

Sex-specific selection on energy metabolism – selection coefficients for winter survival

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Keywords:

body condition;
energetics;
mammals;
Myodes glareolus;
selection;
sexual antagonism.

Abstract

Selection for different fitness optima between sexes is supposed to operate on several traits. As fitness-related traits are often energetically costly, selection should also act directly on the energetics of individuals. However, efforts to examine the relationship between fitness and components of the energy budget are surprisingly scarce. We investigated the effects of basal metabolic rate (BMR, the minimum energy required for basic life functions) and body condition on long-term survival (8 winter months) with manipulated densities in enclosed populations of bank voles (*Myodes glareolus*). Here, we show that survival selection on BMR was clearly sex-specific but density-independent. Both the linear selection gradient and selection differential for BMR were positive in females, whereas survival did not correlate with male characteristics. Our findings emphasize the relative importance of individual physiology over ecological factors (e.g. intra-specific competition). Most current models of the origin of endothermy underline the importance of metabolic optima in females, whose physiology evolved to fulfil demands of parental provisioning in mammals. Our novel findings of sex-specific selection could be related to these life history differences between sexes.

Introduction

Numerous studies have reported inter- and intra-specific variation in the metabolic rates for birds and mammals (Dohm *et al.*, 2001; Lovegrove, 2003; Labocha *et al.*, 2004; Ronning *et al.*, 2007; Gębczyński, 2008; Jetz *et al.*, 2008) and tried to explain the evolutionary and ecological causes behind that variation (Garland & Carter, 1994; Angilletta & Sears, 2003; Rezende *et al.*, 2004; Konarzewski *et al.*, 2005). The focus has especially been on the extreme values of energy metabolism, and mostly on its lower limit: the basal metabolic rate (BMR). BMR reflects the minimum energy absolutely required to keep an individual alive and is determined by the size and metabolic activity of internal organs such as the liver, kidneys and heart (Książek *et al.*, 2004; Russell &

Chappell, 2007). The basal metabolic rate is often assumed to be associated with an individual's fitness (Lovegrove, 2003; Rezende *et al.*, 2004); however, current empirical data have shown that natural selection on BMR is rather weak (Jackson *et al.*, 2001; Boratynski & Koteja, 2009).

It is not certain how natural selection should be linked with BMR (e.g. should higher or lower metabolism be correlated with better survival), and if it is consistent over time (Boratynski & Koteja, 2009). Also, energy expenditure at some life stages (e.g. during reproduction) is extremely different between the sexes, which might generate differential selection on energetic components between males and females (Key & Ross, 1999; Krol *et al.*, 2003). Sexually antagonistic selection has been shown to promote different trait optima in males and females in many traits. Particularly, sexual signalling, weaponry traits, size and behaviour can be divergently selected between sexes (Lindenfors, 2002; Bouteiller-Reuter & Perrin, 2005; Robinson *et al.*, 2006; Foerster *et al.*, 2007; Moller, 2007). Because those traits are often

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energetically costly, one can expect that selection would act on the traits related to energy budgets, such as the metabolic rate. Until recently, there has been little information about the correlation between energy metabolism and fitness, while most of the recent studies on the subject have focused on the indirect measures of an individual's performance, like aggressiveness or disappearance from an open population [for invertebrates: (Watson & Lighton, 1994); fish: (Cutts *et al.*, 2001); mammals: (Hayes & O'Connor, 1999; Jackson *et al.*, 2001)]. Very few studies have discussed possible differences between the sexes in energy metabolism and the influence of this on fitness (Farmer, 2003; Bouteiller-Reuter & Perrin, 2005).

The strength and direction of natural selection can depend on environmental settings, such as the density of conspecifics (Sinervo *et al.*, 2000; Calsbeek *et al.*, 2007; Mappes *et al.*, 2008b). In highly dense populations, there are fewer resources per capita than in lower density populations, which may be especially demanding during the seasons when resources are not renewed. Thus, for many small animals, e.g. many rodents, the strongest survival selection is expected to act in dense populations during the winter (i.e. outside the growing season of vegetation), favouring individuals that are either able to cope with low nutrient availability or who can monopolize the few remaining resources. If the metabolic rate correlates positively with activity level in mammals (Henderson *et al.*, 2002; Rezende *et al.*, 2006), individuals with a higher basal metabolic rate might be better competitors for scarce resources, e.g. through an ability to sustain activity longer. Alternatively, there may not be enough nutrients in the environment, even for a good competitor, to cover the high costs of elevated activity and basic life functions connected to high BMR. Another character potentially affecting performance of individuals and their survival over the winter is body condition. Body condition is usually defined as the residual value of body mass derived from the linear regression of mass on the linear index of a structural (skeletal) size measure. It reveals an individual's nutritional state and reflects variation in all components of body composition including fat, lean dry mass (proteins) and water (Schulte-Hostedde *et al.*, 2005). Individuals with a better condition are expected to have a higher probability of survival, especially during circumstances of food shortage and elevated expenses (Huitu *et al.*, 2007).

Here, we examined the association between BMR, body condition and survival by monitoring small rodents, bank voles (*Myodes glareolus*), in enclosed populations of varying densities over the winter. The experiment was carried out in Central Finland over 8 months (September to May) in harsh conditions where the temperature was below zero Celsius for 5 months. We predicted that a high basal metabolic rate would increase the probability of survival. BMR is mainly generated by the size and activity of internal organs responsible for energy flux,

and faster energy flow should improve an individual's ability to cope with low ambient temperatures through faster heat production (Krol *et al.*, 2003). Alternatively, as individuals with a low level of metabolism carry lower costs of basic life functions (Speakman *et al.*, 2004), it could allow them to spend more time at rest (e.g. in the well-insulated nest), thereby increasing their survival. With respect to condition, individuals with higher body conditions were predicted to have higher survival probabilities. Stored resources of fat and proteins are necessary to support energetically costly functions, such as temperature regulation, when food is scarce. Additionally, by manipulating the density of conspecifics, we addressed whether the potential association between BMR, body condition and survival is mainly associated with the level of intra-specific competition (i.e. density dependent) or is caused by density-independent factors (i.e. individual physiology). Finally, we tested whether the potential effects of measured traits on survival differed between the sexes as recently suggested for several life history and energetic traits [body size: (Toigo & Gaillard, 2003; Bouteiller-Reuter & Perrin, 2005); horn phenotype: (Robinson *et al.*, 2006); arrival date to nesting site: (Moller, 2007); metabolic rate: (Boratynski & Koteja, 2009)]. The differential selection between the sexes could potentially contribute to the additive genetic variation of a trait like BMR, previously shown to be highly heritable in our study species (Sadowska *et al.*, 2005).

Materials and methods

Study organism

The bank vole (*Myodes glareolus*) is a Eurasian murid rodent inhabiting mostly forested, but also other densely vegetated areas (IUCN, 2007). Its variable (mainly herbivorous) diet consists typically of different parts of plants, but also fungi and insects. Population densities are highly variable within and between years, and distinctive density cycles are observed also in Central Finland (Hansson & Henttonen, 1985; Kallio *et al.*, 2009). Fitness of bank vole individuals is affected by population density (Mappes & Ylonen, 1997), usually by decreasing survival probability in dense populations (Oksanen *et al.*, 2007; Mappes *et al.*, 2008b). Bank voles remain active during the winter under the snow. In Fennoscandia, they experience extremely low temperatures and a food shortage during winters, and it is likely that these environmental characters cause strong survival selection especially at high densities. According to previous studies carried out in enclosed populations, usually less than half of the individuals present in the population in autumn survive until spring [enclosures: (Koskela, 1998): approximately 60% (Oksanen *et al.*, 2001): 5–20%, islands: (Kallio *et al.*, 2007): 11–22%].

The bank vole has been used as a noncaptive mammalian model species in several experimental studies

concerning life history evolution and evolutionary physiology (Oksanen *et al.*, 1999; Labocha *et al.*, 2004; Sadowska *et al.*, 2005; Mappes *et al.*, 2008a). It is already known that morphological (body mass, head width) and physiological (BMR) traits in this species are highly variable (Koivula *et al.*, 2003; Labocha *et al.*, 2004) and that a considerable proportion of this variation is heritable (narrow sense heritability of body mass, head width and basal metabolic rate: 0.86, 0.83 and 0.40) (Mappes & Koskela, 2004; Sadowska *et al.*, 2005). The variation in these characteristics is known to also exist in natural populations where individual values remain repeatable even over relatively long time periods [intra-class correlation over an average of 56.5 days for body mass, head width and BMR: 0.67, 0.83 and 0.40 (Boratynski & Koteja, 2009); for comparable repeatabilities of metabolic rate in other species see Nespolo & Franco (2007)].

The bank voles for this study came from a laboratory colony maintained at the University of Jyväskylä, Finland. Laboratory-born animals were 5th generation descendants of wild-trapped individuals that originated from central Finland (62°37' N, 26°20' E). In laboratory, the animals were housed in individual cages (43 × 26 × 15 cm) with bedding of wood shavings and hay, in a 16L:8D photoperiod, at 20 ± 2 °C. Food (Labfor 36; Lactamin AB, Stockholm, Sweden) and water were available *ad libitum*. The colony was artificially selected for small and large litter sizes at birth (voles were affiliated to two lines, that were bred separately in isolated groups); however, the overall variation in the 5th generation was still continuous and within natural variation (E. Schroderus *et al.*, unpublished). All experimental and housing procedures complied with the animal experimentation laws in Finland.

Survival experiment

The study was carried out in 13 outdoor enclosures situated in Konnevesi, central Finland. Each enclosure was 0.2 ha in size, with 20 trapping sites (Ugglan Special multiple-capture live traps; Grahnbab, Hillerstorp, Sweden) every 10 m. Enclosures were fenced with 1.25 m high galvanized metal sheet embedded 0.5 m into the ground. The fences did not necessarily prevent the entry of mammalian and bird predators (e.g. foxes, stoats and owls). For a detailed description of enclosures see Oksanen *et al.* (2002).

Altogether we released 155 voles that had been born during spring and early summer 2004 (70–99 days old at the beginning of experiment). However, only animals that had complete phenotypic measurements recorded were included in the analyses ($n = 136$). Individuals were released to empty enclosures on the 1st September 2004 after 1-week acclimatization to outdoor conditions (housed in cages in an open greenhouse). Within each enclosure, the sex ratio and the proportion of animals were kept as close as possible to 1 : 1. The density in

populations was treated as a continuous variable, and the number of voles released was 4, 5, 6, 7, 8, 9, 10, 11, 14, 15, 17, 18 and 21 individuals in 1–13 enclosures, respectively. Individuals were assigned to the enclosures in such a way that density manipulation was not associated with variation in condition, BMR and body mass (when controlled for sex, line, family and enclosure; $P > 0.29$). Also, no sex-specific correlations were significant ($P > 0.3$). Prior to the experiment, residual values of BMR (corrected for body mass and age during metabolic trials) did not correlate with condition of individuals (when controlled for sex, line and family affiliations; $P > 0.5$) or with density in enclosures (also controlled for enclosure number; $P > 0.5$).

The survival of bank voles was monitored at the beginning of the next breeding season in May by live trapping. During the study winter, the snow cover in Konnevesi lasted from mid November until early April (monthly mean values of snow cover calculated from daily measures, in cm: 3.3, 19.9, 29.3, 38.5, 40.7 and 4.9 for November, December, January, February, March and April). Average temperatures below zero also lasted for approximately 5 months (monthly mean values calculated from daily measures taken at 08:00: -1.3, -3.0, -3.0, -9.7, -13.3 and 0.7 °C; for November, December, January, February, March and April). Trappings started 30 May and were conducted twice a day (in the morning and evening) until no voles were trapped during three consecutive sessions. Most voles, 92%, were trapped during the first session (in the morning of 31 May), and no animals were captured after fourth session. The enclosures were trapped again just before the start of the new experiment conducted in the same enclosures during the following summer, confirming no remaining individuals.

Basal metabolic rate

Oxygen consumption and carbon dioxide production (millilitre per hour) were measured in a four-channel open-flow respirometric system (Sable Systems, Henderson, NV, USA) based on Fc-1B O₂ (Sable Systems) and Li-6252 CO₂ (Li-Cor, Lincoln, NE, USA) analyzers. Multiple channel systems of this kind are proved to give reliable (highly repeatable) estimations of BMR in this species [see: (Labocha *et al.*, 2004; Boratynski & Koteja, 2009) detailed description of procedures]. The system was adapted to measure 12 animals per day in three groups. Prior to measurements, animals were weighed (three groups at: 04:45, 09:15 and 13:15) and placed in Plexiglas chambers (554 mL volume) without water and food at 29.0 ± 1 °C (within the thermal neutral zone). After 3 h, the chambers were connected to a system of dry and CO₂-free air (after passing through columns with Drierite and soda lime chemicals) flow at 350 mL min⁻¹ (Sierra Instruments, Monterey, CA, USA). Each vole was confined with wire baskets to the middle of the chamber, so that it could not exhale near the air in/outlets. Oxygen

consumption was recorded throughout a period of 3 h and 20 min (between: 09:00–12:20, 13:30–16:40 and 17:30–20:40) after 4 h of starvation. The samples of dried air were taken sequentially from the four measurements and the reference chambers every 12.5 min (with flow rate: 150 mL min⁻¹). In each cycle, measurement chambers were active for 2.5 min each, during which O₂ and CO₂ values were sampled every 1 s. Samples, taken from the last 20 s of 2.5 min measure, were used for the calculation of average oxygen consumption. Used desiccant, regenerated Drierite, might cause minor underestimation of the differences in metabolic rate between animals (White *et al.*, 2006). The oxygen consumption (metabolic rate) was calculated according to the following formula [modified from (Koteja, 1996)]:

$$VO_2 = \frac{V_i[FdO_2(1 + FeCO_2) - FdCO_2FeO_2]}{1 - FeO_2 + FeCO_2}$$

where VO₂ is the oxygen consumption rate, V_i is flow rate through the measurement chamber, FdO₂ and FdCO₂ are differences of O₂ and CO₂ fractional concentrations in dry air flow before and after passing through the measurement chamber, whereas FeO₂ and FeCO₂ are fractional concentrations of O₂ and CO₂ in dry air flow after passing the measurement chamber. A total of 16 average oxygen consumption values were calculated for each animal throughout one measurement trial, over a 3 h 20 min. Because the lowest level of oxygen consumption may be highly biased by random effects [low repeatability between two consecutive trials of the lowest values, see (Labocha *et al.*, 2004) for details], we defined BMR as a mean of the second, third and fourth lowest averages of oxygen consumption calculations.

Statistical analyses

Variation in traits

To quantify the possible sources of variation in BMR, we included in generalized linear model (GLM procedure in SAS 9.1; SAS Institute Inc., Cary, NC, USA) line and family (nested within line) affiliations, sex, time (three groups per day), day of measurements and respirometric chamber as factors, while age and body mass during metabolic trials were continuous variables. To obtain the measure of whole-body condition, independent of an individual's structural size, we defined condition as a standardized residual value of body mass regressed against head width. Simple analyses of variation (one-way ANOVA in procedure GLM, SAS 9.1) were used to check for differences among individuals, which survived and did not survive over the winter. The analyses were performed separately for the sexes.

Survival analyses

The association between winter survival of bank voles and variation in their trait values was tested using a generalized linear mixed model (GLIMMIX procedure in

SAS 9.1) with a binary response distribution and logit link function for the survival variable. In the model, we included age, density in enclosures, body mass, BMR and body condition as continuous variables. Sex and line affiliation were included as fixed, whereas family affiliation and enclosure number as random factors. We tested the significance of the two- and three-way interactions between sex and density and the rest of the variables. Whenever interactions were not significant ($P > 0.1$), models were hierarchically reduced. Additionally, simple models after stepwise reduction in nonsignificant main effects as well as separate sex models were tested. Finally, results after reducing nonsignificant random effects are presented (GENMOD procedure in SAS 9.1). Analyses with head width (structural size measure) instead of body condition (residual values of body mass regressed against head width) were also conducted. Both analyses gave comparable results and only those with body condition are presented in detail.

Selection gradients and differentials

To distinguish between selection acting directly on the character and correlative selection, both selection gradients and simple selection differentials are presented. Selection gradients are derived from multiple regression models with a fitness measure, survival, as a dependent variable and all measured individual characters as independent variables (Lande & Arnold, 1983). Selection differentials are derived from simple regression models including only one independent variable, and those do not control for possible correlative selection on other traits (Arnold & Wade, 1984). Coefficients in the regression models are standardized by adopting units of standard deviations of each independent variable (phenotypic trait). This allows for a direct comparison of selection intensity measured in different studies, for different fitness components and between different characters (Hoekstra *et al.*, 2001; Kingsolver & Pfening, 2007). To avoid problems arising when using linear regression analyses with a binary dependent variable (survival), we implemented the method described by Janzen & Stern (1998) to transform logistic regression coefficients into selection coefficients:

$$\beta_{\text{avggrad}} = \alpha_i \times \left\{ \frac{\sum [W_{(z)} \times (1 - W_{(z)})]}{N} \right\}$$

where β_{avggrad} is an average gradient vector based on a logistic regression model; α_i is a logistic regression coefficient for an i character; $W_{(z)}$ is the probability of an individual's survival (predicted probability) given the traits, z , included in the regression model; and N is the number of individuals for which phenotype and survival data were available. Consequent selection coefficients (β_{avggrad}) are comparable to those derived from the linear regression models [(Janzen & Stern, 1998), see also Supporting Information for linear and logistic regression coefficients: Tables S1 and S2]. To test for

disruptive/stabilizing selection in the above described regression models, quadratic terms of all traits were included. Regression coefficients for quadratic terms were multiplied by two to obtain nonlinear selection coefficients (Fairbairn & Reeve, 2001).

Results

Correlation between traits

Basal metabolic rate (BMR) was estimated for 136 individuals (64 females and 72 males). None of the respirometry system variables (time and day of measurements or measurement chamber) significantly affected variation in BMR ($P > 0.28$). Out of all individual characters (sex, line and family affiliations, age and body mass during BMR measurements), only body mass significantly explained part of the variation in BMR ($F_{1,134} = 122.29$, $P < 0.0001$; the rest: $P > 0.25$). Also, in the analyses conducted separately for sexes, only body mass significantly correlated with BMR (for body mass: female: $F_{1,62} = 48.98$, male: $F_{1,70} = 39.04$, $P < 0.0001$, for the other characters $P > 0.14$).

Basal metabolic rate, body mass (BM) and body condition (and head width, HW) were significantly correlated with each other (Pearson correlation coefficients: BM vs. BMR, BM vs. condition, BM vs. HW and condition vs. BMR: $r = 0.71, 0.63, 0.68$ and 0.42 , $N = 136$, $P < 0.0001$). Sex-specific correlations were also significant for all traits ($P < 0.01$, Fig. 1). Only condition correlated with the age of individuals ($r = -0.32$, $P = 0.002$, separate for females and males: $P < 0.043$, Fig. 1).

Survival selection

Observed survival rates between the release and spring trapping were 21.9% and 13.9%, for female and male bank voles, respectively. Altogether, of 136 individuals released, 24 voles survived the period of study (14 females and 10 males; Fig. 2). Females which survived over winter were on average heavier and had higher BMR and body condition values than the females which did not survive (one-way ANOVAs with each trait as dependent and survival as independent variables, procedure GLM, SAS 9.1, Fig. 2). No differences between males that survived and did not survive were found ($P > 0.1$, Fig. 2).

The factors affecting winter survival were further tested using a generalized linear mixed model (GLIMMIX procedure in SAS 9.1) with a binary response distribution and logit link function for the survival variable. In a full model, the survival was significantly affected only by the interaction between BMR and sex ($F_{1,127} = 3.95$, $P = 0.049$, Table 1). After excluding nonsignificant main and random effects, body condition positively ($\chi^2_{1,130} = 4.70$, $P = 0.030$) and density negatively ($\chi^2_{1,130} = 3.97$, $P = 0.046$) affected survival (Table 1). When conducting analyses separately for females, BMR

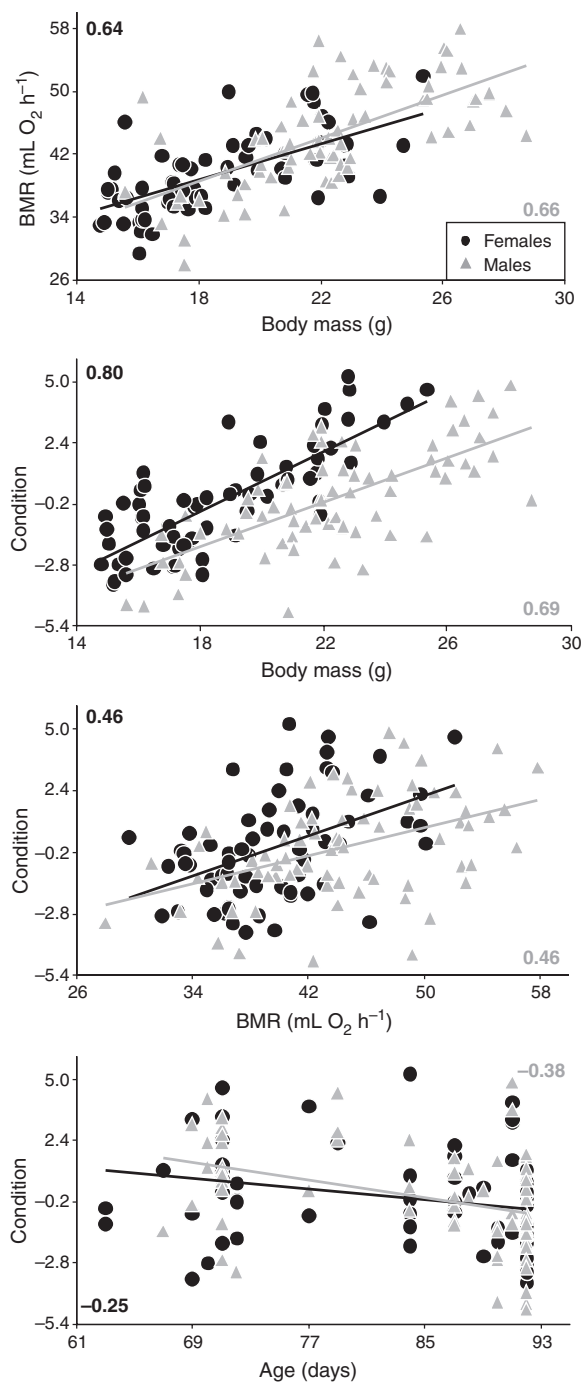


Fig. 1 Correlation between basal metabolic rate (BMR), body mass and condition and between condition and age of female (black circles) and male (grey triangles) bank voles. All Pearson correlation coefficients were significant (correlations with age: $P < 0.043$, and rest: $P < 0.01$).

showed a significant positive correlation with survival, both in a full mixed model ($F_{1,57} = 5.15$, $P = 0.027$) and after excluding nonsignificant main and random effects

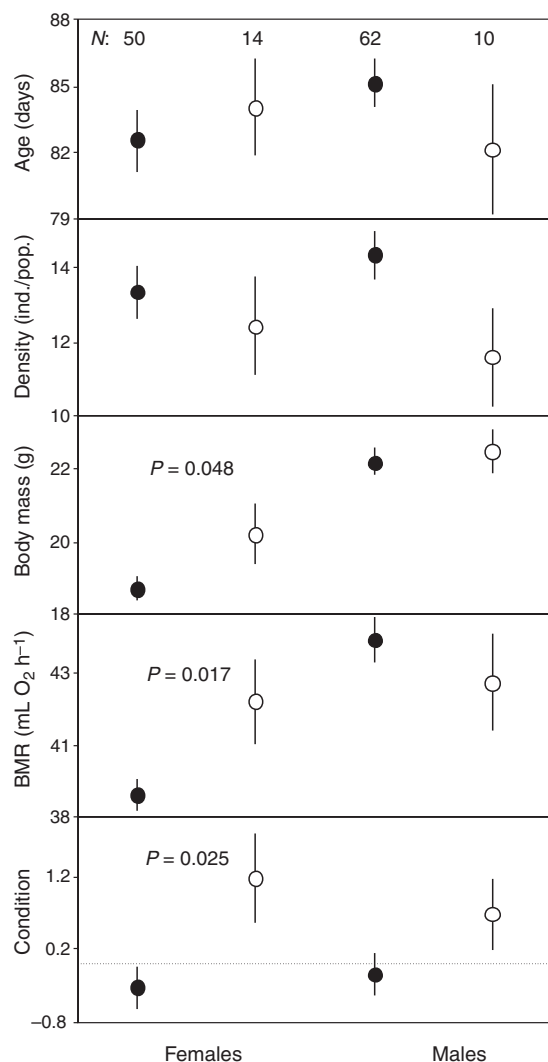


Fig. 2 Mean and standard error of age (days), density (number of individuals per population/enclosure), body mass (g), basal metabolic rate (BMR ml min⁻¹ of O₂ consumption) and condition (residual values from linear regression of body mass on head width) presented separately for females and males and for voles, which survived (white) and did not survive (black circles) over winter. Statistical significances are here derived from simple analyses of variance with trait as dependent and survival as independent variables. *N*, number of individuals.

($t_{62} = 2.46$, $P = 0.017$). None of the studied effects significantly explained male survival, even in the reduced model ($P > 0.1$). Overall, random effect, enclosure, did not affect survival significantly (covariance parameter estimates \pm SE from mixed models for all data: 1.12 ± 0.87 , for females: 1.39 ± 1.65 and for males: 1.61 ± 1.45). Survival was substantially higher in only one enclosure (64%) compared to the average (18%) ($t_{13,92} = 2.45$, $P = 0.028$), whereas it did not vary among the others ($P > 0.2$).

Table 1 Generalized linear mixed and logistic regression analyses of correlation between over winter survival and individual characters. Estimates \pm SE for random effects, enclosure number and family affiliation: 1.12 ± 0.87 and 0.23 ± 0.48 .

Effect	Full model*				Reduced model†		
	d.f.(n)	d.f.(d)	F	P	d.f.	χ^2	P
Sex	1	127.0	3.64	0.059	1	4.79	0.029
Line	1	11.8	0.45	0.514	‡	–	–
Age	1	127.0	0.43	0.512	–	–	–
Density	1	15.0	2.50	0.135	1	3.97	0.046
Body mass	1	127.0	0.67	0.414	–	–	–
BMR	1	127.0	0.66	0.420	1	0.11	0.740
Condition	1	127.0	2.41	0.123	1	4.70	0.030
Sex*BMR	1	127.0	3.95	0.049	1	5.52	0.019

*Includes all main and random (enclosure number and family affiliation) effects and a significant interaction (interactions between sex and density and all other characters were insignificant: $P > 0.1$), †Excludes insignificant main and random effects ($P > 0.1$), ‡ ‘–’ effect not included in the model. d.f., degrees of freedom for numerator (*n*) and denominator (*d*); *F* and χ^2 , test statistics; *P*, probability, BMR, the basal metabolic rate.

Directional selection gradients and selection differentials derived from logistic regression of survival on age, density, body mass, BMR and body conditions are presented (Fig. 3; detailed regression results: Supporting Information: Tables S1 and S2). Regression models for selection gradients include all traits, whereas regression models for selection differentials include only one trait at a time in the analysis. Statistics for directional selection are presented from the models after excluding quadratic trait terms (Fig. 3). None of the quadratic selection gradients or differentials was significant (Tables S1 and S2). The only significant and positive selection gradient found for females was BMR ($\beta_{\text{avgrgrad}} \pm \text{SE} = 0.13 \pm 0.06$, $n = 64$, $P = 0.043$). The selection gradient for condition was also nearly significant and positive for females ($\beta_{\text{avgrgrad}} \pm \text{SE} = 0.20 \pm 0.11$, $n = 64$, $P = 0.072$, Fig. 3). None of the selection gradients were significant for males (Table S1). Both linear selection differentials for BMR and condition were significant and positive (both $\beta_{\text{avgrgrad}} \pm \text{SE} = 0.11 \pm 0.05$, $n = 64$, $P < 0.025$, Fig. 3), and the selection differential for body mass was nearly significant for females ($\beta_{\text{avgrgrad}} \pm \text{SE} = 0.10 \pm 0.05$, $P = 0.055$, Fig. 3). No significant selection differentials were found for males (Fig. 3, Table S2).

Discussion

We conducted a field experiment to examine the relationship between components of the energy budget and fitness using a wild small mammal, the bank vole, as a study species. We monitored the overwinter survival of individuals of known age, body mass, condition and BMR by simultaneously manipulating the density of conspecifics in large outdoor enclosures. BMR was the

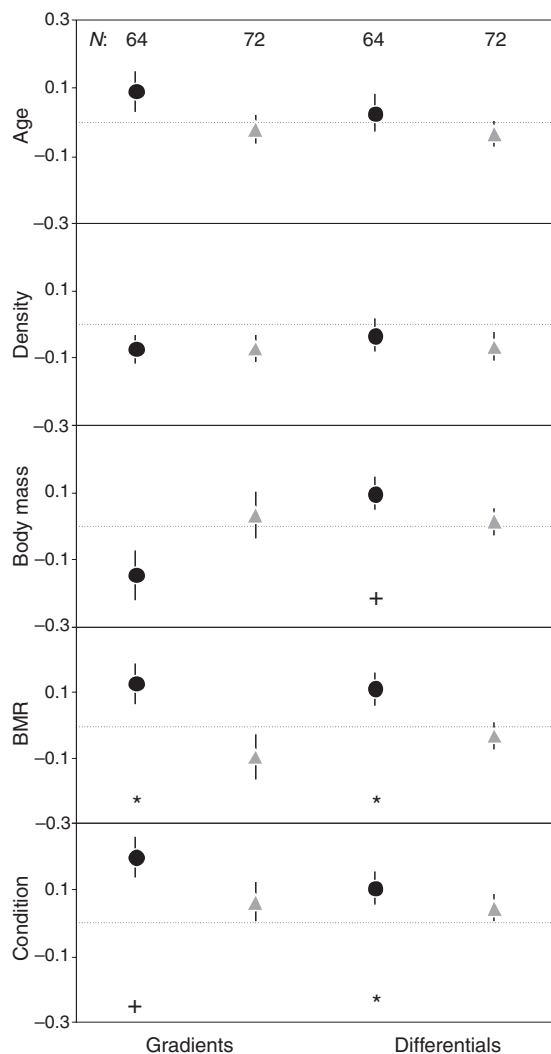


Fig. 3 Directional selection gradients and differentials (\pm SE) of survival on traits calculated in logistic regression models (procedure GENMOD, SAS 9.1) separately for females (black circle) and males (grey triangle). Logistic regression coefficients were calculated on log transformed and standardized independent variables and transformed according to Janzen & Stern (1998) into selection coefficients (see methods for details). Regression models to calculate gradients include all traits in the same analysis, whereas those to calculate differentials were conducted separately for each trait. * $P < 0.05$, $^+P < 0.075$.

only character significantly associated with the probability of survival, and this benefit of higher BMR was evident only in females. Moreover, although survival was in general higher in lower densities, survival selection on BMR was density-independent. Even though our study unavoidably has its limitations (as BMR of individuals was not manipulated, the sources of overwinter mortality, like starvation, predation and hypothermia, were not known, enclosure specific

densities were not replicated), it provides the first experimental attempt to examine the effects of small mammal energy budgets on their long-term survival in harsh natural conditions.

The results of two previous studies that estimated the association between survival and BMR showed either selection on residual values of BMR only (Jackson *et al.*, 2001), or that there was weak, inconsistent selection over time (Boratynski & Koteja, 2009). In the current study, selection (measured by survival) was shown to be associated with both the whole body and mass-corrected basal metabolic rates in females. Because the analysis controlled for age, body mass and condition, it ensured that the results demonstrated the relatedness of BMR (or some unmeasured character closely related to it) to fitness and were not confounded by these factors (both the selection gradient and selection differential were significant, Fig. 3). Independent of the correlation between survival and BMR, a weaker correlation was also found between survival and body condition. This result is consistent with observations and predictions from previous studies (Merilä *et al.*, 2001; Murray, 2002; Huitu *et al.*, 2007). Interestingly, estimates of survival selection were independent of population density (Fig. 3). This result indicates that an individual's physiology is more important than variation in environmental quality in determining survival. However, it should be kept in mind that manipulating the quality and quantity of available resources by setting different densities in natural situations has its limitations.

We found that BMR affected bank vole survival and that the selection on BMR differed between sexes. According to current knowledge, it is still unclear why females, but not males, would benefit from elevated BMR (Fig. 3). It has been proposed that the elevated level of the minimum metabolic rate in endotherms evolved as a consequence of selection acting on characters linked to parental provisioning (Koteja, 2000; Farmer, 2003). Those models explain the origin of endothermy (fuelled by a high level of metabolism) by focusing on fitness components linked to reproductive effort and a high level of sustained activity and energy processing capacities, affected mainly by energetically active internal organs (Książek *et al.*, 2004; Russell & Chappell, 2007). Consequently, in the case of mammals, the relevant processes are related to female reproduction: pregnancy and lactation. The survival of females could also be affected if females with an elevated BMR are better equipped for costly behaviours in general. Outside of the breeding season, females could potentially allocate this surplus energy to other physiological processes, whereas males might not have this opportunity. The possible physiological mechanism responsible for sex-related overwinter survival could be caused by sex differences in energy storage and usage strategies. Beck *et al.* (2003) showed that female phocid seals were risk averse, preparing themselves for reproduction much earlier than males. As

a consequence, females accumulated more energy stores and carried a higher level of insurance against environmental uncertainty than males. Seals are an extreme example of a capital breeding strategy, whereas small rodents are usually considered as income breeders (Jönsson, 1997). However, some studies on laboratory rodents indicated that caloric restriction has a different impact on female than male physiology (Valle *et al.*, 2005, 2007; Shi *et al.*, 2007). Particularly, female rats showed a greater drop in oxygen consumption, better conservation of metabolically active organs and a greater decrease in adipose depots than males, when both were food restricted (Valle *et al.*, 2005). Shi *et al.* (2007) also found that females decreased energy expenditure during caloric restriction and after lipectomy-induced fat loss. As pointed out by the authors, there are differences in the way male and female rodents regulate their body weight, which might be advantageous for female survival in conditions when the food is limited (e.g. during winter). We propose, as a working hypothesis, that the observed sexual differences in the relationship between survival and BMR could be an effect of sex differences in energy storage–usage strategies. However, because our data do not provide all the information (body composition prior to the survival experiment, phenotypic measurements during winter) to test the hypothesis properly, it remains to be further examined.

Estimated selection coefficients showed that the selection was rather weak ($\beta_{\text{avggrad}} \leq 0.2$). This result does not deviate from the other studies of strengths of selection operating on phenotypic traits in wild populations. A review of published directional selection coefficients by Hoekstra *et al.* (2001) showed that the estimates of absolute values of $|\beta|$ have an exponential distribution with median of 0.150 and mean of 0.220, with most (90%) of the values being smaller than 0.5. Moreover, the natural selection measured by survival appears to be even weaker, with a median of 0.088 and mean of 0.153 (Hoekstra *et al.*, 2001; Kingsolver *et al.*, 2001; Kingsolver & Pfening, 2007). Our directional selection coefficients did not differ markedly from those presented in the reviews, with a median of absolute value 0.081 and mean of 0.101. Nonlinear selection coefficients were also low, however, never significant (probably partly because insufficient statistical power needed to detect quadratic regression effects). In general, the results for simple selection differentials and selection gradients, which account for correlational selection, are consistent among traits (Tables S1 and S2). Overall, the selection intensity observed in our study is typical of the intensity of survival selection described in the past.

Conclusions

Our results demonstrate that differential (and/or perhaps antagonistic) survival selection on basal metabolism may

operate between sexes in boreal rodents. Even though the present study design cannot exclude whether the result could be caused by direct selection on some other trait, it does show that BMR has an association with survival either by direct selection for it, or else through indirect selection because of a functional or genetic link (such as with metabolic capacity). Also, the relationship between BMR and survival was independent of all the characteristics measured here. A high level of BMR in birds and mammals is linked with the ability to keep a high and stable body temperature, independent of the ambient temperature they experience. However, it is not certain if the direct benefits of endothermy could outweigh the costs, especially at an early stage of evolution, without a net benefit associated with endothermy. In this regard, some models of the evolution of endothermy are based on the correlative evolution of a high level of BMR with selection acting on other traits, i.e. connected to parental provisioning behaviours (Koteja, 2000; Farmer, 2003; Kemp, 2006). Consequently, these models predict different selection pressures for females and males, as the females usually provide most of the energy to progeny. We showed that the survival selection pressure and direction differed between the sexes and that a high level of BMR was beneficial for females, but not for males. This difference may also affect other life history traits and should be taken into account when studying male–female co-evolution.

Acknowledgments

We gratefully acknowledge Mikael Mokkonen, Eero Schroderus and two anonymous reviewers for helpful comments to manuscript, Pawel Koteja for help in setting up the metabolic laboratory and Konnevesi Research Station and the Experimental Animal Unit of the University of Jyväskylä for providing the facilities. The study was financially supported by the Academy of Finland (grant no 115961 and 119200 to EK; 118603, 109165 and 204284 to TM; 104568 and 108955 to TAO), the Marie Curie Training Sites (Marie Curie Fellowship to ZB) and the Centre of Excellence in Evolutionary Research of the Academy of Finland.

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Supporting information

Additional supporting information may be found in the online version of this article:

Table S1 Selection gradients, coefficients from multiple regression models.

Table S2 Selection differentials, coefficients from simple regression models.

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Received 18 February 2010; revised 24 May 2010; accepted 28 June 2010