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COMMENTARY

Understanding variation in metabolic rate

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ABSTRACT

Metabolic rate reflects an organism's capacity for growth, maintenance and reproduction, and is likely to be a target of selection. Physiologists have long sought to understand the causes and consequences of within-individual to among-species variation in metabolic rates – how metabolic rates relate to performance and how they should evolve. Traditionally, this has been viewed from a mechanistic perspective, relying primarily on hypothesis-driven approaches. A more agnostic, but ultimately more powerful tool for understanding the dynamics of phenotypic variation is through use of the breeder's equation, because variation in metabolic rate is likely to be a consequence of underlying microevolutionary processes. Here we show that metabolic rates are often significantly heritable, and are therefore free to evolve under selection. We note, however, that 'metabolic rate' is not a single trait: in addition to the obvious differences between metabolic levels (e.g. basal, resting, free-living, maximal), metabolic rate changes through ontogeny and in response to a range of extrinsic factors, and is therefore subject to multivariate constraint and selection. We emphasize three key advantages of studying metabolic rate within a quantitative genetics framework: its formalism, and its predictive and comparative power. We make several recommendations when applying a quantitative genetics framework: (i) measuring selection based on actual fitness, rather than proxies for fitness; (ii) considering the genetic covariances between metabolic rates throughout ontogeny; and (iii) estimating genetic covariances between metabolic rates and other traits. A quantitative genetics framework provides the means for quantifying the evolutionary potential of metabolic rate and why variance in metabolic rates within populations might be maintained.

KEY WORDS: Metabolism, Evolution, Selection, Quantitative genetics

Introduction

Metabolic rate reflects the 'pace of life' and is one of the most widely measured physiological traits. Metabolic rate has been linked to key physiological and life-history traits, including survival, growth, immunity, predation and reproductive output. Although metabolic rate is somewhat predictable – allometric scaling (see Glossary) between mass and metabolic rate is widespread, for example – variation is still substantial. Among species, there is a severalfold magnitude of difference in basal metabolic rate among individuals of the same mass (White and Kearney, 2013). At the level at which selection (see Glossary) operates (i.e. within species), basal metabolic rate can also vary considerably (Konarzewski and Ksiazek, 2013). This variation has long intrigued physiologists, and various hypotheses have been proposed to understand it (Glazier, 2005). As such, the field has been dominated by studies that seek to understand the proximal causes of variation – the biochemical and physiological mechanisms underlying the response of metabolic rate to biotic and abiotic drivers (as reviewed by Glazier, 2005). For example, the rate-of-living hypothesis (see Glossary) (Rubner, 1908) proposes that metabolic rate inversely determines longevity, based on observations that species with higher metabolic rates have shorter lifespans, although this remains controversial (Speakman, 2005; Glazier, 2015). More recent mechanistic explanations that seek to link metabolic rate to the pace of life have been proposed (Nilsson, 2002). The 'compensation hypothesis' (or 'allocation hypothesis') (see Glossary) suggests a fitness advantage of lower basal or standard metabolic rates as a result of lower maintenance costs, and thereby greater allocation of energy to reproduction (Gadgil and Bossert, 1970; Steyermark, 2002; Larivée et al., 2010). Alternatively, higher basal or standard metabolic rates allow for greater energy turnover and synthesis and maintenance of larger organs, leading to greater reproductive yield, known as the 'increased-intake hypothesis' (see Glossary) (Bennett and Ruben, 1979; Hayes et al., 1992). While these approaches may assign causation to an immediate response, exclusively mechanistic approaches have had limited success predicting how traits evolve. One key limitation with the mechanistic approach is that it lacks standardized methods to compare across studies. Meanwhile, phenomenological approaches such as those used in evolutionary biology are under-utilized in studies of metabolic rate.

Evolutionary biology seeks to understand the ultimate causes of variation in traits – causes that are a consequence of many generations of selection (Mayr, 1961). Darwin first observed that natural selection operating within populations ultimately shapes heritable differences among species. Estimates of the heritability (see Glossary) of metabolic rate vary widely, but are often more than zero (see 'Genetic variation in metabolic rates' section below). Within-population studies elucidate the selective forces acting on individuals, and the underlying genetic processes that constrain their evolution (see Glossary). To understand patterns in metabolic rates, and predict how they are likely to evolve under selection, it is necessary to measure 'performance' as fitness – the lifetime reproductive output of an individual – and to determine how fitness covaries with metabolic rates throughout ontogeny. Although metabolic rate is likely to evolve in response to selection, underlying genetic constraints may alter its evolution in ways that have yet to be considered in many physiological studies (Arnold, 1988), with some notable exceptions (e.g. Garland and Carter, 1994). We argue that quantitative genetics (see Glossary) provides a powerful framework for understanding the inheritance and evolution of traits, including their responses to selection.

Quantitative genetics partitions the population-level phenotypic variation of quantitative traits (see Glossary) into heritable and non-heritable components through measures of heritability and genetic correlation (see Glossary), and links those components to fitness via measures of selection. We emphasize three key advantages of

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Glossary

Additive genetic variance (V_A)
The magnitude of the total variance, due to the additive effects of each gene. The extent to which the average phenotype of the parent is reflected in the offspring, and the response to selection on a quantitative trait, is proportional to V_A.
Allometric scaling
The relationship between the mass of an organism and its metabolic rate, where the slope of the log-log scaled relationship is less than 1 (i.e. non-isometric).
Breeder's equation
A tool developed to predict the amount of change in a single trait from one generation to the next: $R=h^2S$, where h^2 is narrow-sense heritability (the ratio of additive genetic variance to total phenotypic variance) and S is the selection differential (the change in population mean after selection).
Breeding values
The sum of the average effect of alleles carried by an individual.
Compensation hypothesis ('allocation hypothesis')
Hypothesis whereby lower metabolic rates confer a fitness advantage as a result of lower maintenance costs, and thereby greater allocation of energy to reproduction.
Correlational selection
Form of non-linear, multivariate selection where a combination of two or more traits interact non-additively to affect fitness.
Directional selection
Form of univariate selection characterized by a linear fitness function, causing an increase or decrease in the population mean trait value.
Disruptive selection
Form of non-linear, quadratic selection (see 'Quadratic selection') favouring individuals with extreme trait values. Under constant disruptive selection, the trait variance of a population will increase.
Evolution
The change in heritable traits of a population across generations.
Fitness
The number of surviving offspring produced by an individual after a single generation.
G matrix
Matrix of genetic variances and covariances, which summarizes the inheritance of multiple, phenotypic traits.
Genetic correlation
A standardized version of genetic covariance (see definition below) that varies from –1 to 1.
Genetic covariance
The correlation between the breeding values for different traits.
Genetic variance (V_G)
The value of the effect of all an individual's genes that affect the trait of interest. Genetic variance has three main components: additive genetic variance, dominance variance and interaction (epistatic) variance.
Heritability (H^2 or h^2)
Proportion of variance in a phenotypic character in a population due to individual genetic differences that are inherited by offspring. Broad-sense heritability refers to the ratio of total genotypic variance to phenotypic variance ($H^2=V_G/V_P$), while narrow-sense heritability refers to the ratio of additive genetic variance to phenotypic variance ($h^2=V_A/V_P$).
Increased-intake hypothesis
Hypothesis relating performance with metabolic rates, where higher metabolic rates allow for greater energy turnover and synthesis of larger organs, leading to greater reproductive yield.
Indirect selection
Selection on one trait that arises from selection on another trait that is genetically correlated.
Linear selection
See 'Directional selection'.
Microevolution
Within-species evolutionary change over short time scales, e.g. changes in gene frequencies within a population.

Glossary (cont.)

Non-linear selection
Univariate (see 'Quadratic selection') or multivariate (see 'Correlational selection') selection that is non-linear.
Quadratic selection
A form of non-linear, univariate selection that can also be positive (convex/disruptive) or negative (concave/stabilizing).
Quantitative genetics
The study of inheritance of genetically complex traits.
Quantitative trait
A trait that may be influenced by multiple genes, showing continuous variation in a population.
Rate-of-living hypothesis
Theory proposed by Rubner (1908) that lifespan is inversely related to metabolic rate, based on observations that larger animals with slower metabolic rates outlive smaller organisms with faster metabolic rates.
Selection
The differential survival and reproduction of individuals with varying phenotypes within a population. The covariance between fitness and a trait.
Selection coefficient (s)
Difference in relative fitness.
Selection differential (S)
Difference between the mean trait value of the population before and after selection.
Selection gradient
The slope (linear β, and non-linear γ) of the regression of fitness on a trait value.
Stabilizing selection
Form of non-linear, quadratic selection (see 'Quadratic selection') favouring individuals with intermediate trait values. Under constant stabilizing selection, the trait variance of a population will decrease.

studying metabolic rate within a quantitative genetics framework. (1) Formalism: evolutionary biologists have been thinking about the ultimate processes driving variation in traits since Darwin; microevolutionary theory and the powerful statistical tools developed from this work have been widely applied in the evolution community for over 40 years, and can be leveraged by physiological studies. (2) Predictive: microevolutionary approaches allow us to quantify how traits are likely to evolve given specific selection and genetic parameters. (3) Comparative: quantitative genetics provides standardized estimates of selection and heritability that are directly comparable among populations, species and environments. Here we advocate for wider adoption of the quantitative genetics approach in physiological studies in order to gain insights into evolutionary causes and consequences of variation in metabolic rate.

The breeder's equation as a framework

The breeder's equation (see Glossary) is a fundamental tool used in quantitative genetics for understanding phenotypic evolution in response to selection, and has been used by evolutionary biologists for over 50 years. Quantitative traits have phenotypes that are continuously distributed in natural populations, and include morphological, physiological, behavioural and molecular phenotypes. Like other quantitative traits, metabolic rates are likely to be genetically complex and sensitive to environmental conditions. Quantitative genetic variation underlies phenotypic evolution – measuring the genetic basis of variation in quantitative traits is therefore essential to understanding variation in phenotypes, such as metabolic rates. The univariate breeder's equation predicts the amount of change in a single trait from one generation to the next in response to selection. The response of a quantitative trait to selection, R, is described by the breeder's equation:

$$R = h^2 S, \quad (1)$$

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where h^2 is narrow-sense heritability [the ratio of additive genetic variance (see Glossary) to total phenotypic variance; see 'Genetic variation in metabolic rates' section below], and S is the selection differential (the change in population mean after selection) (see Glossary). The breeder's equation serves as a simple, but powerful, tool for understanding variation in metabolic rate and other physiological traits.

Univariate selection on metabolic rate

Selection is the phenotypic covariance between a trait and fitness, where fitness of an individual is determined by the contribution of offspring to the next generation (Falconer and Mackay, 1996). If fitness covaries with a trait, then that trait is said to be under selection. This relative difference in fitness among phenotypes (selection) forms one half of the breeder's equation and provides a standardized estimate of the strength and direction in which evolution is expected to occur, if the trait has adequate genetic variation. The slope of the relationship between relative fitness and a particular character (i.e. selection coefficient), weighted by the phenotype distribution, represents standardized estimates of selection.

Two general forms of univariate selection can occur: linear and quadratic selection (Box 1). Linear selection (see Glossary) occurs when fitness (w) consistently increases or decreases with the value of a trait (z), and is fitted by a linear function:

$$w = \alpha + \beta z, \quad (2)$$

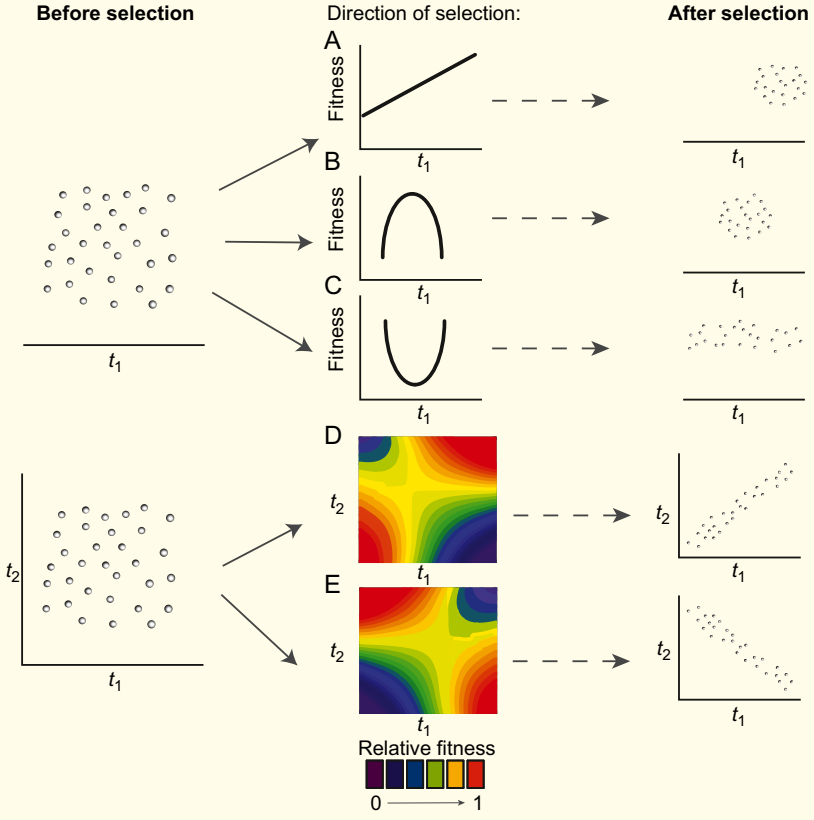
where α is the intercept of the fitness function, and β is the coefficient giving the direction (positive or negative) and magnitude of selection. If a trait exhibits sufficient genetic variation (i.e. if it is heritable) and not constrained by other traits that are also correlated with fitness (see 'Metabolic rate is more than a single trait' section below), persistent directional selection (see Glossary) *should* result in a shift in the mean trait of a population (Kingsolver and Pfennig, 2007). Quadratic selection is characterized by a non-linear fitness function that can also be positive (disruptive) or negative (stabilizing), and is described by the quadratic fitness function:

$$w = \alpha + \beta + (1/2)\gamma z^2, \quad (3)$$

where γ is the degree of curvature in the fitness function. Selection is stabilizing when β is 0 and γ is negative, such that intermediate values of a trait possess highest fitness while extreme trait values have lowest fitness. Selection is disruptive when β is 0 and γ is positive. Where disruptive selection (see Glossary) is maintained across generations, population variance will increase as selection favours trait values on the tail ends of the trait distribution. Under constant stabilizing selection (see Glossary), there is a single optimal

Box 1. Predicted population-level response to persistent univariate and multivariate selection

(A) Directional selection: in this example the linear coefficient of selection β is positive. Over generations, the population mean of trait 1 (t_1) is expected to increase. (B) Stabilizing selection: where the quadratic coefficient γ is negative. Over generations, the population variance will decrease, forming a single optimum for t_1. (C) Disruptive selection: where the quadratic coefficient γ is positive. Over generations, the population variance will decrease, forming two optima for t_1. (D) Positive correlational selection on t_1 and trait 2 (t_2) (where γ is positive) produces an increase in the covariance between t_1 and t_2. (E) Negative correlational selection on t_1 and t_2 (where γ is negative) produces a decrease in the covariance between t_1 and t_2.



value for a phenotype, hence variance in population traits would be expected to decrease over generations. Note that quadratic selection can occur when $\beta \neq 0$; this is termed either concave (stabilizing) or convex (disruptive) selection. By providing comparable estimates of selection on metabolic rate, selection analyses have the potential to leverage comparative data (i.e. comparing values of β and γ) that vary across spatial and temporal scales, study systems and phenotypic characters (Kingsolver et al., 2001). Indeed, the idea that a single value of a trait is consistently beneficial under all circumstances seems unlikely, and the same is true of metabolic rate. Spatial and temporal variation in selection therefore seems likely to be a mechanism by which variance in metabolic rate is maintained. To some extent, selection analyses have already been implemented in the general mechanisms that have been proposed to explain variation in metabolic rates, i.e. covariance between metabolic rate and some measure of performance (fitness). The increased-intake and compensation hypotheses point towards positive and negative directional selection on (basal or standard) metabolic rate, respectively, for example, while the 'context dependent' hypothesis (Burton et al., 2011) points toward selection gradients (see Glossary) that vary in space and time. The approach we advocate is therefore not incompatible with proximate mechanistic approaches; rather selection analyses provide the formalism and standardized measures required to make comparable estimates for the relationship between metabolic rate and fitness.

In order to gain reliable estimates of selection, studies need to measure actual fitness (see Glossary). So far, selection studies on metabolic rate have relied almost exclusively on the use of fitness proxies, such as survival, growth or reproductive traits such as clutch size, rather than the ultimate measure of fitness: lifetime reproductive output (Box 2). This view is illustrated by the compilations of Biro and Stamps (2010), Burton et al. (2011) and White and Kearney (2013). The tables summarizing the known phenotypic correlations between metabolic rate and fitness proxies in these papers do not provide any examples of a correlation between metabolic rate and actual fitness.

Using fitness proxies can create misleading or incomplete interpretations of the strength and direction of selection if these proxies trade-off with actual fitness. For example, Pettersen et al. (2016) show that metabolic rates through ontogeny covary with actual fitness (lifetime reproductive output) as well as several fitness proxies, but the direction and magnitude of the covariance differs among measurements of metabolic rate. Fitness was maximized when individuals had low metabolic rates early in ontogeny (MR_E) but high metabolic rates later (MR_L) (or vice versa). Although we found evidence for correlational selection (see Glossary) alone based on true fitness, estimates based on fitness proxies incorrectly implied that directional selection was operating. For example, individuals with higher MR_E reproduced sooner, but individuals with lower MR_L were longer lived, and growth rate was maximized when MR_E was high and MR_L was low. In this case, using any of the commonly used proxies for fitness (growth rate, longevity, age at the onset of reproduction) would lead to wildly different, and incorrect, conclusions about the expected evolutionary trajectory of metabolic rate.

Genetic variation in metabolic rates

As the breeder's equation elegantly illustrates, selection on a trait will not generate evolution of that trait unless the trait is heritable. The capacity for metabolic rates to evolve thus depends not only on covariation between metabolic rate and fitness, but also on the other half of the breeder's equation – the genetic basis of variation in metabolic rate. The total phenotypic variance of a trait (V_P) is the sum of the variances attributable to genetic (V_G) and environmental (V_E) influences (including maternal effects), and the variance associated with the interaction between genetic and environmental influences (V_{GE}). V_G can be further subdivided into three components: additive (V_A), dominance (V_D) and interaction (V_I) variance, where collectively V_D and V_I are known as non-additive genetic variance and are not easily disentangled using standard quantitative genetics designs. V_A quantifies deviations from the mean phenotype attributable to the additive contribution of particular alleles to the phenotype; V_D quantifies interactions

Box 2. Compilation of studies measuring the relationship between metabolic rates and survival or reproductive output as fitness proxies

Species	MR measure	Fitness proxy	Reference
Laboratory studies			
Microgale dobsoni (shrew tenrec)	RMR	Litter size, neonate mass, litter mass (+)	Stephenson and Racey, 1993
Mus musculus (laboratory mouse)	RMR	Litter size (+), mean offspring mass (–)	Johnson et al., 2001
Taeniopygia guttata (zebra finch)	DEE	Clutch size (+), clutch mass (+), brood mass (+)	Vezina et al., 2006
Field studies			
Bugula neritina (marine bryozoan)	Unspecified	Reproductive output (negative correlational)	Pettersen et al., 2016*
Cornu aspersum (garden snail)	SMR	Survival (stabilizing)	Bartheld et al., 2015*
Cyanistes caeruleus (blue tit)	BMR	Survival (+ and –)	Nilsson and Nilsson, 2016
Helix aspersa (garden snail)	SMR	Juvenile survival (– and stabilizing)	Artacho and Nespolo, 2009*
Microtus agrestis (short-tailed field vole)	RMR	Over-winter survival (+)	Jackson et al., 2001
Microtus oeconomus (root vole)	RMR	Survival (+)	Zub et al., 2014
Myodes glareolus (bank vole)	BMR	Reproductive success (+)	Boratynski and Koteja, 2010*
Myodes glareolus (bank vole)	BMR	Over-winter survival (–)	Boratynski et al., 2010*
Salmo salar (Atlantic salmon)	MR	Survival (+, – and no relationship)	Robertsen et al., 2014
Tamiasciurus hudsonicus (red squirrel)	RMR	Over-winter survival (–)	Larivee et al., 2010*
Tamiasciurus hudsonicus (red squirrel)	DEE	Annual reproductive success (+)	Fletcher et al., 2015*
Tamias striatus (eastern chipmunks)	RMR	Juvenile survival (stabilizing)	Careau et al., 2013*
Zootoca vivipara (common lizard)	RMR	Survival (–)	Artacho et al., 2015*

Symbols in parentheses indicate the direction/form of significant selection on metabolic rates. BMR, basal metabolic rate; SMR, standard metabolic rate; DEE, daily energy expenditure; MR, maintenance metabolic rate. *These studies use a multiple regression framework, providing standardized and comparable estimates of selection [i.e the Lande and Arnold (1983) approach].

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between alleles (dominance) and V_I quantifies interactions between alleles (epistasis). Heritability in the broad sense (H^2) is calculated as V_G/V_P, whereas heritability in the narrow sense (h^2) – the metric of interest for the breeder's equation – quantifies the contribution of additive genetic variance to total phenotypic variance and is calculated as V_A/V_P.

The heritability of a trait can be estimated in multiple ways (Box 3), but a common feature of all approaches is that they require the measurement of usually hundreds or thousands of individuals of known pedigree. The requirement to measure so many individuals means that estimates of h^2 for metabolic rate are historically rare, but are becoming much more common: we are aware of only two estimates published prior to 2000 (Lacy and Lynch, 1979; Lynch and Sulzbach, 1984), and most (43) of the remaining 64 estimates we were able to locate have been published since 2010. The available estimates range from 0 to 0.72; h^2 is significantly higher for endotherms than for ectotherms, and h^2 is significantly higher for active metabolic levels than for resting metabolic levels, defined here as the rate of oxygen consumption of an inactive, non-reproductive, post-absorptive animal (Box 4). These heritability estimates suggest that metabolic rate is, in many cases and especially for endotherms and for active metabolic rates, likely to be free to evolve under selection. In support of this suggestion, artificial selection experiments have yielded responses to selection on basal metabolic rate (Ksiazek et al., 2004) and maximum metabolic rate in laboratory mice (Gebczynski and Konarzewski, 2009; Wone et al., 2015), and maximum metabolic rate in bank voles *Clethrionomys glareolus* (Sadowska et al., 2015).

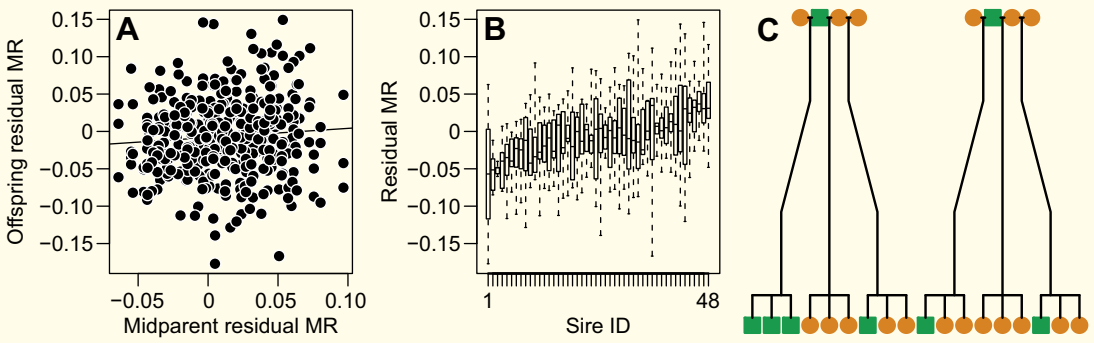
After accounting for genetic contributions to phenotypic variance, there remains a significant proportion of unexplained variation in metabolic rate that needs to be considered. Variation in metabolic rate may also be a consequence of environmental effects, which can affect metabolic rate either directly (e.g. temperature effects on metabolic rate in ectotherms; Angilletta et al., 2002), or indirectly (e.g. nutritional state on standard metabolic rate; Auer et al., 2015). Parental effects are also known to influence physiological traits (e.g. Bacigalupe et al., 2007; Sadowska et al., 2013). For example, brown trout may alter the routine metabolic rates of their offspring in order to control timing of emergence and therefore dispersal in larvae (Régnier et al., 2010). Addressing the relative importance of heritable versus non-heritable components of variation in metabolic rate will provide a more complete picture of how we expect variation in metabolic rate to evolve.

Multivariate breeder's equation

The univariate breeder's equation is a useful heuristic tool for understanding how microevolutionary processes (see Glossary) work. Increasingly, however, it seems that a more complex approach to predicting microevolution is necessary. The univariate breeder's equation necessarily treats each trait in isolation but it has long been recognized that no trait is an island (Dobzhansky, 1956). Traits covary with each other genetically such that evolution in one trait will necessarily cause evolution in another, and selection often acts on multiple traits simultaneously such that the fitness returns of one trait value depend on the value of other traits. The multivariate breeder's equation reflects this complexity and connectedness of traits in terms of both genetics and selection.

Box 3. Methods for estimation of the heritability of metabolic rate (parent–offspring regression, half-sibling–full sibling breeding designs and the 'animal model'), and a compilation of published estimates of the heritability of metabolic rate

(A) Parent–offspring regression showing the relationship between parent and offspring metabolic rate for cockroaches *Nauphoeta cinerea* from a breeding design in which 48 sires were each mated to three dams, and the metabolic rates of all sires and dams, and three of the adult offspring from each clutch, were measured (Schimpf et al., 2013). Narrow sense heritability is estimated as the slope of the line relating offspring and mid-parent trait values; here residual metabolic rates were calculated from a model describing variation in $\log_{10}$-tranformed metabolic rate as functions of $\log_{10}$-transformed body mass and sex (h^2=0.12±0.07, mean±s.e.m.). (B) Among-sire differences in residual resting metabolic rate for cockroaches *Nauphoeta cinerea* from the same experiment (Schimpf et al., 2013). Half-siblings have one-quarter of their alleles in common, so in a half-sibling–full sibling breeding design, the among-sire variance (V_{sire}) is equal to one-quarter of the additive genetic variance (V_A), and $h^2=4V_{sire}/V_P$, where V_P is total phenotypic variance. In the example in (B), which utilizes only the data for the adult offspring (i.e. those individuals with a known sire and dam; sires and dams of the parental generation are unknown), sire and dam variances were calculated for a model describing variation in $\log_{10}$-tranformed metabolic rate as functions of the fixed effects of sex and $\log_{10}$-transformed body mass, with random effects for sire and dam nested within sire estimated using restricted maximum likelihood (REML); h^2=0.10±0.16 [the model was implemented in ASReml-R version 3.0 in R version 3.0.2, with standard errors for variance ratios calculated using the delta method; Gilmour et al. (2009); http://www.homepages.ed.ac.uk/iwhite//asreml/; http://www.R-project.org/]. For presentation, residual metabolic rates were calculated from a model describing variation in $\log_{10}$-tranformed metabolic rate as functions of $\log_{10}$-transformed body mass and sex, and data are shown ranked by the mean value of metabolic rate for each sire. (C) The 'animal model' is a form of mixed-effects model used to partition phenotypic variance into different genetic and environmental sources using knowledge of the relatedness of individuals in a population (Wilson et al., 2010), such as depicted here for the descendants of two sires in the cockroach half-sibling–full sibling breeding design (males are green squares and females are orange circles). Calculated using the animal model, h^2=0.12±0.07 (the model was implemented in ASReml-R version 3.0 in R version 3.0.2, with standard errors for variance ratios calculated using the delta method).



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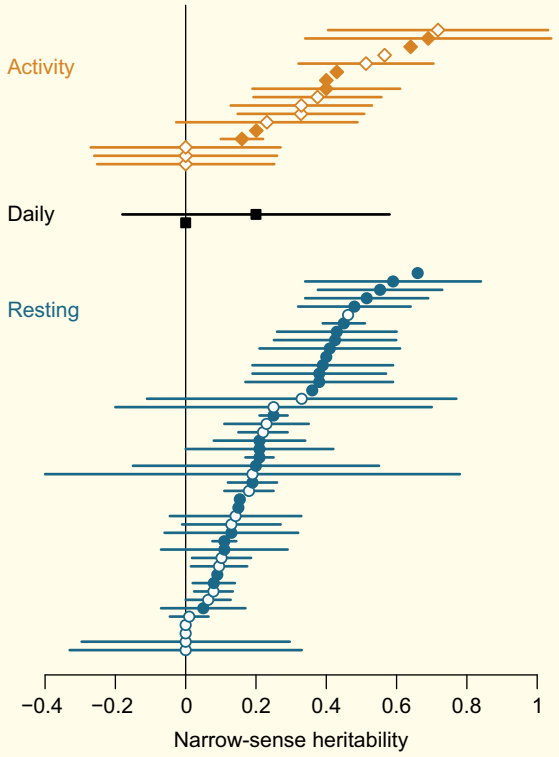
Box 4. Forest plot summarizing published estimates of the narrow-sense heritability (h^2 shown with s.e.m. where possible) of metabolic rate for endotherms and ectotherms, subdivided by activity level

Filled symbols, endotherms; open symbols, ectotherms. Resting: resting, basal or standard metabolic rate (blue circles). Daily: daily rate of energy expenditure or sustained metabolic rate (black squares). Activity: peak metabolic rate, flight metabolic rate, maximum metabolic rate, or maximum rate of oxygen consumption elicited by treadmill exercise or swimming (orange diamonds). With the two values for daily metabolic rate excluded from analysis, there was no significant interaction between activity level (resting or active) and endothermy (endotherm or ectotherm) as predictors of h^2 in a mixed model including random effects of species and publication [$t_{56.4}$=−0.61, P=0.54; the model was implemented in the 'lme4' version 1.1-13 package of R version 3.2.3, with the significance of fixed effects based on Satterthwaite approximation for denominator degrees of freedom from the 'lmerTest' package version 2.0.33: Bates et al. (2015); http://cran.r-project.org/package=lmerTest; http://www.R-project.org/]. With the non-significant interaction removed from the model, h^2 is significantly different from zero (intercept=0.19±0.06, $t_{13.2}$=3.00, P=0.01), endotherms have significantly higher h^2 than ectotherms (parameter estimate=0.19±0.08, $t_{12.8}$=2.38, P=0.03) and h^2 is higher for active metabolic levels than for resting metabolic levels (parameter estimate=0.21±0.05, $t_{54.6}$=4.3, P<0.001); estimates of h^2 for mass-independent metabolic rates were not significantly different from estimates of h^2 for whole-animal metabolic rates (parameter estimate=−0.08±0.05, $t_{56.0}$=−1.67, P=0.10); variance components: species=0.0159, publication <0.0001, residual=0.0197.



Consider the response to selection of a trait, which we will call trait 1 (z_1). As described by the univariate breeder's equation, the evolution of that trait will of course depend on the selection on that trait (β_1) and the genetic variation in that trait (which we will denote as $G_{1,1}$). However, let us suppose that another trait (trait 2) covaries genetically with trait 1; such a covariance would be denoted as $G_{1,2}$. Let us also suppose that trait 2 is under selection (β_2). The response of trait 1 (Δz_1) will therefore be the sum of the evolution due to direct selection on trait 1 and the indirect selection (see Glossary) on trait 1 via the genetic covariance (see Glossary) with trait 2 and selection on trait 2, or formally:

$$\Delta z_1 = (\beta_1 \times G_{1,1}) + (\beta_2 \times G_{1,2}). \quad (4)$$

Furthermore, the covariance between traits 1 and 2 will also be affected by the correlational selection on these traits, formally represented as $\gamma_{1,2}$ (see below). Eqn 4 can be extended to as many traits that genetically covary and experience selection:

$$\begin{bmatrix} \Delta z_1 \\ \Delta z_2 \\ \vdots \\ \Delta z_n \end{bmatrix} = \begin{bmatrix} \beta_1 \\ \beta_2 \\ \vdots \\ \beta_n \end{bmatrix} \begin{bmatrix} G_{1,1} & G_{1,2} & \cdots & G_{1,n} \\ G_{1,2} & G_{2,2} & \cdots & \vdots \\ \vdots & \vdots & \ddots & \vdots \\ G_{1,n} & \cdots & \cdots & G_{n,n} \end{bmatrix}, \quad (5)$$

where the column vector of changes in phenotypic trait values for n traits, $\Delta z = \{\Delta z_1, \Delta z_2, \ldots \Delta z_n\}^{\mathrm{T}}$, is a function of a column vector of selection gradients $\beta = \{\beta_1, \beta_2, \ldots\}^{\mathrm{T}}$ and a matrix of genetic variances and covariances [the G matrix (see Glossary)]. Although more complicated, a quick consideration of a realistic but simple example reveals why the multivariate equation provides a more complete understanding of the microevolutionary forces acting on metabolic rate. Suppose for example that trait 1 is metabolic rate and trait 2 is running speed in a hypothetical lizard species. Further assume that metabolic rate is subjected to strong negative directional selection (i.e. β_1 is negative) and that the heritability of metabolic rate is high, because the trait has significant additive genetic variance ($G_{1,1}$>0). The univariate breeder's equation and the first component of the multivariate breeder's equation ($\beta_1 \times G_{1,1}$) would therefore predict that metabolic rate would decrease from one generation to the next. However, further suppose that metabolic rate covaries positively with running speed ($G_{1,2}$ is positive) and there is strong positive directional selection for faster running speeds (β_2 is positive). The second component of the multivariate breeder's equation ($\beta_2 \times G_{1,2}$) would therefore be highly positive and might 'cancel out' the selection for lower metabolic rate in the first term. Thus by considering more traits, we move from a misleading prediction of an evolutionary response to a more accurate one. Unfortunately, there is no magic number of traits that should be considered; instead we are left with the rather unsatisfying statement that more traits are likely to be more informative than fewer traits. A multivariate view of evolution is particularly important for considerations of metabolic rate specifically for at least two reasons: first, because metabolic rate is likely to be more than just a single trait, and second, because metabolic rate is almost certainly under multivariate selection.

Metabolic rate is more than a single trait

What is metabolic rate? Measures of metabolic rate integrate the rates at which organisms expend energy to do metabolic work, and so incorporate energy expenditure for a wide range of processes including the maintenance of homeostasis, growth and reproduction, movement and digestion. Metabolic rate is measured as the rate of heat production by direct calorimetry, or – more often – is estimated from rates of oxygen consumption or carbon dioxide production measured by indirect calorimetry (Lighton, 2008). Metabolic rate can be measured for animals that are free-living in the field; for animals at rest; for animals experiencing elevated metabolic rates due to exercise, digestion, lactation, thermogenesis or osmoregulation; or for animals exhibiting depressed metabolism due to hibernation or torpor,

hypoxia or anoxia, desiccation or aestivation (Suarez, 2012). The major contributors to whole-organism metabolic rate will change as animals transition through these metabolic states, raising the important question of the extent to which they are constrained to always evolve together ('metabolic rate' is a single trait), or free to evolve independently ('metabolic rate' is many traits). In mammals, most metabolic activity during basal metabolism is associated with the internal organs including liver, kidney, gastrointestinal tract, heart and brain. Whereas during exercise-induced maximal metabolism, most (ca. 90%) metabolic activity is associated with work done by the locomotor muscles and the work done to deliver substrates and oxygen to these (reviewed by White and Kearney, 2013). From a mechanistic perspective, it therefore seems reasonable to conclude that these metabolic states represent different traits. From a quantitative genetics perspective, however, what matters is the extent to which two putative traits covary genetically. Published mass-independent additive genetic correlations between basal and running-induced maximal metabolic rate range from 0.21 to 0.72 (Dohm et al., 2001; Wone et al., 2009, 2015). Thus these traits – basal and maximal metabolic rate – are at least somewhat free to evolve independently, as has been demonstrated in selection experiments (Sadowska et al., 2015; Wone et al., 2015). What is less clear, however, is the extent to which measurements of a single metabolic state, but taken at different times, represent the same trait.

Two measurements of the same phenotype can be considered a single trait genetically only if they covary perfectly. Resting metabolic rate (as defined earlier) is perhaps the most widely measured physiological phenotype. Resting metabolic rate is repeatable (Nespolo and Franco, 2007; White and Kearney, 2013; Auer et al., 2016a) and heritable (see 'Genetic variation in metabolic rates' section above, and Box 4), but not perfectly so. It varies during ontogeny due to changes in size and growth (e.g. Moran and Wells, 2007; Rosenfeld et al., 2015), seasonally (e.g. Smit and McKechnie, 2010), geographically (e.g. Broggi et al., 2007), with food deprivation (e.g. Schimpf et al., 2012), due to changes mitochondrial coupling (Salin et al., 2015), and in response to a range of other biotic and abiotic variables (reviewed by Konarzewski and Książek, 2013; White and Kearney, 2013). Furthermore, not only does metabolic rate vary over time in the same individuals, but individuals can vary in the flexibility of their metabolic rate – in other words, the reaction norm of metabolic rate varies among individuals (Auer et al., 2015, 2016b). Thus an organism has no single metabolic rate, even for a single well-defined metabolic state (e.g. resting metabolic rate), and metabolic rate is therefore likely to be more than one single trait. Even if differences in metabolic rate throughout the life history were trivial, we know from a previous study that selection perceives metabolic rates (and their combinations) differently (Pettersen et al., 2016). In Pettersen et al. (2016), metabolic rate was only measured at two time points in the life history – both during early stages of development, which is unlikely to capture a complete picture of selection. We therefore suggest that the field should work towards gaining multiple measures of metabolic rate if we are to gain an accurate representation of net selection on metabolic rates. We acknowledge the considerable logistical challenges associated with doing so, but we nonetheless advocate treating metabolic rate at different times as separate traits as a useful heuristic for future studies.

Multivariate selection on metabolic rates

Selection acts on combinations of traits, rather than individual traits in isolation (Lande and Arnold, 1983; Blows and McGuigan, 2015). Multivariate (or non-linear correlational) selection examines how selection affects, and is affected by, correlations between traits (Phillips and Arnold, 1989). Studies measuring selection on metabolic rate have largely focused on relationships between fitness and single traits (although see Artacho et al., 2015); however, univariate analyses provide limited scope for predicting change in phenotypic distribution (Phillips and Arnold, 1989). This is because apparent selection on one trait may be due to selection on another unmeasured, correlated trait – resulting in misleading conclusions about selection on the initial trait. Genetically coupled traits will not evolve independently; selection on one trait is likely to cause evolutionary changes in the other trait. For example, selection on metabolic rate early in ontogeny (MR_E) may yield a correlated response in metabolic rate late in ontogeny (MR_L) if MR_E and MR_L are positively genetically correlated, even if there is no direct selection on MR_L. Metabolic rate is known to show additive genetic correlations with a range of traits including body mass (Rønning et al., 2007; Nilsson et al., 2009; Tieleman et al., 2009; Careau et al., 2011; Schimpf et al., 2013), maximum metabolic rate (Sadowska et al., 2005; Wone et al., 2009, 2015), growth rate (Sadowska et al., 2009), the ability to cope with a poor diet (Sadowska et al., 2009) and exploratory behaviour (Careau et al., 2011). These and other additive genetic correlations may constrain the evolution of metabolic rate, but such constraints would not be identifiable in a univariate framework that considers metabolic rate in isolation. If several traits are measured, however, a multivariate approach can determine relative direct and indirect selection acting on each trait through multiple regression.

Correlational selection favours certain combinations of traits, and is measured using second-order polynomial regression to produce a fitness surface that is a function of linear and squared (quadratic) trait values:

$$w = \alpha + z\beta^T + (1/2)z^T\gamma z, \tag{6}$$

where $z = \{z_1, z_2, \ldots z_n\}^T$ is a column vector of phenotypic values for n traits, $\beta = \{\beta_1, \beta_2, \ldots\}^T$ is the column vector of directional selection gradients, and γ is the matrix of non-linear selection (see Glossary) gradients:

$$\gamma = \begin{bmatrix} \gamma_{1,1} & \gamma_{1,2} & \cdots & \gamma_{1,n} \\ \gamma_{1,2} & \gamma_{2,2} & \cdots & \vdots \\ \vdots & \vdots & \ddots & \vdots \\ \gamma_{1,n} & \cdots & \cdots & \gamma_{n,n} \end{bmatrix}, \tag{7}$$

where $\gamma_{i,i}$ is a stabilizing or disruptive selection gradient for trait i, and $\gamma_{i,j}$ is a correlational selection gradient for traits i and j (Stinchcombe et al., 2008). Note that in the univariate case where correlational selection is not considered, Eqn 6 simplifies to:

$$w = a + z_i\beta_i + (1/2)g_{i,i}z_i^2, \tag{8}$$

(i.e. Eqn 1). Despite the importance of estimating correlational selection for providing a more complete visualization of the distribution of phenotypes, studies that measure correlational selection on physiological traits are rare.

In a study on a bryozoan, Pettersen et al. (2016) found significant negative correlational selection between metabolic rates across two life stages [early (MR_E) and late (MR_L) in juvenile development], but positive phenotypic covariance between these traits (individuals with high MR_E generally possessed high MR_L and vice versa). In other words, there is a positive covariance between the two metabolic rates but selection 'wants' to decrease this covariance. Furthermore, under persistent correlational selection across

generations, we might expect the positive covariance among metabolic rates to decrease and become negative over time. However, without an understanding of the degree of genetic covariance among traits (such as metabolic rates across ontogeny), our capacity to make such predictions remains limited.

Conclusions and future directions

Metabolic rate is perhaps the most widely measured physiological trait, and has long been argued to have important implications for life history, ecology and evolution. We argue that more widespread adoption of a microevolutionary quantitative genetics framework is valuable for understanding variation in metabolic rate. In adopting such an approach, we should consider metabolic rate as a multivariate trait and measure actual fitness (lifetime reproductive output) in the field, in order to estimate the genetic covariance between metabolic rates and fitness throughout ontogeny. Such measurements are needed in order to understand the drivers of phenotypic variation in metabolic rate.

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Competing interests

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