Individual (Co)variation in Standard Metabolic Rate, Feeding Rate, and Exploratory Behavior in Wild-Caught Semiaquatic Salamanders

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ABSTRACT

Repeatability is an important concept in evolutionary analyses because it provides information regarding the benefit of repeated measurements and, in most cases, a putative upper limit to heritability estimates. Repeatability (R) of different aspects of energy metabolism and behavior has been demonstrated in a variety of organisms over short and long time intervals. Recent research suggests that consistent individual differences in behavior and energy metabolism might covary. Here we present new data on the repeatability of body mass, standard metabolic rate (SMR), voluntary exploratory behavior, and feeding rate in a semiaquatic salamander and ask whether individual variation in behavioral traits is correlated with individual variation in metabolism on a whole-animal basis and after conditioning on body mass. All measured traits were repeatable, but the repeatability estimates ranged from very high for body mass ($R = 0.98$), to intermediate for SMR ($R = 0.39$) and food intake ($R = 0.58$), to low for exploratory behavior ($R = 0.25$). Moreover, repeatability estimates for all traits except body mass declined over time (i.e., from 3 to 9 wk), although this pattern could be a consequence of the relatively low sample size used in this study. Despite significant repeatability in all traits, we find little evidence that behaviors are correlated with SMR at the phenotypic and among-individual levels when conditioned on body mass. Specifically, the phenotypic correlations between SMR and exploratory behavior were negative in all trials but significantly so in one trial only. Salamanders in this study showed individual variation in how their exploratory behavior changed across trials (but not body mass, SMR, and feed in-

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take), which might have contributed to observed changing correlations across trials.

Introduction

Evolutionary biologists are typically interested in how the traits of organisms and the functions accomplished by these traits are related to fitness (Arnold 1983). Natural selection acts on differences among individuals, and, for that reason, individual variation is usually seen as the "raw material" on which selection can act. Individual variation can also be seen as the result of selection itself, as both natural selection and sexual selection sometimes favor the coexistence of alternative morphs or strategies within a population (Wilson et al. 1994; Wilson 1998; Calsbeek et al. 2002; Dingemanse and Réale 2005; Angilletta et al. 2006; Corl et al. 2010). As a result, the study of individual variation is pivotal to our understanding of evolution (Bennett 1987; Bauwens et al. 1995; Careau and Garland 2012).

An important first step of any evolutionary analysis is to quantify the repeatability (R) of the traits measured, defined as the ratio of among-individual variance to total phenotypic variance (Falconer and Mackay 1996). R is an important feature to quantify on both practical grounds and empirical grounds because it provides information regarding the benefit of repeated measurements and a putative upper limit to heritability estimates (Boake 1989; Falconer and Mackay 1996; Lynch and Walsh 1998; but see Dohm 2002). An extensive literature has accumulated documenting repeatability of numerous morphological, physiological, behavioral, performance, and life-history traits in a great diversity of organisms (Garland and Losos 1994; Versteegh et al. 2008; Bell et al. 2009; Careau and Garland 2012; Wolak et al. 2012; White et al. 2013). As natural selection is thought to act more directly on life-history and behavioral traits than performance, physiological, and morphological traits (Arnold 1983; Careau and Garland 2012), one may expect to find marked differences in the repeatability of traits across trait categories. However, as shown by Bell et al. (2009) for behavioral traits, comparing repeatability estimates may be confounded by differences in the representation of taxa, time interval over which R is estimated, environmental conditions (e.g., laboratory vs. wild), age or sex groups, and number of observations per individual. Our first objective was to control for these sources of variation and compare R across trait categories and different time intervals in a set of wild-caught salamanders.

Just as phenotypic variance can be partitioned into among- and within-individual variances, phenotypic correlations (r_P)

can be partitioned into among- and within-individual correlations. An among-individual correlation (r_{ind}) is present when individual mean values of trait y correlate with individual mean values of trait z. A within-individual correlation (r_e) exists when an individual's change in y between time period t and $t + 1$ is correlated with its change in z over the same period (Dingemanse and Dochtermann 2013). A focus on r_P without consideration of r_{ind} and r_e may lead to inappropriate conclusions. For example, a behavioral syndrome describes the situation where the among-individual components in two behavioral traits are correlated (Dingemanse et al. 2012b). Given that the average repeatability of behavioral traits is 0.37 (Bell et al. 2009), r_e should influence r_P 1.7 times more than r_{ind} (Dingemanse and Dochtermann 2013).

The pace-of-life syndrome (POLS) concept stipulates that species, populations, or individuals should differ in a suite of physiological traits and be coadapted with the life-history particularities favored under different ecological conditions (Ricklefs and Wikelski 2002; Wikelski et al. 2003; Martin et al. 2006). Although the POLS concept is potentially applicable across multiple levels of biological organization (i.e., species, populations, individuals), its relevance at the individual level remains poorly explored (Réale et al. 2010). Moreover, studies on the POLS concept have largely neglected behavioral traits, most likely due to the challenge associated with measuring behavior in a way that it is comparable across species (but see Careau et al. 2009). Therefore, our second objective was to test whether two behavioral traits, namely, feeding rate and exploratory behavior, were correlated with standard metabolic rate (SMR) at different levels of variation (i.e., we estimated r_P, r_{ind}, and r_e).

The empirical studies conducted on this topic so far indicate that the relationship between maintenance metabolism (i.e., SMR in ectotherms and basal metabolic rate in endotherms) and exploratory behavior can be positive, absent, or negative, depending on the taxon studied, level of analysis (i.e., interspecific correlations vs. intraspecific correlations and phenotypic correlations vs. genetic correlations), sex, and environmental contexts (reviewed in Careau and Garland 2012; see also Le Galliard et al. 2012; Maldonado et al. 2012; Bouwhuis et al. 2013). One reason why a general pattern has not yet emerged from these studies might be that all but one of them (Careau et al. 2011) focused on r_P. Given that the repeatability of both maintenance metabolism and exploratory behavior is typically low (i.e., $R < 0.4$), it is very likely that r_e obfuscates any potentially informative relationships at the among-individual level (i.e., r_{ind}).

Open-field and other novel-environment tests are widely used in personality research as it is shown that these tests measure components of an individual's behavior that are predictive of its behavior in free-ranging conditions, including space use (Boon et al. 2008; Boyer et al. 2010; van Overveld and Matthysen 2010; Montiglio et al. 2012) and dispersal (Fraser et al. 2001; Dingemanse et al. 2003). However, it has also been shown that individuals differ in how their exploratory behavior changes across repeated trials (possibly related to habituation;

Dingemanse et al. 2012a). Such changes occurring within individuals could also obfuscate any potentially informative relationships at the among-individual level (i.e., r_{ind}). The extent to which individuals differ in how their SMR changes across trials, in relation to habituation to respirometry procedures (Careau et al. 2008), is currently unknown.

In this study, we present new data on body mass, SMR, voluntary exploratory behavior, and feeding intake in a set of wild-caught semiaquatic salamanders (*Desmognathus brimleyorum*). Our approach was multifaceted and aimed at estimating consistency of individual differences for each trait and the temporal pattern of repeatability. In addition, we tested whether behavioral traits were correlated with SMR at multiple levels (i.e., the phenotypic level, among-individual level, and within-individual level). Finally, we asked whether individuals significantly differed in how their body mass, SMR, feeding rate, and exploratory behavior changed over the three trials.

Material and Methods

Salamander Maintenance and Sampling Time Line

The University of Arkansas at Little Rock Institutional Animal Care and Use Committee approved all experimental methods and procedures (protocol R-11-02). We collected 19 adult salamanders from the field (16 females and 3 males) and maintained them individually in plastic containers (21 cm × 13 cm × 5 cm) housed in a temperature-controlled incubator set at 15°C with a photoperiod of 14L : 10D. Plastic containers contained moist paper toweling to prevent desiccation. Salamanders received approximately 100 fruit flies (*Drosophila hydei*) at weekly intervals (except for the week preceding trials to ensure that animals were postabsorptive). We first quantified SMR, exploratory behavior, and feeding rate in all individuals after 2 wk of captivity (week 0). To measure repeatability over different time periods, we remeasured SMR, exploratory behavior, and feeding rate during the fifth and eleventh weeks of captivity. Hence, we had pairs of measurements for all traits that were separated by 3 wk (week 0 vs. week 3), 6 wk (week 3 vs. week 9), and 9 wk (week 0 vs. week 9).

Metabolic Rate Measurements

We measured SMR of salamanders in an automated flow-through system (Qubit Systems, Kingston, Ontario) at 15°C. Metabolic chambers consisted of 60-mL cylinders each containing a small length of moist sponge to prevent salamander desiccation during measurement. Source gas was pulled through Drierite and soda lime columns prior to entering a mass flow controller (G246, Qubit Systems), which regulates the flow rate through metabolic chambers. We maintained flow rates during measurement at 100 mL min^{-1}. The air stream exiting the chambers flowed into a gas switcher (G244, Qubit

Table 1: Repeatability (R) of $\log_{10}$-transformed body mass (g), $\log_{10}$-transformed standard metabolic rate (SMR), feeding rate (flies consumed), and exploratory behavior (squares crossed in a novel environment) in 19 wild-caught semiaquatic salamanders (*Desmognathus brimleyorum*)

| | Fixed effects | | | | | | Random effects | | | | |
| | Trial | | | Body mass | | | Intercept | | | Residual | Repeatability |
Data/interval	F	df	P	F	df	P	$V_I \pm$ SE	$\chi^2_{0:1}$	P	$V_R \pm$ SE	$R \pm$ SE
Body mass:											
All data	23.88	36.0	<.001	NA	NA	NA	.998 ± .335	120.43	<.001	.021 ± .005	.980 ± .008
3 wk	17.00	18.0	.001	NA	NA	NA	1.012 ± .339	69.80	<.001	.011 ± .004	.990 ± .005
6 wk	14.01	18.0	.001	NA	NA	NA	.996 ± .336	55.85	<.001	.023 ± .008	.977 ± .011
9 wk	34.30	18.0	.000	NA	NA	NA	.972 ± .329	51.76	<.001	.029 ± .010	.971 ± .013
SMR:											
All data	.93	37.5	.403	50.04	18.3	<.001	.148 ± .079	7.27	.004	.227 ± .054	.394 ± .159
3 wk	1.30	18.5	.268	48.11	17.6	<.001	.176 ± .094	5.32	.011	.169 ± .056	.510 ± .187
6 wk	1.32	18.5	.266	45.64	17.7	<.001	.125 ± .094	2.16	.071	.240 ± .080	.342 ± .226
9 wk	.24	19.7	.631	39.76	18.0	<.001	.129 ± .104	1.84	.087	.281 ± .094	.316 ± .230
Feeding rate:											
All data	4.25	38.6	.021	7.653	19.5	.012	.448 ± .192	16.99	<.001	.324 ± .077	.580 ± .137
3 wk	8.44	19.2	.009	6.163	18.4	.023	.592 ± .234	16.54	<.001	.169 ± .056	.778 ± .101
6 wk	.70	18.9	.412	7.562	18.3	.013	.444 ± .226	6.16	.007	.373 ± .125	.543 ± .192
9 wk	5.91	20.4	.025	5.357	18.3	.033	.351 ± .206	4.01	.023	.428 ± .143	.451 ± .217
Exploratory behavior:											
All data	2.84	37.2	.071	2.513	18.0	.130	.226 ± .164	2.92	.044	.684 ± .161	.248 ± .175
3 wk	.38	18.4	.546	3.299	17.4	.087	.366 ± .243	2.90	.044	.574 ± .191	.389 ± .237
6 wk	4.71	18.6	.043	1.570	17.7	.226	.275 ± .229	1.68	.097	.636 ± .212	.302 ± .251
9 wk	2.27	19.3	.148	1.638	17.7	.217	.138 ± .228	.39	.267	.800 ± .267	.147 ± .258

Note. Table shows data from all trials ("All data") and different pairs of trials separated by different time intervals (3, 6, and 9 wk) and parameters from univariate mixed-effect models with fixed effects of trial (categorical) and body mass and a random effect for individual identity. Estimates of between- and within-individual variances (V_I and V_R, respectively) and repeatability (R) are reported with their standard errors ($\pm$ SE). The significance of V_I was tested using a log-likelihood ratio test with a χ^2 statistic distributed as an equally weighted mixture of χ^2 distributions with 1 and 0 df ($\chi^2_{0:1}$). Each trait was standardized to a mean of 0 and a phenotypic variance of 1 prior to analysis. NA = not applicable.

Systems), which directed the stream from a focal chamber through the gas analyzers. The effluent gas stream was subsampled in parallel through H_2O scrubbers prior to entering an O_2 (S104 [DOX], Qubit Systems) and a CO_2 (S157, Qubit Systems) analyzer. We quantified gas exchange rates using equations of Withers (2001) to account for dilution and concentration effects. These calculations were performed in the Multi Channel Gas Exchange software (C950, Qubit Systems).

We placed six animals individually in metabolic chambers at 0900 hours and recorded gas exchange continuously for 24 h. Because animals were measured sequentially, each cycle through six animals (interspersed with measurement of a reference chamber) required 2 h (10 min recording each animal followed by a 10-min recording of the reference chamber). Therefore, we obtained 12 measurements for each animal throughout the 24-h period. Salamanders were typically active during the first cycle, so we treat this as an acclimation period and exclude this initial measurement for each animal from analysis. We calculated SMR for each animal as the mean 120 s of the lowest continuous stable O_2 and CO_2 recordings over the final 11 cycles. We measured body mass immediately prior to and after SMR trials. We used the average of these two body mass measurements in all analyses (see below).

Voluntary Exploratory Behavior

Forty-eight hours after metabolic rate measurement, each salamander was subjected to an open-field behavioral trial to quantify exploratory behavior. For each open-field assay a salamander was placed in a disinfected, naive arena (21 cm × 13 cm × 5 cm) in a random position inside the arena. The floor of the arena was covered with a 2 × 2-cm grid. We initiated a behavioral trial by placing a salamander in an arena and placing the arena and salamander in an environmental chamber with no illumination and set at 15°C. We digitally recorded four salamanders simultaneously (each in separate arenas) using infrared security cameras connected to a digital video recorder. After initiation, each salamander was recorded for 15 min. Voluntary behavior was quantified as the total number of grid squares a salamander crossed throughout the entire 15-min trial. Repeat visits to the same square were counted; thus,
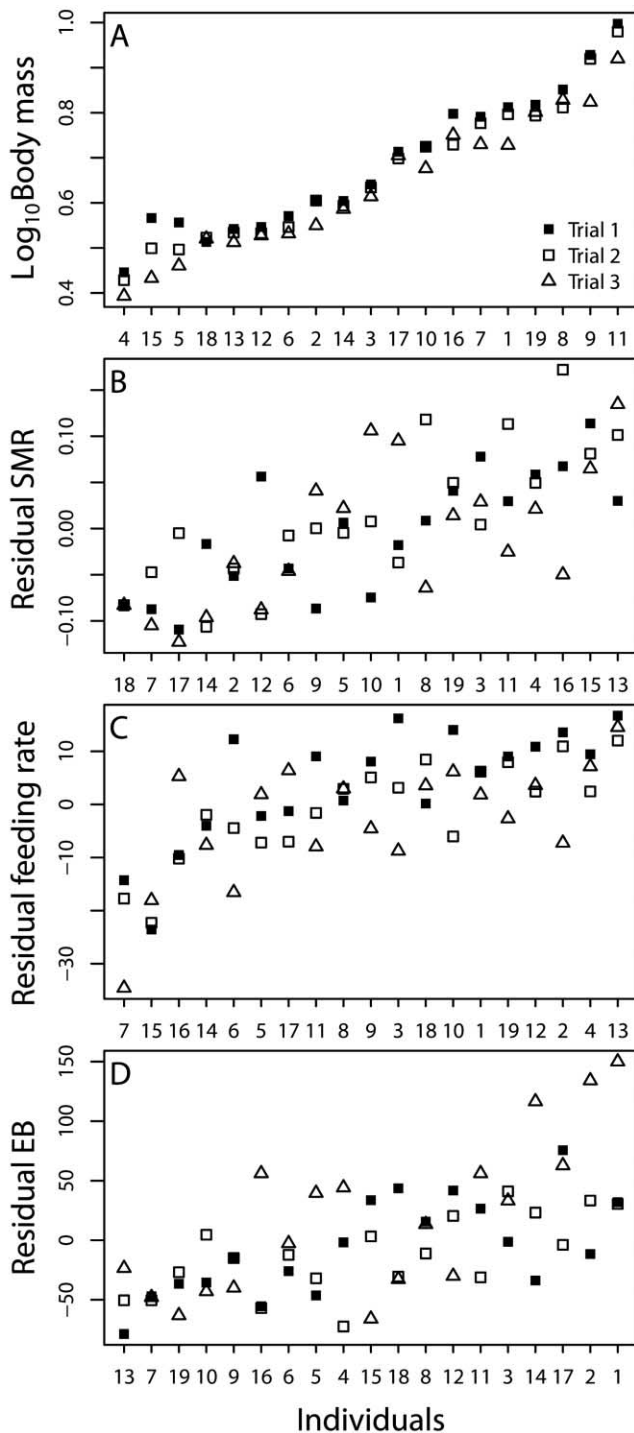
number of squares crossed represents the rate of movement in a novel environment.

Feeding Trials

Twenty-four hours after each set of behavioral assays was completed, we measured voluntary feeding rate for each salamander at 15°C. We conducted feeding trials in plastic containers (21 cm × 13 cm × 5 cm) over 4 d. We offered fasted salamanders 100 fruit flies (*D. hydei*). Each subsequent day we counted the number of flies consumed and replenished flies to the original number. Most animals consumed a large number of flies on the first day (mean = 83 flies) with lower numbers on the following 3 d. We quantified feeding rate (flies d^{-1}) as the number of flies consumed over the final 3 d of the trial divided by 3.

Analysis: Allometry

We analyzed all data in the R statistical programming language (ver. 3.0.0) and ASReml-R (Butler et al. 2007). Because of the extremely biased sex ratio in our sample (16 females, 3 males), we did not include sex in analyses. Body mass and SMR were normalized by log$_{10}$ transformation. We first examined whether SMR, feeding rate, and exploratory behavior were influenced by body mass using least squares regressions applied on data from each trial separately. We used a linear mixed model (LMM; with individual identity as a random effect) to test whether allometric scaling exponents were different from one trial to another.

Analysis: Repeatability

We tested whether individuals differed significantly in body mass, SMR, feeding rate, and exploratory behavior using mixed models with individual identity fitted as a random effect. In all mixed models, the dependent variable was standardized to a mean of 0 and variance of 1, and trial number was fitted as a categorical variable. All models for SMR, feeding rate, and exploratory behavior included a fixed-effect body mass recorded for that trial to account for changes in body mass over the study. Hence, our repeatability estimates should be interpreted as being conditioned on body mass (see Wilson 2008). Significance of fixed effects was tested with a conditional Wald F statistic, and the denominator degrees of freedom (df) were calculated following Kenward and Roger (1997).

In a first step, we provided an overall estimate of repeatability across trials by including all data in the mixed model. In a second step, we included different pairs of trials to estimate repeatability over different time intervals (3, 6, and 9 wk; see above). We calculated R as the ratio of among-individual variance (V_I) to total phenotypic variance (V_P). V_I is quantified as the variance attributed to individual identity as a random effect and V_P as the sum of V_I and residual variance (V_R; conditioned on fixed effects). Approximate standard errors for repeatability

Figure 1. Individual variation in log$_{10}$-transformed body mass (g; *A*), log$_{10}$-transformed standard metabolic rate (SMR, μL O$_2$ h^{-1}; *B*) at 15°C, feeding rate (no. flies consumed d^{-1}; *C*), and exploratory behavior (EB, no. squares crossed in a novel environment; *D*) in 19 wild-caught semiaquatic salamanders (*Desmognathus brimleyorum*) in three temporally separated trials. All traits are shown as residuals from a linear regression against log$_{10}$-transformed body mass (except in *A*). Individuals were ordered along the *X*-axis according to their mean value (i.e., order differs across panels). See table 1 for statistical results.
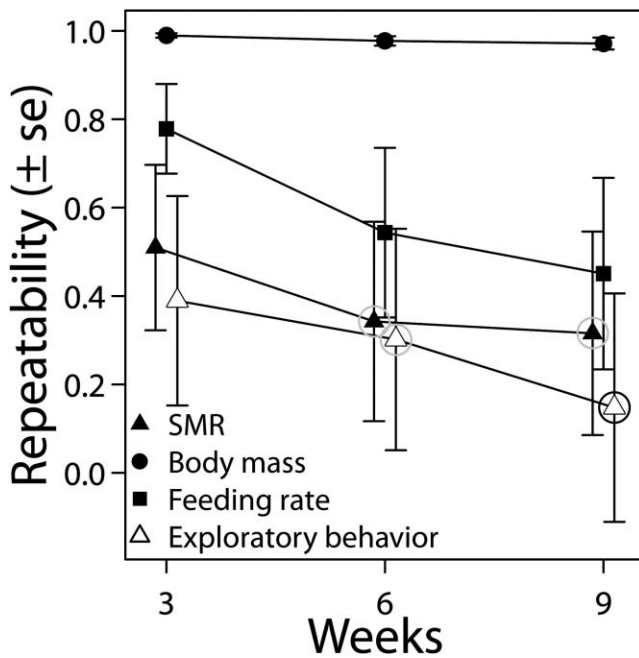
Figure 2. Temporal changes in repeatability ($\pm$SE) for $\log_{10}$-transformed body mass (g; filled circles), $\log_{10}$-transformed standard metabolic rate at 15°C (SMR, μL O_2 h^{-1}; filled triangles), feeding rate (no. flies consumed d^{-1}; filled squares), and exploratory behavior (no. squares crossed in a novel environment; open triangles) in 19 wild-caught semiaquatic salamanders (*Desmognathus brimleyorum*). Symbols enclosed in circles denote repeatabilities that were not statistically significant (black circle, $P > 0.10$) or marginally nonsignificant (gray circle, $P < 0.1 > 0.05$; see table 1).

estimates were obtained using the delta method (see app. 1 in Lynch and Walsh 1998).

We tested for the statistical significance of V_I using a likelihood ratio test (LRT) comparing the log likelihoods of a full model that included V_I and a reduced model that excluded it. The LRT statistic is equal to twice the difference in log likelihoods between the two nested models and is assumed to follow a χ^2 distribution with df equal to the difference in the number of parameters estimated. However, when testing a single component against a boundary of its parameter space (e.g., $V_I > 0$), the χ^2 statistic is distributed as an equally weighted mixture of χ^2 distributions with 1 and 0 df ($\chi^2_{0:1}$; Self and Liang 1987). In practice, this is equivalent to halving P values obtained from a χ^2 distribution with 1 df (Dominicus et al. 2006).

Analysis: Phenotypic Correlations

Using data from all trials, we fitted a three-trait multivariate model to estimate the phenotypic correlation (r_p) between SMR, feeding rate, and exploratory behavior on a whole-animal basis and after traits were conditioned on body mass. The multivariate model allowed a correlation (*corgh* structure in AS-Reml-R) between the residual variance of each trait. Such an

analysis is accomplished in a one-step process, which is more conservative than a two-step analysis (e.g., when residuals are first calculated and then used for testing correlations).

To estimate the r_p values between SMR, feeding rate, and exploratory behavior within each of the three trials, we ran a second three-trait multivariate model in which we allowed trial-specific residual variances and correlations. In addition to providing r_p values within each trial, we could compare this model with a reduced model in which the correlations for a pair of traits (e.g., SMR and feeding rate) were constrained to be equal across all trials. Because this model estimates two fewer parameters, we could use a LRT with 2 df to test whether the correlations were significantly different across trials. Each multivariate model was computed twice, one in which trial number (categorical) was the only fixed effect to estimate whole-animal correlations and another in which $\log_{10}$-transformed body mass was included to estimate correlations conditioned on body mass.

Analysis: Among- and Within-Individual Correlations

Because r_p values are shaped by correlations at two distinct levels of variance, among (V_I) and within (V_e) individuals, they provide limited information about the nature of the association between traits (Dingemanse and Dochtermann 2013). An among-individual correlation (r_{ind}) reflects the effects (i.e., genetic, epigenetic, or other permanent environmental effects) that are responsible for the association between the two traits, whereas a within-individual correlation (r_e) represents combined, reversible changes in the two traits (i.e., phenotypic plasticity) occurring within an individual (Ferrari et al. 2013).

We estimated r_{ind} and r_e between SMR, feeding rate, and exploratory behavior using data from all trials and fitting a three-trait multivariate model. This model included a random effect of individual identity (V_I) fitted to all dependent variables and an unstructured correlation matrix between them, which estimated r_{ind}. An unstructured correlation matrix between the residual variances (V_e) estimated the r_e values among traits. Again, the correlations between SMR, feeding rate, and exploratory behavior were estimated on a whole-animal basis and after conditioning on body mass by excluding or including a fixed effect of $\log_{10}$-transformed body mass, respectively.

Analysis: Individual Variation across Trials

Using data from all trials, we fitted random regression models to test whether individuals differ significantly in how their body mass, SMR, feeding rate, and exploratory behavior changed over the three trials (for detailed explanation of the approach, see Singer and Willett 2003). Random slope models describe the pattern of among-individual variation over a gradient (trial in our case) by estimating the variance in elevation (intercept; V_I), the variance in slope (V_S), and the covariance between V_I and V_S (Cov_{I-S}). We therefore tested for the presence of random

Table 2: Phenotypic correlation estimates ($r_p \pm$ SE) from a three-trait multivariate model of standard metabolic rate (SMR), feeding rate (flies consumed), and exploratory behavior (squares crossed in a novel environment) in 19 wild-caught semiaquatic salamanders (*Desmognathus brimleyorum*)

Data	Whole-animal correlations			Mass-conditioned correlations		
	$r_p \pm$ SE	χ_1^2	P	$r_p \pm$ SE	χ_1^2	P
SMR vs. feeding rate:						
All	**.39 ± .12**	**9.06**	**.003**	.04 ± .14	.07	.796
Trial 1	.31 ± .21	1.87	.172	−.09 ± .24	.13	.720
Trial 2	.41 ± .20	3.28	.070	−.14 ± .24	.36	.549
Trial 3	**.47 ± .18**	**4.39**	**.036**	.28 ± .22	1.51	.219
SMR vs. exploratory behavior:						
All	**−.32 ± .12**	**5.93**	**.015**	−.20 ± .13	2.17	.140
Trial 1	−.29 ± .22	1.61	.204	−.06 ± .24	.07	.787
Trial 2	**−.58 ± .16**	**7.38**	**.007**	**−.52 ± .18**	**5.30**	**.021**
Trial 3	−.22 ± .22	.88	.347	−.13 ± .23	.31	.576
Feeding rate vs. exploratory behavior:						
All	−.05 ± .14	.14	.706	.08 ± .14	.35	.555
Trial 1	−.23 ± .22	1.00	.317	−.10 ± .24	.19	.666
Trial 2	−.10 ± .23	.18	.671	.15 ± .23	.38	.538
Trial 3	.08 ± .23	.13	.720	.17 ± .23	.52	.471

Note. Statistically significant correlations are indicated in bold. An unstructured correlation matrix was included in the residuals to estimate phenotypic correlation for each pair of traits. Models were run with trial number (categorical) as the only variable (whole-animal correlations) and with $\log_{10}$-transformed body mass (mass-residual correlations). The significance of each r_p was tested using a log-likelihood ratio test with a χ^2 statistic with 1 df (χ_1^2).

slopes by comparing a model that included all three parameters (V_I, V_S, and Cov_{I-S}) against a model that included V_I only. Since V_S is bounded to 0 but Cov_{I-S} is not, the χ^2 statistic is distributed as an equal mixture of mixture of χ_1^2 and χ_2^2 distributions ($\chi_{1:2}^2$), which is obtained by adding half the P value obtained for a χ_1^2 distribution and half the P value obtained for a χ_2^2 distribution.

Results

Allometry

As expected, SMR was significantly and positively correlated with body mass in each trial (separate linear regressions: $r^2 > 0.57$, df = 17, $P < 0.0002$), but the nonsignificant interaction term between trial and body mass (LMM: $F_{2, 33.9} = 2.126$, $P = 0.135$) indicated that the differences in scaling across trials were not significant. Similarly, feeding rate was positively correlated with body mass across all trials, but the linear regression was significant only in trial 2 ($r^2 = 0.32$, df = 17, $P = 0.012$) and marginally nonsignificant in trials 1 and 3 ($r^2 < 0.15$, df = 17, $P > 0.098$). The differences in scaling of feeding rate across

trials were not significant (LMM: $F_{2, 34} = 0.268$, $P = 0.767$). By contrast, exploratory behavior tended to be negatively correlated with body mass in trial 2 ($r^2 = 0.15$, df = 17, $P = 0.105$), but the relationships were nonsignificant in trials 1 and 3 ($r^2 < 0.10$, df = 17, $P > 0.19$). The differences in scaling of exploratory behavior across trials were not significant (LMM: $F_{2, 34.2} = 0.007$, $P = 0.993$).

Repeatability across All Trials

Using all repeated measures, we obtained significant estimates of among-individual variance (V_I) in all traits (table 1). The repeatability estimates ranged from very high for body mass ($R = 0.98$; table 1; fig. 1A), to intermediate for SMR ($R = 0.39$; table 1; fig. 1B) and feeding rate ($R = 0.58$; table 1; fig. 1C), to low for exploratory behavior ($R = 0.25$; table 1; fig. 1D).

Repeatability over Time

The repeatability in body mass remained high independent of the time period over which it was estimated (table 1; fig. 2).

Table 3: Whole-animal and mass-conditioned correlation estimates ($\pm$ SE) from three-trait multivariate mixed models of standard metabolic rate (SMR), feeding rate (flies consumed), and exploratory behavior (squares crossed in a novel environment) in 19 wild-caught semiaquatic salamanders (*Desmognathus brimleyorum*)

Level	Whole-animal correlations			Mass-conditioned correlations		
	$r \pm$ SE	χ_1^2	P	$r \pm$ SE	χ_1^2	P
SMR vs. feeding rate:						
r_{ind}	**.60 $\pm$ .19**	**5.87**	**.015**	.25 $\pm$ .33	.54	.461
r_e	$-.17 \pm .16$	1.04	.307	$-.17 \pm .16$	1.09	.296
SMR vs. exploratory behavior:						
r_{ind}	$-.60 \pm .27$	3.41	.065	$-.41 \pm .41$	.84	.359
r_e	$-.15 \pm .16$	.89	.345	$-.15 \pm .16$	.82	.365
Feeding rate vs. exploratory behavior:						
r_{ind}	$-.09 \pm .36$	.07	.798	.25 $\pm$ .39	.40	.527
r_e	$-.01 \pm .16$	.00	.965	.00 $\pm$ .16	.00	.992

Note. Statistically significant correlations are indicated in bold. Unstructured correlation matrices were included at among-individual and residual levels, which yielded estimates of among-individual correlations (r_{ind}) and within-individual correlations (r_e) for each pair of traits. Models were run with trial number (categorical) as the only variable (whole-animal correlations) and with $\log_{10}$-transformed body mass (mass-conditioned correlations). The significance of each r_{ind} and r_e was tested using a log-likelihood ratio test with a χ^2 statistic with 1 df (χ_1^2).

By contrast, repeatability declined over time in all other traits when conditioned on body mass (table 1; fig. 2). Although the V_I estimates for feeding rate were significant over all time periods, the repeatability decreased from 0.78 when estimated over 3 wk to 0.45 when estimated over 9 wk, which represents a 42% decrease (table 1; fig. 2). Even higher decreases were observed in SMR (50% decrease, from 0.49 to 0.25) and exploratory behavior (62% decrease 0.39 to 0.15), with the V_I estimates becoming marginally nonsignificant over 6 wk and nonsignificant over 9 wk (table 1; fig. 2).

Phenotypic Correlations

Allowing trial-specific residual variances and correlations revealed that the r_p values between SMR, feeding rate, and exploratory behavior did not significantly vary from one trial to the next on a whole-animal basis (LRT: $\chi_2^2 < 1.96$, $P > 0.38$ for all pairwise combinations; table 4). When conditioned on body mass, however, the r_p values between SMR, feeding rate, and exploratory behavior differed significantly from one trial to the next (table 2). Although none of the trial-specific r_p values between SMR and feeding rate was significantly different from 0 (table 2), the r_p values were significantly different from each other as they ranged from -0.14 to 0.28 (LRT: $\chi_2^2 = 17.64$, $P < 0.001$). The only trial-specific r_p that was significantly different from 0 was between SMR and exploratory behavior during trial 2 (table 2). Again, the r_p values ranged from -0.52 to

-0.06, which was a significant difference (LRT: $\chi_2^2 = 20.54$, $P < 0.001$).

Among- and Within-Individual Correlations

Using data from all trials, we further partitioned r_p into among-individual (r_{ind}) and within-individual (r_e) correlations. None of the whole-animal and mass-conditioned correlations was statistically significant at the within-individual level (table 3). At the among-individual level, there was a statistically significant whole-animal r_{ind} between SMR and feeding rate (table 3) and a marginally nonsignificant whole-animal r_{ind} between SMR and exploratory behavior (table 3; fig. 3C). When conditioned on body mass, however, r_{ind} was weaker and not significantly different from 0 (table 3; fig. 3D).

Individual Variation in Phenotypic Change across Trials

Adding V_S and Cov_{I-S} did not improve model fit for body mass, SMR, and feeding rate (table 4), indicating that the changes in these traits across trials did not significantly differ across individuals (fig. 4A–4C). By contrast, adding V_S and Cov_{I-S} did significantly improve model fit for exploratory behavior (table 4), suggesting that individuals differed in their habituation to the testing procedure (fig. 4D). Indeed, some individuals remained consistent in their exploratory behavior across trials,
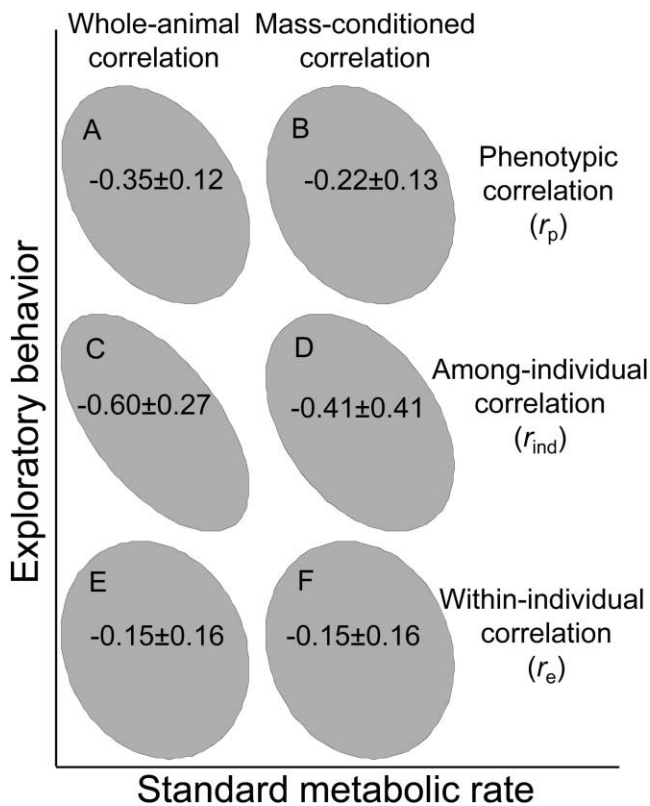
Figure 3. Ellipse representation of the correlations between standard metabolic rate and exploratory behavior in 19 wild-caught semiaquatic salamanders (*Desmognathus brimleyorum*) at different levels of variation (phenotypic correlation: top row; among-individual correlation: middle row; within-individual correlation: bottom row) and on a whole-animal basis (left column) and conditioned on body mass basis (right column). Ellipses were made using the "ellipse" package in R.

whereas others clearly decreased or increased (fig. 4D). The Cov_{I-S} was negative, indicating that individuals with high and low initial exploratory behavior tended to show decreases and increases, respectively, in exploratory behavior across trials (fig. 4D). The negative Cov_{I-S} (table 4) also indicates that differences among individuals changed across trials.

Discussion

Our results demonstrate that body mass, SMR, exploratory behavior, and feeding rate are repeatable in a lungless salamander and that the repeatability varied substantially across trait categories. Indeed, repeatability estimates ranged from very high for body mass ($R = 0.98$), to intermediate for SMR ($R = 0.39$) and feeding rate ($R = 0.58$), to low for exploratory behavior ($R = 0.25$). Moreover, repeatability was not constant over time; it decreased in all traits (except body mass) with the time elapsed between measurements (from 3 to 9 wk). It is possible that the decline in repeatability and the loss of statistical significance in some estimates were the consequence of low

statistical power (19 individuals). Despite significant repeatability in all traits, we find little evidence that behaviors are correlated with SMR at the phenotypic and among-individual levels when conditioned on body mass. Specifically, the phenotypic correlations between SMR and exploratory behavior were negative in all trials but significantly so in one trial only. Finally, individuals showed variation in how their exploratory behavior changed across trials (but not body mass, SMR, and feed intake), which might have contributed to observed changing correlations across trials.

At first sight, our repeatability estimates for SMR may appear lower than values typically reported for this trait (mean ± SE: $R = 0.67 \pm 0.05$, $n = 13$) as compiled by Nespolo and Franco (2007). However, metabolic rate is intimately tied to variation in body mass, and the relatively high repeatabilities for metabolic rates reported in Nespolo and Franco (2007) might reflect artificial inflation due to high body mass repeatability. In fact, the repeatability of whole-animal SMR was 0.79 ± 0.08 in our population, showing good agreement with repeatability for whole-animal estimates (see above). Here we emphasize our repeatability estimate of SMR using mixed models that included a fixed effect of body mass (i.e., conditioned on body mass). In the most recent compilation of repeatability of metabolic rates, White et al. (2013) considered only studies that accounted for variation in body mass and obtained an average ($\pm$SE) repeatability for mass-conditioned SMR of $R = 0.44 \pm 0.05$ ($n = 31$), which is very close to our estimate ($R = 0.39 \pm 0.16$). Moreover, the values reported here are similar in magnitude to those measured over a similar time frame in brown trout (5 and 10 wk; Norin and Malte 2011). Although the compilation by White et al. (2013) included estimates based on a variety of taxa (invertebrates [insects, spiders, snails], fishes, and reptiles), there are currently no comparable repeatability estimates for SMR in an amphibian. Repeatabilities of similar magnitude (range = 0.20–0.34) were found for locomotor performance in tiger salamanders (*Ambystoma californiense*; Austin and Shaffer 1992).

Exploratory behavior is a trait frequently associated with animal behavioral syndromes and has been shown to correlate with life-history variation across muroid rodent species (Careau et al. 2009) and with important ecological characteristics such as resources and predation risk (Mettke-Hofmann et al. 2002; Dingemanse et al. 2007). Feeding rate is another important behavioral trait as it is a key component regulating the energy budget of individuals and hence should be related to fitness. The existence of consistent individual differences in behaviors suggests that many behaviors are not as phenotypically plastic as previously thought and that they may often be heritable (Boake 1994; Stirling et al. 2002; Careau et al. 2011). Using all three repeated measures on each individual, we found that both exploratory behavior and feeding rate were significantly repeatable, although the repeatability of the former ($R = 0.25$) was about half that of the latter ($R = 0.58$). Bell et al. (2009) compiled repeatability estimates for a broad range of behaviors and over varying time periods and reported that the average repeatability across all estimates was 0.37. In addition, Bell et
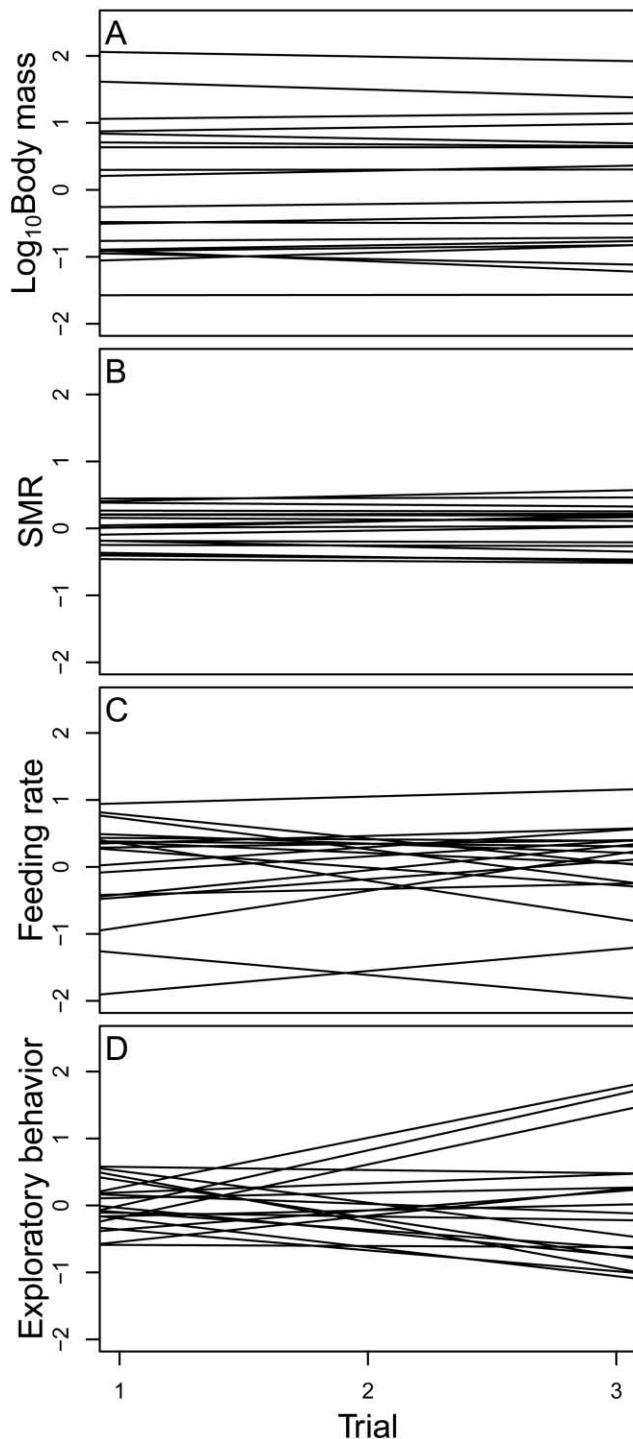
Figure 4. Individual variation in $\log_{10}$-transformed body mass (*A*), $\log_{10}$-transformed standard metabolic rate at 15°C (SMR; *B*), feeding rate (flies consumed d^{-1}; *C*), and exploratory behavior (squares crossed in a novel environment; *D*) in 19 wild-caught semiaquatic salamanders (*Desmognathus brimleyorum*) in three temporally separated trials. Lines represent predicted individual trajectories from the random regression models in table 4. Dependent variables were transformed to a mean of 0 and a variance of 1 prior to analysis.

al. (2009) found large differences in repeatability across types of behaviors, but the meta-analysis was complicated by the sparse nature of their data set. To our knowledge, this study is among the first demonstrations of significant repeatability of feeding rate and exploratory behavior in amphibians.

Repeatability for mass-conditioned SMR and exploratory behavior declined over time, and we did not detect significant repeatability over longer time periods (although 6-wk repeatability estimates were marginally nonsignificant). By contrast, feeding rate was significantly repeatable over all time periods, but repeatability still declined substantially from 0.78 to 0.45. A pattern of declining repeatability of SMR with time has been frequently reported in studies of birds, mammals, lizards, and fishes (Chappell et al. 1995, 1996; De Vera and Hayes 1995; Broggi et al. 2009; Norin and Malte 2011; White et al. 2013); our study extends this phenomenon to amphibians. Bell et al. (2009, p. 777) also found that repeatability estimates were higher for behaviors measured close together in time, but their time category (greater than or less than 1 yr) was a "fairly coarse measure, and one which does not take differences in life span into consideration." Here, we have shown a general decline in repeatability, and the controlled nature of our experiment eliminated potential confounding variables.

Biro and Stamps (2010) recently hypothesized that energy metabolism could contribute to consistent individual differences in behavior through the effects of behaviors on an individual's energy budget. Behaviors contribute to the energy budget by consuming energy produced via metabolism or by affecting energy intake, which fuels metabolism (Biro and Stamps 2010). Thus, behaviors that influence food intake rates or energy expenditure should be correlated with metabolic rate. Movement (exploratory) behavior could be correlated with food intake rates via higher prey encounter rates for those individuals that are more active (Zollner and Lima 1999; Avgar et al. 2008). Despite large and consistent individual differences in feeding rates, we found that this trait was not significantly correlated with SMR and exploratory behavior. The absence of a correlation between organ size and SMR might, in part, explain the results presented here, but in a larger sample of *Desmognathus brimleyorum*, internal organ masses are significantly correlated with SMR after controlling for body size (M. E. Gifford, unpublished data). Therefore, our study does not support the metabolic engine model proposed by Biro and Stamps (2010), also referred to as the " increased-intake or the performance model (Nilsson 2002; Careau et al. 2008).

An alternative explanation considers exploratory behavior (and other personality traits) as being energetically costly to express. From the allocation principle, which posits that animals must divide a fixed quantity of energy among competing processes, one could expect to find a negative correlation between SMR and exploratory behavior because individuals with higher SMR have less energy to allocate to activity (Careau et al. 2008). It must be noted that all of the correlations (r_p, r_{ind}, and r_e) between SMR and exploratory behavior were negative, which is consistent with the allocation model proposed by Careau et al. (2008). However, using data from all trials yielded

Table 4: Parameters from univariate random regression models with fixed effects of trial (categorical) and body mass and random effects of intercept (V_I), slope (V_S), and the covariance between intercept and slope (Cov$_{I-S}$) for log$_{10}$-transformed body mass (g), log$_{10}$-transformed standard metabolic rate (SMR), feeding rate (flies consumed), and exploratory behavior (squares crossed in a novel environment) in 19 wild-caught semiaquatic salamanders (*Desmognathus brimleyorum*)

| | Variance components | | | | V_I model | |
Trait	$V_I \pm$ SE	$V_S \pm$ SE	Cov$_{I-S} \pm$ SE	$V_R \pm$ SE	$\chi^2_{1:2}$	P
Body mass	1.088 ± .373	.008 ± .005	−.030 ± .032	.013 ± .004	3.10	.145
SMR	.164 ± .278	.013 ± .054	−.016 ± .112	.213 ± .071	.14	.824
Feeding rate	1.141 ± .542	.147 ± .084	−.308 ± .193	.178 ± .059	4.14	.084
Exploratory behavior	.817 ± .641	.307 ± .177	−.430 ± .315	.377 ± .126	6.98	.019

Note. Dependent variables were transformed to a mean of 0 and a variance of 1 prior to analysis. The random variance component for slopes (V_S) captures the extent to which individuals differ in how their phenotype changed across trials. The Cov$_{I-S}$ component captures how the initial expression of the phenotype (intercept) covaries with the change (slope) across trials. The significance of the random regression models was tested using a log-likelihood ratio test by comparing the full model (including V_S and Cov$_{I-S}$) with a model that included V_I only (table 1).

only nonsignificant relationships, which could be a consequence of the relatively small sample size used in this study.

In fact, the only significant correlation was between mass-conditioned SMR and exploratory behavior in trial 2. The lack of consistency among trials might suggest that this one significant result is anomalous. To the extent that it is not, the differences in the relationship between mass-conditioned SMR and exploratory behavior appear context dependent (Burton et al. 2011; Careau and Garland 2012). This result is also supported by all empirical studies that specifically tested for a link between resting or basal metabolic rate and exploratory behavior in a novel environment. These studies, conducted on small rodents (Careau et al. 2011; Lantová et al. 2011; Timonin et al. 2011) and birds (Maldonado et al. 2012; Bouwhuis et al. 2013), reported that the relationship between resting or basal metabolic rate and exploratory behavior can vary from positive, to nil, to negative according to reproductive status, sex, and population (reviewed in Careau and Garland 2012). Hence, our study extends this observation to ectotherms on a trial-to-trial basis. Furthermore, quantitative genetic analyses that estimate both phenotypic correlations and additive genetic covariances can result in different conclusions regarding how (or even whether) particular traits are correlated (see Sadowska et al. 2009; Careau et al. 2011). This stresses the importance of applying the analytical methods developed within the quantitative-genetics framework to estimate, as a first step, r_{ind} separate from r_P (Brommer 2013).

Perhaps a more likely explanation for the lack of, or context-dependent, correlations obtained in this study concerns maintenance of body condition throughout the experiment. From a theoretical standpoint, repeatability might vary depending on other environmental characteristics (Dohm 2002). Variation in environment between measurements has been shown to influence repeatability estimates. For example, O'Connor et al. (2000) found that a period of food deprivation caused a loss of repeatability of metabolic rate in Atlantic salmon. Furthermore, Norin and Malte (2011) demonstrated declining re-

peatability in brown trout kept under a reduced feeding regime. Our study was not planned to impose food restriction, and food was offered on a weekly basis between trials. However, animals in this study showed a net loss of body mass over the course of the experiment (10.4% ± 0.017%). Therefore, loss of body condition could explain the decline in repeatability of all traits over the course of 9 wk and the overall lack of correlations among physiological and behavioral traits. Using the random regression approach, however, we found that individuals did not differ in how their body mass, SMR, and feeding rate changed over the three trials, such that the reaction norms were mostly parallel. By contrast, individuals significantly differed in how their exploratory behavior changed over the three successive trials, perhaps related to experience or habituation to a novel environment. Such a pattern was found in four different populations of great tits (Dingemanse et al. 2012a) but not in a population of eastern chipmunks (Martin and Réale 2008).

Morphological traits (e.g., body mass, lean mass, leg length) generally are less closely associated with Darwinian fitness than higher-level traits (e.g., life-history and behavioral traits; Careau and Garland 2012) and generally have higher heritability (Mousseau and Roff 1987; Kruuk et al. 2000; Walsh and Blows 2009). One could expect repeatability estimates for different traits to reflect the pattern observed for heritability. Results from our study are roughly consistent with this prediction (body mass > feeding rate > SMR > exploratory behavior). Feeding rate is arguably a behavioral trait, but nevertheless it showed relatively high repeatability, perhaps because it is somewhat less directly associated with fitness than SMR and exploratory behavior in salamanders. Although repeatability provides a reasonable proxy for the upper limit to heritability, there are many reasons why two equally heritable traits have different repeatabilities. By contrast to heritability, which includes only additive genetic effects, repeatability includes nonadditive genetic effects (dominance) and permanent environment effects (conditions an individual experienced during its lifetime, ma-

ternal or natal effects). It is the sum of all these effects that generates consistent among-individual variation (Falconer and Mackay 1996; Lynch and Walsh 1998). Further studies should use special breeding designs and data analyses to further partition among-individual (co)variances into permanent, nonadditive, and additive genetic components.

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