Genetic variances and covariances of aerobic metabolic rates in laboratory mice

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The genetic variances and covariances of traits must be known to predict how they may respond to selection and how covariances among them might affect their evolutionary trajectories. We used the animal model to estimate the genetic variances and covariances of basal metabolic rate (BMR) and maximal metabolic rate (MMR) in a genetically heterogeneous stock of laboratory mice. Narrow-sense heritability (h^2) was approximately 0.38 ± 0.08 for body mass, 0.26 ± 0.08 for whole-animal BMR, 0.24 ± 0.07 for whole-animal MMR, 0.19 ± 0.07 for mass-independent BMR, and 0.16 ± 0.06 for mass-independent MMR. All h^2 estimates were significantly different from zero. The phenotypic correlation of whole animal BMR and MMR was 0.56 ± 0.02 , and the corresponding genetic correlation was 0.79 ± 0.12 . The phenotypic correlation of mass-independent BMR and MMR was 0.13 ± 0.03 , and the corresponding genetic correlation was 0.72 ± 0.03 . The genetic correlations of metabolic rates were significantly different from zero, but not significantly different from one. A key assumption of the aerobic capacity model for the evolution of endothermy is that BMR and MMR are linked. The estimated genetic correlation between BMR and MMR is consistent with that assumption, but the genetic correlation is not so high as to preclude independent evolution of BMR and MMR.

Keywords: heritability; animal model; metabolic rate; genetic correlation; aerobic capacity; endothermy

1. INTRODUCTION

Energy metabolism, or what Kleiber (1961) called the fire of life, is perhaps the most important physiological attribute of an animal. Energy metabolism has intrigued biologists for more than a century (Rubner 1883; McNab 1992; Boratyński & Koteja 2009; Gebczyński & Konarzewski 2009), and a recent series of influential papers on the scaling of metabolic rate with body mass has rekindled a keen interest in energy metabolism (West *et al.* 1997; Brown *et al.* 2004; White *et al.* 2006; Makarieva *et al.* 2008). Thousands of studies have been published on energy metabolism which attests to its central biological importance for both domestic and wild animals (Houston *et al.* 1993; Hayes & O'Connor 1999; Speakman 2008). Almost all of these studies investigated energy metabolism at the phenotypic level. Studies of the genetic underpinnings of energy metabolism are surprisingly uncommon (Konarzewski *et al.* 2005). Energy metabolism is strongly influenced by temperature, and as concern about global climate change grows, information on the genetic architecture of metabolic rates may become valuable, not only from the perspective of basic biological understanding, but also in modelling how species distributions may be impacted by climate change.

Information on the genetic architecture of aerobic metabolism is available from at least three sources: (i) direct estimates that rely on information about relatedness of individuals, (ii) interstrain comparisons, and (iii) artificial selection experiments. In terms of direct estimates the majority of the data are for rodents (e.g. mice (*Mus musculus*), leaf-eared mice (*Phyllotis darwini*) and bank voles (*Clethrionomys glareolus*)) probably because it is tractable to conduct experiments with appropriate breeding designs (Lacy & Lynch 1979; Bacigalupe *et al.* 2004; Leamy *et al.* 2005; Nespolo *et al.* 2005; Sadowska *et al.* 2005). Besides data for rodents, the heritability of basal metabolic rate (BMR) has been estimated for zebra finches (*Taenopygia guttata*; Rønning *et al.* 2007), and the heritability of several measures of aerobic metabolism, including maximal metabolic rate (MMR), has been estimated for humans (Bouchard *et al.* 1998; Perusse *et al.* 2001).

Other information on the genetics of aerobic metabolism comes from comparing the exercise performance of strains of rats or mice. When raised under common environmental conditions, phenotypic differences among strains indicate genetic differences. In turn these differences in exercise performance imply differences in the genetics underlying aerobic metabolism (Barbato *et al.* 1998; Koch *et al.* 1999; Lightfoot *et al.* 2001; Lerman *et al.* 2002; Billat *et al.* 2004; Massett & Berk 2005). Besides studies related to exercise performance, interstrain comparisons of laboratory mice have identified presumptive genetic differences in resting metabolism and

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in the partitioning of energy metabolism into resting and active components (Sacher & Duffy 1979).

Artificial selection experiments also provide information on the genetic basis of metabolism (MacLaury & Johnson 1972). Metabolic rates can evolve as a result of direct or correlated responses to selection. In rodents, significant direct responses of metabolic rates to artificial selection have been reported for MMR elicited by swimming in bank voles, for endurance running in rats, and for BMR in mice (Koch & Britton 2001; Ksiazek *et al.* 2004; Sadowska *et al.* 2005). Metabolic rates can also evolve as a correlated response to selection on another phenotypic trait, such as artificial selection for high voluntary-wheel running (Swallow *et al.* 1998; Rezende *et al.* 2005). In general, these selection experiments suggest that aerobic metabolism is heritable, although heritability may not always be particularly high (Ksiazek *et al.* 2004).

As suggested by the studies reported in the previous paragraphs, the emergence of evolutionary physiology as a distinct discipline has been accompanied by increased interest in the genetic architecture of metabolic traits (Feder *et al.* 2000; Henderson *et al.* 2002; Konarzewski *et al.* 2005; Gebczynski & Konarzewski 2009). Information on genetic architecture helps us understand how metabolic traits might respond to artificial or natural selection (Houle 1992; Lynch & Walsh 1998; Hansen *et al.* 2003), and how covariances among traits might affect evolutionary trajectories (Arnold 1987; Sadowska *et al.* 2008). Another reason to be interested in genetic covariances and their evolutionary effects is that a pervasive genetic correlation between minimal and maximal aerobic metabolism is an implicit feature of one model for the evolution of endothermy (Bennett & Ruben 1979; Hayes & Garland 1995). Herein, we used quantitative genetics analyses to estimate the genetic variance of BMR and MMR and their covariance in a large sample of mice (*M. musculus*) from an artificial selection study. We tested the hypotheses that BMR and MMR were heritable and hence able to respond to selection. We also tested the hypothesis that the genetic covariance between BMR and MMR was significant and hence could influence the joint evolution of these traits. Our study is noteworthy for its large sample size, for obtaining rigorous measures of MMR during treadmill exercise, for obtaining robust estimates of the genetic variances and covariances of important physiological traits, and for its potential relevance to the aerobic capacity model for the evolution of endothermy.

2. MATERIAL AND METHODS

(a) *Study organism*

We used the laboratory house mouse, *M. musculus*, because of the extensive background information on its physiology, morphology and life history, and because it is a feasible organism in which to conduct the large-scale physiological measurements needed to estimate quantitative genetic parameters. Data presented here represent mice from the base population and control groups of a larger artificial selection experiment on aerobic metabolism. The starting population comprised 49 male and 49 female mice representing 35 families from a random-bred HS/IBG (heterogeneous stock/Institute of Behavioral Genetics) stock of mice

obtained from the University of Colorado, Boulder, CO, USA. Mice were divided in a stratified random fashion such that 12–13 mating pairs were allocated to each of four breeding blocks (hence four replicates) where no family was represented more than once (regardless of sex). We created four breeding blocks to accommodate the time consuming physiological measurements to be completed. The breeding and measurement of blocks were separated in time by approximately four weeks. The initial group of mice (from all four blocks) represented G_{-1} , where G stands for generation. These mice were bred to increase population size, so no metabolic measurements were made on these mice. Mice produced from these initial breeding pairs comprised G_0 (see figure S1 in the electronic supplementary material). Mice from G_0 had their MMR and BMR measured as described below. This manuscript reports data from the baseline population (G_0) and control mice through to generation 6 (G_6).

In general, mice were weaned at 21 days of age and housed five per cage. Cages were filled with corn cob bedding. Owing to logistical constraints, weaned mice were typically caged with siblings. Food and water were available ad libitum. A constant 12:12 h photoperiod and approximately 22°C ambient temperatures were maintained where the cages are kept. Environmental temperature control in our mouse rooms in the first few generations was not particularly good, however, so we sometimes had appreciably colder or warmer temperatures. In latter generations, environmental temperature controls and emergency backup systems were improved to provide more stable temperatures, but in general mice experienced ambient room temperatures in the building, not a precisely regulated thermal environment.

(b) *Timing of physiological measurements*

Measurement of BMR and MMR generally began when the mice from a particular block were approximately eight weeks old, but owing to various logistical constraints (available analyzers, personnel time, and so on) there was some range in when measurements were completed for each mouse (see §3 for details). In almost all cases MMR was measured at least two days before BMR. All metabolic trials were completed between 08.00 and 17.00.

(c) *Maximal metabolic rate*

We used forced exercise on a motorized treadmill with an incremental step test to measure MMR (Hayes *et al.* 1992; Swallow *et al.* 1998). Two custom built treadmills were used. Each was enclosed in an open-flow respirometry chamber. A fan was mounted at the front of the treadmill to ensure that air in the chamber was well mixed. The internal dimensions of the treadmill chambers were approximately 45 × 11 × 5.7 cm. The metabolic rate of exercising mice can change rapidly but the excurrent air leaving an enclosed chamber does not reach steady-state rapidly. Hence, 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. Each motorized treadmill was independently controlled with a Dart Micro-Drive Controller (Dart Controls, Zionsville, ID, USA), which could incrementally increase the treadmill speed. Each treadmill had an electrical stimulator attached to a shocking grid at the end of the treadmill to motivate the mouse to run. Although, the stimulators were from the

same manufacturer (Harvard Apparatus Co., Dover, MA, USA), they were different models (Models 340 and 343). We detected and statistically corrected for a difference between treadmills until generation five, when we were able to replace the stimulators with identical custom-built stimulators that eliminated differences between the treadmills.

MMR was measured once for each mouse. Prior to placing a mouse in a treadmill, body mass was measured. Ambient air was dried with Drierite and filtered before being delivered to each treadmill. Air flow was regulated at 600 ml min^{-1} with a mass flow controller (Sensirion, Zurich, Switzerland) interfaced with LABVIEW v. 7.1 (National Instruments, Austin, TX, USA) computer software. Excurrent air was passed through a column of Drierite and Ascarite II to remove water vapour and CO_2 , respectively, prior to analysis. Oxygen concentration of the excurrent air was measured with a Sable Systems Oxilla II oxygen analyser (Sable Systems, Las Vegas, NV, USA) interfaced to a LABVIEW data acquisition system. Oxygen concentration was sampled at 100 Hz, and this output averaged and recorded each second. Following a baseline measurement of the ambient oxygen concentration, the mouse was placed in the chamber with the shocking grid off and the treadmill belt stationary. After a 2 min acclimation period the shocking grid was turned on, and after an additional 2 min the test was started with the treadmill set to 20 m min^{-1} . Every 2 min the treadmill speed was increased by 4 m min^{-1} (i.e. 20 m min^{-1} for 2 min, then 24 m min^{-1} for 2 min, etc.) until either the mouse showed no increase in MMR with increased treadmill speed or refused to run. After generation four, because the treadmill measurements were very time consuming, the step increments in speed were increased to 8 m min^{-1} every 2 min (i.e. 20 m min^{-1} then 28 m min^{-1} , etc) to reduce the duration of the trial. A test with 51 mice, each run with both methods, showed that MMR was slightly (2.3%), and significantly ($p = 0.02$) higher when using the original test than when using the modified step increment. After the trial was completed, the incurrent air flow was diverted from the treadmill to obtain a second baseline measurement, and the mouse was removed from the treadmill. Following the trial, body mass was measured again to the nearest 0.01 g. MMR body mass was calculated as the average of the pre- and post-MMR mass measurements. MMR was defined as average metabolic rate during the highest 1 min period of oxygen consumption during forced exercise. We used the instantaneous MMR data for all genetic analyses.

(d) Basal metabolic rate

We used flow-through respirometry to measure BMR. For almost every mouse, BMR was measured at least two days after the MMR trial. Mice were fasted overnight and were measured at approximately 30°C , which is within the thermal neutral zone for *M. musculus* (Hart 1971). BMR was measured with a 16 chamber open-circuit system in which up to 12 mice can be measured. Four empty chambers were used to measure baseline concentrations of oxygen in ambient air. Each chamber was 590 ml in volume and received dry air at 200 ml min^{-1} STP from upstream mass flow controllers (Sensirion, Zurich, Switzerland). Water and CO_2 were scrubbed from excurrent air with Drierite and Ascarite II, respectively. LABVIEW was used to control incurrent air flow rates and sampling order for all chambers.

Excurrent air was monitored by two Oxilla II dual channel oxygen analysers. With two analysers and two channels per analyser, the system could monitor four chambers at a time. Every 15 min the system switched excurrent airflows going to the analysers to measure the next four chambers. BMR was measured for 6 h giving six separate 15 min measurements for each mouse. Empty chambers were sampled for 5 min between each 15 min period to obtain initial and final baselines of ambient oxygen concentration for each measurement. Excurrent oxygen concentration was recorded at 1000 Hz and recorded as 5 s averages. BMR was calculated using eqn. 4 from Hill (1972). Body mass was measured to the nearest 0.01 g just before the mouse was placed in the BMR chamber and again when the animal was taken out. Body mass was calculated as the average of the two measurements. BMR for each mouse was calculated as the lowest 5 min metabolic rate from the six sampling periods.

(e) Genetic models

Before genetic analyses of metabolic traits, we screened the data for each generation separately for outliers using least-squares multiple regression of BMR or MMR on body mass and covariates, such as treadmill (1 or 2), age, observer (i.e. person who conducted the treadmill test), and which particular BMR chamber was used. Statistical analyses were performed using SAS, v. 9.1 (SAS Institute, Cary, NC, USA). If standardized residuals from these regressions were $> |3.0|$, observations were omitted from the genetic analyses. We eliminated mice with these residuals because we think they most probably resulted from measurement errors. For example, most BMR outliers were positive which probably resulted from mice that were not at rest, so that their metabolism was not basal but instead was elevated by activity within the chamber. Similarly, most MMR outliers were negative, which we think probably resulted from mice that did not reach their MMR. Significant covariates were included in the genetic models as fixed effects. While results are not reported here, our results were not appreciably influenced by including or excluding outliers, with the exception that in general residuals were normally distributed when outliers were excluded but they sometimes deviated from normality when outliers were included.

We used the animal model to estimate quantitative genetic parameters. The animal model is a mixed-model approach that uses the relatedness between all individuals in a pedigree to estimate the genetic variance components of a trait. These estimates are considered robust given a complete pedigree (Lynch & Walsh 1998). We implemented the animal model using ASREML v. 2.0 (Gilmour *et al.* 2006) a mixed-model software program that uses restricted maximum-likelihood (REML) estimation.

We used univariate animal models to calculate narrow-sense heritability (h^2) for BMR and MMR and to obtain starting values for subsequent bivariate analyses. For the univariate animal models, we started with a full model that included six variance components. These were the additive genetic variance, V_A , the common environmental variance attributable to natal cage, V_{C1} , the common environmental variance attributable to post-weaning cage, V_{C2} , the maternal genetic variance, V_{Mg} , the maternal environmental variance, V_{Me} , and the environmental variance unique to individuals, V_E (Kruuk 2004). The common environmental variance

attributable to post-weaning cage, the maternal genetic variance and the maternal environmental variance were quite small and did not explain significant variation for either metabolic trait, and hence those results were not included in the h^2 analyses presented. Instead for simplicity, we estimated h^2 from a reduced model (i.e. ACE) that contained three variance components. These were the additive genetic variance, V_A , the common environmental variance attributable to natal cage, V_C , and environmental variance unique to individuals, V_E . One of the main advantages of the animal model is the ability to simultaneously include fixed and random effects in the model (Lynch & Walsh 1998). For all the univariate animal models, generation and sex were fitted as fixed effects (see table S1 in the electronic supplementary material). In addition, block by generation interaction was fitted as a random effect to account for possible generational effects across blocks. For the univariate BMR animal models, body mass, age and chamber in which the animal was measured also were included as fixed effects. For the univariate MMR animal models, body mass, age, treadmill, observer and stimulator also 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 generation four. As stated earlier, after generation four we used identical custom built stimulators to eliminate this design flaw.

We estimated the significance of h^2 as the probability that the additive genetic variance component was greater than zero by using a log-likelihood ratio test (Lynch & Walsh 1998). For example, the log-likelihood of additive genetic variance (ACE model) was compared to the log-likelihood of a constrained model (CE model) with the additive genetic component set to zero. The test compares twice the difference in log-likelihoods with a one-tailed chi square (χ^2) distribution with the degrees of freedom equal to the number of parameters constrained to zero (Shaw & Geyer 1997). If the additive genetic effects (i.e. V_A) were significant in the univariate models, we constructed bivariate animal models to evaluate the significance of the covariance of the traits (i.e. genotypic correlation). We tested whether genetic correlations were significantly different from both zero and one. To test whether the genetic correlation was significantly different from one, we used an approximation suggested by A. R. Gilmour (2009, personal communication). That is we fixed the correlation to 0.999, and then we compared the log-likelihood from that analysis with the log-likelihood of the model where the genetic correlation was unconstrained (Dingemanse *et al.* 2009). The significance test was a log-likelihood ratio test (Lynch & Walsh 1998), where twice the difference in log-likelihood was compared to a one-tailed chi square (χ^2) distribution with one degree of freedom. We performed analyses for whole-animal BMR and MMR by omitting body mass as a covariate, and we also performed mass-independent analyses by including body mass as a covariate.

In addition to h^2 , we computed the additive genetic coefficient of variation, CV_A , because it is a more meaningful scale-free measure of evolvability and genetic variability of a trait (Houle 1992; Hansen *et al.* 2003). This measure is based on the additive genetic variances scaled by the trait mean and is reported as a percentage. The CV_A is thus computed as the square root of additive genetic variance scaled by the trait mean. Likewise, we also reported the

environmental coefficient of variation unique to individuals, CV_E , which is computed as the square root of the environmental variance unique to individuals scaled by the trait mean (Houle 1992). For these calculations, we used the additive genetic variance estimates from their corresponding ACE models. Similarly we used the environmental variance unique to individuals from their corresponding ACE models to compute the environmental coefficient of variation unique to individuals.

3. RESULTS

BMR or MMR was measured in 1642 laboratory mice (see table S2 in the electronic supplementary material). On average, these mice had a mean body mass of 21.8 g (3.25 s.d.; range 13.4–32.2) when BMR was measured and a mean body mass of 23.7 g (3.43 s.d.; range 14.4–34.8) when MMR was measured. Mean BMR was 0.60 ml O_2 min^{-1} (0.11 s.d.; range 0.30–0.95), and mean MMR was 4.69 ml O_2 min^{-1} (0.74 s.d.; range 2.74–6.87). Complete pedigree information was available for all mice bred for the experiment regardless of whether metabolic trials were successfully completed or not. Measurements were completed at an average age of 69.9 days (9.81 s.d.; range 49–106) for BMR and 64.6 days (6.99 s.d.; range 52–84) for MMR. The univariate BMR animal models were based on a pedigree of 1334 animals. The univariate MMR models were based on a pedigree of 1539 animals. The bivariate models were based on a pedigree of 1334 animals. Whole-animal metabolic traits varied between individuals and increased with body mass. In addition, BMR increased with MMR as expected (figure 1).

(a) *Narrow-sense heritability*

All traits had significant additive genetic variation (table 1). Based on the ACE model, h^2 of body mass at the time BMR was measured was 0.40 (ACE versus CE, $\chi^2 = 29.2$, $p < 0.001$) and of body mass at the time MMR was measured was 0.36 (ACE versus CE, $\chi^2 = 26.2$, $p < 0.001$). Based on the ACE model, h^2 for whole-animal BMR was 0.26 (ACE versus CE, $\chi^2 = 10.2$, $p < 0.001$) and for whole-animal MMR was 0.24 (ACE versus CE, $\chi^2 = 13.1$, $p < 0.001$). Based on the univariate ACE models, h^2 for mass-independent BMR was 0.19 (ACE versus CE, $\chi^2 = 6.28$, $p < 0.001$), and h^2 for mass-independent MMR was 0.16 (ACE versus CE, $\chi^2 = 10.5$, $p < 0.001$). The additive genetic coefficient of variation, CV_A , showed that most traits have greater than 5 per cent additive genetic coefficients of variation with whole-animal BMR having the largest additive genetic coefficient of variation.

(b) *Phenotypic and genetic correlations*

Owing to difficulties with model convergence for some bivariate models, we do not report the genetic correlations between BMR and body mass at the time BMR was measured, between MMR and body mass at the time MMR was measured, or between body mass at the time BMR was measured and body mass at the time MMR was measured. The phenotypic and genetic correlations between whole-animal BMR and whole-animal MMR and between mass-independent BMR and mass-independent MMR were positive. The genetic

Figure 1. Scatter plot of whole-animal maximal metabolic rates (MMR) in relation to whole-animal basal metabolic rates (BMR). The figure shows data from the latest generation only (i.e. control mice of G₆) to make the data easier to visualize. Filled circle, female; cross, male.

correlations of these bivariate models were significantly different from zero, but not significantly different from one (table 2).

4. DISCUSSION

(a) *Narrow-sense heritability*

Our results indicate significant h^2 and modest genetic variance for mass-independent BMR and MMR. Other studies of BMR in rodents have reported results ranging from low h^2 that was not statistically significant (Lacy & Lynch 1979; Dohm *et al.* 2001; Nespolo *et al.* 2003; Bacigalupe *et al.* 2004) to high h^2 that was statistically significant (Konarzewski *et al.* 2005; Sadowska *et al.* 2005). Previous estimates of h^2 for MMR were generally fairly high (≥ 0.4), although as far as we know only one previous study in mice has estimated heritability of MMR elicited by running (Dohm *et al.* 2001; reviewed in Konarzewski *et al.* 2005). Variation in estimates of h^2 is not surprising because estimates of h^2 are expected to vary among populations, environmental conditions and statistical methods (Lynch & Walsh 1998). Relatedly, estimates will depend on which fixed and random effects are included in the model (Wilson 2008).

Heritability was higher for whole-animal metabolism than mass-independent metabolism (table 1). These results have implications for selection on whole-animal traits versus selection on mass-independent traits. Given the greater genetic variances for mass and whole-animal metabolism than for mass-independent metabolism, selection on whole animal metabolism should progress more rapidly than selection on mass-independent metabolism. An example of this phenomenon may be Sprague-Dawley rats selected for low and high running capacities. The running capacity of low and high selected lines differed by 70 per cent after just three generations of divergent selection. This result suggests that selection on whole-animal traits (as opposed to mass-independent

traits) may have major effects on performance, although these differences in performance may be caused largely by differences in mass (Koch & Britton 2001).

(b) *Phenotypic and genetic correlations*

Phenotypically, mass-independent BMR and MMR were significantly positively correlated (figure 2). The mass-independent correlation of BMR or resting metabolic rate with MMR (measured either by cold exposure or by strenuous exercise) has been estimated interspecifically for anurans, lizards, passerines, birds, shrews, rodents and mammals (e.g. Taigen 1983; Koteja 1987; Bozinovic 1992; Hinds & Rice-Warner 1992; Sparti 1992; Walton 1993; Dutenhoffer & Swanson 1996; Thompson & Withers 1997; Rezende *et al.* 2002; Wiersma *et al.* 2007). Three of those correlations were negative but none was significantly different from zero. The significant positive correlations across species for anurans, birds and rodents suggest (i) that genetic variation for the correlation (i.e. a genetic covariance) exists or used to exist, (ii) that genetic variation for each trait exists or used to exist and that evolutionary differences among species resulted from correlated selection (e.g. selection for both high BMR and high MMR), or (iii) that both those things were true (cf. Rezende *et al.* 2004).

Besides estimates of interspecific correlations, mass-independent intraspecific correlations of resting metabolism and MMR have been reported for a variety of amphibians, squamates, birds and mammals. Variation in methodology, age and other factors would make a thorough discussion of these studies overly long so herein we provide a very brief overview of those studies. For endotherms, significant positive intraspecific correlations have been reported for at least three species of mammals (*Dasyurus novemcinctus*, *Peromyscus maniculatus* and *Spermophilus beldingii*) and one passerine bird, *Passer domesticus* (Hayes 1989; Chappell & Bachman 1995; Chappell *et al.* 1999; Boily 2002). Non-significant correlations (both positive and negative) have been reported for several other mammals (*Meriones unguiculatus*, *Mus domesticus*, *Myodes glareolus* and *P. darwinii*) and for some birds (*Calidris canutus* and *Gallus gallus*; Hammond *et al.* 2000; Dohm *et al.* 2001; Nespolo *et al.* 2005; Sadowska *et al.* 2005; Vézina *et al.* 2006; Chappell *et al.* 2007; Boratyński & Koteja 2009). In ectotherms, the intraspecific correlation has been estimated for at least nine species of squamates, and at least 21 species of amphibians (see table 1 in Hayes & Garland 1995; Thompson & Withers 1997; Gomes *et al.* 2004), but for most species the number of individuals studied was small; hence power to detect correlations has generally been low. Two significant positive correlations for squamates and two significant negative correlations for anurans have been reported. The significant phenotypic correlations found in some species suggest that mass-independent resting metabolism and MMR may be genetically correlated (Cheverud 1988; Roff 1995).

While phenotypic correlations are important, our focus is on the genetic correlation between BMR and MMR. Genetic correlations have been estimated between basal metabolism and at least three other measures of energy metabolism: MMR during running, MMR during cold exposure and MMR during swimming. These three

Table 1. Variance components, heritability (h^2) and standard errors obtained from univariate animal models for laboratory mice as estimated by ASREML v. 2.0. (Univariate model tested was the ACE model, where A is the additive genetic variance, C is the common environmental variance attributable to natal cage, and E is the environmental variance. Tests of statistical significance of h^2 for the models were determined by constraining V_A to zero and obtaining the log-likelihood (LL) of subsequent reduced models. Twice the difference in LL was compared to a one-tailed chi square (χ^2) distribution with the degrees of freedom equal to the number of parameters constrained to zero. Additive genetic coefficient of variation (CV_A) and environmental coefficient of variation unique to individuals (CV_E) of body mass and metabolic trait values presented are in percentages. *statistically significant at $p < 0.05$; **statistically significant at $p < 0.001$.)

trait	univariate ACE models				coefficient of variation	
	h^2	V_A	V_C	V_E	CV_A	CV_E
body mass at BMR	0.40** (0.078)	1.90** (0.43)	1.26** (0.21)	1.57 (0.23)	6.35	5.77
body mass at MMR	0.36** (0.077)	1.83** (0.44)	1.44** (0.22)	1.76 (0.24)	5.71	5.59
whole-animal BMR	0.26** (0.080)	0.0021** (0.00067)	0.0012** (0.00031)	0.0047 (0.00042)	7.54	11.36
whole-animal MMR	0.24** (0.074)	0.055** (0.019)	0.059** (0.010)	0.12 (0.011)	5.03	7.39
mass-independent BMR	0.19* (0.071)	0.00089* (0.00034)	0.00022 (0.00016)	0.0036 (0.00025)	4.98	9.95
mass-independent MMR	0.16* (0.059)	0.014* (0.0052)	0.0081** (0.0029)	0.063 (0.0038)	2.50	5.38

Table 2. Additive genetic (r_A), phenotypic (r_P) and environmental (r_E) correlations and standard errors between pairs of traits from a bivariate animal model as estimated by ASREML v. 2.0. (Analyses are from the ACE model, where A is the additive genetic variance, C is the common environment variance attributable to natal cage, and E is the environmental variance. Tests of statistical significance were determined by constraining the correlation to zero or 0.999 (i.e. $r_A = 1$) and obtaining the log-likelihood (LL) of the subsequent models. Because it is not possible to obtain estimates with the genetic correlation exactly equal to one, the test for significant difference from one was approximated by constraining the correlation to 0.999. Twice the difference in LL was compared to a one-tailed chi square (χ^2) distribution with one degree of freedom.)

pairs of traits	r_A	r_P	r_E	test of $r_A = 0$ p -value	test of $r_A = 1$ p -value
whole-animal BMR–whole-animal MMR	0.79 (0.12)	0.56 (0.023)	0.38 (0.051)	<0.001	0.08
mass-independent BMR–mass-independent MMR	0.72 (0.28)	0.13 (0.030)	0.02 (0.048)	<0.05	0.42

measures of MMR may be genetically correlated, but they probably represent different traits, because within the same population responses of the traits to environmental changes may not be commensurate. For example, deer mice (*P. maniculatus*) acclimatized to cold (3°C) increased their MMR during cold exposure by 31 per cent but their MMR during running increased by only 9 per cent (cf. Harri *et al.* 1984; Hayes & Chappell 1986).

Few studies have estimated the genetic correlation between BMR and MMR. The one previous study of the genetic correlation between BMR and MMR measured during running was performed on an outbred strain (ICR) of *M. domesticus* (Dohm *et al.* 2001). The additive genetic correlation was significantly positive for some, but not all models that were fitted (e.g. including or excluding dominance or common, postnatal environmental effects).

The genetic correlation between BMR and MMR during cold exposure has also been estimated in the leaf-eared mouse (Nespolo *et al.* 2005). The correlation was positive ($r_A < 0.23$) but not statistically significant, despite a sample size of about 360 mice. The lack of statistical significance may be an issue of statistical power, and the results illustrate the need for very large sample sizes in such studies.

One of the largest studies to date (>1000 bank voles) found a positive genetic correlation between BMR and MMR during swimming (Sadowska *et al.* 2005). MMR during swimming might have included a thermoregulatory component, as indicated by modest hypothermia at the end of the test. Interestingly, BMR and MMR during cold exposure were not genetically correlated, even though there was a high genetic correlation between MMR during swimming and MMR during cold exposure. This important study showed that the correlations among various measures of metabolism and with mass may be complex.

Besides estimating genetic correlations from pedigrees, genetic correlations can be estimated from correlated responses to selection. That is, a positive correlated response to selection implies a positive genetic correlation and vice versa. Swiss-Webster mice have been selected for low and high BMR (Ksiazek *et al.* 2004). Because the demands of achieving a response to selection for BMR were very high, that the experiment was unable to use replicate lines, so the responses to selection are more challenging to interpret statistically (Henderson 1997; Ksiazek *et al.* 2004). Mice selected for low BMR had slightly higher MMR during swimming and slightly lower MMR during cold exposure than mice selected for high BMR. However, the

Figure 2. Scatter plot of residual basal metabolic rates (BMR) in relation to residual maximal metabolic rates (MMR). The figure shows data from the latest generation only (i.e. control mice of G_6) to make the data easier to visualize. Phenotypic correlation of the base population (generation 0 and 1) and control mice (i.e. G_2 – G_6) is significant by conventional correlation ($r = 0.159$, $p < 0.0001$). Filled circle, female; cross, male.

standardized between line differences were sufficiently small that a reasonable interpretation is that there was no correlated response to selection and that the small observed differences can be accounted for by genetic drift (Ksiazek *et al.* 2004).

(c) *Aerobic capacity model*

Our study is potentially germane to the aerobic capacity model for the evolution of endothermy (Bennett & Ruben 1979). This model postulates that high BMR evolved as a correlated response to selection on aerobic capacity (i.e. MMR during vigorous exercise). The aerobic capacity model is difficult to test because endothermy evolved in mammals (or more probably their ancestors) more than 100 Myr ago. It is not particularly common to estimate the strength of selection in extant populations. To do so in extinct populations may be impossible. Nonetheless, the aerobic capacity model argues that endothermy evolved as a correlated response to selection on MMR. Indeed, it has been postulated that a positive correlation between BMR and MMR may be an inescapable attribute of the design of all endotherms. If that postulation is true, then all endotherms ought to have a positive genetic correlation between BMR and MMR. We think that despite the challenges posed, this hypothesis, which we call the strong form of the aerobic capacity model, is testable.

The strong form of the aerobic capacity model postulates that all endotherms and their ancestors that evolved endothermy had a positive genetic correlation between BMR and MMR and that this correlation is ubiquitous in this group of organisms. In addition the hypothesis incorporates the idea that endothermy evolved as a correlated response to selection on MMR. Genetic correlations may evolve owing to selection, drift and other factors, so this is a demanding hypothesis. The notion that a genetic correlation would be ubiquitous and persist over tens of

millions of years would only be plausible if it reflected a fundamental design constraint.

Even if there was a fundamental design constraint, that constraint might have existed only in the ancestors of endotherms that evolved endothermy. If the design constraint (genetic correlation) was lost subsequently, we refer to this scenario as the weak form of the aerobic capacity model. We do not think that existing methods can falsify this hypothesis.

If the strong form of the aerobic capacity model is true, then BMR and MMR during exercise should be highly genetically correlated in every extant endotherm. Finding that any extant endotherm did not have a highly positive genetic correlation would falsify the strong form of the aerobic capacity model. Indeed, if this can be demonstrated, then the strong form of the aerobic capacity model would be falsified. While this argument is indirect, we argue that science operates by seeking to falsify hypotheses. Our test of the genetic correlation does exactly that, at least for the strong form of the aerobic capacity model.

Our genetic analyses indicate that the genetic correlation between BMR and MMR is significantly different from zero, but not significantly different from one. Hence, direct selection on either BMR or MMR would be expected to result in a correlated response in the other trait. If the genetic correlation was significantly different from one but still very high (e.g. 0.9), then the capacity for independent evolution would be fairly limited. Our estimate of the genetic correlation for mass-independent metabolic traits is high ($r_A = 0.72$) and not significantly different from one, but if the true correlation is in fact 0.72 (not 1) then these traits could evolve independently because selection could act on the genetic variance unique to each trait. As such our results argue against the strong form of the aerobic capacity model *sensu stricto*. Indeed, it would be surprising in the extreme if a perfect genetic correlation was ubiquitous between any two traits, so the strong form of the aerobic capacity model may be overly restrictive. Nonetheless, we think discussion of models of this sort may be a useful springboard to stimulate research into this difficult evolutionary problem. Whether our quantitative genetic results have any bearing on the evolution of endothermy, they do add to our knowledge of the genetic architecture of metabolic traits, an area in which much remains to be learned.

(d) *Conclusions*

To summarize, we found significant additive genetic variance for BMR and MMR at both the whole-animal and mass-independent levels, and a strong positive genetic correlation between mass-independent BMR and MMR. This latter result supports the possibility that this correlation may be a pervasive or nearly pervasive feature of the design of tetrapods, but our analyses suggest that the correlation does not constitute an absolute genetic constraint and that these traits are capable of independent evolution to some degree. A remaining challenge is establishing the functional connection between BMR and MMR and what accounts for their correlation, because the main contributors to BMR are the liver and kidney while the main contributor to MMR is the musculature (Weibel *et al.* 2004).

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