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Cite this article: Sadowska ET, Stawski C, Rudolf A, Dheyongera G, Chrząścik KM, Baliga-Klimczyk K, Koteja P. 2015 Evolution of basal metabolic rate in bank voles from a multidirectional selection experiment. *Proc. R. Soc. B* **282**: 20150025.
http://dx.doi.org/10.1098/rspb.2015.0025

Received: 6 January 2015
Accepted: 19 March 2015

Subject Areas:
evolution, physiology, ecology

Keywords:
endothermy, food habits, aerobic metabolism, experimental evolution

Author for correspondence:
Paweł Koteja
e-mail: pawel.koteja@uj.edu.pl

†Present address: Centre for Behavioural and Physiological Ecology, Zoology, University of New England, Armidale, New South Wales 2351, Australia.
‡Present address: Directorate of Fisheries Resources, Ministry of Agriculture, Animal Industry and Fisheries, PO Box 4, Lugard Avenue, Entebbe, Uganda.

Electronic supplementary material is available at http://dx.doi.org/10.1098/rspb.2015.0025 or via http://rspb.royalsocietypublishing.org.

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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 $O_2\,h^{-1}$, respectively), and the values were intermediate in P lines (59.0 ml $O_2\,h^{-1}$). 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

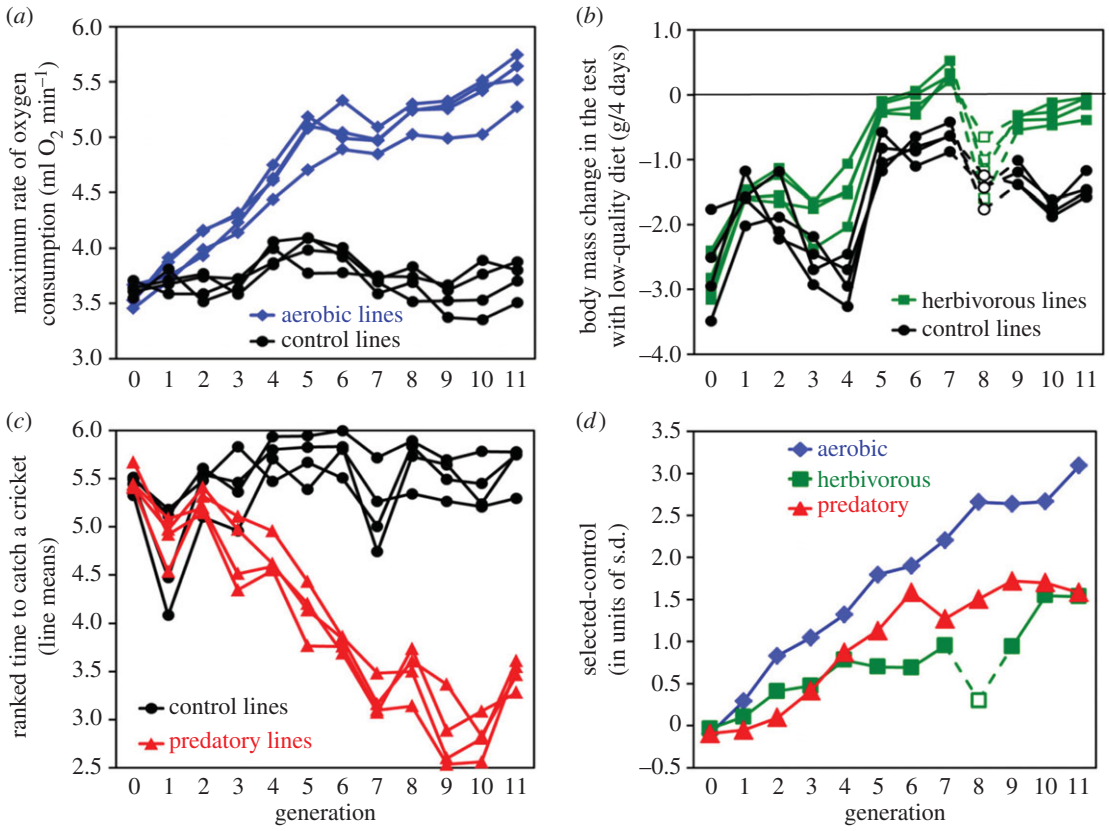

Figure 1. Direct phenotypic responses to 11 generations of selecting bank voles towards (*a*) high swim-induced aerobic metabolism, (*b*) herbivorous capability measured as ability to maintain body mass in a test with low-quality diet, (*c*) predatory propensity measured as ranked time to attack a cricket, 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.)

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 ($\dot{V}O_2$swim), 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

measurements of swim-induced aerobic metabolism and the predatory behaviour tests were performed on adults (about 75–95 days old), and the tests with low-quality diet on young, still growing animals (32–36 days). All the trait values used as selection criteria were mass-adjusted (residuals from ANCOVA including also other covariates and cofactors). Four replicate lines for each selection direction and an unselected control (C) were maintained (to allow valid tests of the effects of selection [47]), with 15–20 reproducing families in each of the 16 lines (which avoided excess inbreeding). The selection was applied mostly within families, but if more than 16 families were available, families in which all individuals scored below the line mean were excluded.

In generation 11, differences between all selection directions and the unselected C lines were highly significant (ANCOVA mixed nested models, with lines as a random effect nested in fixed selection factor, and random family effect nested in lines: $p < 0.0001$). $\dot{V}O_2$swim was 49% higher in A lines (mean $\pm$ s.d.: 333.1 ± 39.2 ml $O_2\,h^{-1}$; $n = 824$) in comparison to C lines (223.37 ± 31.83 ml $O_2\,h^{-1}$; $n = 98$; figure 1*a*). Voles from H lines were nearly able to maintain body mass during the 4 day test with low-quality diet (body mass loss: 0.15 ± 0.83 g; $n = 1019$), whereas those from C lines lost more mass (1.42 ± 0.826 g; $n = 107$; figure 1*b*). In P lines, 75.3% of individuals attacked a cricket in at least one of the tests and the ranked time to catch a cricket averaged 3.46 ± 1.81 ($n = 837$), whereas in C lines only 14.4% of individuals behaved as predators, and the ranked time to catch averaged 5.64 ± 0.94 ($n = 837$; figure 1*c*).

The animals were maintained in standard plastic mouse cages with sawdust bedding, at a constant temperature (20 ± 1°C) and photoperiod (16 L : 8 D; light phase starting at 2.00). Water and food (a standard rodent food: 24% protein, 3% fat, 4% fibre; Labofeed H, Kcynia, Poland) were provided ad libitum. More detailed information about the housing conditions and animal welfare is provided in the electronic supplementary material.

Measurements of BMR were performed on 313 individuals of both sexes from all 16 lines: 22–28 from each of the A lines, 21–22 from each of the P lines and 14–18 from each of the C and H lines. Animals were chosen randomly from 12 to 15 families per line, with the condition that no more than two males and two females from a full-sibling family were chosen. Individuals were born in litters one to four of a given female (only six were from the fourth litter, and in statistical analyses they were merged with the third litter), and they were not used in the test with low-quality diet. The animals were adults at ages ranging from 69 to 155 days (mostly 90–130 days; mean 109 days), which allowed us to determine if BMR changed with age.

(b) Measurement of basal metabolic rate

Measurements of BMR were performed similarly as described in Labocha *et al.* [48]. Details of the respirometric set-up, the measurement protocol and calculation of the rate of oxygen consumption ($\dot{V}O_2$) are described in the electronic supplementary material. Briefly, animals were weighed and placed in respirometric chambers without water or food (BMR is defined as minimum resting metabolism at post-absorptive state [1]). Two types of chambers were used: glass 550 ml with 300 ml min^{-1} air flow rate or plastic 850 ml with 350 ml min^{-1} air flow rate. The chambers were placed in a climate-controlled room at 28°C (at the lower side of thermal neutral zone [49]). Only dim red lights were left on in the room.

To check if hypothetical differences in BMR are not associated only with a particular time of day, the BMR trials were performed in three 'timing' groups (the actual timing varied ± 0.45 h from the following values): 'night' (20.30–06.00), 'morning' (06.00–14.00) and 'afternoon' (09.00–20.30). In the morning and night groups, the chambers were connected to the respirometric system at the start of the trial, and measurements lasted until the end of their measurement period. When voles from the afternoon group started the trial, the respirometric system continued to record data for the morning group. Therefore, their chambers were connected to the system only at 14.00. Thus, in the afternoon group, $\dot{V}O_2$ was not measured during the initial 5 h, which is typically considered a period of acclimatization and fasting not included in BMR trials (cf. [48]).

Of the 313 voles measured, 15 died (all from A lines). These incidents were not caused by fasting, because sometimes death occurred at the beginning of the trials. The size of respirometric chambers was large enough to allow free movement (animals were not force-constrained; see the electronic supplementary material). Animals did not suffer from inadequate ventilation, because even at moments of intensive activity CO_2 concentration did not exceed 1.2%, and nearly all the time was below 0.6%, which causes no adverse effects in burrowing rodents. Apparently, the deaths resulted from episodes of hyperactivity and a resulting hyperthermia, occurring at any time during the trials. Unfortunately, however, the signal from activity sensors or gas analysers could not be used to anticipate and prevent death, because many individuals showed such periods of intensive activity without any adverse effects.

$\dot{V}O_2$ was measured with an eight-channel respirometric system. Samples of air flowing out of an empty reference and seven animal measurement chambers were analysed sequentially, in a 13 min cycle. Oxygen and CO_2 concentrations were recorded every second. $\dot{V}O_2$ 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 were 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 $\dot{V}O_2$. 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. $\dot{V}O_2$ 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) mixed 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 $\times$ sex interaction and random interactions sex $\times$ line and timing $\times$ 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 ($t_{246} = 4.21$, $p < 0.0001$), 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 2*a*; 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 2*a*): 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

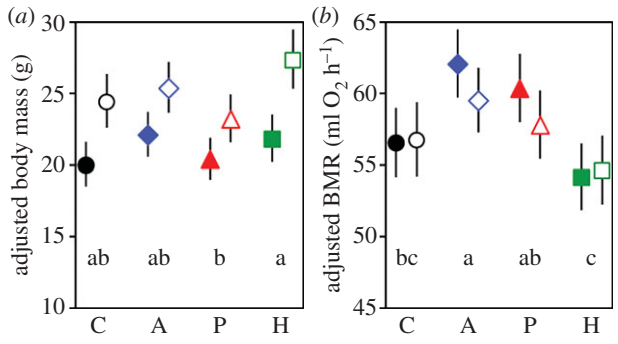

Figure 2. Adjusted least-square means from mixed ANCOVA models (± 95% CIs) of (*a*) body mass measured before BMR trials (g; all individuals) and (*b*) basal metabolic rate (ml O_2 h^{-1}), in male and female voles from lines selected in three directions (A, Aerobic; P, Predatory and H,Herbivorous) and unselected control (*C*) lines. The values are back-transformed from the analyses on log-transformed data. Lowercase letters (abc) indicate selection groups not statistically different at $p = 0.05$ (Tukey–Kramer *post hoc* pairwise comparisons). Open symbols denote males, and filled symbols females. (Online version in colour.)

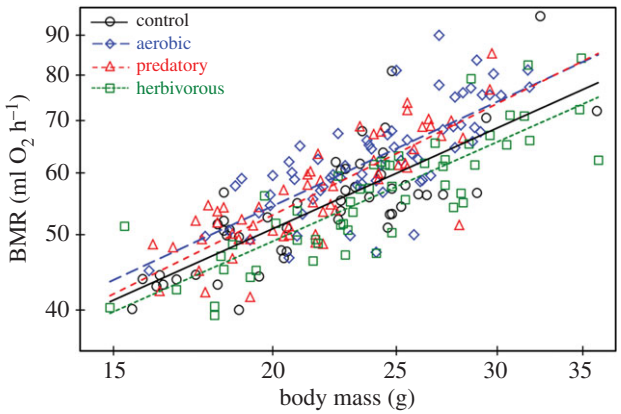

Figure 3. The relationship between BMR and body mass (note the log–log scale) in voles from all four selection directions. The slopes of the lines did not differ significantly (note that the values are not adjusted for any other effects used for calculation of the adjusted means presented on figure 2*b*; common slope from the ANCOVA model ± s.e. = 0.78 ± 0.04). (Online version in colour.)

effects differed among the selection directions ($F_{3,12} = 12.87$, $p = 0.0005$; figures 2*b* 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}; Tukey–Kramer: $t_{12} = 3.62$, $p = 0.016$) 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 $\dot{V}O_2$swim and BMR. However, the voles in our previous study swam at 30°C and therefore $\dot{V}O_2$swim also comprised a thermoregulatory burden. In the current experiment, however, the voles were selected for $\dot{V}O_2$swim 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 $\dot{V}O_2$ 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 $\dot{V}O_2$swim, even though maximum forced-running $\dot{V}O_2$ was increased [38]. However, even if BMR were increased in that study, interpretation of the result would be unclear, because $\dot{V}O_2$swim 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 $\dot{V}O_2$, 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, the directly selected trait (maximum $\dot{V}O_2$) was only about 11% higher in the selected than in the control lines [17, table 2]. So, considering the large individual variation in those traits, the chance of detecting a correlated response in BMR was small. On the other hand, the quantitative genetic analyses were based on several thousand observations, which gave enough power to reliably estimate genetic correlation.

The selection experiments mentioned here, as well as most selection experiments on mammals, were performed on laboratory species. Thus, the pattern of direct and correlated responses to selection could be strongly affected by domestication (e.g. [56]) and previous selection for peculiar traits, such as high reproductive output under no food restriction. Conversely, our selection experiment has been performed on a wild rodent that had been maintained under laboratory conditions for only five to seven generations (see the electronic supplementary material for details) before the selection protocol began. Thus, it can be assumed that the standing genetic variation resembles that in a wild population. Certainly, we can expect that in addition to the intended selection, the freshly established laboratory colony was subject to an unintentional 'laboratory natural selection' to laboratory conditions, i.e. underwent a process of domestication [58]. However, because all our inferences are based on comparisons across the selected and control lines within one generation, rather than a comparison of the selected line with the base population, the plausible process of domestication does not undermine the inferences.

In their influential review, Hayes & Garland [3] advocated to test for a presence of an additive genetic correlation between BMR and the capacity for exercise-induced aerobic metabolism as a crucial test of the main assumption of the aerobic capacity model of the evolution of endothermy. From this perspective, our results could be treated as an elegant corroboration of the hypothesis. However, for two reasons, this conclusion should be treated with caution. First, a presence or absence of such a correlation in an extant species should not be treated as evidence concerning the state in remote ancestral species [19,20,34,42,43]. Second, we should note that the nearly 50% increase of $\dot{V}O_2$swim in A lines was accompanied by just a 15% increase of the absolute values of BMR (and just 7.3% of the values adjusted for all covariates; figure 2*b*). Thus, at least in voles, the level of aerobic capacity can evolve to a large extent with only a small change of BMR. Therefore, while the results are consistent with the idea that selection for the ability to endure high aerobic locomotor activity was a trigger for the evolution of endothermy, they also suggest that other factors must have been involved to produce a 10-fold difference in the level of resting metabolism, such as that between ectotherms and endotherms (cf. [5–9,11–16,59]).

Although BMR was not statistically significantly higher in P lines than in C lines, BMR in P lines was closer to that of A than C lines, and it was significantly higher than in the 'lowest' H lines (figure 2*b*). 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 2*b* 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 1*a*,*c*,*d*), in H lines we observed large fluctuations among generations (figure 1*b*,*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 1*c*,*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.

Ethics statement. All the procedures were approved by the decision of the Local Ethical Committee for Experiments on Animals in Kraków, Poland (no. 99/2006).

Data accessibility. The raw results are provided as an electronic supplementary material, appendix, and are available from the Dryad depository: http://dx.doi.org/10.5061/dryad.kh312.

Acknowledgements. We are grateful to the many students and technicians who helped with animal maintenance and measurements, especially to R. Czuchnowski, K. Sęk, M. Skrzypczak, K. Ścigajło, K. Wilk and A. Zawada.

Funding statement. The project was supported by grants from the Polish Ministry of Science and Higher Education N303 2752 33 and Jagiellonian University DS/BW UJ INoS 757.

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Supplementary Information to an article in *Proceedings B*:

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, Kraków, Poland
pawel.koteja@uj.edu.pl

I. Supplementary Materials

The following supplementary materials complement information about the selection experiment presented in Sadowska et al. 2008.

1. Animals: history of the colony, maintenance, breeding, and welfare

The rationale of the selection experiment and of choosing bank voles (*Myodes* = *Clethrionomys glareolus* Schreber 1780), history of the colony, and results of the direct responses in the first three generations was presented in our earlier work (Sadowska et al. 2008). Briefly, two founding groups (generation 0_1 and 0_2) were caught in the Niepołomice Forest in southern Poland in 2000 and 2001, and six subsequent captive-born generations were produced as in the following simplified diagram:

$$\begin{array}{rl} 0_1 \rightarrow 1 \rightarrow 2 \rightarrow & \left.\begin{array}{l}\\\\\end{array}\right\} \\ & \left.\begin{array}{l}\\\end{array}\right\} 3 \rightarrow 4 \rightarrow 5 \rightarrow 6 \\ 0_2 \rightarrow & \left.\begin{array}{l}\\\\\end{array}\right\} \end{array}$$

At that time the colony was first used as a basis of research on individual variation and quantitative genetics of metabolic traits (Labocha et al. 2004, Sadowska et al. 2005, 2009).

In 2004 we produced a large base population for the selection experiment: about 1000 individuals reared from 159 pairs. An average inbreeding coefficient in the base population (nominally 7th laboratory-bred generation) was 0.0085.The individuals were randomly assigned to 16 lines, with the condition that each individual from a full-sib family was assigned to a different line. In February of 2005 we mated the animals within lines and their offspring (pre-selection generation 8, according to the scheme above) formed generation 0 of the selection experiment (i.e., the last generation before applying selection).

In subsequent generations, in 12 selected lines the best 1-2 males and 1-2 females from a family were selected as breeders, according to respective selection criteria (see below). In the four control lines breeders were chosen randomly from each family. Selection was not strictly "within-family": more individuals for reproduction were taken from families with high scores in the selected traits, and, if the number of available families in a line was large enough (above 16),

offspring from families in which all individuals had very low scores were not used for breeding. In consequence, the inbreeding coefficient was slightly higher in the selected than in the control lines, but because the number of reproducing families was larger than in most selection experiments on rodents, the inbreeding was still low (mean values in generation 11: control lines - 0.098, selected lines - 0.111-0.120). Average litter size of voles is only about 4.5, and to get enough individuals for effective selection we tried to obtain a few subsequent litters from each pair (in practice, 1 to 4 litters). Because voles do not reproduce well in winter (even under long photoperiod), we produced only two generations per year.

Animals were maintained in standard plastic mouse cages (mostly opaque, polypropylene) with sawdust bedding, at a constant temperature (20±1°C) and photoperiod (16h:8h light:dark; light phase starting at 2:00am). Air humidity in the animal rooms was precisely controlled only during particular experiments (e.g. during the test with low-quality diet; see below); otherwise it varied from about 20 to 80%, depending on outside ambient conditions. Breeding pairs and pairs with offspring (up to 17 days old) were maintained in model 1290D cages (Tecniplast, Bugugiatte, Italy; dimensions L×W×H: 425×266×155 mm, floor area 800 cm^2), equipped with a shelter (ceramic pot), additional nest material (paper towels) and cardboard tubes (environment enrichment). At the age of 17 days the animals were weaned, marked temporarily by fur clipping and kept in family groups until the age of 30-35 days. At the age of about 34 days, all individuals were marked permanently with mouse ear tags (model 10005-1; National Band and Tag, Newport, KY; mass 0.18g) and later maintained in same-sex groups of three individuals in model 1264C cages (Tecniplast, Bugugiatte, Italy; dimensions L×W×H: 267×207×140 mm; floor area 370 cm^2) or up to five (usually four) individuals in the larger model 1290D cages (described above). Cages were changed every 5-14 days, depending on the number of animals in the cage, size of the cage and their cleanliness.

Water and food (a "breeding type" rodent chow: 24% protein, 3% fat, 4% fibre; Labofeed H, Kcynia, Poland) was provided ad libitum. Every day all cages were visually inspected for presence of food and water and dead animals (because of large amount of shavings, dead animals in some cases could not be detected instantly, but only during cage changing).

The colony was under supervision of a qualified veterinary surgeon. During any kind of measurements if symptoms of poor condition were observed in an animal (problems with breathing or moving, injury, etc.), it was removed from the experiment. Depending on judgment of the observer or animal care personnel, it was either allowed to recover or was euthanized. Subject to circumstances, one of three methods of euthanasia was used: exposure to a rising concentration of CO2, cervical dislocation, or isoflurane inhalation (AEranne, Baxter; applied using open-drop technique).

After completing the BMR measurements (in generation 14, not reported here) we learned that the colony had been infected with *Puumala* hantavirus, and the colony was probably infected already at the time when the BMR measurements were performed. Virus was not detected earlier because under normal housing conditions infection does not inflict any pathological effects in bank voles (Bernshtein et al. 2009; some data suggest, however, that it may result in decreased survival under harsh winter conditions: Kallio et al. 2007). We also checked that the parameters of reproduction (litter mass and litter size during weaning), mortality, and condition (adult body mass) in the ‘infected generations’ did not differ from the preceding ‘uninfected generations’.

2. Measurement protocols of the selected traits

2.1. High aerobic metabolism during swimming

In the *high aerobic metabolism* (A) lines we measured the rate of oxygen consumption achieved by the voles during swimming ($\dot{V}O_2$swim). The measurements were performed in a positive pressure open-flow respirometric system, as described in our earlier report (Sadowska et al. 2008). Typically the measurements were performed simultaneously on two respirometric systems, which used a few types of oxygen analyzers (S-3A/II - AMETEK, Pittsburgh, PA, USA; FC1, FC2, or FOX - Sable Systems Inc., Las Vegas, NV, USA; details of the systems changed across several years of the project). The voles swam in glass chambers partly filled with water. The chamber diameter was 15 cm, and 6 cm of space was retained between water surface and the top of the chamber, so the voles had enough space to swim freely. A drop of a shampoo for dogs was added to ensure complete soaking of fur. The measurements were performed at 38°C, to ensure that the increase of metabolism was solely due to locomotor activity and not due to thermoregulatory demand. To stay on the surface of the water the voles did not have to work hard, and many of them were actually able to "hang" for some time in the water without any movement, with just the tip of nose above the water surface. Thus, the animals were not *forced* to work up to their physiological limit, and the level of $\dot{V}O_2$swim certainly depended on behavioral characters, such as motivation to work. Therefore, $\dot{V}O_2$swim did not measure the aerobic capacity in a strict sense of the word; instead, it could be named *a voluntary maximum rate of oxygen consumption*. The selection criterion was the 1-minute maximum instantaneous rate of oxygen consumption and ANCOVA-adjusted for body mass, sex, number of litter, litter size, age, measurement date and the type of respirometer. In the first four generations two tests were performed on each individual, but in subsequent generations only one test was performed (we noticed that selection decisions based on the first of the tests were nearly the same as those based on results averaged from the two trials).

2.2. The ability to cope with a low-quality herbivorous diet

Herbivorous capability was measured as a body mass change during a 4-day trial in which young, growing voles (32-36 days) were fed a low-quality, herbivorous diet made of dried, pulverized grass and flour. Because animals exposed suddenly to a very low-quality diet could not survive, we applied a protocol with two types of pellets: i) an "intermediate" food during the first two days of a trial and ii) a "bad" food in the next two days of a trial. To avoid measuring a reaction to novel food, 5 days before the test the animals were given (in addition to the standard food) a few pellets of both of the two experimental diets. For the first seven generations "intermediate diet" consisted of 30% grass mixed with 70% wheat flour (approx. 11.4% protein, 2.2% fat, 7.7% fiber), and a "bad diet" consisted of 50% grass bonded with 30% wheat flour and 20% potato starch (approx. 11.3% protein, 2.2% fat, 11.1% fiber). In generation 7, we observed that the "bad" food ceased to be a challenge for voles from the selected lines: they were able to maintain a positive body mass balance. Therefore, to allow further differentiation between the selected and control lines, in generation 8 the proportion of ingredients in the "bad diet" was changed (50% grass bonded with 50% potato starch; approx. 8.8% protein, 1.8% fat, 10.5% fiber) to decrease its quality. Also, in an attempt to improve the protocol by making the food pellets more uniform, in this generation we decided to have the pellets (both the "intermediate" and the "bad" diet)

produced by a factory, instead of hand-made in our laboratory, as in previous generations. However, the factory produced-pellets, especially those of the "intermediate" diet, were harder than the hand-made pellets and apparently for that reason the voles reacted to the food in a different way: they were losing more mass on the "intermediate" diet than on the "bad" one, and the voles from the selected lines could not cope with the food better than the control ones. Therefore, in the 9th generation we again hand-made the pellets, and changed the composition of the "bad diet" to 80% grass mixed with 20% potato starch (approx. 13.7% protein, 2.8% fat, 16.8% fiber). In subsequent generations composition of the "bad diet" remained the same.

The selection criterion was the change of body mass during the 4-day trial and ANCOVA-adjusted for body mass at weaning, growth rate between weaning and the start of the trial, sex, consecutive litter number, litter size and the measurement date.

2.3. The intensity of predatory behavior

The intensity of predatory behavior towards crickets (*Gryllus assimilis*) was measured in adult voles (75-105 days, mean varied among generations from about 80 to 95 days), fasted before the trials (pilot observations indicated that non-fasted voles would not attack the crickets). The cricket was placed in each cage with the vole, and then presence of the cricket was checked after 0.5, 1, 3, 6 and 10 minutes. If the cricked had been eaten or caught before or at the time of the observation, the "ranked time to catch" was scored from 1 (0.5min) to 5 (10min). If the cricket was not caught during 10 minutes, the "ranked time to catch" was scored as 6. The number of trials and some details of the protocol were changed during the first four generations (see Sadowska et al. [1] for justification of the changes).

In generation "zero" the tests were repeated twice and in generations 1-2 three times in about 10 day intervals. In generation 0 the voles were fasted overnight and observations were performed in the morning. In generations 1-2 the protocol was reversed and fasting started in the morning and observations were performed soon after the beginning of the subjective night. In generation 3 and the following ones the tests were repeated on two measurement days (10 days apart), but with two trials on each day: first about 2h - 0.5h before, and second about 0.5h - 2h after the beginning of the subjective night. This change was introduced to avoid a possible selection for activity at a single, particular time of the day. Time of fasting before the observations was modified in subsequent generations. In generation 0 voles were fasted for 12h and in generations 1 and 2 for 10-11h before the test. In generations 3 to 7 they were fasted for 8h before the first trial and fasting was continued for the next 3 hours before the second trial (on the same day). In the 8th generation fasting lasted 7h +3h. Shortening the fasting time was necessary to achieve further progress of selection in a situation when most voles from the selected lines attacked the cricket within the first 30 seconds. The selection criterion was the "ranked time to catch," averaged across the repeated trials and ANCOVA-adjusted for sex, body mass, number of consecutive litter, litter size, age and date of measurement.

All the experimental protocols have been approved by the Local Ethical Committee for Experiments on Animals, Kraków, Poland (No. 99/2006).

3. Respirometric system used for measurements of the Basal Metabolic Rate

Animals were weighed and placed in one of two types of respirometric chambers (550 ml gas jars or rectangular 850 plastic containers), with air inlet near the bottom and outlet at the top. The chambers were fitted with wire tops suspended 3-4 cm below the ceiling of the chamber (10-11.5 cm above the bottom). Thus, the voles could not exhale air near the air outlet, but had enough room for movement (i.e., they were not force-restrained).

The rates of oxygen consumption and CO_2 production (ml/h) were measured with an 8-channel open-flow positive-pressure respirometric system based on S-3A/II O_2 analyzer (AMETEK, Pittsburgh, PA, USA) and CA2A CO_2 analyzer (Sable Systems Inc., Las Vegas, NV, USA). Fresh air was dried (silica gel) and pumped into seven chambers with animals and an empty, reference chamber. The rate of air flowing into the chambers was stabilized at either 300ml/min (with the 550 ml chamber) or 350ml/min (with 850 ml chamber) (STPD) with GFC-17 thermal mass-flow controllers (AALBORG, Orangeburg, NY, USA), separately for each channel. The actual flow on each measurement channel was corrected after calibrating the mass-flow controllers against a precise LO 63/33 rotameter (Rota, Germany).

Samples of air flowing out of the one reference and seven measurement channels were taken sequentially through V3 Intelligent Multiplexer (Sable Systems Inc.), pre-dried with ND2 non-chemical drier (Sable Systems Inc.), dried with a small volume of chemical absorbent (magnesium perchlorate) and passed through the CO_2 and O_2 analyzers. In each cycle, lasting 13 minutes, the reference channel and the first measurement channel were active for 111 sec, and the remaining six channels were active for 93 sec, which ensured a complete washout of the system after switching channels (the time was longer for the reference and the first measurement channels because the change of the air composition after switching to those channels is larger than in the case of the other channels). Mean values of analog outputs from the O_2 and CO_2 analysers were recorded once per second with UE-9 AD interface (LabJack Corporation, Lakewood, CO, USA) and a custom-made protocol using DAQ Factory acquisition system (Azotech, Ashlans, OR, USA). After completing the measurements we have found that the oxygen analyzer had underestimated the values of changes of oxygen concentration by a factor of 0.93, and all the raw readings were corrected accordingly.

The values recorded in the last 20 sec before channel switching were used for calculation of $\dot{V}O_2$ and $\dot{V}CO_2$ for a given channel and cycle, according to equation:

$$\dot{V}O2 = \dot{V}i \times \frac{FiO(1 - FeC) - FeO(1 - FiC)}{1 - FeO - FeC}$$

where $\dot{V}i$ is the incurrent air flow rate, and FiO, FeO, FiC, FeC are fractional concentrations (F) of oxygen (O) or CO_2 (C) in incurrent (i) or excurrent (e) air. The equation is basically the same as equation 10.6 in Lighton (2008), but it correctly accounts for the fact that CO_2 concentration in incurrent air differs from zero.

The $\dot{V}O_2$ values were calculated for each second, and averaged for the 20-sec period as a basis for further calculations (as described in Material and Methods of the main text). Because the aim of the measurements was to estimate resting metabolic rates, associated with stable readings, we consciously did not apply an "instantaneous correction" to the readings (c.f., Lighton 2008). The 20 1-sec values were also used to calculate standard deviation of the readings, which provided information about stability of the readings (high variation would indicate that the animal was not resting). Note, that because the washout time of the chambers (i.e., the chamber volume/flow rate) was about 2 min, the mean value calculated for the short, 20 sec recording period, reflects in fact the rate of metabolism in the preceding few minutes.

4. Supplementary References

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II. Supplementary Results

Supplementary Results 1. Descriptive statistics for A) Age and Body Mass of all individuals used in BMR trials, and B) Age, Body Mass, BMR and Activity signal of individuals for which BMR was obtained.

Selection direction:	Control			Aerobic			Herbivorous			Predatory		
Variables	**N**	**Mean**	**SD**	**N**	**Mean**	**SD**	**N**	**Mean**	**SD**	**N**	**Mean**	**SD**
A. All individuals used in BMR trials (N=313)												
Age (days)												
Pooled	62	106.1	9.5	98	110.5	17.2	67	106.6	8.9	86	111.5	12.8
Females	31	105.9	9.2	49	110.6	19.0	33	109.8	9.0	43	110.4	10.8
Males	31	106.2	9.9	49	110.4	15.5	34	103.4	7.7	43	112.6	14.5
Body mass (g)												
Pooled	62	22.51	4.26	98	24.14	3.56	67	24.63	4.94	86	22.15	3.57
Females	31	20.26	3.40	49	22.54	3.13	33	22.15	4.04	43	20.61	3.22
Males	31	24.77	3.84	49	25.75	3.24	34	27.03	4.57	43	23.70	3.24
Litter 1	35	23.82	2.73	13	21.78	5.28	24	23.71	4.61	37	22.04	3.69
Litter 2	52	24.30	4.18	34	23.09	4.07	19	25.84	4.99	33	22.45	3.41
Litter 3	11	24.43	2.79	15	21.85	3.78	24	24.58	5.22	16	21.81	3.78
B. Individuals for which BMR was obtained (N=232)												
Age (days)												
Pooled	51	106.1	10.0	68	111.3	17.5	55	107.0	8.8	58	111.0	13.8
Females	29	105.4	9.2	33	110.3	19.1	26	110.6	8.3	31	110.4	11.6
Males	22	106.9	11.1	35	112.1	16.0	29	103.7	8.1	27	111.8	16.0
Body mass (g)												
Pooled	51	22.11	4.29	68	24.24	3.64	55	24.51	4.96	58	21.98	3.58
Females	29	20.14	3.43	33	22.60	3.43	26	21.65	4.02	31	20.82	3.25
Males	22	24.71	3.96	35	25.79	3.16	29	27.07	4.31	27	23.32	3.52
BMR (mlO2/h)												
Pooled	51	54.92	10.28	68	63.15	9.75	55	56.83	10.17	58	57.31	9.32
Females	29	50.57	6.73	33	61.31	10.29	26	51.25	7.58	31	56.25	9.37
Males	22	60.65	11.42	35	64.89	9.01	29	61.83	9.66	27	58.52	9.29
Activity signal (V)*												
Pooled	51	0.057	0.016	68	0.062	0.016	55	0.058	0.016	58	0.064	0.017
Females	29	0.053	0.014	33	0.064	0.015	26	0.055	0.015	31	0.063	0.018
Males	22	0.062	0.016	35	0.061	0.017	29	0.060	0.016	27	0.065	0.016

* Note that the gravimetric activity detectors return some "noise" signal also when animals are resting. The mean level of the "noise" signal (measured for an empty chamber) recorded in the same 3-min periods from which BMR was obtained was 0.059 ±0.031.

Supplementary Results 2: Output from SAS (v. 9.3) Mixed model analysis for log-transformed Body mass (logBodyMass). The analysis was performed for all individuals used in the study (N=313). REML method of estimation was applied, and variance estimates were constrained to be positive (default option; if variance estimate was negative it was set to zero, but the estimate is not reported in the table with covariance estimations). The results show the final model (preliminary models showed that additional predictors and interactions, described in Methods-Statistics section, were not significant). Graphical analyses of distribution of residuals and boxplots showing the distribution of log-transformed raw values of body mass in the four selection group is also presented.

Model Information	
Data Set	WORK.BMR_DATA
Dependent Variable	logBodyMass
Covariance Structure	Variance Components
Estimation Method	REML
Residual Variance Method	Profile
Fixed Effects SE Method	Model-Based
Degrees of Freedom Method	Containment

Class Level Information		
Class	**Levels**	**Values**
Selection	4	A C H P
Line	16	A1 A2 A3 A4 C1 C2 C3 C4 H1 H2 H3 H4 P1 P2 P3 P4
Sex	2	F M
LitterNumber	3	1 2 3
Timing	3	A M N

Dimensions	
Covariance Parameters	5
Columns in X	22
Columns in Z	144
Subjects	1
Max Obs Per Subject	313

Number of Observations	
Number of Observations Read	313
Number of Observations Used	313
Number of Observations Not Used	0

Iteration History			
Iteration	Evaluations	-2 Res Log Like	Criterion
0	1	-750.50161242	
1	4	-760.11859555	.
2	1	-760.14226509	0.00000022
3	1	-760.14241319	0.00000000

Convergence criteria met.

Covariance Parameter Estimates with 95% Confidence Limits				
Cov Parm	Estimate	Standard Error	Lower	Upper
Line(Selection)	0.000168	0.000267	0.000030	1.0438
Line*Sex(Selection)	0.000267	0.000287	0.000068	0.01705
Line*Timing(Selection)	0.000156	0.000196	0.000034	0.04156
Line*LitterNumber(Selection)	0	.	.	.
Residual	0.003526	0.000322	0.002971	0.004252

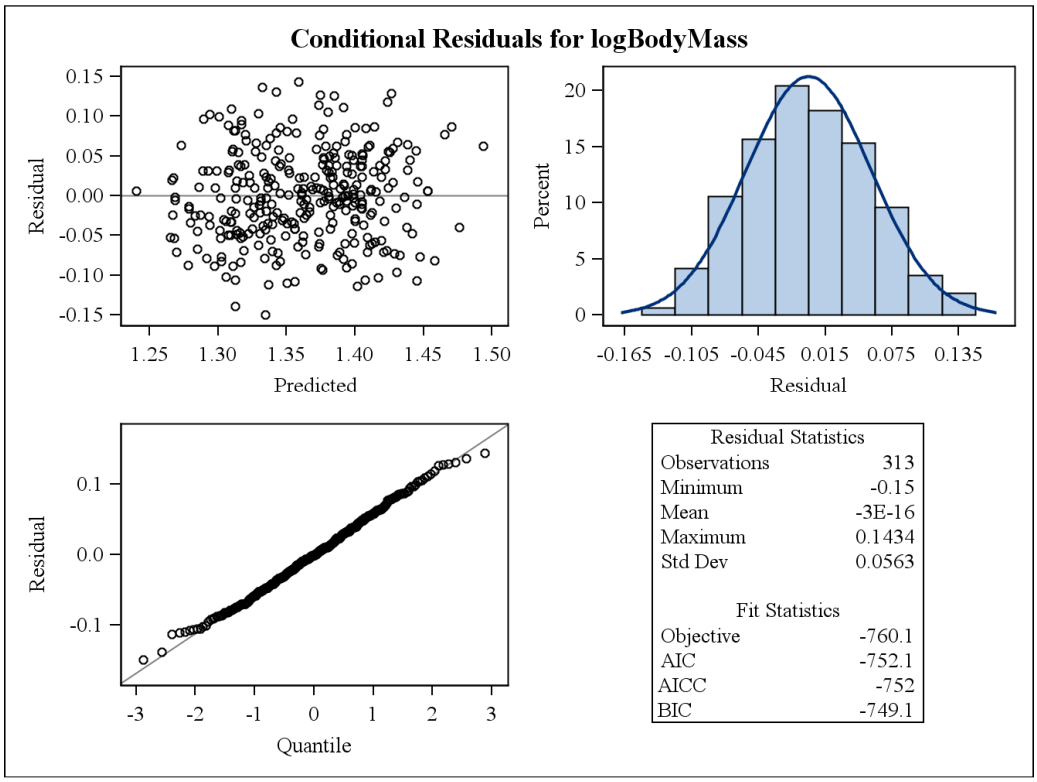
Fit Statistics	
-2 Res Log Likelihood	-760.1
AIC (smaller is better)	-752.1
AICC (smaller is better)	-752.0
BIC (smaller is better)	-749.1

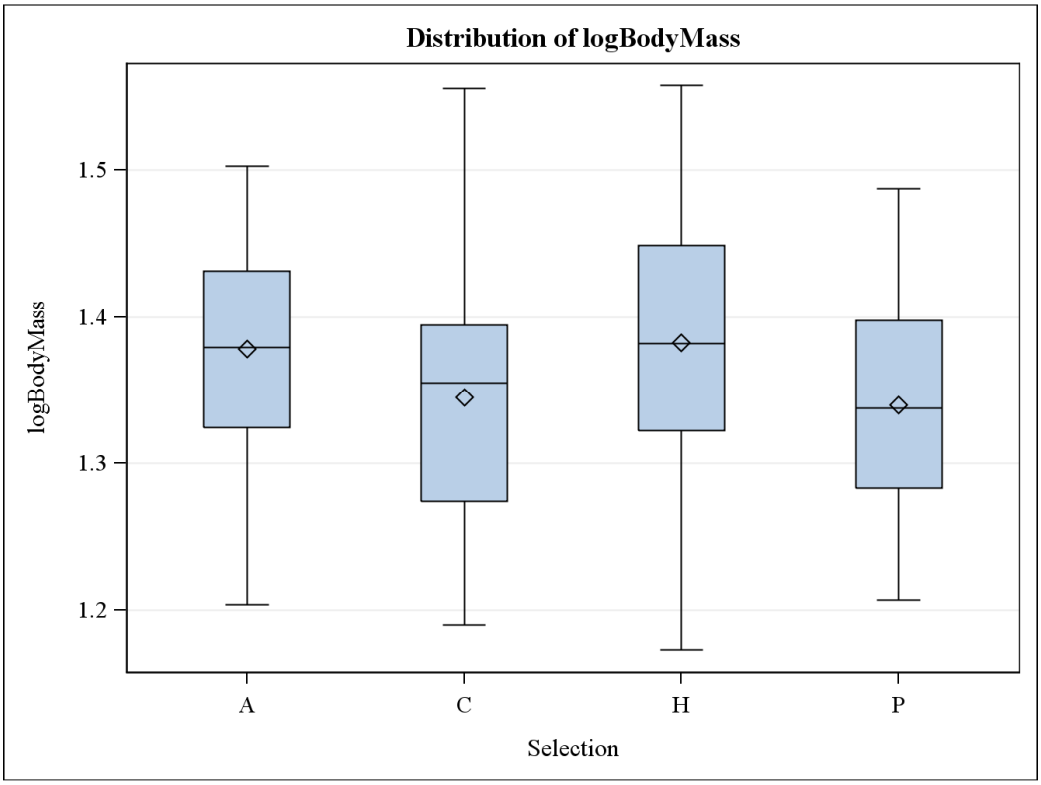
Solution for Fixed Effects									
Effect	**Selection**	**Sex**	**Timing**	**LitterN**	**Estimate**	**Standard Error**	**DF**	**t Value**	**Pr > |t|**
Intercept					1.2233	0.03505	12	34.90	<.0001
Selection	A				0.03872	0.02000	12	1.94	0.0767
Selection	C				0.02243	0.02109	12	1.06	0.3086
Selection	H				0.07130	0.02101	12	3.39	0.0053
Selection	P				0	.	.	.	.
Sex		F			-0.05600	0.01733	12	-3.23	0.0072
Sex		M			0	.	.	.	.
Timing			A		0.01644	0.009439	30	1.74	0.0918
Timing			M		0.01046	0.009404	30	1.11	0.2747
Timing			N		0	.	.	.	.
Selection*Sex	A	F			-0.00404	0.02413	12	-0.17	0.8697
Selection*Sex	A	M			0	.	.	.	.
Selection*Sex	C	F			-0.03104	0.02577	12	-1.20	0.2516
Selection*Sex	C	M			0	.	.	.	.
Selection*Sex	H	F			-0.04236	0.02557	12	-1.66	0.1234
Selection*Sex	H	M			0	.	.	.	.
Selection*Sex	P	F			0	.	.	.	.
Selection*Sex	P	M			0	.	.	.	.
LitterNumber				1	-0.01347	0.01028	30	-1.31	0.2002
LitterNumber				2	0.01438	0.009609	30	1.50	0.1450
LitterNumber				3	0	.	.	.	.
Age					0.001219	0.000289	216	4.21	<.0001

Type 3 Tests of Fixed Effects				
Effect	**Num DF**	**Den DF**	**F Value**	**Pr > F**
Selection	3	12	4.17	0.0307
Sex	1	12	70.48	<.0001
Timing	2	30	1.56	0.2267
Selection*Sex	3	12	1.31	0.3157
LitterNumber	2	30	5.63	0.0084
Age	1	216	17.72	<.0001

Least Squares Means with 95% Confidence Limits								
Effect	**Selection**	**Sex**	**Timing**	**LitterN**	**Estimate**	**Standard Error**	**Lower**	**Upper**
Selection	A				1.3742	0.01136	1.3494	1.3989
Selection	C				1.3444	0.01218	1.3179	1.3709
Selection	H				1.3876	0.01192	1.3616	1.4136
Selection	P				1.3375	0.01146	1.3125	1.3624
Sex		F			1.3232	0.007442	1.3070	1.3395
Sex		M			1.3986	0.007406	1.3825	1.4147
Timing			A		1.3684	0.008075	1.3519	1.3849
Timing			M		1.3624	0.008021	1.3460	1.3788
Timing			N		1.3519	0.008043	1.3355	1.3684
Selection*Sex	A	F			1.3442	0.01415	1.3133	1.3750
Selection*Sex	A	M			1.4042	0.01406	1.3736	1.4348
Selection*Sex	C	F			1.3009	0.01546	1.2672	1.3346
Selection*Sex	C	M			1.3879	0.01547	1.3542	1.4216
Selection*Sex	H	F			1.3384	0.01513	1.3055	1.3714
Selection*Sex	H	M			1.4368	0.01517	1.4037	1.4698
Selection*Sex	P	F			1.3095	0.01434	1.2783	1.3407
Selection*Sex	P	M			1.3655	0.01440	1.3341	1.3968
LitterNumber				1	1.3471	0.007753	1.3313	1.3630
LitterNumber				2	1.3750	0.007128	1.3604	1.3895
LitterNumber				3	1.3606	0.009121	1.3420	1.3792

Differences of Least Squares Means (pairwise *a posteriori* tests)								
Effect	**Selection**		**Litter Number**		**Estimate**	**Standard Error**	**DF**	**t Value**	**Tukey-Kramer adjusted P**
Selection	A	C			0.02978	0.01651	12	1.80	0.3181
Selection	A	H			-0.01342	0.01651	12	-0.81	0.8470
Selection	A	P			0.03669	0.01598	12	2.30	0.1535
Selection	C	H			-0.04321	0.01705	12	-2.53	0.1044
Selection	C	P			0.006911	0.01668	12	0.41	0.9750
Selection	H	P			0.05012	0.01656	12	3.03	0.0453
LitterNumber			1	2	-0.02784	0.008341	30	-3.34	0.0062
LitterNumber			1	3	-0.01347	0.01028	30	-1.31	0.4009
LitterNumber			2	3	0.01438	0.009609	30	1.50	0.3069


Conditional Residuals for logBodyMass
Residual
Predicted
Percent
Residual
Residual
Quantile
Residual Statistics
Observations 313
Minimum -0.15
Mean -3E-16
Maximum 0.1434
Std Dev 0.0563
Fit Statistics
Objective -760.1
AIC -752.1
AICC -752
BIC -749.1


Distribution of logBodyMass
logBodyMass
A
C
H
P
Selection

Supplementary Results 3: Output from SAS Mixed model analysis for log-transformed Basal Metabolic Rate (logBMR). The analysis was performed for only those individuals, in which activity signal indicated a resting state at the time when the minimum rate of oxygen consumption was achieved (N=232). REML method of estimation was applied, and variance estimates were constrained to be positive (default option; if variance estimate was negative it was set to zero, but the estimate is not reported in the table with covariance estimations). The results show the final model (preliminary models showed that additional predictors and interactions, described in Methods-Statistics section, were not significant). Graphical analyses of distribution of residuals and boxplots showing the distribution of log-transformed raw BMR values in the four selection group is also presented.

Model Information	
Data Set	WORK.BMR_DATA
Dependent Variable	logBMR
Covariance Structure	Variance Components
Estimation Method	REML
Residual Variance Method	Profile
Fixed Effects SE Method	Model-Based
Degrees of Freedom Method	Containment

Class Level Information		
Class	**Levels**	**Values**
Selection	4	A C H P
Line	16	A1 A2 A3 A4 C1 C2 C3 C4 H1 H2 H3 H4 P1 P2 P3 P4
Sex	2	F M
Timing	3	A M N
ChamberType	2	P S

Dimensions	
Covariance Parameters	4
Columns in X	23
Columns in Z	96
Subjects	1
Max Obs Per Subject	232

Number of Observations	
Number of Observations Read	232
Number of Observations Used	232
Number of Observations Not Used	0

Iteration History			
Iteration	Evaluations	-2 Res Log Like	Criterion
0	1	-720.59048343	
1	3	-721.04940543	0.00000008
2	1	-721.04945057	0.00000000

Convergence criteria met.

Covariance Parameter Estimates with 95% Confidence Limits				
Cov Parm	Estimate	Standard Error	Lower	Upper
Line(Selection)	0	.	.	.
Line*Sex(Selection)	0	.	.	.
Line*Timing(Selection)	0.000060	0.000096	0.000011	0.3549
Residual	0.001656	0.000176	0.001360	0.002062

Fit Statistics	
-2 Res Log Likelihood	-721.0
AIC (smaller is better)	-717.0
AICC (smaller is better)	-717.0
BIC (smaller is better)	-715.5

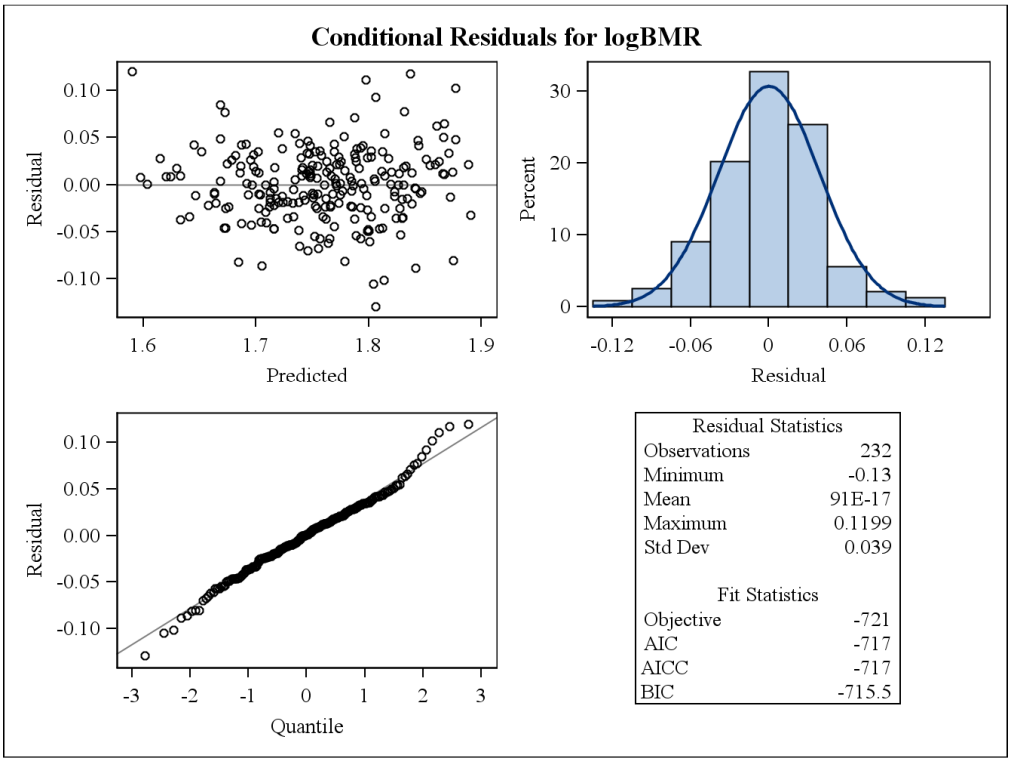
Solution for Fixed Effects									
Effect	Selection	Sex	Timing	Chamber Type	Estimate	Standard Error	DF	t Value	Pr > |t|
Intercept					0.7903	0.07143	12	11.06	<.0001
Selection	A				0.01273	0.01120	12	1.14	0.2779
Selection	C				-0.00793	0.01229	12	-0.64	0.5313
Selection	H				-0.02461	0.01204	12	-2.04	0.0635
Selection	P				0	.	.	.	.

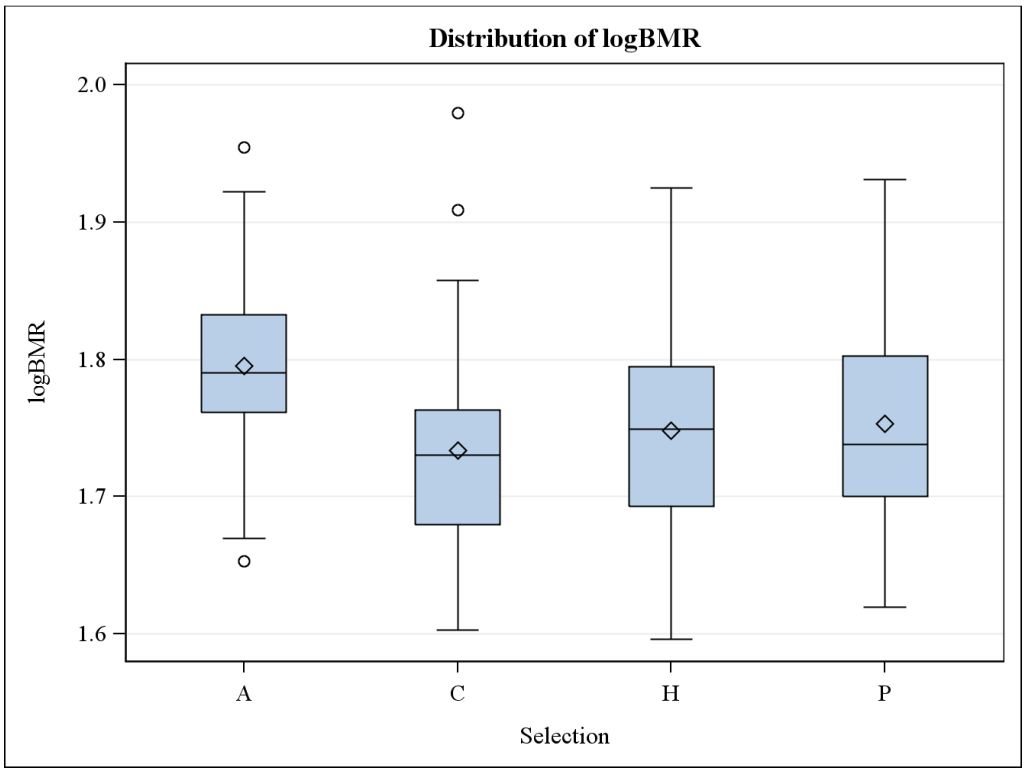
Solution for Fixed Effects									
Effect	**Selection**	**Sex**	**Timing**	**Chamber Type**	**Estimate**	**Standard Error**	**DF**	**t Value**	**Pr > |t|**
Sex		F			0.01885	0.01106	12	1.70	0.1139
Sex		M			0	.	.	.	.
Timing			A		-0.00330	0.007198	30	-0.46	0.6504
Timing			M		-0.00382	0.007175	30	-0.53	0.5981
Timing			N		0	.	.	.	.
Selection*Sex	A	F			-0.00061	0.01478	12	-0.04	0.9676
Selection*Sex	A	M			0	.	.	.	.
Selection*Sex	C	F			-0.02036	0.01611	12	-1.26	0.2301
Selection*Sex	C	M			0	.	.	.	.
Selection*Sex	H	F			-0.02257	0.01573	12	-1.43	0.1769
Selection*Sex	H	M			0	.	.	.	.
Selection*Sex	P	F			0	.	.	.	.
Selection*Sex	P	M			0	.	.	.	.
ChamberType				P	-0.02049	0.007145	164	-2.87	0.0047
ChamberType				S	0	.	.	.	.
logBodyMass					0.7787	0.04242	164	18.36	<.0001
Age					-0.00018	0.000218	164	-0.82	0.4155
logActSignal					0.04479	0.02852	164	1.57	0.1182

Type 3 Tests of Fixed Effects				
Effect	**Num DF**	**Den DF**	**F Value**	**Pr > F**
Selection	3	12	12.87	0.0005
Sex	1	12	1.60	0.2293
Timing	2	30	0.17	0.8467
Selection*Sex	3	12	1.21	0.3470
ChamberType	1	164	8.22	0.0047
logBodyMass	1	164	337.05	<.0001
Age	1	164	0.67	0.4155
logActSignal	1	164	2.47	0.1182

Least Squares Means with 95% Confidence Limits								
Effect	**Selection**	**Sex**	**Timing**	**Chamber Type**	**Estimate**	**Standard Error**	**Lower**	**Upper**
Selection	A				1.7837	0.005640	1.7714	1.7960
Selection	C				1.7531	0.006351	1.7393	1.7670
Selection	H				1.7353	0.006245	1.7217	1.7489
Selection	P				1.7712	0.005960	1.7583	1.7842
Sex		F			1.7648	0.004337	1.7554	1.7743
Sex		M			1.7569	0.004464	1.7471	1.7666
Timing			A		1.7599	0.005210	1.7493	1.7706
Timing			M		1.7594	0.005244	1.7487	1.7701
Timing			N		1.7632	0.005074	1.7529	1.7736
Selection*Sex	A	F			1.7928	0.007608	1.7762	1.8094
Selection*Sex	A	M			1.7745	0.007648	1.7579	1.7912
Selection*Sex	C	F			1.7524	0.008569	1.7337	1.7710
Selection*Sex	C	M			1.7539	0.009111	1.7340	1.7737
Selection*Sex	H	F			1.7335	0.008576	1.7148	1.7522
Selection*Sex	H	M			1.7372	0.008761	1.7181	1.7563
Selection*Sex	P	F			1.7807	0.007968	1.7633	1.7980
Selection*Sex	P	M			1.7618	0.008288	1.7438	1.7799
ChamberType				P	1.7506	0.003942	1.7428	1.7584
ChamberType				S	1.7711	0.005380	1.7605	1.7817

Differences of Least Squares Means (pairwise *a posteriori* tests)							
Effect	**Selection**		**Estimate**	**Standard Error**	**DF**	**t Value**	**Tukey-Kramer adjusted P**
Selection	A	C	0.03054	0.008443	12	3.62	0.0161
Selection	A	H	0.04832	0.008180	12	5.91	0.0004
Selection	A	P	0.01243	0.008225	12	1.51	0.4612
Selection	C	H	0.01779	0.008762	12	2.03	0.2307
Selection	C	P	-0.01811	0.008677	12	-2.09	0.2120
Selection	H	P	-0.03590	0.008777	12	-4.09	0.0071


Conditional Residuals for logBMR
Residual
Predicted
Percent
Residual
Residual
Quantile
Residual Statistics
Observations 232
Minimum -0.13
Mean 91E-17
Maximum 0.1199
Std Dev 0.039
Fit Statistics
Objective -721
AIC -717
AICC -717
BIC -715.5


Distribution of logBMR
logBMR
A
C
H
P
Selection