

RESEARCH ARTICLE

Context-dependent correlation between resting metabolic rate and daily energy expenditure in wild chipmunks

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SUMMARY

Several empirical studies have shown that variation in daily energy expenditure (DEE) and resting metabolic rate (RMR) is influenced by environmental and individual factors, but whether these shared influences are responsible for, or independent of, relationships between DEE and RMR remains unknown. The objectives of this study were to (i) simultaneously evaluate the effects of environmental and individual variables on DEE and RMR in free-ranging eastern chipmunks (*Tamias striatus*) and (ii) quantify the correlation between DEE and RMR before and after controlling for common sources of variation. We found that the influence of individual factors on DEE and RMR is most often shared, whereas the influence of environmental factors tends to be distinct. Both raw and mass-adjusted DEE and RMR were significantly correlated, but this correlation vanished after accounting for the shared effect of reproduction on both traits. However, within reproductive individuals, DEE and RMR remained positively correlated after accounting for all other significant covariates. The ratio of DEE to RMR was significantly higher during reproduction than at other times of the year and was negatively correlated with ambient temperature. DEE and RMR appear to be inherently correlated during reproduction, but this correlation does not persist during other, less energy-demanding periods of the annual cycle.

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INTRODUCTION

Basal metabolic rate (BMR; the lowest measured metabolic rate of a post-absorptive, normothermic adult individual in its thermoneutral zone during its inactive period) or resting metabolic rate (RMR; same criteria as BMR, except animals are not fasted and therefore are not post-absorptive) has been measured in over 1000 species (McNab, 2008; McNab, 2009; Speakman et al., 2004). It has become clear that inter-specific differences in these minimum metabolic rates are related to life-history traits (Wiersma et al., 2007), behaviour (Careau et al., 2009), phylogeny (Hayssen and Lacy, 1985), and several ecological factors such as climate (Lovegrove, 2000), latitude (Rezende et al., 2004), diet (McNab, 1986) and environmental productivity (Mueller and Diamond, 2001).

Variations in BMR and RMR are widely assumed to be correlated with metabolism expressed under different circumstances, including during shorter periods of peak demand (maximum metabolic rate or summit metabolism) and on a daily basis (daily energy expenditure, DEE). Particular attention has been paid to the relationship between BMR and sustained levels of DEE expressed during periods long enough in duration that metabolism is fuelled by food intake rather than by transient depletion of energy reserves (Daan et al., 1990; Hammond and Diamond, 1997; Peterson et al.,

1990). When expressed as a multiple of BMR, levels of sustained DEE are referred to as sustained metabolic scopes, which have long been noted to cluster around 3–4 times BMR and rarely exceed 5 times BMR across many species and a wide variety of energy-demanding activities (Hammond and Diamond, 1997; Peterson et al., 1990; Piersma and van Gils, 2011; Speakman, 2000; Weiner, 1992). The most intuitive explanation for this pattern of constrained variation is that BMR and RMR reflect the energy costs of maintaining the metabolic machinery required for elevated metabolic performance, meaning that an increased DEE will be mechanistically coupled to an increase in BMR and RMR (Drent and Daan, 1980; Speakman et al., 2004). Thus, the relationship between DEE, BMR (or RMR) and sustained metabolic scope has become an issue of central importance to the understanding of organismal design and function, including the evolution of endothermy (Ricklefs et al., 1996; Speakman et al., 2003).

Over the last 30 years, a large body of research has been conducted to investigate the nature of the coupling between DEE and BMR and the limits to sustained metabolic scope (Speakman and Król, 2011). An inherent, positive coupling between DEE and BMR may occur either centrally, because an increase in the size of energetically expensive digestive organs is required to process more

food (Brzęk et al., 2007; Daan et al., 1990; Konarzewski and Diamond, 1995; Selman et al., 2001), or peripherally, if the tissues at the site of energy use reach maximal capacity, which may also require high maintenance costs such as mammary glands (Hammond et al., 1994). However, recent theoretical and empirical work has suggested that DEE should not be limited to a fixed ratio of BMR. Research on limits to heat dissipation predicts that sustained metabolic scope will decline as ambient temperature (T_a) increases (Speakman and Król, 2011). For example, it is possible that DEE but not BMR varies with T_a , such that day-to-day variations in the two traits are not correlated, but the ratio of DEE to BMR increases with decreasing T_a .

In line with an inherent coupling between DEE and BMR, many comparative studies have shown that these traits are positively correlated at the inter-specific level (Daan et al., 1990; Drent and Daan, 1980; Koteja, 1991; Ricklefs et al., 1996; Speakman, 2000; White and Seymour, 2004). Yet, the evidence for a link between DEE and BMR (or RMR) among individuals within populations is equivocal. In mammals, only three previous studies have tested for a correlation between DEE and BMR (or RMR) within species in the field (Fyhn et al., 2001; Meerlo et al., 1997; Speakman et al., 2003). Of those studies, only one (Speakman et al., 2003) found a positive association between mass-residual DEE and RMR in field voles (*Microtus agrestis*). However, this DEE–RMR relationship was driven by a study site effect; there was no evidence of a within-site DEE–RMR correlation after this site effect was accounted for. In free-living garter snakes (*Thamnophis sirtalis*), measures of standard metabolic rate (analogous to BMR in ectotherms) were uncorrelated with DEE (Peterson et al., 1998). In birds, a correlation between DEE and BMR was found in two studies of reproductive individuals subjected to experimental manipulations [i.e. brood enlargement (Nilsson, 2002) or primary feathers clipped (Tieleman et al., 2008)]. Thus, collectively the nature of the intra-specific relationship between DEE and BMR or RMR remains largely unresolved (Speakman et al., 2004).

Beyond characterizing the absence or existence of an intra-specific correlation between DEE and BMR, it is important to assess how and why such a correlation arises. Both DEE and BMR (or RMR) are known to be related to food quantity and quality (Bozinovic et al., 2007; Cruz-Neto and Bozinovic, 2004; Welcker et al., 2009), climate (Lovegrove, 2000) and environmental productivity (Mueller and Diamond, 2001; Thomas et al., 2001). Thus, correlations between DEE and BMR (or RMR) could reflect the shared influence of one or more of these environmental factors. However, both traits are also known to be related to body mass (M_b) (e.g. Fletcher et al., 2012; Speakman et al., 2003; Speakman et al., 2004), reproductive status (Bergeron et al., 2011a; Speakman, 2008), and age (Bergeron et al., 2011a; Bouwhuis et al., 2011; Broggi et al., 2010; Das et al., 2001; Even et al., 2001), meaning that the correlation could arise from covariation with one or more of these individual traits at the whole-animal level. Previous studies examining intra-specific correlations between DEE and BMR (or RMR) had a limited ability to assess potential environmental and individual drivers of the correlation (but see Speakman et al., 2003), and studies assessing multiple drivers have generally focused on a single metabolic trait (e.g. Bouwhuis et al., 2011; Fletcher et al., 2012).

Further attempts at testing the relationship between DEE and BMR (or RMR) in wild animals must therefore be accompanied by a thorough evaluation of the relative importance of likely environmental and individual determinants of both traits and their ratio. Here, we performed this evaluation in free-ranging eastern

chipmunks (*Tamias striatus*). The eastern chipmunk is a burrowing rodent that specializes on mast seed produced by oak (*Quercus* spp.), American beech (*Fagus grandifolia*) and maple (*Acer* spp.) trees (Elliott, 1978). At our study site, American beech is the predominant canopy tree species and chipmunks also forage on seeds (black cherry *Prunus serotina*), fruits (strawberry *Fragaria virginiana*), bulbs (spring beauty *Claytonia caroliniana*, trout lily *Erythronium americanum*), different species of invertebrates (snails, slugs, caterpillars), and mushrooms (V.C., personal observation). Chipmunks experience extreme intra- and inter-annual variation in food availability and quality at this site because of mast seeding by beech trees (involving synchronous production of large amounts of seeds in some autumns and few or no seeds in others), which leads to pronounced annual differences in seasonal activity patterns (Bergeron et al., 2011b; Munro et al., 2008), torpor expression (Landry-Cuerrier et al., 2008), bot fly parasitism (Careau et al., 2010) and the timing and extent of reproduction (Bergeron et al., 2011c).

We assessed how DEE and RMR varied between mast- and non-mast years, and between early summer, late summer and autumn seasons, as well as the effects of T_a and a suite of individual variables (M_b , reproductive state, age and sex). We tested whether the correlation between DEE and RMR persisted after accounting for likely environmental or individual covariates. In so doing, we provide one of the few tests of the alternative hypotheses that (a) DEE and RMR are uncorrelated at an intra-specific level, (b) DEE and RMR are correlated at an intra-specific level, but this correlation arises from the shared influence of individual (whole-animal) and environmental traits, or (c) DEE and RMR are inherently correlated at an intra-specific level (implying an underlying physiological mechanism), such that they remain correlated even after accounting for likely individual and environmental covariates. Finally, we tested whether the ratio of DEE to RMR was correlated with environmental and individual variables (see above), including a recently proposed negative relationship with T_a (Speakman and Król, 2011).

MATERIALS AND METHODS

Study area

From 2004 to 2009, we monitored individually marked free-ranging eastern chipmunks, *T. striatus* (Linnaeus 1758), on a 25 ha study site in the Ruitter Valley Land Trust (Sutton Mountains, Québec; 45°05'N, 72°26'W). Trapping sessions were conducted from early May until early October in all years, which we divided into three seasons: spring (May and June), summer (July and August) and autumn (September and October). Chipmunks were live-trapped daily between 08:00 h and sunset using Longworth traps baited with peanut butter and visited every 2 h. At first capture, individuals were permanently marked with numbered ear tags (1005-1, National Band and Tag Company, Newport, KY, USA) and a PIT-tag (Trovan, Douglas, UK) inserted in the inter-scapular region. At each capture, we noted trap location, M_b , sex and reproductive status (males during mating had a developed scrotum and females during lactation had clearly visible mammae). We also recorded the minimum known age according to the year of first capture and whether the individual was first captured as a juvenile (age 0) or as an adult (age assumed to be 1 year), as detailed in a previous publication (Careau et al., 2010). We differentiated juvenile chipmunks from adults based either on an initial capture within the month following emergence when M_b was <80 g or, for individuals >80 g when first captured, on the absence of a darkened scrotum or developed mammae (Careau et al., 2010). Most of the measurements were taken from 2006 to 2009 on individuals of known age that were first captured between 2004 and 2009. Animals were captured and handled following the

protocol approved by the Animal Welfare Committee of the Université de Sherbrooke and the Ministère des Ressources Naturelles et de la Faune du Québec.

Ambient temperature (T_a)

Daily averages of T_a data were obtained from a meteorological station located *ca.* 20 km from the study site (Environment Canada, Sutton Station, <http://climate.weatheroffice.gc.ca>; 45°04'N, 72°41'W) for each year from November to April. This station only reported the daily average, which we used in all analyses except for DEE because we needed hourly estimates (we used the average of two equally distant meteorological stations from our site: Lac Memphremagog 45°16'00"N, 72°10'00"W, 32 km north; Frelighsburg 45°03'01"N, 72°51'41"W, 37 km west). Both stations gave T_a estimates that were highly correlated with each other and to a weather station located on the middle of our grid that was operational only in 2006 (Lac Memphremagog *versus* Frelighsburg stations: $r=0.96$, $N=3719$; onsite station *versus* Lac Memphremagog: $r=0.97$, $N=3719$; onsite station *versus* Frelighsburg: $r=0.98$, $N=4366$; correlations estimated for each hourly estimate available for each station from 1 May to 1 October 2006).

Tree mast sampling

The annual mast seed production of *F. grandifolia* within the study site was sampled as detailed in previous publications (Bergeron et al., 2011b; Landry-Cuerrier et al., 2008). At 30 sampling points, evenly distributed across the study site, we placed a seed-collecting bucket (0.06 m²) under the canopy of a beech tree with a diameter at breast height (dbh) >10 cm nearest each sampling point. Buckets were installed well before autumn seed fall (in late summer) and the contents were counted twice per autumn. We identified seed coats containing kernels and weighed those seeds. Energy contained in beech seeds was measured using a bomb calorimeter (27.65 kJ g⁻¹ dry mass, data not shown), which was used to calculate energy availability in kJ per m² (see Landry-Cuerrier et al., 2008). We assumed that wet seeds contained 6.6% of water as indicated by USDA Agricultural Research Service data (<http://www.nal.usda.gov/fnic/foodcomp/>). The years 2006 and 2008 were categorized as mast years (see supplementary material Fig. S1).

Daily energy expenditure (DEE)

We measured DEE during three summers (2007–2009) using the doubly labelled water (DLW) technique, which estimates the CO₂ produced by a free-ranging animal based on the differential washout of injected hydrogen (²H) and oxygen (¹⁸O) isotopes (Butler et al., 2004). This technique provides an accurate measure of DEE over periods of several days in small mammals and has previously been used successfully on eastern chipmunks (Humphries et al., 2002). The dataset used in this study is a subset (i.e. excluding juveniles) of the dataset used in previous publications (Bergeron et al., 2011a; Careau et al., 2012a; Careau et al., 2012b). All injections and blood samples were taken in the field by one of us (V.C.) to minimize variation.

We used a two-capture approach to estimate DEE. On initial capture, chipmunks were injected intra-peritoneally with 240 µl of DLW (37.78% and 4.57% enriched with ¹⁸O and ²H, respectively). Following injection, chipmunks were held in the trap for a 1 h equilibration period (Speakman and Król, 2005). Then, a first blood sample was collected *via* a clipped toenail for initial isotope analysis. Chipmunks were then released at the site of capture and recaptured, weighed and bled 1–3 days later, as close as feasible to 24 h intervals, and a final blood sample was taken to estimate isotope

elimination rates. Taking samples over multiples of 24 h periods minimizes the substantial day-to-day variability in DEE (Berteaux and Thomas, 1999; Speakman et al., 1994). The range of absolute deviation from 24 h was 5–180 min (25th percentile, 24 min; median, 40 min; 75th percentile, 62 min). From 2007 to 2009 a total of 10 animals were blood sampled without prior injection to estimate background isotope enrichments of ²H and ¹⁸O [method C in Speakman and Racey (Speakman and Racey, 1987)]. Capillaries were flame sealed immediately after sample collection. Capillaries that contained blood samples were vacuum distilled and water from the resulting distillate was used to produce CO₂ (Speakman et al., 1990) and H₂ (Speakman and Król, 2005). The isotope ratios ¹⁸O:¹⁶O and ²H:¹H were analysed using gas source isotope ratio mass spectrometry (Optima, Micromass IRMS and Isochrom µG, Manchester, UK). Samples were run alongside three lab standards for each isotope (calibrated to international standards) to correct delta values to p.p.m. Isotope enrichments were converted to values of field metabolic rate (FMR) using a single pool model as recommended for this size of animal (Speakman, 1993). We assumed evaporation of 25% of the water flux [equation 7.17 in Speakman (Speakman, 1997)], which minimizes error in a range of conditions (Visser and Schekkerman, 1999). Because chipmunks spend the night in burrows, some re-entry of labelled CO₂ might have occurred (see Speakman, 1997).

We collected a total of 58 DEE measurements on 48 adult individuals. Eight individuals were measured twice and one individual three times in different years. We sampled reproductive individuals in different proportions across seasons (females: spring 5.9%, summer 79.4%, autumn 14.7%; males: spring 100%). Initial models for DEE included covariates related to its measurement to control for sampling methods in the field, but these variables were all not significant [number of days over which DEE was measured ($F=1.04$, $P=0.31$), relative deviation from 24 h cycle ($F=0.32$, $P=0.58$), equilibration time ($F=0.01$, $P=0.91$) and whether the animal was trapped between initial and final samples ($F=0.21$, $P=0.65$)].

Resting metabolic rate (RMR)

We measured RMR from autumn 2006 to 2009 using a computerized open-circuit respirometry system described elsewhere (Careau et al., 2010). For a given metabolic run, four individuals were weighed and then placed individually in a 650 or 850 ml Plexiglas cylindrical metabolic chamber. Chambers were placed in a constant-temperature cabinet regulated at 30°C, which lies within the thermoneutral zone of chipmunks (Wang and Hudson, 1971). A manifold and four mass-flowmeters (Side-track model 844, Sierra Instruments, Monterey, CA, USA) provided a constant flow of 450 ml min⁻¹ of dry (Drierite), CO₂-free (soda lime) air to each chamber, as well as to two baseline airflows. The four mass-flow meters were calibrated with a 'master' flow meter (Brooks model 5850E set to read mode; Brooks, Hatfield, PA, USA) at the start of each year and regularly (approximately every other week) until the end of the field season. The outflows of each chamber and the two baselines were directed to a computer-controlled multiplexor, which allowed us to sequentially sample baselines and the chambers using two oxygen analysers (Model FC-1, Sable Systems International, Henderson, NV, USA). We calibrated oxygen analysers at 20.95% with dry, CO₂-free outside air before each run.

A 100 ml min⁻¹ sub-sample of baseline air or chamber outflow was dried and pulled through the oxygen analysers, alternating between baseline (5 min) and the two chambers (25 min each) over a 3.5 h period. By running two cycles between 20:00 h and 05:00 h, we were able to measure metabolism during the resting phase for

up to eight animals each night. Although we could have monitored metabolic rate for a longer period, this would have reduced the number of animals we could measure per night. We used Expedata software (Sable Systems) to control the multiplexor outputs, read chamber O₂ concentration and temperature at 1 s intervals, correct for drift between consecutive baseline measurements, and calculate individual O₂ consumption according to eqn 4a of Withers (Withers, 1977) without taking CO₂ production into account (Koteja, 1996). We assumed a respiratory exchange ratio of 0.8 (not post-absorptive) to calculate O₂ consumption and convert data to mW. RMR was calculated from the lowest baseline level of O₂ consumption recorded for 5 min during a 3.5 h run. The lowest 5 min (=RMR) normally occurred in the last 25 min period.

All measurements were made on adult individuals (age ≥ 1 year) that settled down in the respirometer (resting state of individuals was confirmed by visual inspection of oxygen consumption rates and of the animals themselves; most of them (95.7%) were sleeping or clearly resting at the end of a trial). The proportion of animals that were resting or sleeping *versus* active at the end of the respirometry run did not differ between the first (97.1%) and second run (95.4%) of the night.

We collected a total of 409 RMR measurements on 204 adult individuals (mean \pm s.d. number of measurements per individual 2 ± 1.2 , range 1–6). We sampled reproductive individuals in different proportions across seasons (females: spring 47.1%, summer 29.4%, autumn 23.5%; males: spring 71.1%, summer 24.8%, autumn 4.1%). Initial models for RMR included covariates related to its measurement to control for sampling methods, but these variables were all not significant [size of chamber ($F=1.11$, $P=0.29$), test sequence within a year ($F=1.16$, $P=0.28$) and whether the run was conducted early or late at night ($F=0.64$, $P=0.42$)].

Limitations

As this study was part of a larger project, we wanted to minimize the time chipmunks were absent from their territory to avoid impacting survival and reproductive success (e.g. losing mating opportunities, or having their food hoards being pilfered). We therefore captured chipmunks as late as possible in the afternoon, transported them to the nearby (~10 km) laboratory facility, measured their metabolism overnight, and released them at their original trap location the following morning, just before sunrise. These constraints on the time we could keep animals in captivity introduced two limitations into our study design.

First, capture success is usually better in the morning and for this reason most captures related to the DLW method were done in the morning to maximize re-capture success for the second blood sample. Therefore, we could not pair a RMR measurement with each DEE measurement, using a single capture, as it would have involved keeping animals in captivity for too long. Instead, we targeted individuals on the days following their DEE measurement, but logistical constraints in the field, variation in above-ground activity and reduced capture success in the afternoon meant that the two measurements were separated by varying time periods (range 1–108 days, median 29 days).

Second, we could not keep animals in captivity for long enough to ensure that they were post-absorptive during respirometry measurements. Therefore, all animals were provided with apple and peanut butter at all times except when in the metabolic chambers. Because animals were not post-absorptive during respirometry measurements, we classified metabolic measurements as RMR rather than BMR. Thus, like many recent metabolic studies on small, wild-caught endotherms (Larivée et al., 2010; Speakman et al., 2004;

Timonin et al., 2011), our RMR measurements include an unquantified metabolic contribution from the heat increment of feeding. Among small, granivorous rodents, RMR typically exceeds BMR by 5–15% with the difference becoming negligible after 3 h of respirometry measurement (Nespolo et al., 2003). Accordingly, the average RMR (2.34 kJ h^{-1} for a M_b of 90.8 g) is 11% higher than the BMR measured in captive eastern chipmunks captured at a similar latitude (2.11 kJ h^{-1}) (Levesque and Tattersall, 2010).

Data analysis

We first tested whether DEE and RMR were influenced by food abundance (mast year or not), season (spring, summer and autumn), T_a , age (in years), sex, reproductive status (reproductive if lactating or testicles descended, otherwise not), and M_b using linear mixed models in JMP (v9.0.0, SAS Institute Inc., Cary, NC, USA), including identity of the animal and year as random effects. Model selection was performed using backward procedures, sequentially removing the least significant term from the model based on its P -value ($\alpha=0.05$). We calculated mean T_a during each DEE sampling interval by taking the mean T_a of the hourly averages between initial and final samples. For RMR, however, we had no *a priori* reason for choosing an exact time period to test for the effect of T_a . We calculated average T_a over different periods preceding RMR measurements and sequentially tested the predictive power for RMR of each period (30, 15, 7, 3 or 1 day), and selected the period with the highest predictive power and included this average in the model. Once the fixed effects structure was determined, we estimated the repeatability of DEE and RMR while controlling for significant covariates (see Table 1) using an intra-class correlation coefficient (τ) calculated on individuals with repeated measures only (Lessells and Boag, 1987).

Seasons that did not statistically differ were pooled in order to test whether RMR increased disproportionately in non-mast *versus* mast years at the population level. A ‘mast \times season’ interaction was included in the model and the model selection was started again (see above). To eliminate the possibility that the seasonal changes in RMR are a sampling artefact (e.g. if only chipmunks with high RMR remain active in autumn), we performed a paired t -test on a subset of individuals with at least one measurement in two different seasons within a year.

RMR and DEE were not measured simultaneously (see above). We therefore selected the closest RMR measurement to any DEE measurement to test whether RMR and DEE were correlated within our population. Restricting the analysis to measurements made within 10 days or fewer considerably reduced the power (sample size) to detect any relationship, whereas using all estimates increased the time elapsed between the two measurements. We therefore performed a series of analyses restricted to different time periods on a whole-animal and mass-independent basis (residuals from the regression against M_b) to find the period that maximized effect size (regression estimate) on a whole-animal basis. Next, we calculated the ratio of DEE to RMR and used this period to assess the significance of the relationship between mass-independent DEE and RMR using a bivariate mixed model in ASReml-R (Butler et al., 2007). This approach allowed us to test the relationship in a one-step process instead of testing a correlation between residuals in a less conservative two-step process. The bivariate model included M_b taken at the time of DEE measurement ($M_{b,DEE}$) and M_b taken at the time of RMR measurement ($M_{b,RMR}$) as separate fixed effects and ID as a random effect to account for the small number of repeated measures. In this model, we also fitted a covariance term between the residuals ($COV_{DEE-RMR}$). Therefore, the correlation

Table 1. Linear mixed effects models for daily energy expenditure and resting metabolic rate in relation to environmental and individual variables in free-ranging eastern chipmunks

	DEE (mW)				RMR (mW)			
	Estimate \pm s.e.m.	d.f.	<i>t</i>	<i>P</i>	Estimate \pm s.e.m.	d.f.	<i>t</i>	<i>P</i>
Mast (no)	0.1 \pm 80.1	23	0.00	1.00	21.2 \pm 19.9	2	1.07	0.40
Season								
Spring	-143.0 \pm 153.1	39	0.93	0.36	-40.8 \pm 6.2	391	6.54	<0.001
Summer	-31.3 \pm 125.4	37	0.25	0.80	-25.6 \pm 6.4	391	4.02	<0.001
T_a	-38.6 \pm 18.7	32	2.06	0.047	-1.3 \pm 1.4	391	0.92	0.36
Age	-160.5 \pm 81.1	51	1.98	0.053	-8.7 \pm 4.9	266	1.76	0.08
Sex (F)	78.8 \pm 104.0	45	0.76	0.45	26.1 \pm 5.2	289	4.99	<0.001
Reproductive (no)	-277.7 \pm 80.8	52	3.44	0.001	-27.4 \pm 5.5	395	5.00	<0.001
M_b	50.0 \pm 11.3	52	4.42	<0.001	4.5 \pm 0.6	312	8.17	<0.001

DEE, daily energy expenditure; RMR, resting metabolic rate. Initial models included effects of mast (yes or no), season (spring, summer and autumn), air temperature (T_a), age (in years), reproductive status (yes or no), body mass (M_b) and sex (F, female), but only significant terms ($P < 0.05$) were retained following model simplification.

Statistics for non-significant variables are shown at the moment they were dropped from the model. The final models explained 87.5% and 47.2% of the variance in daily energy expenditure and resting metabolic rate, respectively.

between mass-residual DEE and RMR can be calculated as the $COV_{DEE-RMR}$ term divided by the square root of the product of the variance of residuals. We tested the significance of $COV_{DEE-RMR}$ using a log-likelihood ratio test (LRT) comparing a full model that included $COV_{DEE-RMR}$ with a reduced model where $COV_{DEE-RMR}$ was constrained to zero. We also constructed a model without $M_{b,DEE}$ and $M_{b,RMR}$ as fixed effects to estimate the correlation on a whole-animal basis. We also ran bivariate models with reproductive status during DEE measurement as a fixed effect (in addition to $M_{b,DEE}$ and $M_{b,RMR}$) to test whether the association between DEE and RMR could relate to their mutual link with reproductive status. Averages and coefficients estimates are reported ± 1 s.e.m., unless mentioned otherwise.

RESULTS

Daily energy expenditure (DEE)

DEE ranged between 990 and 4610 mW (mean 2362 \pm 97 mW; mean M_b 93.8 \pm 1.0 g) and was affected by both individual and environmental factors (Table 1). DEE did not differ between mast and non-mast years or among seasons, but was negatively correlated with T_a (Table 1). DEE was positively correlated with M_b and was higher in reproductive individuals (lactating females or males during mating; Table 1). DEE was marginally and negatively correlated with age (Table 1; $P = 0.053$). The intra-individual correlation coefficient applied to the nine individuals with repeated measures indicated that residual DEE was significantly repeatable ($\tau = 0.57$, $F_{8,10} = 3.83$, $P = 0.03$). The effects of mass and reproductive status remained significant in a linear model after excluding the repeated measures (retaining only the first measurement for each individual), whereas the effect of T_a was no longer significant ($t_{44} = -1.16$, $P = 0.25$).

Resting metabolic rate (RMR)

RMR ranged between 254 and 1019 mW (mean 649 \pm 5.1 mW; mean M_b 90.8 \pm 0.4 g). Like DEE, RMR was higher in reproductive animals than in non-reproductive animals and was positively correlated with M_b (Table 1). In contrast to DEE, RMR varied seasonally (Table 1) and was about 15% higher in autumn than in spring and summer (Fig. 1). RMR was not associated with T_a averaged over various time periods (Table 1). RMR was higher in females than in males by 8% (Table 1). The intra-individual correlation coefficient, applied to the 92 individuals with repeated measurements, also indicated

that mass residual RMR was not repeatable ($\tau = 0.01$, $F_{91,183} = 1.04$, $P = 0.40$).

In a model with spring and summer seasons pooled, estimates of fixed effects remained qualitatively similar except that age was significant in the final model (estimate -10.48 \pm 4.85, $t_{254} = 2.16$, $P = 0.031$). There was a significant interaction between season and mast (estimate -12.74 \pm 5.79, $t_{383} = 2.20$, $P = 0.028$), showing that RMR was 12% higher during the autumn of non-mast years than that during the autumn of mast years at the population level (Fig. 1). At the individual level, RMR significantly increased by an average of 91 \pm 20 mW (i.e. by 14%) during autumn (subset of 63 individuals for which we measured RMR in both spring/summer to autumn of a given year; mean number of days that separates the two measurements 86 \pm 4, paired t -test $t_{62} = 4.52$, $P < 0.001$).

Relationship between DEE and RMR

The following analysis is restricted to RMR and DEE measurements that were made within 50 days of each other because this period maximizes the effect size (regression estimate) on a whole-animal basis (see supplementary material Table S1; the 39 measurements were made on 33 different individuals).

Using a bivariate modelling approach, we found a positive correlation between whole-animal DEE and RMR ($r = 0.51 \pm 0.14$, LRT $\chi^2 = 10.23$, $P = 0.001$). The correlation between DEE and RMR was not solely due to their mutual relationships with M_b , as it remained significant once we accounted for M_b ($r = 0.41 \pm 0.15$, LRT $\chi^2 = 5.99$, $P = 0.014$; Fig. 2). However, because DEE and RMR were both affected by reproductive status (see Table 1), their positive association could be due to the fact that both variables were elevated during reproduction. When the effect of both reproductive status and M_b were accounted for, the relationship between RMR and DEE was no longer significant ($r = 0.28 \pm 0.16$, LRT $\chi^2 = 0.17$, $P = 0.68$). However, analysing reproductive and non-reproductive individuals separately showed that the correlation between DEE and RMR was absent in non-reproductive individuals ($N = 17$, $r = 0.07 \pm 0.26$, LRT $\chi^2 = 0.08$, $P = 0.77$; Fig. 2), but present in reproductive individuals ($N = 22$, $r = 0.48 \pm 0.17$, LRT $\chi^2 = 3.89$, $P = 0.048$; Fig. 2).

Ratio of DEE to RMR

The ratio of DEE to RMR ranged from 1.52 to 5.08 (mean 3.22 \pm 0.13) and did not vary according to season ($t_{31} = 0.29$, $P = 0.77$), sex ($t_{32} = 0.81$, $P = 0.43$), masting events ($t_{33} = 0.94$, $P = 0.36$), M_b

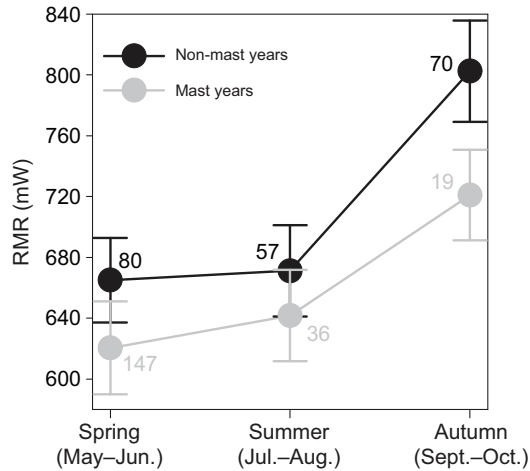


Fig. 1. Seasonal variation (least squares means \pm s.e.m.) in resting metabolic rate (RMR) during mast years (2006 and 2008) and non-mast years (2007 and 2009) in free-ranging eastern chipmunks. Sample size for each category is shown next to the symbols.

($t_{34}=1.57$, $P=0.13$) or age ($t_{35}=1.29$, $P=0.20$). The final model included a significant effect of reproductive state ($t_{36}=2.03$, $P=0.050$) and a marginally non-significant relationship with T_a ($t_{36}=1.89$, $P=0.067$). The interaction between reproductive status and T_a was non-significant ($t_{35}=0.45$, $P=0.66$). The mean T_a was not significantly different in reproductive (mean $17.15\pm 0.87^\circ\text{C}$) and non-reproductive individuals (mean $18.42\pm 0.99^\circ\text{C}$, $t_{37}=0.97$, $P=0.34$). When analysing the effect of reproductive status and T_a separately, the ratio of DEE to RMR was significantly higher in reproductive individuals (mean 3.47 ± 0.17 , median 3.42, 10th and 90th percentile 2.06–4.60) than in non-reproductive individuals (mean 2.89 ± 0.18 , median 2.95, 10th and 90th percentile 1.74–3.88, $t_{37}=2.28$, $P=0.028$; Fig. 3A) and was negatively correlated with T_a ($t_{37}=2.15$, $P=0.039$; Fig. 3B).

Overall, chipmunks maintained a constant M_b during the period over which DEE was measured (i.e. between the initial and final blood samples; 1–3 days). The change in M_b was normally distributed with a mean loss of 0.12 ± 0.59 g (median 0, 10th and 90th percentile -5.0 and 5.0 g). The difference between initial and final M_b was not significantly different for reproductive individuals (mean change in M_b -0.59 ± 0.77 g) versus non-reproductive individuals (mean change in M_b 1.03 ± 0.77 g, $t_{37}=1.38$, $P=0.17$), and nor was it correlated with T_a ($t_{37}=0.32$, $P=0.75$).

DISCUSSION

In free-ranging chipmunks, DEE and RMR vary with environmental and individual factors that, collectively, account for a large portion of the observed variation in both traits. Although environmental variables (seasonal variation and T_a) affected DEE and RMR differentially, two individual factors (M_b and reproductive state) had a common influence on DEE and RMR. After the shared influences of M_b and reproductive state on DEE and RMR were accounted for, the two metabolic traits were uncorrelated. However, we found that within reproductive individuals, DEE and RMR remained positively correlated even after controlling for M_b . Therefore, the correlation between DEE and RMR partly arose from the shared influence of individual traits at the whole-animal level (M_b and reproductive status), but they also appeared to be inherently correlated during reproduction in this species, consistent with the presence of physiological

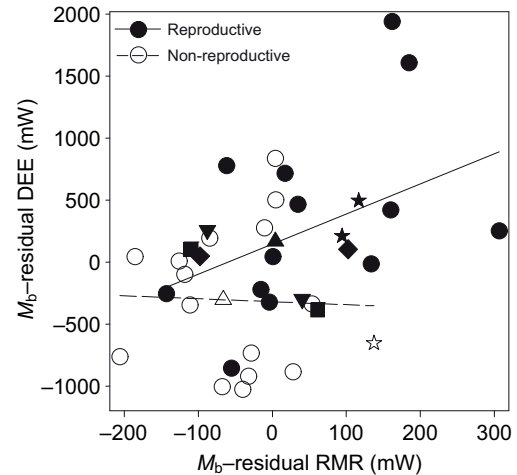


Fig. 2. Relationship between residual daily energy expenditure (DEE) and residual RMR, controlled for body mass (M_b), across 35 free-ranging eastern chipmunks. Individuals sampled repeatedly ($N=6$) are shown by different symbols.

mechanisms linking the two. As reproduction is a period of high energy demand, individual differences in DEE might have been accompanied by differences in the capacity of aerobic pathways of metabolism and in the organ systems (digestion, respiration, circulation and excretion) that support this capacity, of which the cost of maintenance is reflected in the RMR.

Autumn masting events and RMR

We found that RMR increased between spring/summer and autumn at the population level and within individuals measured in both periods. Autumn is a critical period for chipmunks as they must actively forage to accumulate enough energy for winter (Humphries et al., 2002), which is associated with high energy expenditure in other rodents (Fletcher et al., 2012). Thus, in both mast and non-mast years, increased RMR in autumn may support the metabolic demands imposed by the combination of increased foraging effort and increased cold exposure experienced at this time of year. The lack of a corresponding increase in autumn DEE is inconsistent with this interpretation, but our power to detect a seasonal trend in DEE was low because we measured it on only 5 occasions (out of 58 measurements in total) during autumn.

We also found that the magnitude of the spring-to-autumn increase in RMR was greater in non-mast years than in mast years. Thus, eastern chipmunks are characterized by an underlying spring-to-autumn increase in RMR, with the magnitude of this increase affected by variation in mast seed production. That seasonal metabolic responses in this species are affected by mast seed production is unsurprising, given many aspects of the biology of eastern chipmunks are tightly integrated with beech masting events (Bergeron et al., 2011b; Bergeron et al., 2011c; Careau et al., 2010; Landry-Cuerrier et al., 2008; Munro et al., 2008). However, the direction of this mast interaction, involving a greater spring-to-autumn increase in RMR in non-mast years than in mast years, is surprising because it leads to the highest maintenance requirements under the lowest resource conditions. A potential explanation for this is the need to increase the size and activity of the digestive organs involved in assimilating food items with a lower energetic content and/or a higher concentration of secondary compounds (Bozinovic et al., 2007), but more research is required to test this and other alternative explanations.

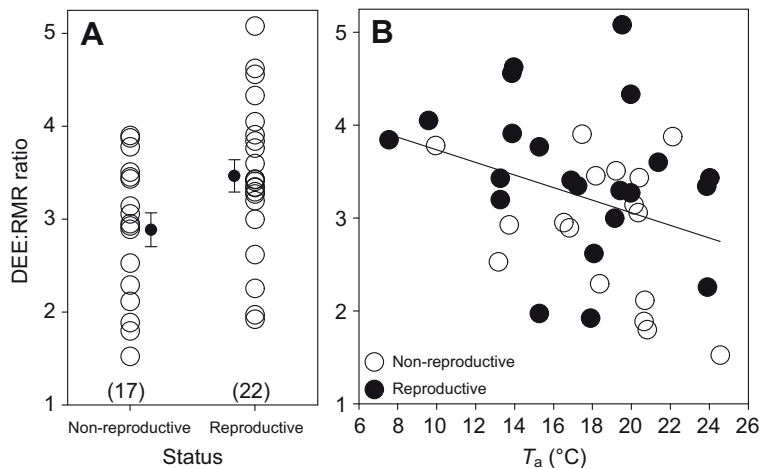


Fig. 3. Ratio of DEE to RMR according to (A) reproductive status (solid dots indicate means \pm s.e.m. for each category) and (B) ambient temperature (T_a) in 35 free-ranging eastern chipmunks.

Repeatability of DEE and RMR

Although our sampling of DEE included few repeated measures, we found that DEE was significantly repeatable ($\tau=0.57$) in an analysis restricted to 9 individuals with repeated measures. It is worth noting here that of these 19 DEE measurements, 14 were made on reproductive individuals. Thus, our results support the conjecture of Berteaux and colleagues (Berteaux et al., 1996) that repeatability of DEE in wild animals is more likely to be obtained during energy-demanding periods, such as during periods of cold exposure or reproduction (see also Fyhn et al., 2001). The significant repeatability of DEE in eastern chipmunks raises the possibility that this trait is heritable and thus may respond to selection (see Bergeron et al., 2011a), or be genetically correlated with other traits such as animal personality (Careau et al., 2008; Careau and Garland, 2012).

An increasingly large number of studies report that RMR is significantly repeatable (reviewed by Duarte et al., 2010; Larivée et al., 2010; Nespolo and Franco, 2007) and heritable (reviewed by Careau et al., 2011). Despite the fact that we made several repeated measures on a large number of chipmunks, RMR was not repeatable in our population (*sensu* Bozinovic, 2007; Russell and Chappell, 2007). Repeatability usually declines with time elapsed between measurements and many of our repeated RMR measurements spanned different seasons and different years. Given the substantial spring/summer-to-autumn changes in RMR that we observed, the absence of repeatability could be influenced by seasonal metabolic flexibility (Piersma and van Gils, 2011).

Assuming our data are accurate, RMR may have changed between repeated measurements because of variation in factors that we did not quantify. Repeatability in highly flexible traits should be expected only when the causes behind flexibility are controlled for (Naya, 2010) and our power to detect repeatability may have been diminished as our successive samples spanned reproductive events, seasons and years. Duarte and colleagues found that RMR was repeatable in female non-reproductive mice over a relatively long interval (110 days), but was not repeatable when a reproductive event separated the measurements (Duarte et al., 2010), and this may also be the case for chipmunks. Food availability in the burrow, the intensity of torpor expression and the heat increment of feeding are some of the un-quantified conditions that may have affected RMR measurements. Although the lack of repeatability in RMR implies a limited potential to respond to selection, it also implies that individuals are highly flexible in their capacity to adjust RMR, even within the same year. Future quantification of an individual's response to changing environments may therefore provide a bridge

between energetics, behaviour, environment and evolution in our population (Goldstein and Pinshow, 2006; Piersma and van Gils, 2011).

Relationship between RMR and DEE

We identified a significant correlation between mass-adjusted DEE and RMR among individuals within our study site. This result differs from previous studies on mammals and reptiles that reported no relationship among individuals within populations (Meerlo et al., 1997; Peterson et al., 1998; Speakman et al., 2003). The most similar study to ours is that conducted by Meerlo and colleagues (Meerlo et al., 1997) who measured DEE and BMR in a heterogeneous sample of reproductive and non-reproductive field voles. DEE and BMR were not influenced by reproductive status nor correlated during reproduction (Meerlo et al., 1997). By contrast, we found that DEE and RMR were elevated during reproduction and that part of the correlation between these variables was attributable to individual differences in reproductive status.

We also found a significant correlation between DEE and RMR among reproductive individuals, which is in agreement with previous studies conducted on reproductive birds (Nilsson, 2002; Tieleman et al., 2008). These studies, however, involved experimental manipulations during breeding to increase the workload required to successfully raise a clutch [Nilsson enlarged and reduced brood size of marsh tits *Parus palustris* (Nilsson, 2002) and Tieleman and colleagues clipped primary feathers of house wrens *Troglodytes aedon* (Tieleman et al., 2008)]. In both studies, the experimental protocol had no significant effect on DEE and BMR, but DEE was positively correlated with BMR. Therefore, our result of a significant correlation between DEE and RMR is the first obtained in unmanipulated, reproductive wild animals. Altogether, our findings and those of previous studies lead to the conclusion that DEE and RMR are inherently correlated during reproduction, but this correlation does not persist during other, less energy-demanding periods of the annual cycle when DEE and RMR are more likely to vary independently in relation to different selective factors. We must note, however, that a weaker correlation during the non-reproductive period could have been clouded by the time elapsed between DEE and RMR measurements.

As we quantified DEE during a relatively long period (i.e. 24–72 h) over which chipmunks maintained a constant M_b , metabolism must have been primarily fuelled by food intake instead of energy reserves. Therefore, our DEE values probably represent sustainable levels of metabolism as initially defined (Peterson et al., 1990). Results from early studies on chick-provisioning birds

(and hard-working lumberjacks) indicated that maximum values of sustained metabolic scope usually approached values of ~4 (Drent and Daan, 1980). Further research in birds and mammals has shown that sustained metabolic scope is rarely higher than 5 (Peterson et al., 1990; Piersma and van Gils, 2011). We found that the ratio of DEE to RMR was significantly higher during reproduction, when it averaged 3.47 and ranged from 1.92 to 5.08 (see Fig. 3), than at other times of year. In our population, DEE is positively correlated with litter size in lactating females (Bergeron et al., 2011a). Hence, increased parental care in chipmunks may raise energy throughput significantly, suggesting that selection towards higher litter size may pull DEE and RMR to higher levels during reproduction (Daan et al., 1990; Farmer, 2000; Koteja, 2000). The hypothesis of an upper limit to sustained metabolic scope (Daan et al., 1990; Hammond and Diamond, 1997; Koteja, 1991) could render RMR a reliable index of the energy expenditure of free-living animals during periods when they must sustain high rates of energy expenditure such as during parental care (Farmer, 2000; Koteja, 2000). Whether this is also true in other species and/or other energetically demanding periods of life, such as during growth (Careau et al., 2012a) and migration (Piersma and van Gils, 2011), requires confirmation.

Our results offer little insight into central *versus* peripheral limits to maximum sustained metabolic scope, as we do not have organ-level measurements. However, we found that the ratio of DEE to RMR tended to increase as T_a decreased, suggesting that the nature of the limit may be affected by external, environmental conditions. Recently, the heat dissipation limit (HDL) theory has emerged as an alternative to the idea that sustained limits to energy expenditure are fixed at some multiple of RMR (Speakman and Król, 2010). Instead of a fixed limit, HDL theory suggests that the maximum sustained metabolic scope will increase with declining T_a as the capacity to dissipate heat increases (Speakman and Król, 2011). Therefore, our results may be interpreted as being consistent with a prediction of the HDL theory. In a previous study, we showed that stress-induced hyperthermia was positively correlated with T_a and DEE in this population (Careau et al., 2012b). It is therefore possible that the sustained metabolic scope may be limited by the risks of hyperthermia in eastern chipmunks. During reproduction, however, when chipmunks are characterized by higher body temperature during handling (Careau et al., 2012b), they may ignore risks associated with hyperthermia (e.g. oxidative stress) to maximize current reproduction (Bergeron et al., 2011a). In this situation, differences in DEE may be more solidly anchored in RMR, leading to the observed positive correlation between DEE and RMR during reproduction.

LIST OF ABBREVIATIONS

BMR	basal metabolic rate
COV _{DEE-RMR}	covariance between the residual DEE and residual RMR
DEE	daily energy expenditure
DLW	doubly labelled water
HDL	heat dissipation limit
LRT	log-likelihood ratio test
M_b	body mass
$M_{b,DEE}$	body mass at DEE measurement
$M_{b,RMR}$	body mass at RMR measurement
RMR	resting metabolic rate
T_a	ambient temperature

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