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ORIGINAL ARTICLE

A strong response to selection on mass-independent maximal metabolic rate without a correlated response in basal metabolic rate

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Metabolic rates are correlated with many aspects of ecology, but how selection on different aspects of metabolic rates affects their mutual evolution is poorly understood. Using laboratory mice, we artificially selected for high maximal mass-independent metabolic rate (MMR) without direct selection on mass-independent basal metabolic rate (BMR). Then we tested for responses to selection in MMR and correlated responses to selection in BMR. In other lines, we antagonistically selected for mice with a combination of high mass-independent MMR and low mass-independent BMR. All selection protocols and data analyses included body mass as a covariate, so effects of selection on the metabolic rates are mass adjusted (that is, independent of effects of body mass). The selection lasted eight generations. Compared with controls, MMR was significantly higher (11.2%) in lines selected for increased MMR, and BMR was slightly, but not significantly, higher (2.5%). Compared with controls, MMR was significantly higher (5.3%) in antagonistically selected lines, and BMR was slightly, but not significantly, lower (4.2%). Analysis of breeding values revealed no positive genetic trend for elevated BMR in high-MMR lines. A weak positive genetic correlation was detected between MMR and BMR. That weak positive genetic correlation supports the aerobic capacity model for the evolution of endothermy in the sense that it fails to falsify a key model assumption. Overall, the results suggest that at least in these mice there is significant capacity for independent evolution of metabolic traits. Whether that is true in the ancestral animals that evolved endothermy remains an important but unanswered question.
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INTRODUCTION

Energy metabolism is one of the most fundamental aspects of biology, and it is key to understanding life histories of living organisms. Its central importance is reflected in the thousands of studies published on energy metabolism (Houston et al., 1993; Hayes and O'Connor, 1999; Speakman, 2008; Burton et al., 2011, Konarzewski and Książek, 2013; White and Kearney, 2013). Despite these studies, many questions about metabolic rates and energy metabolism remain unanswered. For example, is there a universal metabolic scaling law, why is resting metabolism correlated with daily energy use in mammals but not birds, how did the diverse resting and maximal metabolic rates of animals evolve and is there a necessary correlation between resting and maximal aerobic metabolism in vertebrates (Ricklefs et al., 1996; Clavijo-Baque and Bozinovic, 2012)? Some of these questions are very difficult to answer but ecological and evolutionary physiologists have recently made increasing use of artificial selection experiments to test hypotheses about the phenotypic and genetic integration of energy metabolism (Swallow et al., 1998; Koch and Britton, 2001; Książek et al., 2004; Rezende et al., 2004; Sadowska et al., 2005, 2008; Swallow et al., 2009; Wone et al., 2009;

Gebczyński and Konarzewski 2009a, b,). One of the foci of these recent studies has been testing hypotheses about the evolution of endothermy.

Endothermy, the physiological ability to raise the body temperature above environmental temperature via metabolic heat production while at rest, is a key innovation of birds and mammals. It is challenging to explain how vertebrate endotherms evolved from their ectothermic ancestors because this evolutionary change occurred over 100 million years ago (Ruben, 1995) and fossils can rarely be used to determine metabolic status reliably (Seymour et al., 2012; Grady et al., 2014). Nonetheless, two general classes of models attempt to explain how endothermy may have arisen. One class of models suggests that there were direct benefits of initial increases in resting metabolic rate (Bennett and Ruben, 1979; Hayes and Garland, 1995; Farmer, 2000). These models have been questioned because the proposed benefits of initial increases in heat production could be outweighed by elevated energy requirements and would not result in heat production that was rapid enough to enable endothermy (Bennett and Ruben, 1979; Ricklefs et al., 1996; Stevenson, 1985; Angilletta and Sears, 2003). Another set of models suggests that endothermy evolved as a

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correlated response to selection on genetically correlated characters (Bennett and Ruben, 1979; Ruben, 1995; Farmer, 2000; Koteja, 2000). Perhaps the first of these models was the aerobic capacity model, which postulates that endothermy evolved as a correlated response to selection on maximal metabolic rate (MMR or aerobic capacity) during exercise (Bennett and Ruben, 1979). An assumption of the aerobic capacity model is that basal metabolic rate (BMR) and MMR are inescapably correlated in endotherms (Hayes and Garland, 1995). Another model that postulates endothermy evolved as a correlated response to selection is the assimilation capacity model (Koteja, 2000). That model suggests that selection acted on the energy assimilation capacity of the visceral organs (for example, liver and gut) and that the assimilation capacity of the visceral organs is correlated with resting metabolic rate.

A variety of methods can be used to test hypotheses about the evolution of metabolism (Hayes and Garland, 1995; Clavijo-Baque and Bozinovic, 2012). These methods include biophysical modeling to assess the thermal benefits of small initial increases in resting metabolism, fossil studies searching for reliable indicators of physiology, and artificial selection experiments (Stevenson, 1985; Hillenius, 1992; Ruben, 1995; Bennett *et al.*, 2000). Artificial selection is a powerful tool for examining organismal design, genetic architecture and the genetic constraints that underlie the evolution of traits (Brakefield, 2003; Fuller *et al.*, 2005). For example, artificial selection can be used to test which characters evolve in response to selection on resting or maximal metabolic rate. Likewise selection on traits, such as running performance, can be used to test whether metabolism evolves as a correlated response (Rezende *et al.*, 2005, 2009). While each experiment is specific to the animals being studied, the collective information from numerous studies can be used to explore whether phenotypic and genetic integration is ubiquitous, consistent or inconsistent across various species, populations or other units of interest. One question of particular interest in the evolution of energy metabolism is how tightly are resting and maximal aerobic metabolism linked. The aerobic capacity model would suggest that they are tightly correlated and that a high resting metabolic rate is necessary to have a high maximal aerobic metabolic rate. Indeed, the strong form of the model would suggest that genetic correlations resulting from pleiotropic gene action are a pervasive feature of the vertebrate lineages that led to mammals and birds (Wone *et al.* 2009, Hayes 2010). If so, then the physiological design of vertebrates may preclude the possibility of endotherms having a high maximal metabolic rate and simultaneously having a low resting metabolic rate.

Herein, we report the results of an artificial selection experiment (that is, to test for phenotypic responses to selection) and quantitative genetic modeling (that is, to estimate genetic basis of the traits). Both phenotypic and genetic results are reported in an effort to provide a more complete understanding of the relationship between BMR and MMR. Because metabolic rates correlate strongly with mass (Gebczyński and Konarzewski 2009a), we artificially selected mice for high mass-independent maximal aerobic metabolic rates (MMR) and tested whether mass-independent basal metabolic rates (BMR) evolved as a correlated response. That correlated response is what the aerobic capacity model would predict. Undoubtedly, selection on whole-animal MMR would likely progress more rapidly than selection on mass-independent MMR given that there are greater genetic variances for mass and whole-animal MMR than for mass-independent MMR (Wone *et al.*, 2009). Indeed, a selection experiment for low and high running capacities in Sprague–Dawley rats illustrates this phenomenon. In just three generations of divergent selection, the running capacity of low and high selected lines differed by 70% (Koch and Britton, 2001).

However, the differences in running capacity in the Koch and Britton (2001) study are likely attributable largely to differences in mass and motivation as opposed to physiological capacities. In another treatment, we simultaneously selected for increased mass-independent MMR and decreased mass-independent BMR to test how much this additional constraint of selecting for decreased mass-independent BMR influenced the evolution of these two metabolic traits. This combination of traits would seem advantageous because it would reduce the energetic costs associated with BMR, while still providing the possible benefits associated with high MMR. The aerobic capacity model might be construed to suggest that presently unknown constraints associated with the design of endotherms preclude the possibility of animals evolving both increased MMR and decreased BMR. Because all selection protocols and data analyses included body mass as a covariate, so effects of selection on the metabolic rates are mass adjusted (that is, independent of effects of body mass).

MATERIALS AND METHODS
Study organism
We studied the laboratory house mouse, *Mus musculus*, because it is feasible to conduct large-scale breeding and physiological measurements with this organism. In addition, extensive background information on its physiology, morphology and life history is readily available.

Breeding scheme. A starting population of 49 male and 49 female randombred HS/IBG mice, representing 35 families, was obtained from the Institute of Behavioral Genetics at the University of Colorado, Boulder, CO, USA. No more than three mice were from any one family, and no more than two of any one sex were from any one family. Mice were divided in a stratified fashion such that 12–13 mating pairs were randomly allocated to each of the four replicates where no family was represented more than once (regardless of sex). We created four replicates from the starting population so that each selection treatment was replicated four times. Replication of selection treatments is important for drawing inferences from artificial selection experiments (Henderson, 1989). To make the connection between generation and response to selection clearer, we modified the notation for generation from that used in Wone *et al.* (2009) such that the generation subscript used in this paper is one number lower than that used in the previous paper. The initial group of mice (from all four replicates) represented G_{-2}, where G stands for generation (Supplementary Figure A1). Mice produced from these initial breeding pairs produced mice for G_{-1} to increase the population so that there were sufficient mice to assign to treatment groups. All mice that comprised G_{-1} were then randomly assigned to treatment groups. Within each replicate of G_{-1}, mice were allocated to three treatments: a randomly bred control group with no selection (control), directional selection for increased MMR (high-MMR) and antagonistic selection between decreased BMR and increased MMR (antag-MR). Individual replicates were bred ~ 4 weeks apart to allow sufficient time to complete the physiological measurements. The offspring of G_{-1} were designated G_0 (that is, G_0 was the base populations of the treatments). MMR and BMR were measured for all G_0 mice. Selection was first imposed on mice in G_0. So G_1 was the first generation in which the offspring resulted from parents who were artificially selected for their metabolic rates.

Mice were weaned at 21 days of age and housed five per cage, separated by sex. Cages contained a layer of corncob bedding and paper towels for nesting material. Cage assignment was not randomized and mice were typically housed with both siblings and non-siblings. This was due to the logistical constraints of managing a large number of mice. Food and water were available *ad libitum*. Mice were maintained on a 12:12 photoperiod and kept at ambient building temperatures (roughly 21.0–25.5 °C, although occasionally temperatures fell outside that range).

The breeding scheme was previously described (Downs *et al.*, 2013), but is described here with additional detail. As mentioned previously, metabolic rates correlate strongly with mass. Hence, to minimize confounding of metabolic effects with mass effects, we selected on the mass-independent metabolism (that is, on residuals from regressions of metabolism on body mass and other

covariates). Within each of the four replicates, each of the three treatments groups was represented producing a total of 12 lines of mice per generation. Within each line, 13 pairings in general were made to produce offspring for the next generation, with the goal of getting 10 successful litters from each line. For the control lines, breeders were chosen randomly (that is, no artificial selection), and BMR and MMR were measured on randomly chosen animals (20 males and 20 females). The second type of selection was directional selection for increased MMR. Each generation MMR was measured for all the mice in the high-MMR treatment. Selection was on mass-independent MMR (i.e., residual MMR; see preliminary data analysis section for details). In general, 13 females and 13 males with the highest residual MMR were selected as parents for the next generation, out of a total of ~53 scored in each sex. Initially, 13 pairings were made and two other pairs were reserved as backups in cases of unsuccessful breeding in the original 13. The 13 males and 13 females with the highest residual MMR were randomly paired, except that brother–sister mating was avoided. Starting with generation G_4, BMR was measured on 24 randomly selected males and 24 randomly selected females so that we could study the correlated response of BMR to selection on MMR. The third treatment was antagonistic selection (antag-MR) designed to create a line of mice with increased MMR and decreased BMR. In antag-MR lines, each mouse had its BMR and MMR measured. Mass-independent BMR and mass-independent MMR of each mouse were calculated as the residuals from regressions on body mass at the time of measurement. Then the cross product of the residuals was calculated, and mice were ranked on the basis of the cross product with the most negative cross product ranked highest. An additional constraint was that only mice with a positive residual from MMR were selected. This constraint was to avoid the use of mice that might have had large negative residual MMR because, for whatever reason, they did not run at their maximal capacity. Effectively selection was for high (+) residual MMR and low (−) residual BMR. The 13 males and 13 females with the most negative cross products were selected as breeders for the next generation, again with random pairing except that siblings were not paired with one another.

Metabolic trait measurements

The metabolic trait measurements have been previously described in detail (Wone *et al.*, 2009). Briefly, MMR was measured once using an incremental step test during forced exercise on a motorized treadmill. The treadmill was contained within a flow-through respirometry chamber (Hayes *et al.*, 1992; Swallow *et al.*, 1998). Drierite and Ascarite II were used to remove water vapor and CO_2, respectively. Dried, filtered air was pumped through the treadmill chamber at $600 \, ml \, min^{-1}$. Downstream from the chamber, water vapor and CO_2 were removed before O_2 analysis. The mouse was placed on the treadmill and given 4 min to acclimate. At the start of the run, the treadmill was set to $20 \, m \, min^{-1}$ for the first 2 min. The treadmill rate was then increased $2 \, m \, min^{-1}$ every 4 min until the mouse could not keep pace with the treadmill. A shocker grid was located at the rear of the treadmill to motivate mice to run. When mice did not jump off the grid, this behavior was used as an indication that the mouse was exhausted and the trial was ended at this point. After generation four, the step increments in speed were increased to $8 \, m \, min^{-1}$ every 2 min (that is, $20 \, m \, min^{-1}$ then $28 \, m \, min^{-1}$, and so on) to reduce the duration of each trial. A test with 50 mice using both step increments showed that MMR was slightly higher (2.3%, $P < 0.02$) when using the original test than when using the modified step increment. Within any generation, all measurements were made using the same step increments. MMR was determined as the highest 1 min average rate of O_2 consumption during the trial. We used an instantaneous correction for chamber washout to determine MMR (Bartholomew *et al.*, 1981). The effective volume of the treadmill system was 2090 ml.

Basal metabolic rate was measured at least 2 days after the MMR trial. BMR trials were conducted at ~32 °C (within the thermal neutral zone for mice; Hart, 1971; Speakman and Keijer, 2013). Mice were fasted overnight (starting at ~1700 h) to ensure that they were postabsorptive during metabolic measurements and placed in individual metabolic chambers the following morning (at ~0800 h). Each metabolic chamber received dry, filtered air at $200 \, ml \, min^{-1}$. Water and CO_2 were removed from the excurrent air with a column containing Drierite and Ascarite II. LabVIEW 7.1 (National Instruments, Austin, TX, USA)

was used to control incurrent air flow rate for all chambers for the duration of the trial and to switch solenoid valves to allow for sampling of excurrent air. Specifically, the 16 chambers, 12 holding a mouse and 4 empty chambers, were monitored for six cycles of 1 h each. Two dual-channel oxygen analyzers enabled us to monitor each of the 12 mice for 16 min out of every hour. Initial and final baselines samples (2 min each) of ambient air were obtained for each 16-min period from the four empty chambers so that we could correct for any linear drift in the baseline concentration of O_2 during the measurements. Excurrent oxygen concentration was averaged and recorded every 5 s. BMR was estimated as the lowest 5-min steady-state rate of O_2 consumption from the six 16-min measurement periods for each mouse using equation (4) from Hill (1972, p. 261). For both BMR and MMR measurements, air flow was regulated using upstream CMOSens mass flow controllers (Sensirion, Zurich, Switzerland), and oxygen content was analyzed using Oxilla II dual-channel/differential oxygen analyzers (Sable Systems, Las Vegas, NV, USA).

Data analyses

Preliminary statistical analysis. Before phenotypic and genetic trend analyses, we screened the data from each generation for outliers using least squares multiple regression of BMR or MMR on body mass and other covariates (age, treadmill, observer (that is, person who conducted the treadmill test) and BMR chamber number). In addition, treatment and sex were fitted as fixed effects, and line nested in treatment and replicate were each fitted as random effects. Statistical analyses were performed using SAS, v. 9.1 (SAS Institute, Cary, NC, USA). If standardized residuals from these regressions were greater than absolute value 3.0 observations were omitted from the phenotypic and breeding value analyses. Most BMR outliers were positive suggesting that the mice had not been quiescent enough to be considered at their BMR. Similarly, most MMR outliers were negative, which likely resulted from mice that did not reach their MMR due to submaximal running effort or observer error (for example, because observer decisions about precisely when to end a trial might have influenced the estimated MMR). Significant covariates were included in the models as fixed effects.

Phenotypic analysis. For phenotypic trend analyses, we ran separate mixed models for each generation where treatment and sex were fitted as fixed effects. In addition, line nested in treatment and replicate were fitted as two random effects. For the phenotypic analyses of BMR, body mass, age and chamber (CHAM) in which the animal was measured also were included as fixed effects. For the phenotypic MMR analyses, body mass, age, treadmill (TRED) and observer (OBS) were included as fixed effects. Treadmill was included because there was a significant difference between the two motorized treadmills that we think was caused by differences in the stimulators used through G_3. After G_3, we used identical custom-built stimulators to eliminate this design flaw. To determine the differences between treatments, we used *post hoc* contrasts. These contrasts were control versus antag-MR, control versus high-MMR and antag-MR versus high-MMR. Tukey-adjusted *P*-values were calculated to determine significance of *post hoc* pairwise comparisons.

In a phenotypic analysis, which is carried out within generations, an estimate of genetic change is obtained by studying the difference between corrected phenotypic means of a particular selection treatment and the corrected phenotypic means of the control line. This approach does not make strong assumptions about the genetic architecture of the trait analyzed, but results in sampling variances of estimates of response that are considerably larger than those from a mixed-model analysis. In other words, it produces a more erratic picture of the evolution of genetic means.

Bayesian analysis—variance and covariance components and genetic trends. A mixed-model approach extracts more information from the data than the least squares alternative, at the cost of making stronger assumptions about the genetic mechanism operating (Sorensen and Kennedy, 1984). The results reported below indicate that similar qualitative conclusions are drawn from both methods of inference. The Bayesian analysis reported below is based on the method described in Sorensen *et al.* (1994). Essentially, it consists of computing the posterior distribution of the average genetic values (across individuals) over generations, accounting for all other sources of variation included in the model.

In the Bayesian analysis, variance components and genetic trend are computed simultaneously. We have also carried out a restricted maximum likelihood (REML) analysis of variance and covariance components and confirmed that inferences were in excellent agreement. Results from the REML analysis can be found in the Supplementary Tables A4, A5.

The data consists of 4884 records of BMR and 7816 records of MMR. Note that for generations 2–4, we did not collect BMR data from the high-MMR lines in a manner that would allow us to estimate the correlated response of BMR to selection on MMR. The complete pedigree included 8212 individuals. Three sets of analyses were undertaken. First, the data were analyzed separately for each of the 12 lines (12 analyses) using model [1]. Second, the data were analyzed separately for each treatment (three analyses) using model [2]. Third, a single analysis of the 12 lines (three treatments, four replicates per treatment) was conducted using model [2]

$$(1) \begin{bmatrix} BMR \\ MMR \end{bmatrix} = \begin{bmatrix} \mu + SEX + CHAM + CAGE + ANIM + mass\ BMR + age\ BMR + e \\ \mu + SEX + TRED + OBS + CAGE + ANIM + mass\ MMR + age\ MMR + e \end{bmatrix}$$

$$(2) \begin{bmatrix} BMR \\ MMR \end{bmatrix} = \begin{bmatrix} \mu + SEX + CHAM + CAGE + REPLICATE \times GEN + ANIM + mass\ BMR + age\ BMR + e \\ \mu + SEX + TRED + OBS + CAGE + RPELICATE \times GEN + ANIM + mass\ MMR + age\ MMR + e \end{bmatrix},$$

where SEX, CHAM, TRED and OBS are fixed effect classes; mass BMR, age BMR, mass MMR and age MMR are fixed regressions; CAGE (natal cage), REPLICATE × GEN and ANIM (that is, additive genetic effect) are random effects; and e is the random residual.

Assumptions for random effects:

$$\begin{bmatrix} CAGE \\ REPLICATE \times GEN \\ ANIM \\ e \end{bmatrix} \sim \left(\begin{bmatrix} 0 \\ 0 \\ 0 \\ 0 \end{bmatrix}, \begin{bmatrix} C_0 \otimes I_C & 0 & 0 & 0 \\ 0 & LG_0 \otimes I_{LG} & 0 & 0 \\ 0 & 0 & A_0 \otimes S & 0 \\ 0 & 0 & 0 & R_0 \otimes I_e \end{bmatrix} \right)$$

(S is the additive relationship matrix of dimension equal to number of individuals in the pedigree, A represents additive genetic effect, L represents replicates, C represents cage and G represents generations. I_C is an identity matrix of dimension equal to number of cages (for the model across line and treatment, this is equal to 1197), I_{LG} is an identity matrix of dimension equal to number of replicates × generations (equal to 36) and I_e is an identity matrix of dimension equal to the number of observations in the particular analysis) where:

$$C_0 = \begin{bmatrix} \sigma^2_{C_B} & \sigma_{C_{B,M}} \\ \sigma_{C_{B,M}} & \sigma^2_{C_M} \end{bmatrix} LG_0 = \begin{bmatrix} \sigma^2_{BG_B} & \sigma_{BG_{B,M}} \\ \sigma_{BG_{B,M}} & \sigma^2_{BG_M} \end{bmatrix} A_0 = \begin{bmatrix} \sigma^2_{G_B} & \sigma_{G_{B,M}} \\ \sigma_{G_{B,M}} & \sigma^2_{G_M} \end{bmatrix}$$

$$R_0 = \begin{bmatrix} \sigma^2_{e_B} & \sigma_{e_{B,M}} \\ \sigma_{e_{B,M}} & \sigma^2_{e_M} \end{bmatrix}$$

In the above expressions, subscript B represents BMR, and M represents MMR. In A_0, $\sigma^2_{G_B}$ and $\sigma^2_{G_M}$ are the additive genetic variance components for BMR and for MMR, respectively, and $\sigma_{G_{B,M}}$ is the additive genetic covariance between the traits. The structure of the residual covariance matrix R is complicated due to the pattern of missing data. R is a function of the residual variance for MMR, $\sigma^2_{e_M}$, the residual variance for BMR, $\sigma^2_{e_B}$, and the residual covariance between both traits, $\sigma_{e_{B,M}}$. For each analysis, a pedigree file was extracted from the total pedigree file, so the pedigree used in each analysis only included individuals with data for the specific analysis and their ancestors back to generation 0. The number of observations and number of animals in the pedigree for each of the 16 analyses are shown in Table 1. Each analysis was carried out using both Bayesian and REML methods with the DMU-package (Madsen and Jensen, 2010). While REML analyses retrieve inferences about dispersion parameters, Bayesian analyses generate both dispersion parameters and genetic trends. The Bayesian linear mixed models were implemented with a standard Gibbs sampling algorithm (see, for example, Sorensen and Gianola, 2002). A total of 110 000 Gibbs samples were generated, and the first 10 000 were discarded as burn-in. This chain length was adequate as judged by the computation of Monte Carlo variances and effective chain sizes for all dispersion parameters, which were obtained by the method of batching (see Sorensen and Gianola, 2002). Convergence behavior of the Gibbs chains was checked by the inspection of trace plots. The Gibbs chains showed good mixing behavior.

Table 1 Number of observations and number of animals in pedigree for each genetic trend analysis

Treatment	Replicate	BMR	MMR	Pedigree
Control	1	326	326	367
Control	2	303	303	352
Control	3	305	306	363
Control	4	326	327	380
Control	Joint	1260	1262	1462
Antag-MR	1	821	824	850
Antag-MR	2	773	773	796
Antag-MR	3	602	602	627
Antag-MR	4	824	824	844
Antag-MR	Joint	3020	3023	3117
High-MMR	1	152	932	963
High-MMR	2	133	871	896
High-MMR	3	151	833	857
High-MMR	4	168	895	917
High-MMR	Joint	604	3531	3633
Joint	Joint	4884	7816	8212

High-MMR—directional selection for increased mass-independent maximal metabolic rate (MMR). Antag-MR—antagonistic selection for increased mass-independent MMR and decreased mass-independent basal metabolic rate (BMR).

RESULTS
Phenotypic analysis
Body mass, whole-animal BMR and MMR were obtained for over 8000 laboratory mice with a complete pedigree (Supplementary Table A1). Mean mass-adjusted MMR (that is, MMR adjusted for differences in body mass and other factors, hence mass-independent MMR) were very similar in the initial generations across treatments (Table 2). After eight generations of selection, mass-adjusted MMR had increased in all the three treatments, including the controls, which provides an example of why control lines are needed in selection experiments. At G_8, mass-adjusted MMR differed significantly among control, antag-MR and high-MMR treatments ($n = 547$, $F_{2,6} = 24.6$, $P = 0.001$). Mass-adjusted MMR was lowest in the control mice, intermediate in the antagonistically selected mice and highest in the directionally selected mice. High-MMR mice have significantly higher mass-adjusted MMR than control mice ($t = -7.01$, df = 6, Tukey-adjusted $P = 0.001$). Similarly, antag-MR mice have significantly higher mass-adjusted MMR than control mice ($t = -3.35$, df = 6, Tukey-adjusted $P = 0.04$). High-MMR mice also have significantly higher mass-adjusted MMR than antag-MR mice ($t = -3.66$, df = 6, Tukey-adjusted $P = 0.04$). There was no significant difference in body mass at time of MMR measurements among control, antag-MR and high-MMR treatments.

Mean mass-adjusted BMRs were similar in the starting generations across treatments (Table 2). After eight generations of selection, mass-adjusted BMR had decreased in all the three treatments. At G_8, mass-adjusted BMR differed significantly among control, antag-MR and high-MMR treatments ($n = 547$, $F_{2,6} = 7.83$, $P = 0.02$). Recall that we expected a decrease in BMR due to selection for decreased BMR in the antag-MR mice and that we expected an increase in BMR as a correlated response to selection for increased MMR in the high-MMR mice. Consistent with these expectations, mass-adjusted BMR was lowest in antag-MR, intermediate in the controls and highest in the high-MMR mice. After a Tukey adjustment for multiple comparisons, high-MMR mice at G_8 did not have significantly higher BMR when compared with control mice ($t = -1.52$, df = 6, Tukey-adjusted

Table 2 Phenotypic tests for effects of selection for each generation along with the *post hoc* pairwise tests

					Post hoc pairwise tests		
Generation	Control	Antag-MMR	High-MMR	Mixed-model results (P-value)	Control vs antag-MR (adjusted P-value)	Control vs high-MMR (adjusted P-value)	Antag-MR vs high-MMR (adjusted P-value)
MMR							
0	4.54 (0.10)	4.48 (0.10)	4.56 (0.10)	0.08	0.24	0.94	0.08
1	4.94 (0.04)	4.96 (0.04)	4.99 (0.10)	0.62	0.92	0.61	0.81
2	5.10 (0.05)	5.11 (0.04)	5.16 (0.03)	0.60	0.86	0.37	0.44
3	5.10 (0.06)	5.13 (0.05)	5.26 (0.05)	0.08	0.90	0.10	0.13
4	4.95 (0.09)	5.57 (0.17)	5.29 (0.27)	<0.0001	<0.0001	0.27	0.19
5	5.04 (0.05)	5.23 (0.04)	5.44 (0.04)	0.0004	0.01	0.0003	0.008
6	5.04 (0.05)	5.32 (0.05)	5.54 (0.05)	0.003	0.01	0.0006	0.03
7	4.95 (0.05)	5.20 (0.05)	5.46 (0.05)	0.0004	0.01	0.0003	0.009
8	5.01 (0.06)	5.28 (0.06)	5.57 (0.06)	0.001	0.04	0.001	0.04
BMR							
0	0.65 (0.02)	0.64 (0.02)	0.63 (0.02)	0.52	0.51	0.69	0.99
1	0.59 (0.01)	0.59 (0.01)	a	0.98	0.98	a	a
2	0.63 (0.01)	0.60 (0.01)	a	0.17	0.17	a	a
3	0.57 (0.02)	0.56 (0.02)	a	0.36	0.36	a	a
4	0.58 (0.01)	0.56 (0.01)	0.60 (0.01)	0.05	0.27	0.39	0.03
5	0.57 (0.01)	0.57 (0.01)	0.60 (0.01)	0.03	0.99	0.05	0.04
6	0.57 (0.01)	0.56 (0.01)	0.60 (0.01)	0.009	0.51	0.03	0.008
7	0.57 (0.01)	0.56 (0.01)	0.58 (0.01)	0.21	0.78	0.43	0.19
8	0.59 (0.01)	0.57 (0.01)	0.61 (0.01)	0.02	0.12	0.35	0.02

Values are least squares means of BMR and MMR ($\pm$s.e.). See Materials and methods for mixed-model parameters and *post hoc* tests. High-MMR—directional selection for increased mass-independent maximal metabolic rate (MMR). Antag-MR—antagonistic selection for increased mass-independent maximal metabolic rate and decreased mass-independent basal metabolic rate (BMR).
[a]No BMR was measured.

$P = 0.35$). In addition, antag-MR mice did not have significantly lower mean BMR when compared with control mice ($t = 2.43$, Tukey-adjusted $P = 0.12$). However, high-MR mice did have significantly higher mean BMR when compared with antag-MR mice ($t = -3.92$, Tukey-adjusted $P = 0.02$). There was no significant difference in body mass at the time of BMR measurements among control, antag-MR and high-MMR treatments.
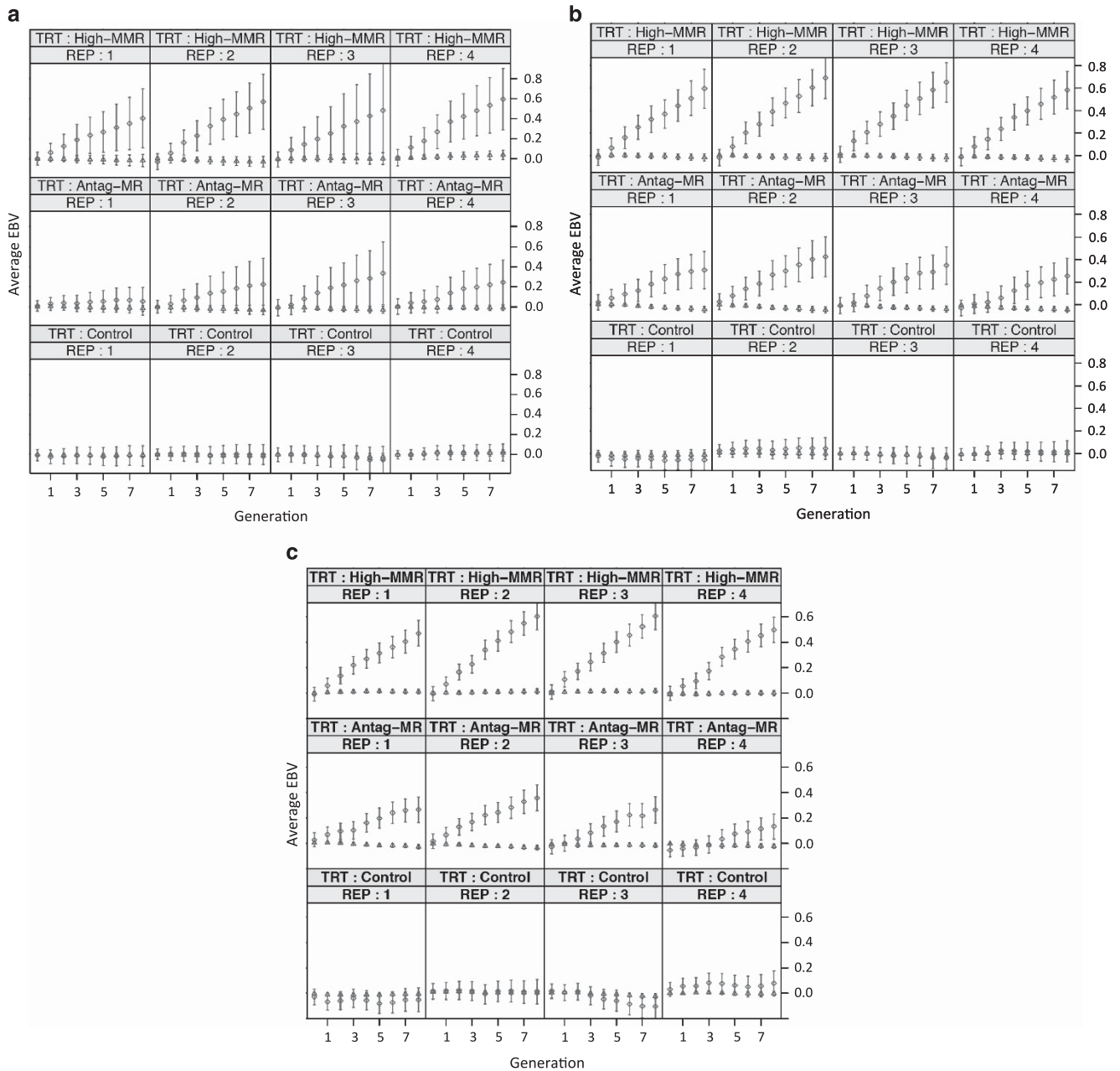
Bayesian analysis
For the three sets of the Bayesian analyses, genetic means per generation (that is, genetic trend) for MMR and BMR were estimated as the average of the posterior means of the additive genetic effects (ANIM in the model (1)) for MMR and BMR, respectively, for each combination of treatment, replicate and generation. In the lines selected for MMR, the genetic trend for MMR represents the direct response to selection for MMR, and the genetic trend for BMR represents the correlated response in BMR. In the lines selected for antag-MR, the genetic trends for MMR and for BMR represent both metabolic rates responses to selection (Figure 1).

Selection of high MMR was effective in changing the mean MMR in all the four replicates at the genetic level. However, none of the replicates showed a correlated genetic response of BMR to selection on MMR. Similarly, the antagonistic selection treatment leads to a marked response in MMR and a lack of response in BMR in all the replicate lines. This general picture is consistent across the three types of analyses. In all the four control replicates, there was no genetic trend for either MMR or BMR. However, the joint analysis displays a small upward trend in MMR in the control lines.

The Bayesian analysis was highly concordant with the results from the REML analysis (see Supplementary Tables A2–A5). In addition, Bayesian analysis displays estimates of dispersion parameters that vary considerably across replicates and lines. Combining the whole data set, the joint Bayesian analysis resulted in estimates of posterior means of genetic correlation (r_A) between BMR and MMR equal to 0.211, and narrow sense heritabilities (h^2) of BMR and MMR equal to 0.154 and 0.201, respectively. Bayesian analysis also indicated that the common environmental variance attributable to natal cage (that is, CAGE effect) was quite small and did not explain significant variation for either metabolic trait.

DISCUSSION
MMR showed a clear response to selection in both the directionally selected and antagonistically selected lines. Pairwise comparisons with control lines show that BMR was not significantly higher in the high MMR lines, nor was it significantly lower in the antagonistically selected lines (Table 2). *A priori* one might have hypothesized an increase in BMR in the high selected lines and a decrease in BMR in the antagonistically selected lines, so the divergence in BMR would be predicted to be largest between those treatments. That pairwise difference in BMR was significant even though neither of the selection treatments was significantly different in BMR from the control lines. The genetic analyses were largely concordant with the phenotypic analyses. Notably, phenotypic analyses during the early phase of selection produced mixed results, but Bayesian analysis offered clearer conclusions. For example, estimates of genetic trend provided no

Figure 1 Genetic trends from Bayesian analyses for basal metabolic rate (BMR) and maximal metabolic rate (MMR). In all the figures, the y axis displays the average genetic means (that is, estimated breeding values—EBV), and the x axis displays generation numbers. Posterior means of genetic means per generation were calculated together with posterior standard deviations. The Monte Carlo estimates of posterior distributions were symmetric, so these can be interpreted as 95% posterior intervals. (**a**) Represents the results of the 12 analyses (within treatments and replicates). Trends are plotted for each treatment and for each replicate. (**b**) Represents the results from the three analyses (within treatments only), but these figures show estimates of trends for each treatment and replicate. (**c**) The single (joint analysis) and the results are shown for each treatment and replicate. TRT, treatment; High-MMR, directional selection for increased mass-independent MMR; Antag-MR, antagonistic selection for increased mass-independent MMR and decreased mass-independent BMR. Blue triangle—BMR, red diamond—MMR. A full color version of this figure is available at the *Heredity* journal online.

statistically significant evidence of a correlated response in BMR in high-MMR mice (Figure 1).

A plausible explanation for the lack of a correlated response in BMR in our lines selected for high MMR is inadequate statistical power. The relatively low heritabilities for BMR and MMR and the small genetic correlation between them would have required more generations before a statistically significant response was likely. We suspect that further selection would have resulted in increased divergence in mass-

adjusted BMR of high-MMR lines and ultimately to a statistically significant difference. Indeed, the expected correlated response in BMR can be approximated using infinite population theory with the formula:

$$CR_y = ti\, r_A \sqrt{h_y h_x} \sigma_{py}$$

(Falconer and Mackay 1996) where CR_y is the correlated response in Y (that is, BMR) when selection is based on X (that is, MMR), t is

generations of selection, i is the selection intensity in X, h_x and h_y are the heritabilities, r_A is the genetic correlation between X and Y and σ_{py} is the phenotypic variance of Y.

$$CR_y = (8)(1.4)(0.21)\sqrt{(0.20 \times 0.15)}0.004 = 0.024$$

Because this is a very small value, a very large experiment (that is, larger number of parentals per replicate, >55 pairs) will be needed to detect such a correlated response. Standard calculations (Hill, 1980) indicate that the experiment had minimal power of detecting a correlated response and that it would have taken ~ 30 generations of selection to obtain a coefficient of variation of the response smaller than 50% (Falconer and Mackay 1996).

A number of studies have suggested that BMR would likely show a correlated response to direct selection on MMR (Koteja, 1987; Bozinovic, 1992; Dohm et al., 2001; Rezende et al., 2004; Nesoplo et al., 2005a; Sadowska et al., 2005; 2008; Wone et al., 2009). Likewise many evolutionary models suggest complex inter-relationships among traits linked to metabolic rates (Ricklefs and Wikelski, 2002; Downs et al., 2013). Although the selection for high MMR might possibly have led to high BMR in the evolutionary past, our results are equivocal with respect to the importance of a genetic covariance constraining the evolution of metabolic rates in the mice we studied.

In this study, MMR and BMR were genetically positively correlated ($r_A = 0.21$), although the precise estimate of the genetic correlation depends on the details of the model that was fitted. This positive correlation is lower than an earlier report of a genetic correlation of 0.72 calculated from a subset of the mice studied herein (Wone et al. 2009). Why these estimates of the genetic correlations differ is not clear, but we suspect that greater confidence should probably be given to the estimate on the basis of the larger sample size (that is, $r_A = 0.21$). Previous studies of the genetic architecture of metabolic rates and theory both suggest that such sensitivity is not necessarily surprising, particularly in models that include numerous covariates and other statistical control factors (Dohm et al. 2001; Wilson 2008). Most importantly, both estimates suggest a positive correlation between MMR and BMR. Taken at face value, the genetic correlation is positive, (as predicted by the aerobic capacity model), so that estimate per se does not falsify the aerobic capacity model (Hayes, 2010; Nespolo and Roff, 2014), but the weak genetic correlation and the lack of a correlated response to the selection in BMR suggest that the constraints imposed by genetic architecture are modest at most.

The results of another selection experiment for high MMR mice are concordant with our study. Gebczyński and Konarzewski (2009a) selected for high MMR during swimming. While MMR increased as a result of selection they did not find a correlated increase in BMR. Hence, Gębczyński and Konarzewski concluded that there was no correlation and no mechanistic linkage between BMR and MMR.

Another study relevant to ours is the Garland laboratory's artificial selection experiment for increased voluntary wheel running in mice. In that study selection was on total distance run. Lines of mice selected to be high runners ran for more hours per day but not at faster speeds (Garland et al., 2011). Mice selected for higher voluntary running had elevated MMR during voluntary exercise (Rezende et al., 2005), but they did not have higher BMR (Kane et al., 2008). In addition, during forced exercise, neither MMR nor BMR was greater in mice selected for high voluntary running than in controls (Rezende et al., 2005, 2009). On the basis of these results, it would be intriguing to see what would happen to MMR and BMR if one selected on the intensity

of exercise; that is, which would likely be more similar to our selection for high MMR. Such selection would presumably lead to increased MMR although it might also select for increased locomotor efficiency.

In contrast to studies which failed to find a link between MMR and BMR, work on bank voles supports the notion that selection on MMR can lead to changes in BMR. In a population of bank voles (Clethrionomys glareolus) with a positive genetic correlation between BMR and MMR (Sadowska et al., 2005), selection for increased MMR was accompanied by a correlated increase in BMR (Sadowska et al., 2008).

Genetic correlations can impose constraints on evolutionary trajectories, and these constraints can sometimes be absolute (Walsh and Blows, 2009). Only a few studies have reported the genetic correlation between BMR and MMR for rodents. The reported correlations range from 0.15 to 0.72 (Dohm et al., 2001; Nespolo et al., 2005b; Sadowska et al., 2008; Wone et al., 2009). Even the highest of these values indicates that the genetic constraint between BMR and MMR is not absolute, and hence that some measure of independent evolution of each of the traits is possible (Beldade et al., 2002). However, evolutionary constraints depend not only on genetic correlations/covariances but also on genetic variances and the relative strength of selection acting on correlated traits. One detailed analysis using the multivariate breeder's equation suggests that even very low correlations between BMR and MMR could have been important in the evolution of endothermy (Hayes, 2010).

Our antagonistic selection treatment was designed to explore the effects of simultaneous selection for a combination of traits (decreased BMR and increased MMR). Clearly in the absence of absolute genetic constraints (that is, the genetic variances do not equal zero and the genetic correlation does not equal 1) independent evolution of BMR and MMR is possible. On average, the difference in BMR between antag-MMR and control mice was 4.2% and the difference in MMR between antag-MMR and control mice was 5.3%. The response to selection in our antag-MR mice might indicate that the physiological design of vertebrates does not preclude animals having an increased MMR and simultaneously having a decreased BMR at least within certain limits. It would be interesting to learn how far those limits could be extended (that is, how low selection could move BMR while simultaneously increasing MMR or at least keeping MMR from decreasing). This idea is consistent with the work on bank voles showing that MMR and BMR are under different selective pressures (Boratynski and Koteja, 2009).

One caveat about our antagonistic selection treatment is that these mice were maintained in a benign laboratory setting that included relatively warm temperatures and ad libitum food. It is possible that our mice selected for decreased BMR and increased MMR might have attributes, which preclude success in a natural environment. In other words, decreased BMR and increased MMR might be achievable in the laboratory but not in nature. However, limited data suggest that some bats and canids have high factorial aerobic scopes (ratio of MMR to BMR) in nature (Koteja, 1987), so the notion of evolving high MMR accompanied by low BMR is plausible.

To summarize, selection for increased MMR led to clear positive responses in MMR, but without a correlated response in BMR. The small and positive genetic correlation between BMR and MMR did not falsify the aerobic capacity model for the evolution of endothermy, but the low genetic correlation suggests that constraints on independent evolution are modest at most. Moreover, the precise estimates of the genetic correlation proved sensitive to sampling (for example, Wone et al., 2009 versus this study) or details of model fitting (Wilson,

2008), consequently our results are rather equivocal with respect to the aerobic capacity model for the evolution of endothermy. Interestingly, in antagonistically selected mice, it was possible to simultaneously achieve increased MMR and decreased BMR. Collectively, our results are in concert with those of others (for example, Sadowska *et al.*, 2008) and suggest that genetic architecture may be important in understanding the evolution of metabolic rates. Nonetheless, in the mice we studied, the genetic correlation is low enough to not preclude the substantial independent evolution of metabolic traits (Pease and Bull, 1988; Walsh and Blows, 2009).

DATA ARCHIVING

Data available from the Dryad Digital Repository: http://doi.org/10.5061/dryad.br506

CONFLICT OF INTEREST

The authors declare no conflict of interest.

Angilletta MJ, Sears MW (2003). Parental care as a selective factor for the evolution of endothermy? *Am Nat* **162**: 821–825.

Bartholomew GA, Vleck D, Vleck CM (1981). Instantaneous measurements of oxygen-consumption during pre-flight warm-up and post-flight cooling in sphingid and saturniid moths. *J Exp Biol* **90**: 17–32.

Beldade P, Koops K, Brakefield PM (2002). Developmental constraints versus flexibility in morphological evolution. *Nature* **416**: 844–847.

Bennett AF, Ruben JA (1979). Endothermy and activity in vertebrates. *Science* **206**: 649–654.

Bennett AF, Hicks JW, Cullum AJ (2000). An experimental test of the thermoregulatory hypothesis for the evolution of endothermy. *Evolution* **54**: 1768–1773.

Boratynski Z, Koteja P (2009). The association between body mass, metabolic rates and survival of bank voles. *Funct Ecol* **23**: 330–339.

Bozinovic F (1992). Scaling of basal and maximal metabolic rate in rodents and the aerobic capacity model for the evolution of endothermy. *Physiol Zool* **65**: 921–932.

Brakefield PM (2003). Artificial selection and the development of ecologically relevant phenotypes. *Ecology* **84**: 1661–1671.

Burton T, Killen SS, Armstrong JD, Metcalfe NB (2011). What causes intraspecific variation in resting metabolic rate and what are its ecological consequences? *Proc R Soc B* **278**: 3465–3473.

Clavijo-Baque S, Bozinovic F (2012). Testing the fitness consequences of the thermo-regulatory and parental care models for the origin of endothermy. *PLoS One* **7**: e37069.

Dohm MR, Hayes JP, Garland T (2001). The quantitative genetics of maximal and basal rates of oxygen consumption in mice. *Genetics* **159**: 267–277.

Downs CJ, Brown JL, Wone B, Donovan ER, Hunter K, Hayes JP (2013). Selection for increased mass-independent maximal metabolic rate suppresses innate but not adaptive immune function. *Proc R Soc B* **280**: 1471–2954.

Falconer DS, Mackay TFC (1996). *Introduction to Quantitative Genetics*. Longman: New York, NY, USA.

Farmer CG (2000). Parental care: the key to understanding endothermy and other convergent features in birds and mammals. *Am Nat* **155**: 326–334.

Fuller RC, Baer CF, Travis J (2005). How and when selection experiments might actually be useful. *Integr Comp Biol* **45**: 391–404.

Garland T, Kelly SA, Malisch JL, Kolb EM, Hannon RM, Keeney BK *et al.* (2011). How to run far: multiple solutions and sex-specific responses to selective breeding for high voluntary activity levels. *Proc R Soc B* **278**: 574–581.

Gebczyński K, Konarzewski M (2009a). Metabolic correlates of selection on aerobic capacity in laboratory mice: a test of the model for the evolution of endothermy. *J Exp Biol* **212**: 2872–2878.

Gebczyński K, Konarzewski M (2009b). Locomotor activity of mice divergently selected for basal metabolic rate: a test of hypotheses on the evolution of endothermy. *J Evol Biol* **22**: 1212–1220.

Grady JM, Enquist BJ, Dettweiler-Robinson E, Wright NA, Smith FA (2014). Evidence for mesothermy in dinosaurs. *Science* **344**: 1268–1272.

Hart JS (1971). Rodents. Whittow GC (ed.). *Comparative Physiology of Temperature Regulation*. Academic Press: New York, NY, USA, vol. 2: 2–149.

Hayes JP, Garland T, Dohm MR (1992). Individual variation in metabolism and reproduction of *Mus*: are energetics and life history linked? *Funct Ecol* **6**: 5–14.

Hayes JP, Garland T (1995). The evolution of endothermy: testing the aerobic capacity model. *Evolution* **49**: 836–847.

Hayes JP, O'Connor CS (1999). Natural selection on thermogenic capacity of high-altitude deer mice. *Evolution* **53**: 1280–1287.

Hayes JP (2010). Metabolic rates, genetic constraints, and the evolution of endothermy. *J Evol Biol* **23**: 1868–1877.

Henderson ND (1989). Interpreting studies that compare high- and low-selected lines on new characters. *Behav Genet* **19**: 473–502.

Hill RW (1972). Determination of oxygen consumption by use of the paramagnetic oxygen analyzer. *J Appl Physiol* **33**: 261–263.

Hill WG (1980). Design of quantitative genetic selection experiments. In: Robertson A (ed.). *Selection experiments in laboratory and domestic Animals*. Commonwealth Agricultural Bureaux: Slough, UK, pp. 1–13.

Hillenius WJ (1992). The evolution of nasal turbinates and mammalian endothermy. *Paleobiology* **18**: 17–29.

Houston AI, Mcnamara JM, Hutchinson JMC (1993). General results concerning the trade-off between gaining energy and avoiding predation. *Phil Trans R Soc Lond B* **341**: 375–397.

Kane SL, Garland T, Carter PA (2008). Basal metabolic rate of aged mice is affected by random genetic drift but not by selective breeding for high early-age locomotor activity or chronic wheel access. *Physiol Biochem Zool* **81**: 288–300.

Koch LG, Britton SL (2001). Artificial selection for intrinsic aerobic endurance running capacity in rats. *Physiol Genomics* **5**: 45–52.

Konarzewski M, Sadowski B, Jówik I (1997). Metabolic correlates of selection for swim stress-induced analgesia in laboratory mice. *Am J Physiol* **273**: R337–R343.

Konarzewski M, Książek A (2013). Determinants of intra-specific variation in basal metabolic rate. *J Comp Physiol B* **183**: 27–41.

Koteja P (1987). On the relation between basal and maximal metabolic rate in mammals. *Comp Biochem Physiol* **87A**: 205–208.

Koteja P (2000). Energy assimilation, parental care, and the evolution of endothermy. *Proc R Soc B* **267**: 479–484.

Ksiażek A, Konarzewski M, Lapo IB (2004). Anatomic and energetic correlates of divergent selection for basal metabolic rate in laboratory mice. *Physiol Biochem Zool* **77**: 890–899.

Madsen P, Jensen J (2010). *A User's Guide to DMU; A Package for Analyzing Multivariate Mixed Models*. Aarhus University: Aarhus, Denmark. Available at http://dmu.agrsci.dk/dmuv6 guide. 5.0. pdf.

Nespolo RF, Bustamante DM, Bacigalupe LD, Bozinovic F (2005a). Quantitative genetics of bioenergetics and growth-related traits in the wild mammal *Phyllotis darwini*. *Evolution* **59**: 1829–1837.

Nespolo RF, Bacigalupe LD, Bozinovic F (2005b). Heritability of energetics in a wild mammal, the leaf-eared mouse (*Phyllotis darwini*). *Evolution* **57**: 1679–1688.

Nespolo RF, Roff DA (2014). Testing the aerobic model for the evolution of endothermy: implications of using present correlations to infer past evolution. *Am Nat* **183**: 74–83.

Pease M, Bull JJ (1988). A critique of methods for measuring life history trade-offs. *J Evol Biol* **1**: 293.

Rezende EL, Bozinovic F, Garland T (2004). Climatic adaptation and the evolution of basal and maximum rates of metabolism in rodents. *Evolution* **58**: 1361–1374.

Rezende EL, Chappell MA, Gomes FR, Malisch JL, Garland T (2005). Maximal metabolic rates during voluntary exercise, forced exercise, and cold exposure in house mice selectively bred for high wheel-running. *J Exp Biol* **208**: 2447–2458.

Rezende EL, Gomes FR, Chappell MA, Garland T (2009). Running behavior and its energy cost in mice selectively bred for high voluntary locomotor activity. *Physiol Biochem Zool* **82**: 662–679.

Ricklefs RE, Konarzewski M, Daan S (1996). The relationship between basal metabolic rate and daily energy expenditure in birds and mammals. *Am Nat* **147**: 1047–1071.

Ricklefs RE, Wikelski M (2002). The physiology/life-history nexus. *Trends Ecol Evol* **17**: 462–468.

Ruben J (1995). The evolution of endothermy in mammals and birds - from physiology to fossils. *Ann Rev Physiol* **57**: 69–95.

Sadowska ET, Labocha MK, Baliga K, Stanisz A, Wroblewska AK, Jagusiak W *et al.* (2005). Genetic correlations between basal and maximum metabolic rates in a wild rodent: consequences for evolution of endothermy. *Evolution* **59**: 672–681.

Sadowska ET, Baliga-Klimczyk K, Chrzascik KM, Koteja P (2008). Laboratory model of adaptive radiation: a selection experiment in the bank vole. *Physiol Biochem Zool* **81**: 627–640.

Seymour RS, Smith SL, White CR, Henderson DM, Schwarz-Wings D (2012). Blood flow to long bones indicates activity metabolism in mammals, reptiles and dinosaurs. *Proc R Soc B* **279**: 451–456.

Sorensen DA, Kennedy BW (1984). Estimation of response to selection using least squares and mixed model methodology. *J Anim Sci* **58**: 1097–1106.

Sorensen DA, Wang CS, Jensen J, Gianola D (1994). Bayesian analysis of genetic change due to selection using Gibbs sampling. *Genet Sel Evol* **26**: 333–360.

Sorensen DA, Gianola D (2002). *Likelihood, Bayesian, and MCMC Methods in Quantitative Genetics*. Springer-Verlag: New York, NY, USA, pp. 740 Reprinted with corrections, 2006.

Speakman JR (2008). The physiological costs of reproduction in small mammals. *Phil Trans R Soc B* **363**: 375–398.

Speakman JR, Keijer J (2013). Not so hot: optimal housing temperatures for mice to mimic the thermal environment of humans. *Mol Metab* **2**: 5–9.

Stevenson RD (1985). The relative importance of behavioral and physiological adjustments controlling body temperature in terrestrial ectotherms. *Am Nat* **126**: 362–386.

Swallow JG, Garland T, Carter PA, Zhan WZ, Sieck GC (1998). Effects of voluntary activity and genetic selection on aerobic capacity in house mice (*Mus domesticus*). *J Appl Physiol* **84**: 69–76.

Swallow J.G, Hayes JP, Koteja P, Garland T (2009). Selection experiments and experimental evolution of performance and physiology. In: Garland T, Rose MR (eds). *Experimental Evolution: Concepts, Methods, and Applications of Selection Experiments*. University of California Press: Berkeley, CA, USA, pp. 301–351.

Walsh B, Blows MW (2009). Abundant genetic variation + strong selection = multivariate genetic constraints: a geometric view of adaptation. *Annu Rev Ecol Evol Syst* **40**: 41–59.

White CR, Kearney MR (2013). Determinants of inter-specific variation in basal metabolic rate. *J Comp Physiol B* **183**: 1–26.

Wilson AJ (2008). Why h^2 does not always equal VA/VP? *J Evol Biol* **21**: 647–650.

Wone B, Sears MW, Labocha MK, Donovan ER, Hayes JP (2009). Genetic variances and covariances of aerobic metabolic rates in laboratory mice. *Proc R Soc B* **276**: 3695–3704.