Evolution of basal metabolic rate in bank voles from a multidirectional selection experiment

Edyta T. Sadowska, Clare Stawski†, Agata Rudolf, Geoffrey Dheyongera‡, Katarzyna M. Chrząścik, Katarzyna Baliga-Klimczyk and Paweł Koteja

Institute of Environmental Sciences, Jagiellonian University, ul. Gronostajowa 7, Kraków 30-387, Poland

A major theme in evolutionary and ecological physiology of terrestrial vertebrates encompasses the factors underlying the evolution of endothermy in birds and mammals and interspecific variation of basal metabolic rate (BMR). Here, we applied the experimental evolution approach and compared BMR in lines of a wild rodent, the bank vole (Myodes glareolus), selected for 11 generations for: high swim-induced aerobic metabolism (A), ability to maintain body mass on a low-quality herbivorous diet (H) and intensity of predatory behaviour towards crickets (P). Four replicate lines were maintained for each of the selection directions and an unselected control (C). In comparison to C lines, A lines achieved a 49% higher maximum rate of oxygen consumption during swimming, H lines lost 1.3 g less mass in the test with low-quality diet and P lines attacked crickets five times more frequently. BMR was significantly higher in A lines than in C or H lines (60.8, 56.6 and 54.4 ml O₂ h⁻¹, respectively), and the values were intermediate in P lines (59.0 ml O₂ h⁻¹). Results of the selection experiment provide support for the hypothesis of a positive association between BMR and aerobic exercise performance, but not for the association of adaptation to herbivorous diet with either a high or low BMR.

1. Introduction

Successful performance of vital animal functions—such as resource acquisition, maintaining homeostasis, growth or reproduction—depends on a complex network of physiological processes. However, each of these processes involves conversion of energy, and therefore the rate of energy metabolism can be used as a unifying quantitative measure of organismal functioning [1,2]. Obvious sources of variation in the rate of metabolism are changes in body and ambient temperature and the level of physical activity, which result in instantaneous changes in metabolic rate. Therefore, the basal rate of metabolism (BMR), which is measured in resting animals at standardized thermal conditions [1], has received special attention as a trait suitable for interspecific comparisons. Consequently, questions about the factors underlying the huge interspecific variation in BMR have become a major theme in evolutionary and ecological physiology of terrestrial vertebrates [1].

At the macroevolutionary scale, the most striking difference in BMR is between ‘endotherms’ (birds or mammals) and ‘ectotherms’ (reptiles). Benefits of endothermy, which allows maintaining a high body temperature by means of metabolic heat production, are easy to identify. However, evolution of the high level of BMR in birds and mammals, which translates to at least an order of magnitude higher costs of maintenance in comparison to ectothermic reptiles, is puzzling and the selection mechanisms that have led to evolution of such an energetically wasteful strategy remain subject to a vivid discussion (reviews: [1–17]). According to the ‘aerobic capacity model’—one of the main hypotheses—high BMR in endotherms evolved as a correlated response to selection for increased locomotor performance fuelled by aerobic metabolism [18]. Testing the basic assumption of the model—that BMR is positively
correlated with aerobic capacity (maximum rate of oxygen consumption)—has been a motivation for many comparative, experimental, quantitative genetic and conceptual studies, but the issue is not resolved (recent reviews: [13,17,19,20]).

At the level of interspecific comparisons within birds and mammals, many studies have focused on the associations between BMR and food habits (e.g. [1,21–29]). Predation and herbivory are the two most basic, but also opposite, food habit strategies available. Evolutionary selection for one of these strategies has a profound effect on other behavioural, physiological and morphological traits. However, the relationship between the expected BMR and either of these strategies is unclear; in both cases, one can provide theoretical and empirical arguments for a relatively low or high BMR [26–29].

The majority of research on hypothetical correlates of BMR has been based on comparative analyses or intraspecific phenotypic correlations, but more recently quantitative genetic analyses (e.g. [30–34]) and selection experiments [2,35–41] have been recognized as powerful tools in such studies (but see [19,20,42,43] for discussion of limitations of these tools).

Here, we applied the experimental evolution approach and asked: ‘how would BMR in a particular species change in response to controlled selection for traits that comparative analyses have indicated as plausible triggers for the evolution of interspecific variation in BMR?’ To this end, we designed a multidirectional artificial selection experiment, with lines of bank voles, selected in three directions (figure 1): increased maximum rate of exercise-induced aerobic metabolism (A), ability to grow on a low-quality herbivorous diet (H) and intensity of predatory behaviour (P) [44]. In this paper, we present a comparison of the level of BMR of voles from lines selected for 11 generations with that of unselected, control lines (C). Based on results from our earlier quantitative genetic analyses, we predicted that BMR will increase both in lines selected for high swim-induced aerobic metabolism [32] and in lines selected for herbivorous capability [33]. Because of a close connection between predatory propensity and locomotor activity, shown also in other selection experiments [45], and in line with the aerobic capacity model of the evolution of endothermy [16,46], we predicted that BMR will also increase in lines selected for increased predatory behaviour.

2. Material and methods

(a) Animals and the selection experiment

This work was performed on bank voles (Myodes = Clethrionomys glareolus Schreber 1780) from generation 11 of a multivariate artificial selection experiment. The rationale, history and protocols of the ongoing selection experiment have been presented in our earlier work [44] and in the electronic supplementary material of this paper. Briefly, selection was applied based on the following criteria: high aerobic metabolism (A)—the maximum 1 min rate of oxygen consumption ($\dot{V}O_2$), achieved during 17 min of swimming at 38°C; herbivorous capability (H)—body mass change in a 4 day trial, during which voles were fed a low-quality, herbivorous diet (made of dried grass and flour); and predatory behaviour (P)—ranked time to catch a live cricket in a 10 min trial (ranks 1–5: cricket caught in 0.5, 1, 3, 6 or 10 min, respectively; rank 6: cricket not caught). The

Figure 1. Direct phenotypic responses to 11 generations of selecting bank voles towards (a) high swim-induced aerobic metabolism, (b) herbivorous capability measured as ability to maintain body mass in a test with low-quality diet, (c) predatory propensity measured as ranked time to attack a cricket, and (d) comparison of the cumulative effects of selection in the three directions expressed as a difference between the means of four selected (in each direction) and four control lines (expressed in units of phenotypic standard deviation). In generation 8, the food used in selection trial in the ‘herbivorous’ lines was different than in other generations, which resulted in the irregular pattern (marked with dashed lines and open symbols on graphs (b) and (d)). (Online version in colour.)
measuring oxygen consumption (VO₂) was operationally defined as the minimum recorded VO₂ and can be calculated from the values recorded in the last 3 min period. VO₂ was calculated from the values recorded in the last 20 s before switching channels. Activity of the animals and background ‘noise activity’ of the empty reference chamber was monitored continuously with MAD-1 gravimetric detectors (signal of 0–5 V range; Sable Systems, Inc., Las Vegas, NV, USA).

BMR was operationally defined as the minimum recorded VO₂. However, if the mean activity signal in the 3 min period preceding and including the lowest readings exceeded markedly typical background noise (mean reading from the empty chamber), the entire trial was rejected. VO₂ was not dependent on the activity signal only after setting the threshold to 0.095 V, corresponding to the upper 90% confidence limit of the noise readings. This eliminated 68 individuals and the final sample used for analyses comprised 232 individuals (C—51, A—68, P—58 and H—55). For the limited sample, the mean activity signal was similar to that of the noise signal (electronic supplementary material, Results S1). In addition, we used the signal from activity detectors as a covariate in all analyses. We also tried analyses based on BMR calculated from the mean of the two lowest readings, but in this case the sample had to be further reduced (and results were qualitatively similar to those reported here).

(c) Statistical analyses of basal metabolic rate data

For comparisons of BMR across the four selection directions, we used SAS v. 9.3 (SAS Institute, Inc. Cary, NC, USA) with the General Linear Model procedure (with REML method) to estimate cross-nested mixed ANCOVA model, with selection (selected versus control) as the main, top-level fixed factor, replicated lines as random effect nested within selection and body mass as a covariate. Because BMR is known to scale allometrically with body mass, and because the distributions of both BMR and body mass were right-skewed, the analyses were performed on log-transformed values. In all the analyses, age, sex, timing, chamber type and log-transformed activity signal were included as additional fixed covariates or cofactors. The model also included a fixed selection × sex interaction and random interactions sex × line and timing × line. The above variables were a priori considered meaningful predictors either for biological or technical reasons, and therefore were retained in the model irrespective of their significance. Before estimating this final model, we tested
preliminary models that included additional fixed effects of litter number, litter size and date of the measurement and a random effect of channel number (variation among the seven measurement channels), models with timing × selection, timing × sex and timing × sex × line interactions and models with interactions between body mass and the main categorical effects (selection, line, sex and timing; to check homogeneity of slopes). None of these additional effects was significant and we present here results from the final model only.

To compare body mass measured with BMR trials (log-transformed) across the selection, sex and timing groups, we applied similar mixed ANCOVA models, but not including the effects of the technical variables meaningful only for the respirometric measurements.

Note that in all the models described above, significance of the fixed effect of selection is tested by means of an F-test against variation among the replicate lines, and significance of sex, selection × sex and timing factors is tested against respective interactions with line, which protects against spurious recognition of correlated responses to selection [47]. Significance of the random effects of variation among replicate lines and respirometer channels was tested with a likelihood ratio test ([50] unlike in the main analyses, in models estimated for these tests variances were not constrained to be positive). In preliminary analyses, we tried to fit also two-level nested models with family (mother identity) as an additional random effect, nested within lines. However, because in many families only one individual was present, higher level effects could not be properly tested (because of lost degrees of freedom). In those cases where the models could be estimated, the results concerning main effects were qualitatively similar to those from the models not including the family effect. For pairwise a posteriori comparisons between groups of factors with more than two levels (selection, timing, litter number), Tukey–Kramer adjustment was applied.

Complete tables with descriptive statistics and results of the mixed ANCOVA models are presented in the electronic supplementary material, Results, and here we show adjusted least-square means with 95% confidence limits (LSM[CL]), back-transformed to original scale.

### 3. Results

Body mass (measured before BMR trials on all 313 individuals) increased with age ($t_{248} = 4.21$, $p < 0.0001$), was larger in males than in females ($F_{1,12} = 70.5$, $p < 0.0001$) and larger in voles from the second litters compared with those from the first ($F_{2,30} = 5.63$, $p = 0.008$; mass in the third litter was intermediate), but it did not differ between timing groups ($F_{2,30} = 1.56$, $p = 0.23$; figure 2a; electronic supplementary material, Results S1 and S2). Body mass adjusted for all the effects varied significantly among replicate lines within selection directions (LR test: $\chi^2 = 7.44$, $p = 0.006$) and differed among selection directions ($F_{3,12} = 4.17$, $p = 0.031$; figure 2a): it was larger in H than in P lines (Tukey–Kramer pairwise comparisons: $t_{12} = 3.03$, $p = 0.045$), whereas in A and C lines, it was intermediate and did not differ from each other or from the H or P lines ($p > 0.1$). The results limited to the 232 individuals for which BMR was obtained were similar, but the difference between selection directions was marginally not significant ($F_{3,12} = 2.98$, $p = 0.074$).

BMR increased with body mass (common slope + s.e. = 0.78 ± 0.04 on log–log scale; $t_{164} = 18.4$, $p < 0.0001$; figure 3), and it was not significantly correlated with the activity signal ($t_{164} = 1.57$, $p = 0.12$). BMR adjusted for both of these effects differed among the selection directions ($F_{3,12} = 12.87$, $p = 0.0005$; figures 2b and 3; electronic supplementary material, Results S1 and S3). It was higher in A lines (LSM [95% CL] = 60.8 [59.1–62.5] ml O$_2$ h$^{-1}$) in comparison to both C (56.6 [54.9–58.5] ml O$_2$ h$^{-1}$) and H lines (54.4 [52.7–56.1] ml O$_2$ h$^{-1}$; $t_{12} = 5.91$, $P = 0.0004$). In P lines, BMR (59.0 [57.3–60.8] ml O$_2$ h$^{-1}$) tended to be higher, though not significantly, than in C lines ($t_{12} = 2.09$, $p = 0.21$), but it was significantly higher than in H lines ($t_{12} = 4.09$, $p = 0.007$). BMR did not differ significantly between H and C lines ($t_{12} = 2.03$, $p = 0.23$) or between A and P lines ($t_{12} = 1.51$, $p = 0.46$). The adjusted BMR did not differ between sexes ($F_{1,12} = 1.60$, $p = 0.23$) or, surprisingly, among the timing (night–morning–afternoon) measurement groups ($F_{2,23} = 0.17$, $p = 0.85$). Likelihood ratio tests showed also that none of the random effects included in the model (line, sex × line, timing × line) contributed significantly to explaining the variance of adjusted BMR ($p > 0.36$ for all the effects).
4. Discussion

Selection was effective in all directions and resulted in substantial differences between selected and control lines in the 11th generation of bank voles (figure 1). Therefore, the selected lines provide a promising foundation for investigating both underlying molecular mechanisms responsible for differences observed at the organismal level [51,52] and a wide range of possible correlated responses [53–55].

Results of the current study confirmed the prediction that selection for high swim-induced aerobic metabolism would also result in an increase in BMR. In our previous work [32], we reported a positive genetic correlation between VO₂swim and BMR. However, the voles in our previous study swam at 30°C and therefore VO₂swim also comprised a thermoregulatory burden. In the current experiment, however, the voles were selected for VO₂swim achieved at 38°C, i.e. with no thermoregulatory burden involved. Thus, the increased BMR was strictly due to selection for a locomotor-performance trait. Our analyses of complete transcriptome from heart and liver of the A-selected and C lines [52] indicated several candidate genes with differentiated proportion of single nucleotide polymorphism alleles or different levels of gene expression, which could underlie correlation between the traits. Perhaps, the most interesting in this respect are allelic differences in glycogen phosphorylase (PYGL) and glycogen-debranching enzyme (AGL), which catalyse the rate-limiting step in glycogenolysis in the liver, and thus provide fuel for the main energy-metabolism pathway [52]. Further molecular and biochemical analyses based on this unique animal model will allow us to verify if these or other candidate genes are indeed responsible for the link between aerobic exercise performance and the level of BMR.

Our result is consistent with the positive genetic correlation between BMR and the maximum forced-running VO₂ reported in laboratory mice [17,34], but selection experiments on mice did not show the expected correlated response. In laboratory mice selected for high wheel-running activity, BMR did not increase [36], but because aerobic capacity was increased only moderately in selected lines [56,57] this result may not be very informative. BMR was also not increased in laboratory mice selected for high VO₂swim, even though maximum forced-running VO₂ was increased [38]. However, even if BMR were increased in that study, interpretation of the result would be unclear, because VO₂swim was measured at 25°C and resulted in hypothermia (about 7°C [38]), so the selected trait certainly comprised a large component of thermogenesis. Finally, a recent report showed no significant increase in BMR after eight generations of selection for high maximum forced-running VO₂, even though quantitative genetic analyses performed within the framework of the same experiment showed that both of the traits are heritable and genetically correlated [17]. The lack of change in BMR in this case could simply be due to premature termination of this selection experiment. After eight generations of selection for high maximum forced-running VO₂, even though quantitative genetic analyses performed within the framework of the same experiment showed that both of the traits are heritable and genetically correlated [17]. The lack of change in BMR in this case could simply be due to premature termination of this selection experiment. After eight generations of selection for high maximum forced-running VO₂, even though quantitative genetic analyses performed within the framework of the same experiment showed that both of the traits are heritable and genetically correlated [17]. The lack of change in BMR in this case could simply be due to premature termination of this selection experiment. After eight generations of selection for high maximum forced-running VO₂, even though quantitative genetic analyses performed within the framework of the same experiment showed that both of the traits are heritable and genetically correlated [17].

Although BMR was not statistically significantly higher in P lines than in C lines, BMR in P lines was closer to that of A lines, and it was significantly higher than in the 'lowest' H lines (figure 2b). This pattern suggests that with the ongoing progress of selection, BMR in P lines is likely to become significantly higher in comparison to C lines. The result is consistent (with all the reservations outlined above) with the hypothesis linking the evolution of endothermy with an active predatory lifestyle (e.g. [16]) and with results of comparative analyses showing that predators have, on average, a higher BMR [25].

Another group of mammals in which comparative analyses revealed a relatively high BMR are terrestrial grazers [21,23,28]. In line with this observation, our earlier quantitative genetic analyses showed a positive genetic correlation between BMR of bank voles and their ability to cope with a low-quality, herbivorous diet [33]. However, in the current experiment, despite a significant selection progress in the ability of the voles to maintain body mass on the low-quality diet, we observed no increase of BMR (figures 2b and 3). The selection experiment was formed on the same laboratory colony that was the basis for the earlier quantitative genetic study, and therefore the
discrepancy between results of the earlier quantitative genetic analysis and the selection experiment could not be due to a different genetic background. We suspect that the explanation for this discrepancy may be in a high sensitivity of the selected trait to changes of the measurement conditions. It is striking that, while the progress of selection was quite consistent in A and P lines (figure 1a,c,d), in H lines we observed large fluctuations among generations (figure 1b,d). The fluctuations observed between generations 1 and 5 were parallel for the H and C lines, so the difference between selected and control lines steadily increased (figure 1). Such among-generation fluctuations are common in selection experiments (e.g. [60]), and the actual reason is usually not identified. We suspect that in our experiment, the fluctuations were due to inevitable differences in the experimental food composition: even though the nominal composition (proportion of grass and flour; see the electronic supplementary material) was not changed, the chemical composition (e.g. of secondary plant compounds) could change. We noted that in generation 7, the experimental food was no longer challenging to H lines, therefore, in generation 8 the composition of the food was slightly changed to worse (food pellets were also harder; see the electronic supplementary material). Surprisingly, voles from H lines could not cope with the modified food better than those from C lines. In generation 9, the food was changed again, and the difference between H and C lines was again present (figure 1c,d). Thus, even the direct effect of selection turned out to be very sensitive to changes in food properties, which may also explain why a correlated response in BMR was not as predicted based on the earlier estimate of a genetic correlation.

5. Conclusion

— Results of the current selection experiment, taken together with results of our previous quantitative genetic analyses [32], provide ‘steady’ support for the assumption that selection for increased aerobic capacity should lead to increased BMR. However, the results also indicate that it is unlikely that such a selection alone could result in the roughly 10-fold difference in BMR between endotherms and ectotherms.

— On the other hand, our results suggest that even a small change in the properties of a diet may change the correlation between the ability to grow on a low-quality diet and the level of BMR. Thus, considering the complexity of the variation of natural diets, it is not surprising that wide-scale patterns of association between a general type of diet and either a high or low BMR are difficult to identify.

References


