Behaviour, metabolism and size: phenotypic modularity or integration in Acheta domesticus?

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Behavior, metabolism, and size: phenotypic modularity or integration in *Acheta domesticus*?

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Abstract

The pace-of-life hypothesis predicts that among-individual differences in behavior should integrate with a wide variety of morphological, metabolic, and life-history traits along a slow to fast pace-of-life continuum. Support for the pace-of-life hypothesis has been mixed, in part because most empirical tests have been conducted strictly at the phenotypic level and have thus conflated genetic and environmental sources of covariance among traits. In the present study, we tested the hypothesis that, according to the predictions of the pace-of-life hypothesis, body-mass, routine metabolic rate, activity, and exploratory propensity would be positively integrated in the house cricket *Acheta domesticus* (Orthoptera: Gryllidae). Using modified open field behavioral tests and flow-through respirometry, we determined whether among-individual differences correlate across morphology, behavior and metabolism in 50 male house crickets. All traits were repeatable, but we found poor evidence for overall integration across traits. Instead we found evidence for modularity, with behavioral traits covarying independently from mass and routine metabolic rate. Modularity, like that found here between activity and exploratory propensity, has been suggested to facilitate adaptive evolutionary change by coupling functionally related traits into suites on which selection can more rapidly act.

Introduction

Phenotypic integration—correlations among phenotypic traits (Armbruster et al., 2014) — can have numerous ecological and evolutionary impacts. For example, evolutionary constraints are an expected product of phenotypic integration (Blows and Hoffmann, 2005), resulting from the inability of traits to respond independently to selection. These correlations thereby generate fitness trade-offs (Careau et al., 2008; Roff, 2002; West-Eberhard, 2003). Such trade-offs are necessarily frequent, as the number of traits expressed by organisms relative to the number of genes commonly present necessitates pleiotropy (Walsh and Blows, 2009). Phenotypic integration can be contrasted with modularity wherein traits are integrated within developmental (West-Eberhard, 2003) or functional units (Araya-Ajoy and Dingemanse, 2014) independently from other traits or groups of traits.

Recent interest in behavioral syndromes, which represent integration of behavioral traits, is demonstrative of an increased appreciation for the importance of phenotypic integration in behavioral ecology (Sih et al. 2004a, Sih et al. 2004b). Behavioral syndromes elicit many of the same questions as phenotypic integration at large. For example, as with other phenotypic traits, behavioral syndromes are of sufficient strength to have the ability to constrain evolutionary responses available to populations (Dochtermann and Dingemanse, 2013). Importantly our understanding of how behavioral responses integrate with other phenotypic domains (e.g. with physiology and life-history) continues to lag behind our understanding of integration within domains.

A variety of physiological and life-history traits are expected to covary with behaviors. For example, Biro and Stamps (2010) and Careau et al. (2008) have suggested that consistent individual differences in metabolic rate (and other physiological processes) should promote consistent individual differences in behavior. These same differences in physiology are also expected to integrate consistent individual differences in growth, reproduction, and other life-history processes into a "pace-of-life syndrome" (Biro and Stamps, 2008, 2010; Careau and Garland, 2012; Careau et al., 2008; Reale et al., 2010). Phenotypic integration will be modulated by physiology within a pace-of-life syndrome, as argued by Ricklefs and Wikelski (2002), due to several underlying assumptions likely general to animals (and, indeed, plants). In particular, integration should arise if: (1) organisms respond to environmental variation; and (2) these responses are constrained by limited resources (Ricklefs and Wikelski, 2002). Under the pace-of-life hypothesis behavior, physiology and life-history are thus considered non-independent components of an integrated phenotype.

Within a pace-of-life syndrome, behavioral responses are expected to correlate with several aspects of life-history and physiology in predictable ways (Reale et al., 2010). For example, higher resting metabolic rates may allow individuals to more rapidly increase energy expenditure (Reinhold, 1999). As a result, individuals with higher resting metabolic rates may be able to engage in more costly behaviors (Mathot et al., 2015). More generally, under the pace-of-life hypothesis, metabolism is expected to positively correlate with activity, exploratory rate, dispersal, "boldness", and aggression (Reale et al., 2010 but see Houston, 2010).

Current support for integration of behavior with metabolic rate according to the pace-of-life hypothesis is mixed (Careau et al., 2015; Mathot and Dingemanse, 2015). Supporting the pace-of-life hypothesis Careau et al. (2010) found that energy expenditure, growth rate, and longevity were all positively correlated with "boldness" and aggression in domestic dogs (*Canis lupus familiaris*); Mathot et al. (2015) found that metabolic rate and risk-taking were correlated—albeit in a context dependent manner—in great tits (*Parus major* (but see Bouwhuis et al., 2014 where a negative relationship was found between metabolic rate and exploratory behavior in female great tits); Shearer and Pruitt (2014) found that heart-rate, a purported proxy for metabolic rate, and "boldness" were positively correlated; likewise heart-rate and social dominance were positively correlated in red deer (*Cervus elaphus*; Turbill et al., 2013); growth rate and boldness were positively correlated in crayfish (*Cherax destructor*; Biro et al., 2014); and fast and slow reproductive strategies corresponded to fast and slow exploration in eastern chipmunks (*Tamias striatus*; Montiglio et al., 2014). In contrast to this support, Mathot et al. (2013) found that the genetic correlation between metabolic rate and courtship was effectively zero in zebra finches (*Taenipygia guttata*); in yellow mealworm beetles (*Tenebiro molitor*) metabolic rate positively correlated with predator response latency and negatively correlated with time immobile, supporting and contradicting the pace-of-life hypothesis respectively (Krams et al., 2014); correlations between metabolic rate and behavior in viviparous lizards (*Zootaca vivipara*) did not differ from zero, although there were slight relationships between behavior and locomotor performance (Le Galliard et al., 2013). Likewise, Gifford et al (2014) found no relationship between metabolic rate and exploratory behavior in salamanders (*Desmognathus brimleyorum*). This mixed support may stem, in part, from

85 difficulties in distinguishing differences among individuals in acquisition—the general
86 basis for pace-of-life relationships—and allocation trade-offs imposed by resource
87 availability (van Noordwijk and de Jong 1986; see also Careau et al. 2014).

88 Regardless of this mixed support, the majority of research into phenotypic
89 integration within a pace-of-life framework has focused on vertebrates. This discrepancy
90 reflects an overall vertebrate bias in the study of behavioral variation (Kralj-Fišer and
91 Schuett, 2014) and the study of behavior more broadly. Nonetheless, the assumptions of
92 the pace-of-life hypothesis will typically be met in invertebrate groups like arthropods. For
93 example, in *Acheta domesticus* , standard metabolic rate accounts for 78% of daily energetic
94 expenditure (Hack, 1997). A consequence of this relationship is that allocation of energy to
95 other purposes will be constrained, a necessary requirement for the pace-of-life hypothesis
96 (Ricklefs and Wikelski, 2002). Further, an important limitation of pace-of-life research is
97 that empirical tests are often conducted strictly at the phenotypic level, thus conflating
98 genetic and environmental effects (Dingemanse and Dochtermann, 2013, 2014;
99 Dingemanse et al., 2012). This conflation means that phenotypic correlations can differ
100 substantially from, for example, among-individual or genetic correlations (Dingemanse and
101 Dochtermann, 2013, 2014; Dingemanse et al., 2012; Downs and Dochtermann, 2014 but
102 see Dochtermann, 2011) and thereby lead to inappropriate evolutionary or ecological
103 inferences.

104 Within this framework of phenotypic integration and the pace-of-life hypothesis, we
105 were interested in whether behaviors covary with physiology and morphology in house
106 crickets (*Acheta domesticus*). We have previously demonstrated that *A. domesticus* exhibits

repeatable behavioral variation with respect to activity and exploratory propensity (Dochtermann and Nelson, 2014), and Wilson et al. (2010) demonstrated that several behaviors of *A. domesticus* were correlated at the phenotypic level. Here we sought to determine whether individual differences in activity and exploration propensity were correlated with adult mass and routine metabolic rate at the among-individual level. Support for the pace-of-life hypothesis is expected to be confirmed if all traits are found to be positively correlated.

Methods

Male five-week old *Acheta domesticus* were commercially obtained (Fluker Farms) and initially housed communally with shelter, *ad libitum* food (ground Purina chick starter), and water. At least 7 days prior to any behavioral and metabolic testing subjects were moved from communal to individual housing. Crickets were individually housed in 0.71 liter containers and provided with *ad libitum* food and water, as well as egg carton pieces for shelter. All individuals were maintained under a 12:12 light/dark photoperiod.

Behavioral tests

We measured behavioral responses using a modified open field test, in which individuals had to navigate around multiple obstacles to explore the entire arena (Figure 1). The arena was 60 cm × 60 cm × 10 cm, constructed of sealed and painted plywood with a Plexiglas lid. This obstacle course behavioral protocol was previously used with *A. domesticus* to evaluate exploratory behavior (Dochtermann and Nelson, 2014) and, here, is being used to assess exploratory propensity and activity levels.

Individuals were introduced into the lower right section of the arena (Z1, Figure 1) and allowed to move throughout the arena for 180 seconds after introduction. Recording started upon introduction for all individuals. We digitally recorded all behavioral trials and used Noldus Ethovision (Noldus Information Technology) to track movements of these individuals from digital videos. Using Ethovision we superimposed a 5 × 5 grid on the arena (Figure 1) and recorded the location and movement of individuals through the resulting 25 zones. As a measure of "exploratory propensity" we recorded the number of unique zones visited. As a measure of "activity" we recorded the total distance moved by an individual. Individual mass was measured immediately prior to behavioral tests. Arenas were cleaned with alcohol wipes and allowed to air-dry between trials.

Exploratory propensity and activity as operationally defined here are expected to exhibit some degree of structural correlation; i.e. individuals that visit more unique zones necessarily move greater distances and individuals that move greater total distances might, but not necessarily, incidentally visit more unique zones. To address this issue, we developed an individually-based simulation model described in greater detail in the supplemental materials. In short, we modeled the movement of individuals through a 60 cm × 60 cm area as a random walk and then calculated the correlation between activity (total distance moved) and unique zones visited. Using this model, we estimated the null structural correlation as 0.199. Unfortunately there is no *a priori* basis on which to determine how this correlation is expected to be divided between the among- and within-individual levels.

Individuals were generally tested in the obstacle course twice, although due to natural mortality some were only able to be tested once ($N_{twice} = 42$, $N_{once} = 10$).

Routine metabolic rate

We used CO_2 emission rate as an index of aerobic metabolic rate, as we have previously done with other invertebrates (Yocum et al., 2011; Greenlee and Harrison, 2004, 2005; Owings et al. 2014). Crickets were weighed on an analytical balance to the nearest 0.01 mg (Mettler Toledo, Columbus, OH) just prior to and immediately following metabolic measurements. Crickets (n = 42) were placed individually into 20 ml respirometry chambers constructed from 50 ml syringes plumbed with Tygon tubing. Chambers were covered and the room was kept dark during recording to minimize activity. Using a multiplexor (Intelligent Multiplexor V3, Sable Systems, Inc., Las Vegas, NV), seven crickets plus a baseline chamber were run concurrently. Dry, CO_2 free air (Balston purge gas generator, Haverhill, MA) was pushed through the measurement chamber and directed to the CO_2 analyzer (LiCor 6252, Lincoln, NE) at a flow rate of 500 ml min^{-1} using a mass flow meter (Sierra Instruments, Monterey, CA) controlled by a mass flow controller (MFC-4; Sable Systems, Inc., LasVegas, NV) . While not being measured, remaining chambers were flushed with dry, CO_2 free air (140 ml min^{-1}, Ametek R2 pump). We used Sable Systems software (Expedata version 1.4.15) and hardware (UI2) to control switching between chambers and to record data. One round of sampling from the multiplexed animals began with recording from the baseline chamber (identical, but lacking a cricket) for 1 min. After this time, the sample airstream was switched to chamber 1, and data were recorded for 5 min. The multiplexor was programmed to switch to the baseline chamber between each of the subsequent animal chambers, which were sampled in series for 5 min each. Crickets were each sampled four times for 5 min each. We calculated mass loss during the time that animals were in the chambers.

We used Expedata to calculate the mean CO_2 emission for each sampling period, trimming the first and last 30 sec of each sampling period from each recording. Because animals were not immobilized, we could not ensure a true resting metabolic rate. Instead our measurement of metabolic rate represents an estimate of "routine metabolic rate" (*sensu* Makarieva et al. 2008). We calculated routine metabolic rate (RMR) from baseline-corrected CO_2 emission data as in Greenlee and Harrison (2004, 2005):

$$MCO_2\ (\mu mol\ CO_2\ h^{-1}) = Vin \times \frac{(FECO_2 - FECO2) \times 60 \times 1000}{22.4^{-1}}$$

where Vin is the upstream flow rate in ml min-1, FECO2 = 0. ml $g^{-1}\cdot min^{-1}$ were converted to $\mu mol\cdot g^{-1}\cdot h^{-1}$ using the following conversion factors: $1000\cdot\mu l\cdot ml^{-1}$, $60\cdot min\cdot h^{-1}$ and $22.4\cdot\mu l\cdot\mu mol^{-1}$. MCO_2 was calculated for each of the four sampling periods and these MCO_2 estimates used in subsequent analyses.

Data analysis

To estimate the cross-domain relationships and overall phenotypic integration we employed a two part analysis (see also Sprenger et al., 2012). First, we estimated the among-individual and within-individual variances and covariances for exploratory propensity, activity, routine metabolic rate, and mass. Second, we used Structural Equation Modeling (SEM) to test a priori hypotheses about how morphological, physiological and behavioral traits may be integrated. Among-individual and within-individual variances and covariances were estimated using multiresponse mixed-effects models (Dingemanse and Dochtermann, 2013, 2014; Dingemanse et al., 2012). We estimated among-individual and within-individual components separately, because phenotypic correlations can be

misleading as to the direction and magnitude of trait relationships at the level of individuals when individuals can vary their own responses (Dingemanse and Dochtermann, 2013; Downs and Dochtermann, 2014).

In our mixed-effects models we included individual as a random factor. Condition (injured or not, four individuals had minor appendage injuries), time of testing and temperature (centered within individuals; van de Pol and Wright, 2009) were included as fixed effects to control for potential confounds and "pseudo-repeatability" or "pseudo-personality" (Nakagawa and Schielzeth, 2010; Westneat et al., 2011). Because we were only interested in the variance components, we will not discuss the fixed effects results (see Table S1). We modeled all variables according to a Gaussian distribution and scaled them to standard deviation units. Mass and RMR were $\log_{10}$-transformed to linearize the exponential relationship between these variables. Analyses were conducted using the MCMCglmm package (Hadfield, 2010) of *R* (R Development Core Team 2014) with 1.3×10^6 iterations, with a 3×10^5 iteration burn-in and thinning intervals of 1000. We used a prior that was flat and uninformative for the correlations.

From these mixed-effects models, we estimated behavioral, morphological, and physiological repeatabilities and the among- and within-individual correlations across the traits. Because they were assessed during separate testing events, we could not estimate the within-individual correlation of RMR with either activity or exploratory propensity. Similarly, the within-individual correlation between mass and RMR could not be estimated. While our sample size for estimating among-individual correlations had low power to distinguish estimates from zero, our correlation should have had relatively low bias (see Figure 1 in Dingemanse and Dochtermann 2013) making these estimates useful in SEM

219 comparisons. The posterior modal estimates of the among- and within-individual
220 correlation matrices were used in the second part of the analysis.

221 For the second part of the analysis we used a structural equation model comparison
222 approach to assess how the different traits might be linked (Dingemanse et al., 2010a;
223 Dochtermann and Jenkins, 2007). We compared *a priori* models using Akaike's Information
224 Criterion (AIC). Eleven models of trait covariance were evaluated at the among-individual
225 level and four at the within-individual level (Figure 2):

226 *model 1:* all traits independent (evaluated for among- (A) and within-individual (W)
227 correlation matrices)

228 *model 2*: only behaviors correlated (A & W)

229 *model 3*: only mass and RMR correlated (A)

230 *model 4*: behaviors are correlated but independent from mass and RMR, which are also
231 correlated (A)

232 *model 5*: behaviors integrated with RMR via an underlying latent variable (A)

233 *model 6*: behaviors integrated with mass via an underlying latent variable (A & W)

234 *model 7*: all four traits integrated via an underlying latent variable (A)

235 *model 8*: both behaviors arise causally from the influence of RMR and mass (A)

236 *model 9*: both behaviors arise causally from the influence of RMR (A)

237 *model 10*: both behaviors arise causally from the influence of mass (A & W)

model 11: mass causally influences RMR and both behaviors arise causally from the influence of RMR and mass (A)

Of these eleven models, model 1 represents null expectation, models 5-7 represent cross-domain trait integration, and models 8-11 represent causal influences of morphology and physiology on behavior.

Results

The four phenotypic measures showed repeatabilities (R) ranging from moderate to high (Table 1), with mass showing the highest repeatability (R = 0.89) and behavior and routine metabolic rate showing moderate repeatabilities (0.28 < R < 0.61, Table 1). At the among-individual level, activity (distance moved) and exploratory propensity (unique zones visited) were positively correlated while separately RMR and mass were positively correlated (among-individual correlations: r = 0.56 and 0.53 respectively). Both of these correlations had 95% credibility intervals excluding 0 (Table 1). Remaining among-individual correlations did not differ from 0 (Table 1).

At the within-individual level only activity and exploratory propensity were correlated (r = 0.75, Table 1). In addition, the phenotypic correlation after controlling for fixed-effects and repeated measures between activity and exploratory propensity (calculated following Dingemanse et al. 2012) was substantially higher than the expected correlation derived from null expectations (r_P = 0.70 (0.57 : 0.79), r_{PNULL} = 0.19; see Supplementary Information).

Consistent with the bivariate correlations, SEM model comparison results suggest that model 4 (Figure 2) best explains the data at the among-individual level (Table 2). This

model suggests behavioral integration separate from the expected relationship between RMR and mass. At the within-individual level, the model in which only behaviors covaried (model 2) was best supported by the data (Table 2). However, since behavioral and physiological measurements were not taken within the same time-spans, several of the proposed models could not be fit to the within-individual correlation matrix.

Discussion

We sought to determine whether *A. domesticus* exhibits phenotypic integration of behaviors, metabolic rate, and morphology as expected according to the pace-of-life hypothesis. Ultimately, we did not find support for integration of behavior and metabolism but found substantive correlations between activity and exploratory behaviors and, separately, between routine metabolic rate and mass. The relationship between mass and metabolic rate has previously been observed in *A. domesticus* (Hack, 1997) and is expected for allometric reasons (e.g. Downs et al., 2008). We also found that all four of the traits we measured exhibited considerable repeatable variation (Table 1), suggesting underlying genetic variation is present in each (Boake, 1989). Meta-analyses suggest that, on average, about half of the repeatable variation present in behaviors corresponds to additive genetic variation with the other half being attributable to permanent environmental differences (Dochtermann et al., 2015). How genetic variation and permanent environmental variation might influence *A. domesticus* behavioral variation is unclear and future research should address the heritability of and genetic correlations among these traits.

Our results also build on previous work by Wilson et al. (2010), who found that *A. domesticus* exhibited significant phenotypic correlations among several behavioral

282 measures. Specifically, our results extend those previous findings by demonstrating that
283 behavioral measures of presumably similar ecologically relevant behaviors demonstrate
284 repeatable variation. Our results therefore suggest that among-individual correlations
285 likely contribute to the phenotypic correlations reported by Wilson et al. (2010).

286 Importantly we did not find evidence for integration of behavior with metabolism.
287 Identifying correlations between behavior and metabolic rate is potentially problematic as
288 under most testing conditions the later cannot be measured independent of the former
289 (Mathot and Dingemanse, 2015). For example, activity in behavioral assays is expected to
290 positively correlate with routine metabolic rate simply because more active individuals will
291 also be more active during metabolic measurements. Such a correlation might be
292 incorrectly viewed as support for the pace-of-life hypothesis if routine metabolic rate is a
293 poor predictor of daily energy expenditure (Mathot and Dingemanse, 2015). This concern
294 is less valid for our results for two reasons. First, standard metabolic rate accounts for 78%
295 of the daily energy expenditure of *A. domesticus* (Hack, 1997) and thus necessarily strongly
296 correlates with routine metabolic rate. Second, in our case the concern about RMR being a
297 poor predictor of daily energy expenditure is not likely to be valid, because the estimated
298 among-individual correlation between routine metabolic rate and activity did not differ
299 from zero (Table 1). Finally, because all individuals were provided with *ad libitum* food, we
300 also do not consider it likely this lack of a connection between behavior and physiology
301 reflects a balancing of allocation and acquisition trade-offs.

302 Our failure to detect phenotypic integration of behavior and physiology is
303 particularly interesting given the considerable theoretical and conceptual literature that
304 suggests such links are to be expected (Biro and Stamps, 2010; Careau et al., 2009; Careau

and Garland, 2012; Careau et al., 2008). In particular, pace-of-life models have posited that among-individual differences in behavior, i.e. personality (sensu Dingemanse and Dochtermann, 2013; Dingemanse et al., 2010b), might arise from underlying differences in energy use (Careau et al., 2009; Careau and Garland, 2012; Reale et al., 2010). Such a connection with physiology might then integrate behavioral variation with aspects of life-history and slow versus fast-paced strategies (Reale et al., 2010). Here we found that neither a causal relationship from metabolic rate to behavior nor general covariance of behaviors and metabolic rate were supported. This lack of support for such cross-domain connections does, however, fit with some recent failures to support pace-of-life predictions. For example, in brown trout (*Salmo trutta*), behavioral variation was correlated to life-history variation opposite to the direction predicted (Adriaenssens and Johnsson, 2011). Our results and corresponding findings elsewhere suggest that arguments such as a general connection among behaviors and physiology due to "pace-of-life" and other conceptual constructs should be reevaluated.

Integration of traits exists on a continuum with "modularity", i.e. independence or "discreteness" of traits (West-Eberhard, 2003). Integration may also exist within modularity; specifically, traits that show integration due to shared developmental or causal pathways (West-Eberhard, 2003) or that have been jointly shaped by selection for a particular function (Araya-Ajoy and Dingemanse, 2014) may be integrated within modules independent from other traits. Here, activity and exploratory propensity can be considered as a module independent of metabolic rate and mass. Our observation of modularity rather than integration across phenotypic domains is important to consider in terms of the potential ecological and evolutionary implications of behavioral syndromes. While

behavioral syndromes might have direct effects on evolutionary outcomes for behavior (Dochtermann and Dingemanse, 2013), our results here suggest that these evolutionary consequences might not carry-over across phenotypic domains. Our findings here that behavior often exists in an integrated module (e.g. as a behavioral syndrome) separate from physiology affords populations with greater adaptive potential, allowing functionally related traits to respond rapidly to changing evolutionary pressures (West-Eberhard, 2003).

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References

Adriaenssens B, Johnsson JI, 2011. Shy trout grow faster: exploring links between personality and fitness-related traits in the wild. Behavioral Ecology 22:135-143.

Araya-Ajoy YG, Dingemanse NJ, 2014. Characterizing behavioural 'characters': an evolutionary framework. Proceedings Of The Royal Society B-Biological Sciences 281:20132645.

Armbruster WS, Pélabon C, Bolstad GH, Hansen TF, 2014. Integrated phenotypes: understanding trait covariation in plants and animals. Philosophical Transactions of the Royal Society B: Biological Sciences 369:20130245.

Biro PA, Adriaenssens B, Sampson P, 2014. Individual and sex-specific differences in intrinsic growth rate covary with consistent individual differences in behaviour. Journal of Animal Ecology 83:1186-1195.

Biro PA, Stamps JA, 2008. Are animal personality traits linked to life-history productivity? Trends in Ecology & Evolution 23:361-368.

356 Biro PA, Stamps JA, 2010. Do consistent individual differences in metabolic rate promote
357 consistent individual differences in behavior? Trends in Ecology & Evolution
358 25:653-659.

359 Blows MW, Hoffmann AA, 2005. A reassessment of genetic limits to evolutionary change.
360 Ecology 86:1371-1384.

361 Boake CRB, 1989. Repeatability - Its Role in Evolutionary Studies of Mating-Behavior.
362 Evolutionary Ecology 3:173-182.

363 Bouwhuis S, Quinn JL, Sheldon BC, Verhulst S, 2014. Personality and basal metabolic rate in
364 a wild bird population. Oikos 123:56-62.

365 Careau V, Bininda-Emonds ORP, Thomas DW, Reale D, Humphries MM, 2009. Exploration
366 strategies map along fast-slow metabolic and life-history continua in muroid
367 rodents. Functional Ecology 23:150-156.

368 Careau V, Garland T, 2012. Performance, Personality, and Energetics: Correlation,
369 Causation, and Mechanism. Physiol Biochem Zool 85:543-571.

370 Careau V, Montiglio P-O, Garant D, Pelletier F, Speakman JR, Humphries MM, Réale D, 2015.
371 Energy expenditure and personality in wild chipmunks. Behavioral Ecology and
372 Sociobiology:1-9.

373 Careau V, Reale D, Humphries MM, Thomas DW, 2010. The Pace of Life under Artificial
374 Selection: Personality, Energy Expenditure, and Longevity Are Correlated in
375 Domestic Dogs. American Naturalist 175:753-758.

376 Careau V, Thomas D, Humphries MM, Reale D, 2008. Energy metabolism and animal
377 personality. Oikos 117:641-653.

378 Dingemanse NJ, Dochtermann N, Wright J, 2010a. A method for exploring the structure of
379 behavioural syndromes to allow formal comparison within and between datasets.
380 Animal Behaviour 79:439-450.

381 Careau V, Buttemer WA, Buchanan KL, 2014. Developmental stress can uncouple
382 relationships between physiology and behaviour. Biology Letters 10:20140834

383 Dingemanse NJ, Dochtermann NA, 2013. Quantifying individual variation in behaviour:
384 mixed-effect modelling approaches. Journal of Animal Ecology 82:39-54.

385 Dingemanse NJ, Dochtermann NA, 2014. Individual behaviour: behavioural ecology meets
386 quantitative genetics. In: Charmantier A, Garant D, Kruuk LEB, editors. Quantitative
387 genetics in the wild: Oxford University Press.

Dingemanse NJ, Dochtermann NA, Nakagawa S, 2012. Defining behavioural syndromes and the role of "syndrome" deviation in understanding their evolution. Behavioral Ecology and Sociobiology 66:1543-1548.

Dingemanse NJ, Kazem AJN, Réale D, Wright J, 2010b. Behavioural reaction norms: animal personality meets individual plasticity. Trends in Ecology & Evolution 25:81-89.

Dochtermann NA, 2011. Testing Cheverud's conjecture for behavioral correlations and behavioral syndromes. Evolution 65:1814-1820.

Dochtermann NA, Dingemanse NJ, 2013. Behavioral syndromes as evolutionary constraints. Behavioral Ecology 24:806-811.

Dochtermann NA, Jenkins SH, 2007. Behavioural syndromes in Merriam's kangaroo rats (*Dipodomys merriami*): a test of competing hypotheses. Proceedings of the Royal Society B-Biological Sciences 274:2343-2349.

Dochtermann NA, Nelson AB, 2014. Multiple facets of exploratory behavior in house crickets (*Acheta domesticus*): Split personalities or simply different behaviors. Ethology 120:1110-1117.

Dochtermann NA, Schwab T, Sih A, 2015. The contribution of additive genetic variation to personality variation: heritability of personality. Proceedings Of The Royal Society B-Biological Sciences 282:20142201.

Downs CJ, Dochtermann NA, 2014. Testing hypotheses in ecoimmunology using mixed models: disentangling hierarchical correlations. Integr Comp Biol 54:407-418.

Downs CJ, Hayes JP, Tracy CR, 2008. Scaling metabolic rate with body mass and inverse body temperature: a test of the Arrhenius fractal supply model. Functional Ecology 22:239-244.

Hack MA, 1997. The effects of mass and age on standard metabolic rate in house crickets. Physiological Entomology 22:325-331.

Hadfield JD, 2010. MCMC Methods for Multi-Response Generalized Linear Mixed Models: The MCMCglmm R Package. J Stat Softw 33:1-22.

Houston AI, 2010. Evolutionary models of metabolism, behaviour and personality.

Gifford ME, Clay TA, Careau V, 2014. Individual (co) variation in standard metabolic rate, feeding rate, and exploratory behavior in wild-caught semiaquatic salamanders. Physiological and Biochemical Zoology. 87:384-396

Greenlee KJ and Harrison JF, 2004. Development of respiratory function in the American locust, *Schistocerca americana* I. Across-instar effects. Journal of Experimental Biology. 204: 497-508.

Greenlee KJ and Harrison JF, 2005. Respiratory changes throughout ontogeny in the tobacco hornworm caterpillar, *Manduca sexta*. Journal of Experimental Biology. 208: 1385-1392.

Kralj-Fišer S, Schuett W, 2014. Studying personality variation in invertebrates: why bother? Animal Behaviour 91:41-52.

Krams I, Kivleniece I, Kuusik A, Krama T, Freeberg TM, Mänd R, Sivacova L, Rantala MJ, Mänd M, 2014. High Repeatability of Anti-Predator Responses and Resting Metabolic Rate in a Beetle. Journal of insect behavior 27:57-66.

Le Galliard J-F, Paquet M, Cisel M, Montes-Poloni L, 2013. Personality and the pace-of-life syndrome: variation and selection on exploration, metabolism and locomotor performances. Functional Ecology 27:136-144.

Makarieva AM, Gorshkov VG, Li B-L, Chown SL, Reich PB, Gavrilov VM (2008) Mean mass-specific metabolic rates are strikingly similar across life's major domains: evidence for life's metabolic optimum. Proceedings of the National Academy of Sciences. 105:16994-16999

Mathot KJ, Dingemanse NJ, 2015. Energetics and behavior: unrequited needs and new directions. Trends in Ecology & Evolution. 30:199-206.

Mathot KJ, Martin K, Kempenaers B, Forstmeier W, 2013. Basal metabolic rate can evolve independently of morphological and behavioural traits. Heredity 111:175-181.

Mathot KJ, Nicolaus M, Araya-Ajoy YG, Dingemanse NJ, Kempenaers B, 2015. Does metabolic rate predict risk-taking behaviour? A field experiment in a wild passerine bird. Functional Ecology 29:239-249.

Montiglio P-O, Garant D, Bergeron P, Messier GD, Réale D, 2014. Pulsed resources and the coupling between life-history strategies and exploration patterns in eastern chipmunks (Tamias striatus). Journal of Animal Ecology 83:720-728.

Nakagawa S, Schielzeth H, 2010. Repeatability for Gaussian and non-Gaussian data: a practical guide for biologists. Biol Rev 85:935-956.

Reale D, Garant D, Humphries MM, Bergeron P, Careau V, Montiglio PO, 2010. Personality and the emergence of the pace-of-life syndrome concept at the population level. Philos Trans R Soc B-Biol Sci 365:4051-4063.

Reinhold K, 1999. Energetically costly behaviour and the evolution of resting metabolic rate in insects. Functional Ecology 13:217-224.

Ricklefs RE, Wikelski M, 2002. The physiology/life-history nexus. Trends in Ecology & Evolution 17:462-468.

Roff DA, 2002. Life history evolution. Sunderland: Sinauer Associates, Inc.

Sih, A., A. Bell, and J. C. Johnson. 2004a. Behavioral syndromes: an ecological and evolutionary overview. Trends in Ecology & Evolution 19:372-378.

Sih, A., A. M. Bell, J. C. Johnson, and R. E. Ziemba. 2004b. Behavioral syndromes: An integrative overview. Quarterly Review of Biology 79:241-277.

Shearer TA, Pruitt JN, 2014. Individual differences in boldness positively correlate with heart rate in orb-weaving spiders of genus Larinioides. Curr Zool 60:387-391.

Sprenger D, Dingemanse NJ, Dochtermann NA, Theobald J, Walker SPW, 2012. Aggressive females become aggressive males in a sex-changing reef fish. Ecology Letters 15:986-992.

Turbill C, Ruf T, Rothmann A, Arnold W, 2013. Social Dominance Is Associated with Individual Differences in Heart Rate and Energetic Response to Food Restriction in Female Red Deer. Physiol Biochem Zool 86:528-537.

van de Pol MV, Wright J, 2009. A simple method for distinguishing within- versus between-subject effects using mixed models. Animal Behaviour 77:753-758.

Van Noordwijk AJ, de Jong G, 1986. Acquisition and allocation of resources: their influence on variation in life history tactics. American Naturalist 128:137-142

Walsh B, Blows MW, 2009. Abundant genetic variation + strong selection = multivariate genetic constraints: a geometric view of adaptation. Annual Review of Ecology, Evolution, and Systematics 40:41-59.

West-Eberhard MJ, 2003. Developmental Plasticity and Evolution. New York: Oxford University Press.

Westneat DF, Hatch MI, Wetzel DP, Ensminger AL, 2011. Individual Variation in Parental Care Reaction Norms: Integration of Personality and Plasticity. The American Naturalist 178:652-667.

Wilson ADM, Whattam EM, Bennett R, Visanuvimol L, Lauzon C, Bertram SM, 2010. Behavioral correlations across activity, mating, exploration, aggression, and antipredator contexts in the European house cricket, *Acheta domesticus*. Behavioral Ecology and Sociobiology 64:703-715.

Yocum, G. D., Greenlee, K. J., Rinehart, J. P., Bennett, M. M., and Kemp, W. B. (2011). Cyclic respiration during the high temperature pulse of fluctuating thermal regime in the alfalfa leafcutting bee, *Megachile rotundata.* Comparative Biochemistry and Physiology Part A. 160: 480-485.

Tables and Figures

Table 1. Trait repeatabilities (shaded diagonal), among-individual correlations (above diagonal), and within-individual correlations (below diagonal, italicized). Values in bold indicate correlations with 95% credibility intervals (in parentheses) that do not overlap zero.

	Activity	Exploratory Propensity	RMR	Mass
Activity	0.28 (0.20 : 0.51)	**0.56** (0.17 : 0.77)	0.02 (-0.43 : 0.34)	0.16 (-0.26 : 0.51)
Exploratory Propensity	***0.75*** *(0.49 : 0.80)*	0.33 (0.18; 0.48)	0.15 (-0.27 : 0.48)	0.13 (-0.24 : 0.52)
RMR	*-0.04** *(-0.49 : 0.45)*	*-0.16** *(-0.43 : 0.42)*	0.61 (0.48 : 0.75)	**0.53** (0.28 : 0.76)
Mass	*0.05* *(-0.08 : 0.22)*	*-0.06* *(-0.14 : 0.17)*	*0.02* *(-0.22 : 0.29)*	0.89 (0.84 : 0.93)

*These values could not be estimated and, as reflected by their credibility intervals, roughly center on zero. Variation around zero is due to stochasticity in the MCMC process.

Table 2. Model comparison results at the among- and within-individual levels. For model descriptions see Figure 2.

Among-individual			Within-individual		
Model	AIC	ΔAIC	Model	AIC	ΔAIC
Model 4	**540.07**	**0.00**	**Model 2**	**531.22**	**0.00**
Model 2	554.29	14.22	Model 6	535.09	3.87
Model 6	556.80	16.72	Model 1	571.51	40.29
Model 5	557.11	17.04	Model 10	575.21	43.99
Model 3	557.29	17.22			
Model 7	557.83	17.75			
Model 11	562.21	22.14			
Model 1	571.51	31.44			
Model 10	573.23	33.15			
Model 9	574.31	34.24			
Model 8	576.43	36.35			

Figures

Figure 1. Obstacle course arena. Individuals were introduced into zone 1 (Z1) and allowed 180 s to explore the arena. Shaded areas represent the placement and size of obstacles within the arena.

Figure 2. *A priori* models of how the four traits might covary. In model 1, all traits are independent. In model 2 (path a active), the two behavioral measures covary. In model 3 (path b active), routine metabolic rate (RMR) and mass covary. In model 4 (paths a and b active), the two behavioral measures covary while separately RMR and mass covary. In model 5 (path c active) the two behaviors covary with routine metabolic rate (RMR) while mass varies independently. In model 6 (path d active), mass covaries with the two behavioral measures while RMR varies independently. In model 7 (paths c & d active), RMR and mass covary with each other and with the two measured behaviors. In model 8 (paths e through h active), the two behaviors are hypothesized to covary due to the joint effects of mass and RMR. In model 9 (paths e and f active), the two behaviors are hypothesized to covary due to the effects of RMR. In model 10 (path g and h active), the two behaviors are hypothesized to covary due to the effects of mass. In model 11 (paths e through i active), the two behaviors covary due to the effects of both RMR and mass while variation in RMR arises (in part) due to variation in mass.

Figures

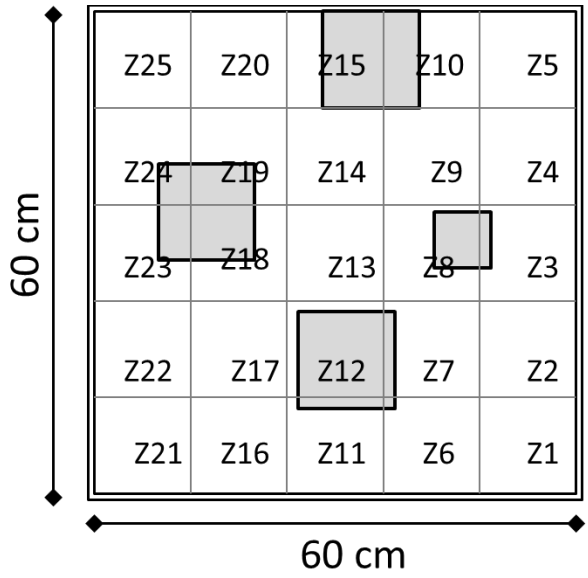
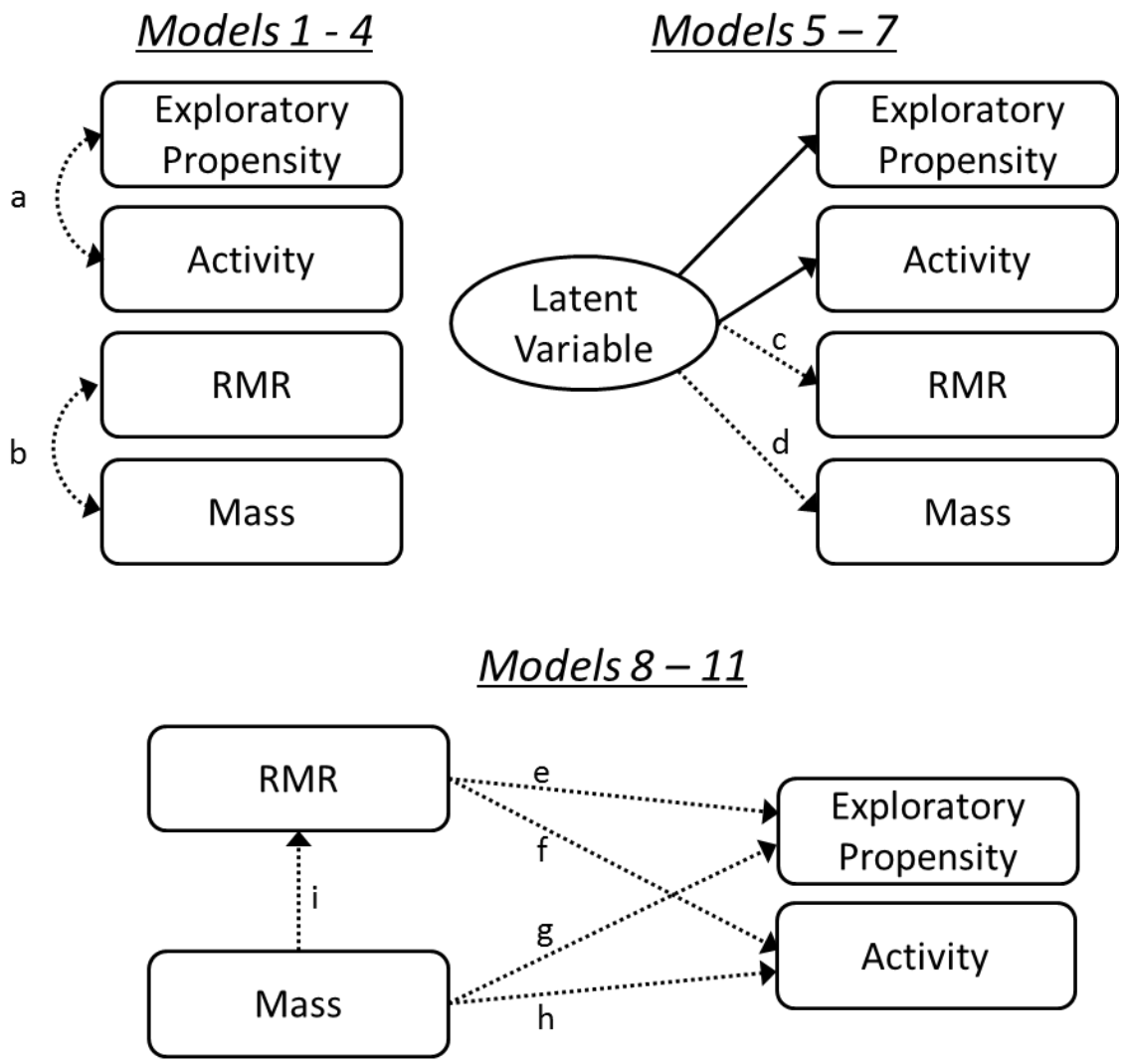


Figure 1.



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528 Figure 2.

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