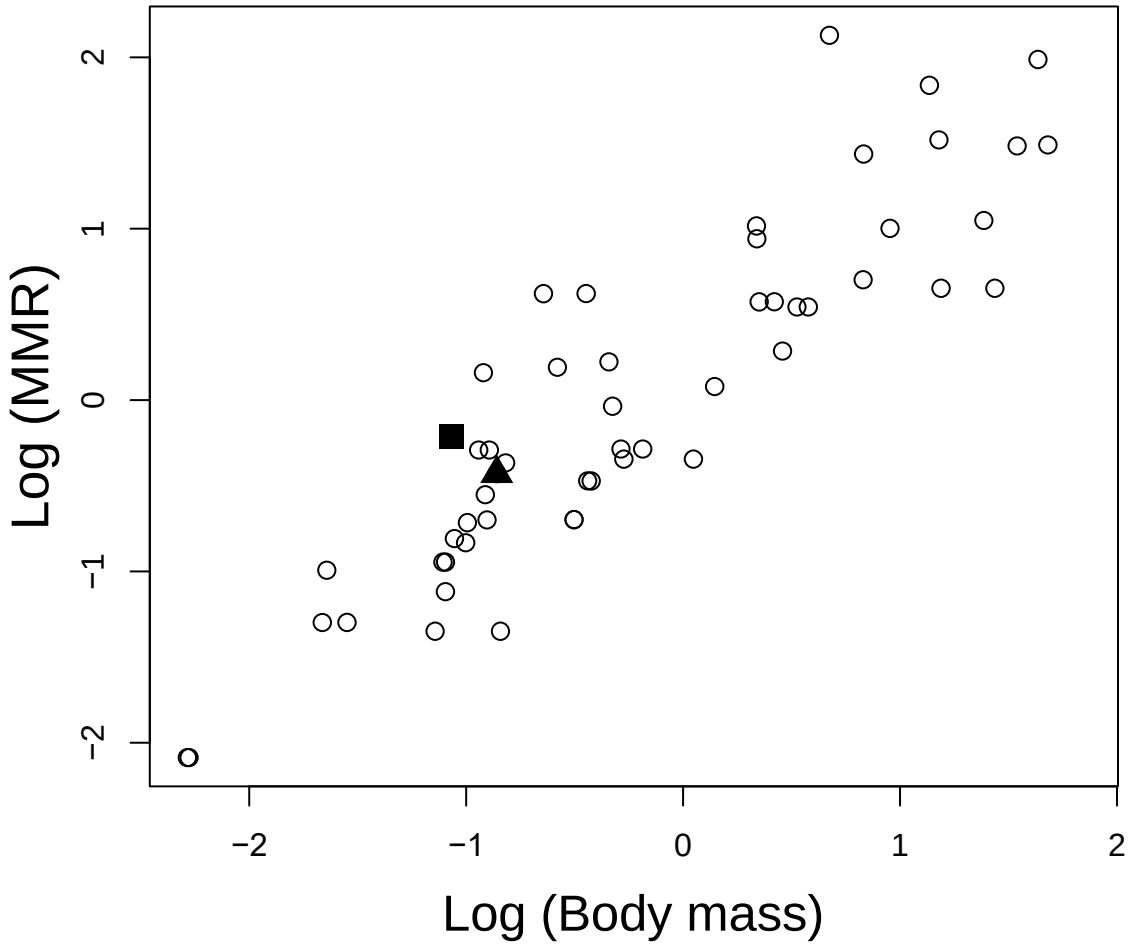

Figure S2. Average maximal metabolic rate (MMR; mL $O_2 \cdot min^{-1}$) as a function of body mass (g) in variety of similar sized rodent and other small mammals, taken from (Dlugosz et al., 2013; Nespolo et al., 2017). The average MMR measurement for *Peromyscus leucopus* in the current study is represented by the solid square, while the average MMR measurement another study (Segrem and Hart, 1967) on *Peromyscus leucopus* is represented by the solid triangle.