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SHORT COMMUNICATION

Among- and within-individual correlations between basal and maximal metabolic rates in birds

Vincent Careau[1,‡], Bethany J. Hoye[1], Terence W. O'Dwyer[2,*] and William A. Buttemer[1]

ABSTRACT

The aerobic capacity model proposes that endothermy is a by-product of selection favouring high maximal metabolic rates (MMR) and its mechanistic coupling with basal metabolic rate (BMR). Attempts to validate this model in birds are equivocal and restricted to phenotypic correlations (r_P), thus failing to distinguish among- and within-individual correlations (r_{ind} and r_e). We examined 300 paired measurements of BMR and MMR from 60 house sparrows before and after two levels of experimental manipulation – testosterone implants and immune challenge. Overall, repeatability was significant in both BMR (R=0.25±0.06) and MMR (R=0.52±0.06). Only the testosterone treatment altered the r_P between BMR and MMR, which resulted from contrasting effects on r_{ind} and r_e. While r_{ind} was high and significant (0.62±0.22) in sham-implanted birds, r_e was negative and marginally non-significant (−0.15±0.09) in testosterone-treated birds. Thus, the expected mechanistic link between BMR and MMR was apparent, but only in birds with low testosterone levels.

KEY WORDS: Endothermy, Multivariate mixed models, Performance, Resting metabolic rate, RMR, $\dot{V}_{O_2,max}$

INTRODUCTION

Metabolic rate (MR) is a fundamental measure in ecology and evolution, because it represents the rate at which an animal oxidizes substrates to produce the energy required to grow, behave, reproduce and survive. In endotherms, basal metabolic rate (BMR) represents the minimum rate of release and use of energy required for self-maintenance. By contrast, maximum metabolic rate (MMR) represents the highest aerobic MR expressed over short periods (i.e. 0.5–10 min) by an animal undergoing maximal physical exertion. MMR sets the upper limit of O_2 consumption and thereby sustained aerobic heat production and vigorous activity. Thus, natural selection may favour higher aerobic capacity because it could facilitate expanded thermal niches, increased energy assimilation capacity and/or enhanced parental care (Hayes, 2010). In turn, selection favouring high MMR may also lead to an elevated BMR through a causal, mechanistic link, and has therefore been postulated to explain the evolution of one of the greatest features of birds and mammals – endothermy (Hayes and Garland, 1995).

In birds, the few studies that have tested the phenotypic correlation (r_P) between BMR and MMR have yielded inconsistent results. For example, r_P tended to be negative in dark-eyed juncos [*Junco hyemalis*; r_P=–0.37, P=0.09 (Swanson et al., 2012)] and male red junglefowl (*Gallus gallus*; r_P=–0.33, P=0.049), but not in female fowl (Hammond et al., 2000). By contrast, juvenile house sparrows (*Passer domesticus*) had a positive correlation (r_P=0.44, P=0.02), whereas this was statistically insignificant in adults [r_P=0.23, P=0.07 (Chappell et al., 1999)]. Such inconsistencies may be due to these studies focusing on r_P, which is shaped by correlations at two distinct levels – the among-individual correlation (r_{ind}) and the within-individual correlation (r_e) (Dingemanse and Dochtermann, 2013). While r_e represents combined, reversible changes in two traits occurring within an individual (i.e. phenotypic flexibility), r_{ind} reflects the genetic and permanent environmental effects that are responsible for the association between the two traits. If BMR and MMR are physiologically (and genetically) coupled, this should be reflected in a positive r_{ind}. However, it is possible that variations in BMR and MMR occurring within individuals (r_e) obfuscate the potentially informative relationship among individuals, resulting in nil or even negative r_P.

Here, we exploited a large dataset to partition the r_P between BMR and MMR into r_{ind} and r_e in house sparrows, *P. domesticus* (Linnaeus 1758). The dataset consists of 300 paired measurements of BMR and MMR made on 60 individuals (i.e. five times each), which affords a unique opportunity and sufficient power to partition r_P into r_{ind} and r_e using multivariate mixed models (Dingemanse and Dochtermann, 2013). Because this dataset was gathered as part of a study examining the interactive effects of testosterone and immune challenge on metabolism, it allowed us to evaluate the extent to which r_P, r_{ind} and r_e were influenced by these experimental manipulations.

RESULTS AND DISCUSSION

Using the entire dataset, the r_P between BMR and MMR was not significant (r_P=0.037±0.058, χ^2_1=0.40, P=0.53). Bivariate models with heterogeneous residuals revealed that the r_P was not significantly different according to sex (χ^2_1=0.93, P=0.36) or immune challenge treatment (χ^2_1=0.90, P=0.34). However, the r_P estimates were significantly different according to testosterone treatment (χ^2_1=9.14, P=0.003). While r_P was positive and significant in birds implanted with empty capsules (r_P=0.204±0.083, χ^2_1=5.73, P=0.011), it was negative and marginally non-significant in birds implanted with testosterone-filled capsules (r_P=–0.150±0.079, χ^2_1=3.51, P=0.061). Thus, by manipulating testosterone levels, we generated contrasting relationships at the phenotypic level, as previously shown in comparisons across sexes (Hammond et al., 2000) and age groups (Chappell et al., 1999). To gain further insight into potential mechanisms underlying this finding, we determined whether the change in r_P caused by testosterone occurred at the among- and/or within-individual levels.

Across the entire dataset, repeatability was substantially higher in MMR than in BMR (Table 1A, Fig. 1A,B), which has previously been reported in birds (White et al., 2013). In the bivariate mixed model that included all data, the r_{ind} was positive and the r_e was negative, but

[1]Centre for Integrative Ecology, Deakin University, 75 Pigdons Road, Waurn Ponds, VIC 3216, Australia. [2]The Institute for Conservation Biology & Environmental Management, School of Biological Sciences, University of Wollongong, Wollongong, NSW 2522, Australia.
*Present address: Australian Museum, Sydney, NSW 2010, Australia.

‡Author for correspondence (vcareau@deakin.edu.au)

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Table 1. Estimates from two bivariate mixed models of basal metabolic rate (BMR) and maximal metabolic rate (MMR) in house sparrows (*Passer domesticus*)

Group	Component–trait	Estimate (means ± s.e.)	χ^2_1	*P*
A. All data				
	$V_{ind,BMR}$	0.169±0.053		
	r_{ind}	0.277±0.182	2.10	0.148
	$V_{ind,MMR}$	0.385±0.089		
	$V_{e,BMR}$	0.510±0.047		
	r_e	–0.079±0.065	1.47	0.226
	R_{BMR}	0.249±0.064		
	$V_{e,MMR}$	0.355±0.033		
	R_{MMR}	0.520±0.064		
B. Birds implanted with testosterone-filled capsules				
	$V_{ind,BMR}$	0.164±0.065		
	r_{ind}	−0.131±0.256	0.26	0.612
	$V_{ind,MMR}$	0.281±0.095		
	$V_{e,BMR}$	0.411±0.052		
	r_e	−0.147±0.088	2.68	0.101
	$V_{e,MMR}$	0.383±0.049		
	R_{BMR}	0.285±0.089		
	R_{MMR}	0.423±0.091		
C. Birds implanted with empty capsules				
	$V_{ind,BMR}$	0.172±0.086		
	r_{ind}	0.618±0.216	5.38	0.020
	$V_{ind,MMR}$	0.499±0.160		
	$V_{e,BMR}$	0.624±0.085		
	r_e	−0.011±0.096	0.01	0.910
	$V_{e,MMR}$	0.324±0.044		
	R_{BMR}	0.216±0.092		
	R_{MMR}	0.606±0.085		

(A) The first model was run using a homogeneous variance structure across the entire dataset (60 individuals measured five times each; total *N*=300). The second model was run with a heterogeneous variance structure across testosterone treatments, including (B) the group of 32 individuals (total *N*=160) that were implanted with testosterone-filled capsules, and (C) the group of 28 individuals (total *N*=140) that were implanted with empty capsules. Individual identity was fitted as a random effect to estimate the among-individual variance (V_{ind}) in both traits and the among-individual correlation between the two (r_{ind}). Residual variance (V_e) represents the within-individual variation, with a within-individual correlation (r_e) fitted to both models. The significance of r_{ind} and r_e was estimated with a log-likelihood ratio test. Repeatability (*R*) was also calculated for each model.

neither was significantly different from zero (Table 1A). At first sight, the non-significant r_{ind} when all data are included suggests a weak physiological coupling of BMR and MMR. However, our dataset is highly heterogeneous and comprises individuals with experimentally manipulated testosterone levels and immune status. Importantly, modelling heterogeneous variances according to testosterone treatment significantly improved the fit (χ^2_6=12.88, *P*=0.045), but immune treatment did not (χ^2_6=7.94, *P*=0.24).

Among the individuals implanted with testosterone-filled capsules, r_e was negative and marginally non-significant (Table 1B, Fig. 1C), which yielded a negative r_P in this group (see above). Thus, when testosterone levels were experimentally elevated, flexible changes in BMR within individuals tended to have opposing effects on MMR. This could represent a testosterone-mediated trade-off, occurring within individuals, between the energy allocated (or oxygen delivered) to systems primarily influencing BMR (e.g. visceral organs and the brain) versus those affecting MMR (e.g. heart, muscles) (Chappell et al., 1999). This could also explain the results obtained in red junglefowl, where the r_P was not significant in females but negative and significant in males (Hammond et al., 2000), which have much higher testosterone levels (Chappell et al., 1997).

Among the individuals implanted with empty capsules, r_{ind} was positive and significant (Table 1C, Fig. 1D), which yielded a significant and positive r_P in this group (see above). Thus, low testosterone levels revealed the expected mechanistic link between BMR and MMR. This suggests that BMR and MMR may be genetically correlated and/or permanently affected by the same environmental factors, but that the individual reaction norms of BMR and MMR are such that the r_{ind} disappears when testosterone increases. This scenario could explain the positive and significant r_P obtained in juvenile house sparrows (Chappell et al., 1999), as these birds were sexually immature and would have minimal testosterone levels. However, testosterone levels would also be low in the adult females they studied, yet a sex-specific reanalysis of adult sparrows yielded a non-significant relationship (r_P=–0.14±0.25, *P*=0.60) (M. A. Chappell, personal communication). Although a positive trend was evident in adult males of that dataset (r_P=0.38±0.21, *P*=0.10), testosterone levels were not measured and are known to vary substantially during the breeding season (Hegner and Wingfield, 1987).

The aerobic capacity model postulates that the evolution of endothermy occurred through directional selection towards higher aerobic capacity, allowing for higher sustained vigorous activity, and an entrained increase in rates of resting heat production because of a genetic correlation (r_A) between MMR and BMR (Hayes and Garland, 1995). The strong form of the aerobic capacity model proposes that the positive r_A between BMR and MMR not only was present in proto-endotherms but also should continue to be present in all their descendants (Hayes, 2010). Hence, the strong form of this model should be tested at the genetic level in birds and mammals. Although the r_A between BMR and MMR has been tested several times in mammals (Gębczyński and Konarzewski, 2009; Nespolo et al., 2005; Sadowska et al., 2005; Wone et al., 2009), it has never been tested in birds, which leaves an incomplete knowledge of one of the most significant developments in vertebrate evolution.

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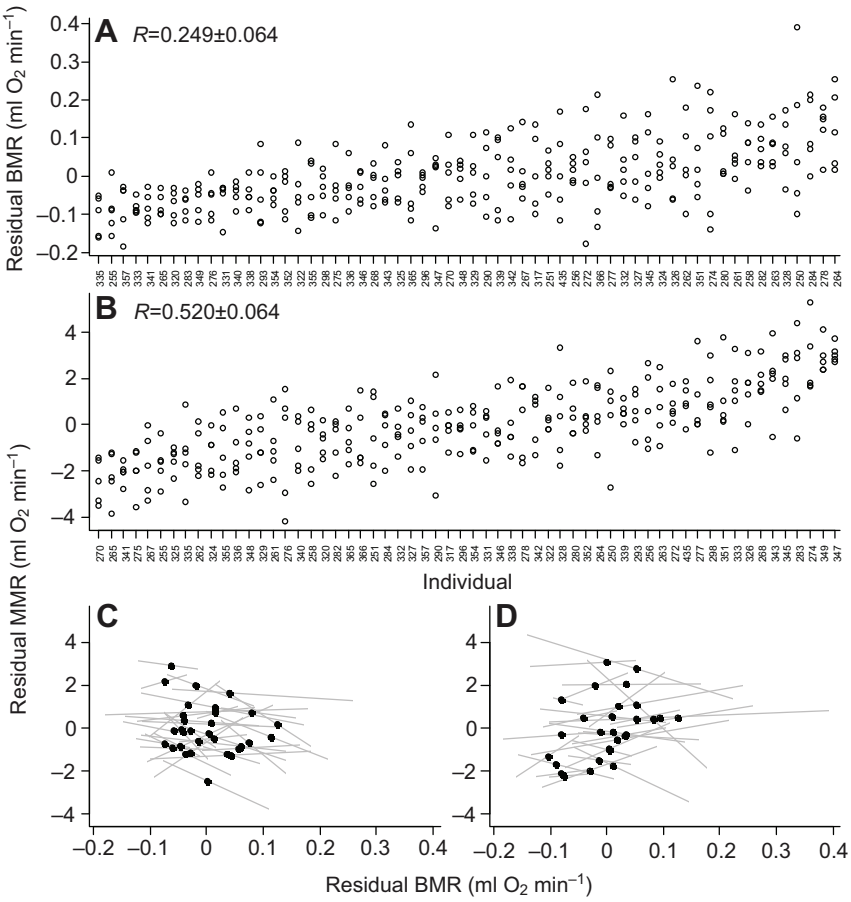

Fig. 1. Among- and within-individual (co)variance in basal metabolic rate (BMR) and maximal metabolic rate (MMR) in house sparrows (*Passer domesticus*). (A,B) Individual variation and repeatability (R ± s.e.) in BMR (A) and MMR (B) in 60 individuals measured five times each, ordered along the *x*-axis according to their mean value (i.e. order differs between panels). BMR and MMR are shown as residuals from a multiple regression model including the same fixed effects as in the bivariate mixed model (see Table 1). (C,D) MMR as a function of BMR in individuals implanted with testosterone-filled capsules (C) and individuals implanted with empty capsules (D). Points in C,D indicate the mean residual BMR and MMR for each individual, thus illustrating the among-individual correlation. Grey lines show linear regression applied for each individual, thus illustrating the within-individual correlation.

Future research should test the presence of a r_A between BMR and MMR in birds by using special breeding designs or extensive pedigree information. In the meantime, our r_{ind} estimate better identifies a potential r_A than do estimates of r_P (Dingemanse and Dochtermann, 2013). To the extent that our relatively high r_{ind} (0.62±0.22) reflects r_A (as opposed to permanent environmental effects), our results suggest that the independent evolutionary potential of BMR and MMR is limited under low testosterone levels. In other words, natural selection towards high MMR would result in an elevated BMR as a correlated response, as assumed by the aerobic capacity model for the evolution of endothermy (Hayes and Garland, 1995). When testosterone levels are high, however, there seems to be a trade-off occurring within individuals that alters the link between BMR and MMR.

MATERIALS AND METHODS

Experimental animals

As part of a study examining the interactive effects of testosterone and immune challenge on metabolic rates (W.A.B., T.W.O., B.J.H., K. C. Klasing and L. B. Astheimer, unpublished observations), we captured 60 (30 males and 30 females) free-living house sparrows residing in the Illawarra region of New South Wales (Australia) between July and August 2005. Birds at this time of year comprise adults and fully mature first-year birds. Birds were distributed with equal sex ratios among four outdoor flight cages (4.5×3.6×2.5 m), with free access to commercial finch seed mix, shell grit and water.

Experimental manipulations

Based on MMR rankings, male and female birds were divided into four experimental groups of individuals that were given: (i) empty capsule and sham injection, (ii) testosterone-filled capsule and sham injection, (iii) empty capsule and immune challenge and (iv) testosterone-filled capsule and immune challenge, with MMR performance distributed evenly among these groups. All male birds were surgically castrated, under anaesthetic within 2 days of initial capture. They were then returned to the flight aviaries and, along with female birds, allowed 2 weeks to adjust to captivity before their BMR and MMR were measured (see below) and blood samples for hormonal and immune characterization were taken (W.A.B., T.W.O., B.J.H., K. C. Klasing and L. B. Astheimer, unpublished observations). Those assigned to testosterone-treatment groups received a single testosterone-filled Silastic subcutaneous implant, while control birds received an empty Silastic implant. MMR and BMR were remeasured 2 weeks later and a blood sample collected to determine testosterone levels and immunity characteristics (trial 2). Males and females implanted with testosterone-filled capsules had plasma testosterone levels that were at physiological levels throughout the study (5.20±0.35 and 5.34±0.50 ng ml^{-1} in males and females, respectively). By contrast, castrated males and females implanted with empty capsules had plasma testosterone levels of 0.49±0.08 and 0.45±0.05 ng ml^{-1}, respectively. Three more rounds of metabolic evaluations and blood collections were made at 2–3 week intervals (trials 3, 4 and 5), with immune challenges (concurrent intra-muscular injection of keyhole limpet haemocyanin and intra-abdominal injection of sheep red blood cells) occurring 1–2 weeks before metabolic measurements. For the sham injections, birds received an intra-muscular and intra-abdominal injection of the same volume of vehicle as the treated birds. Although the treatments did not influence BMR, birds receiving an immune challenge maintained a higher MMR throughout the study compared with sham-treated birds (W.A.B., T.W.O., B.J.H., K. C. Klasing and L. B. Astheimer, unpublished observations).

Respirometry

We measured BMR using a computerized open-circuit respirometry system. Birds had been fasted for 3 h at the beginning of BMR measurements, which started at ~18:00 h (local time) and continued for an additional 12–14 h. For

a given metabolic run, birds were weighed on a digital balance (±0.01 g) and then placed in individual 2 l metabolic chambers fitted with inlet and outlet ports and a perch. Metabolic chambers were placed in a constant-temperature cabinet regulated between 29 and 31°C, which lies within the thermoneutral zone for house sparrows (Chappell et al., 1999). A manifold and mass-flowmeters (Tylan Model FC-280S) provided a constant flow of 500 ml min^{-1} of dry, CO_2-free air to each chamber. Excurrent air from each chamber, along with inlet air from a parallel circuit, was sequentially sampled via an electronic stream selector (Sable Systems Respirometer Multiplexer V 2.0). A 100 ml min^{-1} sub-sample of inlet air or chamber outflow was aspirated from the multiplexer and pulled through Drierite and soda lime before entering the O_2 analyser, which allowed us to sequentially sample baselines and the chambers using Sable Systems Oxzilla II O_2-analysers. We used Warthog Systems LabHelper software to control the multiplexer outputs and record chamber O_2 concentration and chamber temperatures. We used Warthog Systems LabAnalyst to correct the metabolic data for drift between consecutive baseline measures and calculate individual O_2 consumption. BMR was calculated as the mean of the two lowest 5 min averages of O_2 uptake recorded during two separate sampling periods during the 12 h measurement period.

We measured MMR during intense exercise within an enclosed flight wheel. The system is nearly identical to that described elsewhere (Chappell et al., 1999), which reliably elicits maximal rates of exercise metabolism. The setup consists of a revolving 5 l drum with clear sides and carpet lining the inner rim. A mass-flow controller (Tylan Corp.) supplied air to the chamber at 5 l min^{-1} and the O_2 content of inlet and outlet ports was measured with an O_2 analyser (Sable Systems FC-1). Birds were introduced into the chamber, allowed 2 min to settle, and the motor was then activated. The flight drum also contained ping-pong balls, which encouraged birds to maintain a series of rapid take-offs and short-term flights. The O_2-consumption rates were transformed to 'instantaneous' values. The highest instantaneous O_2-consumption rate averaged over a continuous 60 s interval was designated as MMR.

All individuals had their BMR and MMR measured once before the first treatment and again following each of four treatment rounds. Here, our objective was not to evaluate the effect of these experimental manipulations on BMR and MMR (W.A.B., T.W.O., B.J.H., K. C. Klasing and L. B. Astheimer, unpublished observations), but to exploit this dataset to estimate the r_P, r_{ind} and r_e between BMR and MMR after controlling for these potential sources of variance. Moreover, we were interested in evaluating the effect of these experimental manipulations on the r_P, r_{ind} and r_e.

Statistical analysis

We *z*-transformed BMR and MMR (mean=0, variance=1) and analysed them in ASReml-R. All models included fixed effects of body mass, sex, measurement order (five trials), hormone treatment (empty or testosterone-filled capsule), immune treatment (sham or challenge), and an interaction between the two treatments, each fitted separately to BMR and MMR. Using data from all trials, we estimated the r_P between BMR and MMR by fitting a bivariate model that allowed a correlation between the residual variance (V_e) of each trait. Such an analysis is accomplished in a one-step process, which is preferable to a two-step analysis such as when residuals are first calculated and then used for testing correlations (Hayes and Shonkwiler, 1996).

To test whether r_P was affected by testosterone implants and/or immune challenge, we ran a subsequent model in which we allowed treatment-specific V_e and correlation. In addition to providing r_P for each treatment, we compared this heterogeneous model with a reduced model in which r_P was constrained to be equal. Because the reduced model estimates one fewer parameter, we used a likelihood-ratio test with 1 d.f. to test whether r_P was significantly different in males versus females or in individuals that received empty versus testosterone-filled implants, or a sham injection versus an immune challenge.

It is possible to partition r_P into an r_{ind} and r_e whenever two traits are repeatedly assayed simultaneously in a set of individuals (Dingemanse and Dochtermann, 2013), as in our study. We estimated r_{ind} and r_e between BMR and MMR using data from all trials in a bivariate mixed model that included a random effect of individual identity (V_{ind}) fitted to both dependent variables and a correlation between them (i.e. r_{ind}). In this model, the correlation between V_e for each trait provided an estimate for r_e. While the r_{ind} will indicate whether individual mean values of BMR correlate with individual mean values of MMR, the r_e will indicate when an individual's change in BMR between time period t and t+1 is correlated with its change in MMR over the same period (Dingemanse and Dochtermann, 2013). We tested the significance of r_P, r_{ind} and r_e using likelihood-ratio tests.

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Competing interests

The authors declare no competing financial interests.

Author contributions

W.A.B. designed the study; W.A.B., B.J.H. and T.W.O. conducted the experiments; V.C. analysed the data and drafted the manuscript; W.A.B. and B.J.H. revised the manuscript.

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References

Chappell, M. A., Zuk, M., Johnsen, T. S. and Kwan, T. H. (1997). Mate choice and aerobic capacity in red junglefowl. *Behaviour* **134**, 511-529.

Chappell, M. A., Bech, C. and Buttemer, W. A. (1999). The relationship of central and peripheral organ masses to aerobic performance variation in house sparrows. *J. Exp. Biol.* **202**, 2269-2279.

Dingemanse, N. J. and Dochtermann, N. A. (2013). Quantifying individual variation in behaviour: mixed-effect modelling approaches. *J. Anim. Ecol.* **82**, 39-54.

Gębczyński, A. K. and Konarzewski, M. (2009). 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.

Hammond, K. A., Chappell, M. A., Cardullo, R. A., Lin, R. and Johnsen, T. S. (2000). The mechanistic basis of aerobic performance variation in red junglefowl. *J. Exp. Biol.* **203**, 2053-2064.

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

Hayes, J. P. and Garland, T., Jr (1995). The evolution of endothermy: testing the aerobic capacity model. *Evolution* **49**, 836-847.

Hayes, J. P. and Shonkwiler, J. S. (1996). Analyzing mass-independent data. *Physiol. Zool.* **69**, 974-980.

Hegner, R. E. and Wingfield, J. C. (1987). Effects of experimental manipulation of testosterone levels on parental investment and breeding success in male house sparrows. *Auk* **104**, 462-469.

Nespolo, R. F., Bustamante, D. M., Bacigalupe, L. D. and Bozinovic, F. (2005). Quantitative genetics of bioenergetics and growth-related traits in the wild mammal, *Phyllotis darwini*. *Evolution* **59**, 1829-1837.

Sadowska, E. T., Labocha, M. K., Baliga, K., Stanisz, A., Wróblewska, A. K., Jagusiak, W. and Koteja, P. (2005). Genetic correlations between basal and maximum metabolic rates in a wild rodent: consequences for evolution of endothermy. *Evolution* **59**, 672-681.

Swanson, D. L., Thomas, N. E., Liknes, E. T. and Cooper, S. J. (2012). Intraspecific correlations of basal and maximal metabolic rates in birds and the aerobic capacity model for the evolution of endothermy. *PLoS ONE* **7**, e34271.

White, C. R., Schimpf, N. G. and Cassey, P. (2013). The repeatability of metabolic rate declines with time. *J. Exp. Biol.* **216**, 1763-1765.

Wone, B., Sears, M. W., Labocha, M. K., Donovan, E. R. and Hayes, J. P. (2009). Genetic variances and covariances of aerobic metabolic rates in laboratory mice. *Proc. Biol. Sci.* **276**, 3695-3704.