



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



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The pace-of-life hypothesis predicts that among-individual differences in behaviour 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 are positively integrated in the house cricket *Acheta domesticus* (Orthoptera: Gryllidae). Using modified open field behavioural tests and flow-through respirometry, we determined whether among-individual differences are correlated across morphology, behaviour 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 behavioural 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 act more rapidly.

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Phenotypic integration, or correlations among phenotypic traits (Armbruster, Pélabon, Bolstad, & Hansen, 2014), can have numerous ecological and evolutionary impacts. For example, evolutionary constraints are an expected product of phenotypic integration (Blows & Hoffmann, 2005), resulting from the inability of traits to respond independently to selection. These correlations thereby generate fitness trade-offs (Careau, Thomas, Humphries, & Réale, 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 necessitates that pleiotropy will be common (Walsh & Blows, 2009). Phenotypic integration can be contrasted with modularity wherein traits are integrated within developmental (West-Eberhard, 2003) or functional units (Araya-Ajoy & Dingemans, 2014) independently from other traits or groups of traits.

Recent interest in behavioural syndromes, which represent integration of behavioural traits, is demonstrative of an increased appreciation for the importance of phenotypic integration in behavioural ecology (Sih, Bell, & Johnson, 2004; Sih, Bell, Johnson,

& Ziemba, 2004). Behavioural syndromes elicit many of the same questions as phenotypic integration at large. For example, as with other phenotypic traits, behavioural syndromes are of sufficient strength to have the ability to constrain evolutionary responses available to populations (Dochtermann & Dingemans, 2013). Importantly, our understanding of how behavioural 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 behaviours. For example, Biro and Stamps (2010) and Careau et al. (2008) suggested that consistent individual differences in metabolic rate (and other physiological processes) should promote consistent individual differences in behaviour. 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 & Stamps, 2008, 2010; Careau & Garland, 2012; Careau et al., 2008; Réale 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

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(Ricklefs & Wikelski, 2002). Under the pace-of-life hypothesis behaviour, physiology and life history are thus considered non-independent components of an integrated phenotype.

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

Current support for integration of behaviour with metabolic rate according to the pace-of-life hypothesis is mixed (Careau et al., 2015; Mathot & Dingemanse, 2015). Supporting the pace-of-life hypothesis, Careau, Réale, Humphries, and Thomas (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, Quinn, Sheldon, & Verhulst, 2014, where a negative relationship was found between metabolic rate and exploratory behaviour 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, Ruf, Rothmann, & Arnold, 2013); growth rate and boldness were positively correlated in crayfish, *Cherax destructor* (Biro, Adriaenssens, & Sampson, 2014); and fast and slow reproductive strategies corresponded to fast and slow exploration in eastern chipmunks, *Tamias striatus* (Montiglio, Garant, Bergeron, Messier, & Réale, 2014). In contrast to this support, Mathot, Martin, Kempenaers, and Forstmeier (2013) found that the genetic correlation between metabolic rate and courtship was effectively zero in zebra finches, *Taeniopygia guttata*; in yellow mealworm beetles, *Tenebrio 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 behaviour in viviparous lizards (*Zootaca vivipara*) did not differ from zero, although there were slight relationships between behaviour and locomotor performance (Le Galliard, Paquet, Cisel, & Montes-Poloni, 2013). Likewise, Gifford, Clay, and Careau (2014) found no relationship between metabolic rate and exploratory behaviour in salamanders (*Desmognathus brimleyorum*). This mixed support may stem, in part, from difficulties in distinguishing differences among individuals in acquisition (the general basis for pace-of-life relationships) and allocation trade-offs imposed by resource availability (Van Noordwijk & de Jong, 1986; see also Careau, Buttemer, & Buchanan, 2014).

Regardless of this mixed support, the majority of research into phenotypic integration within a pace-of-life framework has focused on vertebrates. This discrepancy reflects an overall vertebrate bias in the study of behavioural variation (Kralj-Fišer & Schuett, 2014) and the study of behaviour more broadly. Nevertheless, the assumptions of the pace-of-life hypothesis will typically be met in invertebrate groups like arthropods. For example, in the house cricket *Acheta domesticus*, standard metabolic rate accounts for 78% of daily energetic expenditure (Hack, 1997). A consequence of this relationship is that allocation of energy to other purposes will be constrained, a necessary requirement for the pace-of-life hypothesis (Ricklefs & Wikelski, 2002). Furthermore,

an important limitation of pace-of-life research is that empirical tests are often conducted strictly at the phenotypic level, thus conflating genetic and environmental effects (Dingemanse & Dochtermann, 2013, 2014; Dingemanse, Dochtermann, & Nakagawa, 2012). This conflation means that phenotypic correlations can differ substantially from, for example, among-individual or genetic correlations (Dingemanse & Dochtermann, 2013, 2014; Dingemanse et al., 2012; Downs & Dochtermann, 2014; but see Dochtermann, 2011) and thereby lead to inappropriate evolutionary or ecological inferences.

Within this framework of phenotypic integration and the pace-of-life hypothesis, we were interested in whether behaviours covary with physiology and morphology in house crickets (*A. domesticus*). We have previously demonstrated that *A. domesticus* exhibits repeatable behavioural variation with respect to activity and exploratory propensity (Dochtermann & Nelson, 2014), and Wilson et al. (2010) demonstrated that several behaviours of *A. domesticus* were correlated at the phenotypic level. Here we sought to determine whether individual differences in activity and exploration propensity are 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 5-week-old *A. domesticus* were commercially obtained (Fluker Farms, Port Allen, LA, U.S.A.) and initially housed communally with shelter, ad libitum food (ground Purina chick starter chow, Purina Mills[®], St Louis, MO, U.S.A.) and water. At least 7 days prior to any behavioural and metabolic testing, subjects were moved from communal to individual housing. Crickets were individually housed in 0.71-litre 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 h light:dark photoperiod.

Behavioural Tests

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

Individuals were introduced into the lower right section of the arena (Z1, Fig. 1) and allowed to move throughout the arena for 180 s after introduction. Recording started upon introduction for all individuals. We digitally recorded all behavioural trials and used Noldus Ethovision (Noldus Information Technology, Wageningen, The Netherlands) to track movements of these individuals from digital videos. Using Ethovision, we superimposed a 5 × 5 grid on the arena (Fig. 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 behavioural 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; that is, individuals that visit more unique zones necessarily move greater distances, and individuals that move greater total distances

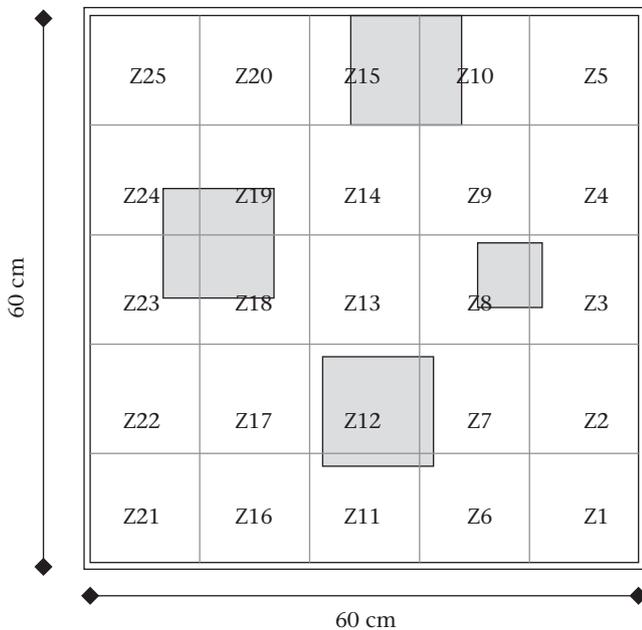


Figure 1. Obstacle course arena. Individual *Acheta domestica* were introduced into zone 1 (Z1) and allowed 180 s to explore the arena. Shaded areas represent the placement and size of obstacles within the arena.

might, but not necessarily, incidentally visit more unique zones. To address this issue, we developed an individually based simulation model (see [Supplementary Material](#)). In short, we modelled the movement of individuals through a 60×60 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 ($N = 42$), although some were only tested once ($N = 10$) due to natural mortality.

Routine Metabolic Rate

We used CO_2 emission rate as an index of aerobic metabolic rate, as we have previously done with other invertebrates ([Greenlee & Harrison, 2004, 2005](#); [Owings et al. 2014](#); [Yocum, Greenlee, Rinehart, Bennett, & Kemp, 2011](#)). Crickets were weighed on an analytical balance to the nearest 0.01 mg (Mettler Toledo, Columbus, OH, U.S.A.) 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, U.S.A.), seven crickets plus a baseline chamber were run concurrently. Dry, CO_2 free air (Balston purge gas generator, Haverhill, MA, U.S.A.) was pushed through the measurement chamber and directed to the CO_2 analyzer (LiCor 6252, Lincoln, NE, U.S.A.) at a flow rate of 500 ml/min using a mass flow meter (Sierra Instruments, Monterey, CA, U.S.A.) controlled by a mass flow controller (MFC-4; Sable Systems, Inc., Las Vegas, NV). While not being measured, remaining chambers were flushed with dry, CO_2 free air (140 ml/min, Ametek R2 pump). We used Sable Systems software (Expedata v.1.4.15, Sable Systems, Inc., Las Vegas, NV, U.S.A.) 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 s 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 \(2004, 2005\)](#):

$$\text{CO}_2 (\mu\text{mol CO}_2/\text{h}) = \text{Vin} \times (\text{FECO}_2 - \text{FICO}_2) \times 60 \times 1000 \times (1/22.4),$$

where CO_2 is the CO_2 emission rate, Vin is the upstream flow rate in ml/min and FECO_2 and FICO_2 are, respectively, the excurrent and incurrent carbon dioxide fractions. Values for CO_2 emission were converted to $\mu\text{mol per g per h}$ using the following conversion factors: 1000 $\mu\text{l/ml}$, 60 min/h and 22.4 $\mu\text{l}/\mu\text{mol}$. CO_2 emission was calculated for each of the four sampling periods and these CO_2 estimates were used in subsequent analyses as routine metabolic rate.

Data Analysis

To estimate the cross-domain relationships and overall phenotypic integration, we used a two-part analysis (see also [Sprenger, Dingemans, Dochtermann, Theobald, & Walker, 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 modelling (SEM) to test a priori hypotheses about how morphological, physiological and behavioural traits may be integrated. Among-individual and within-individual variances and covariances were estimated using multiresponse mixed-effects models ([Dingemans & Dochtermann, 2013, 2014](#); [Dingemans 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 ([Dingemans & Dochtermann, 2013](#); [Downs & 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 (centred within individuals; [van de Pol & Wright, 2009](#)) were included as fixed effects to control for potential confounds and 'pseudorepeatability' or 'pseudopersonality' ([Nakagawa & Schielzeth, 2010](#); [Westneat, Hatch, Wetzel, & Ensminger, 2011](#)). Because we were only interested in the variance components, we will not discuss the fixed effects results (see [Table S1](#)). We modelled all variables according to a Gaussian distribution and scaled them to standard deviation units. Mass and RMR were log transformed to linearize the exponential relationship between these variables. Analyses were conducted using the MCMCglmm package ([Hadfield, 2010](#)) of R (R Foundation for Statistical Computing, Vienna, Austria) 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 behavioural, morphological and physiological repeatabilities and 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 & Dochtermann, 2013](#)), making these estimates useful in SEM comparisons. The posterior modal estimates of the among- and within-individual correlation matrices were used in the second part of the analysis.

For the second part of the analysis we used a structural equation model comparison approach to assess how the different traits might be linked ([Dingemanse, Dochtermann, & Wright, 2010](#); [Dochtermann & Jenkins, 2007](#)). We compared a priori models using Akaike's Information Criterion (AIC). We evaluated 11 models of trait covariance at the among-individual level and four models of trait covariance at the within-individual level ([Table 1, Fig. 2](#)). Of these 11 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 behaviour.

RESULTS

The four phenotypic measures showed repeatabilities (R) ranging from moderate to high ([Table 2](#)), with mass showing the highest repeatability ($R = 0.89$) and behaviour and routine metabolic rate showing moderate repeatabilities ($0.28 < R < 0.61$; [Table 2](#)). 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 2](#)). Remaining among-individual correlations did not differ from 0 ([Table 2](#)).

At the within-individual level, only activity and exploratory propensity were correlated ($r = 0.75$; [Table 2](#)). 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 Material).

Consistent with the bivariate correlations, SEM model comparison results suggest that model 4 ([Fig. 2](#)) best explained the data at the among-individual level ([Table 3](#)). This model suggests behavioural integration separate from the expected relationship between RMR and mass. At the within-individual level, the model in which

only behaviours covaried (model 2) was best supported by the data ([Table 3](#)). However, since behavioural and physiological measurements were not taken within the same time spans, several of the proposed models could not be fitted to the within-individual correlation matrix.

DISCUSSION

We sought to determine whether *A. domesticus* exhibits phenotypic integration of behaviours, metabolic rate and morphology as expected according to the pace-of-life hypothesis. Ultimately, we did not find support for integration of behaviour and metabolism but found substantive correlations between activity and exploratory behaviours 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, Hayes, & Tracy, 2008](#)). We also found that all four of the traits we measured exhibited considerable repeatable variation ([Table 2](#)), suggesting that underlying genetic variation is present in each ([Boake, 1989](#)). Meta-analyses suggest that, on average, about half of the repeatable variation present in behaviours corresponds to additive genetic variation, with the other half being attributable to permanent environmental differences ([Dochtermann, Schwab, & Sih, 2015](#)). How genetic variation and permanent environmental variation might influence *A. domesticus* behavioural 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* exhibits significant phenotypic correlations among several behavioural measures. Specifically, our results extend those previous findings by demonstrating that behavioural measures of presumably similar ecologically relevant behaviours demonstrate repeatable variation. Our results therefore suggest that among-individual correlations likely contribute to the phenotypic correlations reported by [Wilson et al. \(2010\)](#).

Importantly we did not find evidence for integration of behaviour with metabolism. Identifying correlations between behaviour and metabolic rate is potentially problematic as under most testing conditions the latter cannot be measured independent of the former ([Mathot & Dingemanse, 2015](#)). For example, activity in behavioural assays is expected to correlate positively with routine metabolic rate simply because more active individuals will also be more active during metabolic measurements. Such a correlation might be incorrectly viewed as support for the pace-of-life hypothesis if routine metabolic rate is a poor predictor of daily energy expenditure ([Mathot & Dingemanse, 2015](#)). This concern is less valid for our results for two reasons. First, standard metabolic rate accounts for 78% of the daily energy expenditure of *A. domesticus* ([Hack, 1997](#)) and thus necessarily strongly correlates with routine metabolic rate. Second, in our case, the concern about RMR being a

Table 1
Models of trait covariance for behaviours (activity, exploratory propensity), adult body mass and routine metabolic rate (RMR) in *Acheta domesticus*

Model	Hypothesis
Model 1*	All traits are independent
Model 2*	Only behaviours are correlated
Model 3	Only mass and RMR are correlated
Model 4	Behaviours are correlated but independent from mass and RMR, which are also correlated
Model 5	Behaviours are integrated with RMR via an underlying latent variable
Model 6*	Behaviours are integrated with mass via an underlying latent variable
Model 7	All four traits are integrated via an underlying latent variable
Model 8	Both behaviours arise causally from the influence of RMR and mass
Model 9	Both behaviours arise causally from the influence of RMR
Model 10*	Both behaviours arise causally from the influence of mass
Model 11	Mass causally influences RMR and both behaviours arise causally from the influence of RMR and mass

All models were tested at the among-individual level. Four of the models were also tested at the within-individual level (as indicated by an asterisk).

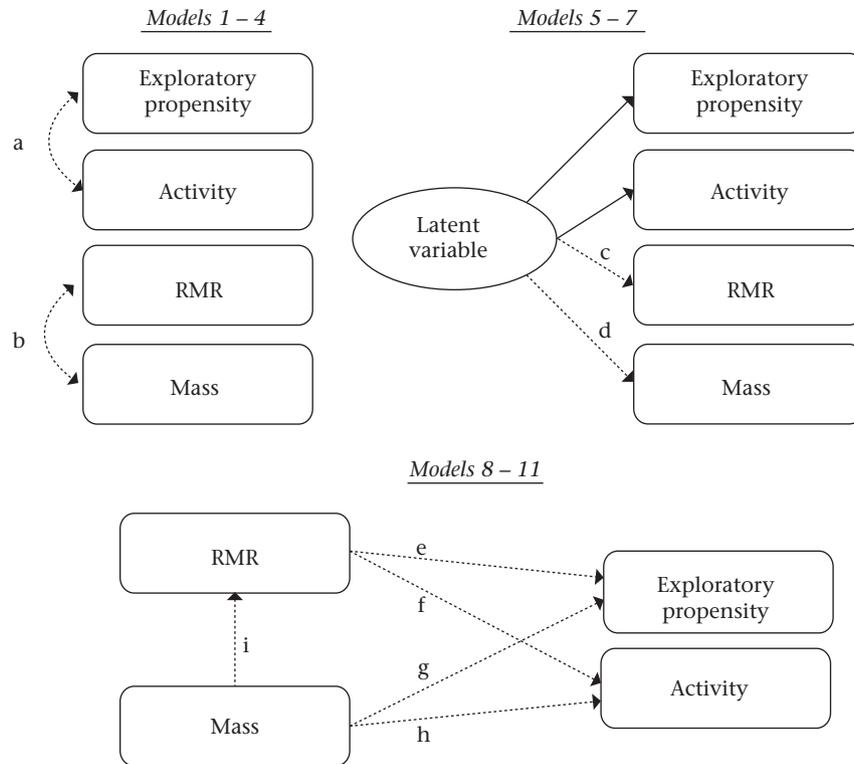


Figure 2. A priori models of how the four traits in *Acheta domestica* might covary. In model 1, all traits are independent. In model 2 (path a active), the two behavioural measures covary. In model 3 (path b active), routine metabolic rate (RMR) and mass covary. In model 4 (paths a and b active), the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

Table 2

Trait repeatabilities (diagonal, underlined), among-individual correlations (above diagonal) and within-individual correlations (below diagonal, italicized) in *Acheta domestica*

	Activity	Exploratory propensity	RMR	Mass
Activity	<u>0.28</u> (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)	<u>0.33</u> (0.18, 0.48)	0.15 (–0.27, 0.48)	0.13 (–0.24, 0.52)
RMR	–0.04 ^a (–0.49, 0.45)	–0.16 ^a (–0.43, 0.42)	<u>0.61</u> (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)	<u>0.89</u> (0.84, 0.93)

RMR: routine metabolic rate. Values in bold indicate correlations with 95% credibility intervals (in parentheses) that did not overlap zero.

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

Table 3

Model comparison results at the among- and within-individual levels

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			

AIC: Akaike's Information Criterion. Bold font denotes the best-supported models. For model descriptions, see Table 1, Fig. 2.

poor predictor of daily energy expenditure is not likely to be valid, because the estimated among-individual correlation between routine metabolic rate and activity did not differ from zero (Table 2). Finally, because all individuals were provided with ad libitum food, we also do not consider it likely that this lack of a connection between behaviour and physiology reflects a balancing of allocation and acquisition trade-offs.

Our failure to detect phenotypic integration of behaviour and physiology is particularly interesting given the considerable theoretical and conceptual literature that suggests such links are to be expected (Biro & Stamps, 2010; Careau, Bininda-Emonds, Thomas, Réale, & Humphries, 2009; Careau & Garland, 2012; Careau et al., 2008). In particular, pace-of-life models have posited that among-individual differences in behaviour, i.e. personality (sensu Dingemans & Dochtermann, 2013; Dingemans, Kazem, Réale, & Wright, 2010), might arise from underlying differences in energy use (Careau et al., 2009; Careau & Garland, 2012; Réale et al., 2010).

Such a connection with physiology might then integrate behavioural variation with aspects of life history and slow versus fast-paced strategies (Réale et al., 2010). Here we found that neither a causal relationship from metabolic rate to behaviour nor general covariance of behaviours 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*, Adriaenssens and Johnsson (2011) found that the correlation between behavioural variation and life-history variation was opposite to that predicted. Our results and corresponding findings elsewhere suggest that arguments such as a general connection among behaviours and physiology due to 'pace-of-life' and other conceptual constructs should be re-evaluated.

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

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Supplementary Material

Supplementary material associated with this article is available, in the online version at <http://dx.doi.org/10.1016/j.anbehav.2015.09.027>.

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