Environmental and genetic influences on body mass and resting metabolic rates (RMR) in a natural population of weasel *Mustela nivalis*

**KAROL ZUB,** STUART PIERTNEY,** PAULINA A. SZAFRAN**ŠKA** and MAREK KONARZEWSKI**‡

*Mammal Research Institute PAS, Białowieża, Poland, †School of Biological Sciences, University of Aberdeen, Aberdeen, UK, ‡University in Białystok, Białystok, Poland*

**Abstract**

Body mass (BM) and resting metabolic rates (RMR) are two inexorably linked traits strongly related to mammalian life histories. Yet, there have been no studies attempting to estimate heritable variation and covariation of BM and RMR in natural populations. We used a marker-based approach to construct a pedigree and then the ‘animal model’ to estimate narrow sense heritability ($h^2$) of these traits in a free-living population of weasels *Mustela nivalis*—a small carnivore characterised by a wide range of BM and extremely high RMR. The most important factors affecting BM of weasels were sex and habitat type, whereas RMR was significantly affected only by seasonal variation of this trait. All environmental factors had only small effect on estimates of additive genetic variance of both BM and RMR. The amount of additive genetic variance associated with BM and estimates of heritability were high and significant in males ($h^2 = 0.61$), but low and not significant in females ($h^2 = 0.32$), probably due to small sample size for the latter sex. The results from the two-trait model revealed significant phenotypic ($r_P = 0.62$) and genetic correlation ($r_A = 0.89$) between BM and whole body RMR. The estimate of heritability of whole body RMR (0.54) and BM corrected RMR (0.45) were lower than estimates of heritability for BM. Both phenotypic and genetic correlations between BM corrected RMR and BM had negative signals ($r_P = -0.42$ and $r_A = -0.58$). Our results indicate that total energy expenditures of individuals can quickly evolve through concerted changes in BM and RMR.

**Keywords**: ‘animal model’, body mass, heritability, *Mustela nivalis*, resting metabolic rate

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**Introduction**

Variation in body mass (BM) and metabolic rates are fundamentally important for understanding many ecological patterns and processes (Anderson & Jetz 2005; Humphries *et al.* 2005), and form the empirical backbone of the so-called Metabolic Theory of Ecology (Brown *et al.* 2004). Surprisingly however, physiological ecologists have almost exclusively concentrated on interspecific comparisons or phenotypic variation of these traits and typically often discuss their adaptive significance based solely on non-genetic data (e.g. McNab 2002). Only recently is there a growing awareness of the need to study heritable variation of physiological traits (Nespolo *et al.* 2003; Sadowska *et al.* 2005). This has become exceptionally important in the context of microevolutionary responses to climate change and the paucity of data for disentangling the genetic (evolutionary) and phenotypic (plastic) components of physiological mechanisms underlying those responses (for review see Gienapp *et al.* 2008).

Genetic variation is usually quantified as narrow sense heritability ($h^2$), which is the ratio of additive genetic variance to total phenotypic variance (Falconer & Mackay 1996). The additive genetic variance is a key quantitative genetic parameter, which indicates the ability of a trait to respond to selection, the potential to
evolve (Falconer & Mackay 1996), and thus has direct impact on evolutionary (Lynch & Walsh 1998; Roff 2002) and physiological ecology (reviewed in Swallow et al. 2009). Despite a growing interest in evolutionary studies on mammalian BM and metabolic rates, the number of studies reporting heritabilities is very limited and these are primarily restricted to classical laboratory model rodents (Dohm et al. 2001; Konarzewski et al. 2005) or wild species bred under laboratory conditions (Nespolo et al. 2003, 2005; Sadowska et al. 2005). However, constant laboratory-controlled conditions are likely to inflate heritability estimates, because effects of environmental variation are relatively lower than in natural populations (Riska et al. 1989). Although studies on wild populations are badly needed, hitherto there are only three papers reporting heritability of BM in wild mammals (Roåle et al. 1999; Thomas et al. 2002; Coltman 2005) and no studies on heritability of mammalian metabolic rates in the wild. In this context, by a ‘wild mammal’ we mean individuals of a species that did not undergo domestication (laboratory mice, Ksiazek et al. 2004), were not artificially selected (bank voles, Sadowska et al. 2005) or bred under laboratory conditions (Bacigalupe et al. 2004).

The scarcity of studies on heritability of morphological and physiological traits is due in part to the lack of reliable pedigree information, which is a primary requirement when calculating \( h^2 \), but is difficult to reconstruct under natural conditions (Lynch & Walsh 1998; Coltman 2005). This difficulty has been only recently circumvented by the application of methods of pedigree reconstruction utilizing information derived from the analysis of highly polymorphic molecular markers (for review see Garant & Kruuk 2005; Pemberton 2008). This reconstructed structure of relatedness can be then fed to a statistical ‘animal model’, which allows estimation of heritability from an extended pedigree, even when that pedigree may be insufficient to allow the more classical full-sib, half-sib designs or parent-offspring regression, traditionally used in heritability estimates (Kruuk 2004). The animal model is a linear mixed model, composed with a mixture of both ‘fixed’ and ‘random’ effects (Shaw 1987). The use of the animal model based on the residual maximum likelihood (REML) techniques has revolutionized quantitative genetic analysis (for review see Kruuk 2004; Thompson 2008).

Here we applied molecular data to construct a pedigree and then the ‘animal model’ to separate genetic and environmental components of BM and resting metabolic rates (RMR) of weasels (Mustela nivalis) under natural conditions. Our study population is characterized by an almost threefold phenotypic variation in adult BM similar to that found in the rest of the geographic range of this species in Europe (King 1989; Reig 1997; Abramov & Baryshnikov 2000). BM is a very important factor determining reproductive success of male weasels given they display a polygynous mating system and thus face a high intraspecific competition (Moors 1980; King 1989). BM is also inexorably linked with the rate of energy expenditures (Nagy et al. 1999; Speakman 2000; McNab 2002), which underpins many other traits, such as fasting endurance and cold-tolerance that will have direct impact on individual survival and lifetime reproductive success. RMR of weasels is very high, on average twice as high as in mammals of similar size (Moors 1977; Casey & Casey 1979) and constitutes up to 70% of their daily energy expenditures (Zub et al. 2009). That said, energetic constrains may limit growth of small mustelids in the periods of low prey abundance (Powell & King 1997). Furthermore, they negatively affect weasels’ winter survival and drive spatial segregation (Zub et al. 2011).

The studied weasel population is characterised by a high within-individual repeatability of BM and lower, though still statistically significant, repeatability of RMR (Szafrańska et al. 2007). We predicted that heritability of these traits will also be high, though higher in BM than in RMR, reflecting the between-trait differences in potential to undergo rapid evolutionary changes. This prediction is based upon generally higher heritability of morphological than physiological traits (Roff 2002). Moreover, the most straightforward way that natural selection can alter energy expenditures is through changes of size/mass of animals (Melton 1993; Ergon et al. 2003; Wikelski 2005). However, these changes are most likely mediated by effects of weasels’ prey size, which is associated with different habitat occupied by weasels (Zub et al. 2011) whereas selection on RMR can act through seasonal changes in energy expenditures (Szafrańska et al. 2007; Zub et al. 2009). In our analyses we have therefore also incorporated major environmental effects, which may constrain full expression of weasels’ BM and RMR genotypes.

Materials and methods

Study area and sampling

The study area encompassed the central part of the Białowieża Forest, NE Poland (23°52’E, 52°42’N), including wet sedge marshes in the Narewka river valley, moist meadows and abandoned fields on the Bialowieza glade, as well as neighbouring pristine forest protected in the Białowieża National Park. Weasels were captured according to procedure described by Jędrzejewski et al. (1995) in three habitats (river valleys, meadows and forest), characterised by different plant communities and
associated rodent communities. The total trapping area encompassed approximately 10 km² (for details see Zub et al. 2008). In two core areas (river valley and meadows) we trapped animals every 2 months. All weasels were individually marked and results from Capture–Mark–Recapture models indicated that we were able to capture and genotype most individuals from studied population (Zub et al. 2008, 2011). From autumn 2002 until winter 2005/2006 we captured 128 individuals (93 males and 35 females): 11 in autumn 2002; 25 in 2003; 47 in 2004; and 45 in 2005. The weasels were collected and analyzed under permit (refs: DOPweg-4201-04-06/03/jr, DOPog-4201-04-43/05/aj, LKE 2003/04 and LKE 2004/06). Captured animals were immediately transported to the laboratory, sexed and weighted. After completing all procedures related to measurements of RMR animals were anaesthetized and marked by ear punching. To allow for the restoration of body condition weasels were provided with water ad libitum and 1–2 laboratory mice, depending on BM. On the next day RMR was measured. Upon completion of all measurement procedures the animals were released at the location of capture.

Measurements of BM and RMR

Each animal was weighed to the nearest 0.1 g prior to the metabolic trial. This BM was usually slightly higher than the BM determined immediately after capture. However, both measures were highly correlated (r = 0.87, P < 0.0001), so we elected to use BM taken before RMR measurements for all further analyses of BM variation.

In total, we collected 285 BM measurements of 93 males and 35 females (BM averaged 84.2 g, SD = 26.5). We observed significant sexual dimorphism (t = 17.06, P < 0.0001), because mean BM of males (95.4 g, SD = 17.58, range: 58.0–147.9 g) was much higher than this of females (43.2 g, SD = 5.71, range: 32.0–56.0 g).

For metabolic measurements we used a positive-pressure, open-circuit respirometry system. Outside atmospheric air was pushed through a column of Drierite to remove water vapor, and forced through a copper coil submerged along with metabolic chamber in a water bath to equalize and control the temperature. The airstream was then divided to control and measurement stream, each fed to a separate mass flow controller (Sierra Instruments, Monterey, CA or ERG-1000, Warsaw). The measurement stream was forced through a metabolic chamber (2300 cm³ of the volume) at a mean rate of 900 mL/min. The air streams were then directed to a computer-controlled Sable Systems TR-1 setup (Las Vegas, NV). The analyzed gas stream was redried (Drierite), subsampled at a rate of 200 mL/min with a subsampler, and then passed through the sensor of an FC-10b oxygen analyzer. Digital signals from the analyzer were stored using WINWEDGE 3.0 software (Taltech, Philadelphia, PA, USA) and subsequently analyzed with Sable System DATACAN V software. We calculated oxygen consumption rates using equation (5) of Hill (1972).

All metabolic trials were carried out at night, starting from 6 pm in winter and 8 pm in summer. Before a trial, weasels were fed only in the morning, so they were not fasted longer than 12 h before measurement—(longer fasting would both compromise an animal’s welfare and/or increase their restlessness). We cannot therefore consider our measurements as taken in a truly post-absorptive state, and thus we refer to them as resting, rather than basal metabolic rate. Measurements were taken during the last 2 h of the 3–6 h trial period at 30 °C, a temperature within the thermoneutral zone. We defined RMR as the lowest readout that did not change during 4 min by more than 0.01% of oxygen concentration. Repeatability of this estimated RMR was statistically significant (intraclass correlation coefficient τ = 0.63) over time scale of 400 days (Szafranska et al. 2007).

We successfully measured RMR in 61 individuals (8 females and 53 males). In most of them we managed to repeat RMR measurements over several months, so the total number of measurements was 117 (Fig. 1). RMR in females averaged 137.5 mL O₂/h (SD = 27.70, range: 98.1–174.9 mL O₂/h) whereas in males it was 205.7 mL O₂/h (SD = 26.63, range: 152.9–268.7 mL O₂/h, Fig. 1).

Mean BM of these weasels was not different from mean values calculated for all individuals (89.5 g, SD = 23.1, range: 36.9–129.3).

Before statistical analyses, BM and RMR were log-transformed and scaled to have a zero mean and unit variance.

Microsatellite genotyping

DNA was extracted from tissue samples using the Qiagen Genomic DNA Extraction Kit (Qiagen Ltd) according to the manufacturer’s instructions, and then diluted to approximately 10 ng/µL. All individuals were genotyped at 12 microsatellite loci: Mer 0005, Mer 0009, Mer 0022, Mer 0041, Mer 0082, Mvi 0111, Mvi 1321, Mvi 1381, Mvi 1843, Mvis 0002, Mvis 0022, Mvis 0072 (O’Connell et al. 1996; Fleming et al. 1999; Vincent et al. 2003).

PCR amplifications were performed in a total volume of 10 µL using an MJ Research PTC-100 thermal cycler. Each reaction mix contained approximately 20 ng of template DNA, 2.5 mM MgCl₂, 75 mM Tris–HCl (pH 9.0), 20 mM (NH₄)₂SO₄, 0.01% (v/v) Tween-20, 0.2 mM
of each nucleotide, 5 pmoles of each primer (forward primer end-labelled with either HEX, NED or 6-FAM fluorescent dyes) and 0.5 units Taq polymerase. PCR profiles followed a ‘touchdown’ (Don et al. 1991) procedure, whereby after an initial denaturation step of 2 min at 92 °C, 20 cycles of PCR were performed, each cycle consisting of 15 s denaturation at 90 °C, and 15 s of annealing starting at 60 °C and dropping by 0.5° per cycle. A further 17 cycles were then performed with 15 s denaturation at 90 °C and 15 s annealing at 50 °C. Only 1 min extension step at 72 °C followed the final annealing. PCR fragments were resolved by electrophoresis on an Applied Biosystems 3730 automated DNA sequencer.

For analyses we used 11 loci, which did not exhibited significant departure from Hardy–Weinberg equilibrium, thus we assumed that presence of null-alleles did not affect results of parentage and relatedness analyses. Mean number of alleles per locus was 11.73, and mean expected heterozygosity 0.80. Departure from Hardy–Weinberg equilibrium and presence of null-alleles was calculated in Cervus 3.0 (Marshall et al. 1998; Kalinowski et al. 2007).

Pedigree reconstruction

For reconstructing pedigrees we used the ‘blind search algorithm’, implemented in program MOL_COANC 2.0 (Fernández & Toro 2006). This algorithm finds the genealogy yielding a co-ancestry matrix with the highest correlation with the molecular co-ancestry matrix calculated using the markers. This method does not require direct assumptions about allelic frequencies or Hardy–Weinberg equilibrium and linkage disequilibrium. The software we used for analysis estimates the genealogical co-ancestry between groups of contemporaneous individuals, thus none of them can be a parent of other individuals. This is the main limitation of the algorithm, but fortunately it allows including previously known genealogy. We therefore first identified potential parents using Cervus 3.0 (Marshall et al. 1998; Kalinowski et al. 2007). During breeding season we performed trapping every 2 months thus we were able to distinguish between potential parents and their offspring. Therefore in Cervus we ran year specific analyses, including as potential parents only individuals captured in previous trapping sessions. When identifying parents we used only strict confidence intervals (95%) and allowed for one mismatching locus. We then included all parents identified by Cervus (known parents) to the initial pedigree and let the MOL_COANC algorithm assign unknown (virtual) parents. We relaxed the conditions of compatibility within a full-sibs family making MOL_COANC accept families with incompatibilities in one locus. All animals captured between 2002 and 2005 were treated as contemporary individuals. Please note that by incorporating known parents we avoided erroneous identification of parent-offspring pairs as full-sibs. Final genealogy file contained all individuals and their known as well as virtual parents. We relaxed the conditions of compatibility within a full-sibs family making MOL_COANC accept families with incompatibilities in one locus. All animals captured between 2002 and 2005 were treated as contemporary individuals. Please note that by incorporating known parents we avoided erroneous identification of parent-offspring pairs as full-sibs. Final genealogy file contained all individuals and their known as well as virtual parents. We reconstructed pedigree assuming two generations (25 males and 25 females in each generation) preceding contemporary individuals. The number of virtual parents per generation was comparable to the number of weasels captured each year in our study area, which averaged 32 individuals (range 11–47 individuals).

To examine the effect of pedigree on estimates of variance components we reconstructed a series pedigrees and then ran ‘animal models’, omitting each time one known parent identified by Cervus. This procedure, which can be treated as delete-1 observation jackknife,
affected both structure of pedigree and estimates produced by animal model. Finally we obtained 20 estimates of variance components for BM and whole-body RMR, and 17 estimates of variance components for BM and BM-corrected RMR. The numbers of whole-body and mass-corrected models differ, because for few models did not converge. We then averaged values across models and calculated means and confidence intervals for estimates of variance components.

*Animal model analyses*

We partitioned the phenotypic variances (V_P) of BM and RMR into the additive (co)variances and other random variance components (permanent environment effects) using the pedigree reconstructed in MOL COANCE and fitting REML mixed-effect models with ASREML 3.0 software (Gilmour et al. 2009). In all those models we fitted a permanent environment effect (individual identity ID), even if it was not significant, because we used repeated measurements. Thus, the ID of an individual was included in the model both in association with the pedigree (to obtain an estimate of additive genetic variance, V_A) and independent of the pedigree (to estimate variance due to permanent environmental effect, V_PE). We also accounted for environmental (non-genetic) effects, which are characterised in the next paragraph and incorporated them as fixed factors.

To obtain estimates of genetic variation we fitted series of two-trait models, assuming that genetic covariances between sexes as well as between BM and RMR are different from zero. For BM we fitted two-trait model using standardized BM of males and females and including gender as a fixed effect. To account for the effect of habitat quality we fitted habitat type (three levels—river valley sustaining the largest prey and the largest weasels; meadows—intermediate; and forest—inhabited by smallest prey and smallest weasels, for details see Zub et al. 2011) as additional fixed effect for both sexes. We also ran an additional animal model and treated standardized BM of males and females as separate traits.

For RMR we fitted two variants of the two-trait model, with BM and RMR as separate traits. In the first variant we used sex and habitat type as fixed factors fitted for BM only, and seasonal variation (two levels—summer and winter) fitted as a fixed effect for RMR only. In the second variant we estimated variance components and correlations for BM-corrected RMR. We corrected for BM by fitting the model as in the first variant, but with BM added as a fixed effect. In both variants, however, we did not fit BM and RMR of males and females as separate traits, due to small sample size of females (eight individuals only).

To estimate of the significance of random effects (additive genetic effect, maternal effect and permanent environment effect sensu Wilson et al. 2009) we used likelihood ratio tests. The value of the log-likelihood difference between the model with and without additional random effect was compared to the chi-square distribution with one degree of freedom (every time we fitted only one additional random effect). The same method was used to test the significance of covariance between traits. In this case we fitted one model with covariance and in the next model we fixed covariance parameters of the random effects to zero.

The significance of fixed effects was obtained using Wald statistics. The significance of heritability and correlation estimates was based on standard errors (SE), provided by ASREML. We divided the value of each estimate by corresponding SE and compared it with Student’s *t* distribution (using appropriate number of degrees of freedom, which was always >100). We used a one-tailed test, since estimates of variance components should be greater than zero.

*Results*

*Pedigree structure and its effect on variance components*

The final structure of the pedigree included two generations of ancestors (virtual grandparents and parents) and three generations of contemporary individuals. The whole population originated from 27 individuals (virtual grandparents), whereas the next generation (virtual parents) consisted of 50 individuals (25 males and 25 females)—the maximum initial number assumed.

The population of contemporary (genotyped) individuals consisted of 28 half-sib families and 15 full-sib families. The offspring of 37 individual males were distributed over the 32 matrilines, creating an interconnected pedigree containing a total of 128 individuals. Paternity was assigned from genetic data for 16 individuals, maternity for 18 individuals, and both parents were known only for three weasels. For remaining individuals only virtual parents were assigned. Complete pedigree is presented in Appendix S1 (Supporting information).

Mean values of variance components and heritability estimates obtained by jackknife procedure (Table 1) were only slightly different than those obtained from the animal models based on the complete pedigree, which included all known parents (Tables 2 and 3). Estimates of heritability were significantly different from zero for BM (*P* < 0.01) for both models (without and with BM as covariate for RMR) and for RMR (*P* < 0.01 for whole body RMR and *P* = 0.04 for BM corrected RMR). Permanent environment effect was
The above results allowed us to adopt the complete reconstructed pedigree as a basis for the remaining detailed analyses of variance reported below.

Animal model analyses

Final models did not include maternal effects, which were not significant in any model. When fixed effects were removed from the final model, estimates of heritability slightly changed, but none of these changes was significant as revealed by log-likelihood test (Table 2). In the first group of models (without BM as covariate for RMR) it was caused by higher estimates of $V_A$ and lower estimates of $V_{PE}$ for BM, whereas in the case of RMR it was caused by lower estimates of $V_A$ and $V_{PE}$. For both traits, estimates of permanent environment effect were lower, when fixed effects were removed from the respective models (Table 2). In the second group of models (with BM as a covariate for RMR) the influence of fixed effect on heritability of BM was also negligible, and in the case of RMR permanent environment effect was not estimable after removing the effect of season (Table 2).

The estimates of $V_A$ associated with BM and RMR (whole body and BM corrected) were significant in both groups of models (Table 2). The estimates of heritability from the first group of models were high and significant for both traits, whereas permanent environment effects were low and not significant (Table 2). Phenotypic correlation between BM and whole body RMR was significant ($r_P = 0.62$, SE = 0.07, $P < 0.001$), and accompanied by high genetic correlation ($r_A = 0.89$, SE = 0.13, $P < 0.001$). The covariance between these traits estimated from first group of models was also significant ($\text{cov}_{BM-RMR} = 0.34$, likelihood ratio test, $\chi^2 = 26.88$, d.f. = 1, $P < 0.001$).

The estimates of heritability of BM from the second group of models were similar to estimates from the first model, whereas estimates of heritability of BM corrected RMR were lower, but also significant (Table 2). Estimates of permanent environment effect for both traits were low and not significant (Table 2). Covariance between BM and RMR estimated from the second model was lower ($\text{cov}_{BM-RMR} = -0.12$), but still significant (likelihood ratio test, $\chi^2 = 4.19$, d.f. = 1, $P = 0.04$). The genetic correlation between BM and RMR also had negative sign ($r_A = -0.58$, SE = 0.22, $P = 0.006$), similarly to phenotypic correlation ($r_P = -0.42$, SE = 0.11, $P < 0.001$). Repeatability of BM calculated from animal model was 0.73 (SE = 0.17) and repeatability of BM corrected RMR was 0.47 (SE = 0.11).

### Table 1

<table>
<thead>
<tr>
<th>Trait</th>
<th>$VA$ (95% CI)</th>
<th>$V_{PE}$ (95% CI)</th>
<th>$V_P$ (95% CI)</th>
<th>$r_P$ (SE)</th>
<th>$r_A$ (SE)</th>
<th>$\chi^2$ (d.f.)</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td>BM (whole)</td>
<td>0.237 (0.223–0.251)</td>
<td>0.129 (0.110–0.148)</td>
<td>0.143 (0.123–0.164)</td>
<td>0.36 (0.31–0.41)</td>
<td>-0.58 (0.22)</td>
<td>4.19 (1)</td>
<td>0.04</td>
</tr>
<tr>
<td>RMR (whole)</td>
<td>0.150 (0.139–0.162)</td>
<td>0.34 (0.25–0.43)</td>
<td>0.60 (0.52–0.68)</td>
<td>0.36 (0.31–0.41)</td>
<td>-0.58 (0.22)</td>
<td>4.19 (1)</td>
<td>0.04</td>
</tr>
<tr>
<td>BM (corrected)</td>
<td>0.166 (0.154–0.179)</td>
<td>0.129 (0.109–0.150)</td>
<td>0.143 (0.123–0.164)</td>
<td>0.36 (0.31–0.41)</td>
<td>-0.58 (0.22)</td>
<td>4.19 (1)</td>
<td>0.04</td>
</tr>
<tr>
<td>RMR (corrected)</td>
<td>0.144 (0.133–0.156)</td>
<td>0.34 (0.25–0.43)</td>
<td>0.60 (0.52–0.68)</td>
<td>0.36 (0.31–0.41)</td>
<td>-0.58 (0.22)</td>
<td>4.19 (1)</td>
<td>0.04</td>
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</table>

Estimates based on jackknife procedure; see text for details.
Heritability of BM in males was higher than in females (Table 3). A permanent environment effect was low and not significant in males, but high and nearly significant in females (Table 3). The genetic covariance between BM of males and females was not significant (covM–F = 0.20, likelihood ratio test, \( \chi^2 = 0.38 \), d.f. = 1, \( P = 0.54 \)). Genetic correlation between these traits, despite it was quite high \( r_A = 0.46 \), but also not significant (SE = 0.73, \( P = 0.27 \)).

**Discussion**

Confounding environmental effects are likely to be particularly prevalent in wild populations, and therefore they are of particular concern for studies of their quantitative genetics (Wilson et al. 2009). Having controlled for the major environmental factors, such as habitat quality and seasonal variation, we have provided the first simultaneous analysis of the heritability of RMR and BM in wild-caught individuals from natural populations. For BM, a significant proportion of the variance was attributable to additive genetic effects, whereas permanent environmental effects were not significant. Thus, under natural conditions \( h^2 \) estimates were similar to those obtained under controlled laboratory situation. Also, the estimates of heritability of RMR were less affected by environmental factors, and even higher than those under from laboratory conditions. Heritabilities of both traits were only slightly lower than their repeatability estimates (Szafranka et al. 2007).
Pedigree reconstruction

Effective quantitative genetic analyses in wild populations require two key steps—the correct reconstruction of genealogical relationships among individuals and the formulation of proper statistical models that partition genetic variance among additive, maternal and environmental effects (Wilson et al. 2009). The MOL_COANC software was well suited for pedigree reconstruction in this system, because it not only classifies individuals to half and full-sibs families, but also enables incorporation of known parents to the pedigree. The number of markers used in the calculations has significant effect on estimated correlation between true and estimated co-ancestry. The algorithm implemented in MOL_COANC able to detect the correct co-ancestry for at least 70% of the couples in unbalanced data set based only on five markers (Fernández & Toro 2006). When Fernández & Toro (2006) used information on only 10 randomly chosen microsatellites from real pig data then correlations between real and estimated genealogy was 0.5, whereas for 18 markers exceeded 0.9. For our data set the correlation between true and estimated co-ancestry was 0.70. This figure was comparable with correlation found for unbalanced data set simulated for 40 founders (20 males and 20 females) with 10 markers and 10 alleles per locus, which was 0.63 (Fernández & Toro 2006). This simulated data set had similar structure to our real data, thus we can assume that our estimates did not depart from the expected, considering the limited amount of information available. Finally, a close correspondence between estimates of heritability obtained from the jackknife analysis (Table 1) and the remaining animal models (Tables 2 and 3) reassured us about the robustness of the reconstructed pedigree.

Partitioning of phenotypic variance of BM

Habitat type (and average BM of potential prey associated with this habitat) is the most significant predictor of phenotypic variation of BM in the studied population (Zub et al. 2011). This finding is particularly interesting in the context of spatial segregation of small and large individuals according to average prey mass. Furthermore, observations of over 50 radiotracked individuals from the same population revealed their high habitat fidelity (Zub 2006; Zub et al. 2008). That said, under favorable environmental conditions (high prey abundance) large individuals may temporary settle in otherwise sub-optimal habitats (Zub et al. 2008). We could therefore surmise that relatives are not distributed randomly with respect to space in our study population. For these reasons we incorporated the habitat type as a fixed effect in the ‘animal model’ (Table 2). Although this effect was statistically significant in all model variants, it only slightly reduced of $V_A$ and $h^2$ of BM. Thus, we can safely conclude that our estimates of the components of BM variation are not biased due to the effect of habitat quality. We postulate that spatial differences in BM results from segregation of individuals of different size, which select habitats best satisfying their energy needs (Zub et al. 2011).

Our estimates of heritability of BM falls within the upper range of estimates reported for this trait in other mammals, which usually varied between 0.2 and 0.6 (Réale et al. 1999; Dohm et al. 2001; Thomas et al. 2002; Coltman 2005; Nespolo et al. 2005). High heritability of weasel BM may be therefore partly maintained by the changing directions of selection dependent on high or low mass of prey characteristics for a particular habitat. Our study therefore supports the existence of the positive correlation between weasels’ BM and the average size of their prey, which was proposed to underlie geographical variation of BM in Mustelidae in Europe (Rellinge 1987; King 1991). However, its existence on a local scale suggests that it does not necessary result from co-variation of longitudinal gradients of BMs of mustelids and their prey.

The importance of nutritional condition on the BM of small Mustelids has been highlighted in several studies (Frank 1985; Powell & King 1997). Observations of captive weasels also revealed that well-fed animals, especially males, attained much higher BM than their parents (Frank 1985). This finding might limit the usefulness of laboratory estimates of BM heritability in this group of animals, because such conditions substantially reduce natural variation of this trait.

Heritability of BM was much higher for male than female weasels. BM of females is probably more constrained then BM of males, mainly because only females are raising pups and limited growth of body size enable them to allocate more energy resources to the offspring (Moors 1980). In contrast, males are subject to strong intraspecific competition for mates, which selects for largest possible body size under given environmental conditions, setting the upper limit on individual energy needs (Zub et al. 2011). However, we are not able to exclude the possibility that lower estimates of additive genetic variance in females were only due to small sample size causing low statistical power of our analyses.

Partitioning of phenotypic variance of RMR

Measurements of RMR are technically complicated and inherently burdened with measurement error of 15–20% (Konarzewski et al. 2005). Furthermore, RMR is an extremely plastic trait, strongly affected by all major environmental factors, such as temperature, food,
availability, photoperiod etc. (McNab 2002). This propensity of RMR to environmental influences restricted its quantitative genetic analyses to well controlled laboratory conditions (e.g. Dohm et al. 2001; Nespolo et al. 2003). However, despite their unquestionable advantages, laboratory conditions yield estimates obtained in an artificial environment, and on animals that are likely to be more inbred than in natural populations. These issues may bias heritability estimates, though the direction and magnitude of this bias is debatable (Riska et al. 1989 vs. Weigensberg & Roff 1996). In contrast to the laboratory studies, we measured RMR on the next day upon capturing each animal, so we can safely assume that their RMR reflects genuine environmental effects, rather than acclimation to the laboratory conditions (Szafrąńska et al. 2007).

Many published estimates of heritability of resting (or basal) metabolic rate suggest that it is very low, usually close to zero (Lacy & Lynch 1979; Dohm et al. 2001; Nespolo et al. 2003; Bacigalupe et al. 2004; but see Nilsson et al. 2009). Furthermore, heritability of some traits closely related to RMR, such as body temperature, are also effectively zero (Lacy & Lynch 1979; Lynch & Sulzbach 1984; Bacigalupe et al. 2004; but see Nespolo et al. 2003). However, some recent laboratory studies reported much higher and significant estimates of heritability of RMR, within the range of 0.1–0.4 (Dohm et al. 2001; Nespolo et al. 2003; Konarzewski et al. 2005; Sadowska et al. 2005). Our estimates of the heritability of RMR in a wild population fall within this range. This was most likely due to the effective control of environmental factors, which brought our estimates of the additive genetic variance into line with those obtained under laboratory controlled conditions.

Unlike many other small mammals, weasels adapt to seasonal variation through adjustment of RMR, rather than BM (Speakman 2000). However, we also found highly significant genetic correlations between BM and whole-body as well as mass-corrected RMR, which were in line with phenotypic ones as well as with general patterns well documented in the physiological literature (e.g. McNab 2002). These genetic correlations indicate that BM and RMR do not vary independently, and can evolve in a concerted fashion (Arnold et al. 2008). Lower heritability of RMR implies its higher phenotypic plasticity, which enables individual weasels to adjust to short-term changes in climatic conditions and unpredictable variation of food resources. Indeed, subsequent generations of the studied population experience very diverse climatic and nutritional conditions (Jędrzejewski et al. 1995).

Lower estimates of additive genetic variance associated with RMR in comparison to BM also suggest that we were probably not able to identify all factors affecting RMR. Recently, Careau et al. (2008) highlighted inter-individual differences in the rate of energy metabolism caused by personality of individuals, who may consistently differ in their stress response, exploration or activity levels. The likely candidate factor contributing to estimate of permanent environment effect is therefore behavioral response to measurements of RMR incurred by individual response to handling during metabolic trials (Lantová et al. 2011). This possibility fits into a developing paradigm of evolutionary physiology, which sees natural selection acting primarily on behavioral traits as the mechanism of evolution of complex physiological adaptations, such as endothermy (for review see Careau et al. 2009; Hayes 2010; Nespolo et al. 2011). In weasels permanent environment effect associated with RMR was low and not significant, thus lower estimates of RMR heritability are probably not caused by personality of individuals.

We cannot exclude that high phenotypic and genetic variation of BM and RMR observed in our population of weasels is exceptional. Abramov & Baryshnikov (2000) showed that the coefficient of variation (CV) of condylobasal length of weasels’ skulls increased southwards in Europe. It is therefore possible that at northern latitudes unidirectional selection is stronger due to limited resources, decreasing both, phenotypic and genetic variation. In contrast, at lower latitudes more variable climate and fluctuating rodent populations may maintain higher genetic variation of BM and RMR in weasels and indeed, in one of our previous studies we were able to demonstrate that BM may significantly affect survival of weasel males, but the strength of selection differ among years (Zub et al. 2011).

In conclusion, we have demonstrated the application of molecular techniques and quantitative genetics to estimate variance components and heritability in a wild mammalian population that could not have been analyzed using traditional methods. We have also demonstrated that both BM and RMR are heritable and that their $h^2$ are comparable to estimates obtained under laboratory conditions. Our findings emphasize the relative importance of genetic background of individual physiology over ecological factors (such as habitat quality).

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References


K.Z. is interested in ecology, physiology and genetics; particularly how variation of morphological and physiological traits, and genetic diversity affect individual fitness and population dynamics of mammals. S.P. is a molecular ecologist with interests in understanding the causes and consequences of variation in genetic diversity among populations, and how this influences individual fitness and population processes. P.A.S. is a postdoctoral researcher studying influence of physiological traits on ecological processes. M.K. research interests interface evolutionary ecology, eco-physiology and quantitative genetics of vertebrates. He uses both laboratory and natural settings to study physiological traits (mostly related to metabolism) from molecular to whole-animal levels.

Data accessibility

The data supporting all analyses and results of this paper have been deposited in Dryad repository under DOI: 10.5061/dryad.54j79463.

Supporting information

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

Appendix S1 Pedigree of weasels used in ‘animal model’ reconstructed based on molecular markers.

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