When the mean no longer matters: developmental diet affects behavioral variation but not population averages in the house cricket (*Acheta domesticus*)

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Abbreviated title: Diet effects on personality differences

ABSTRACT

Despite recent progress elucidating the genetic basis for behavioral variation, the effects of the developmental environment on the maintenance and generation of behavioral variation across multiple traits remains poorly resolved. We investigated how nutritional status during development affected behavioral variation and covariance between activity in an open field test and response to cues of predator presence in the house cricket (*Acheta domesticus*). We provided 98 juvenile crickets with either a high or low quality diet during development throughout which we measured body mass, activity in a modified open-field and response to predator excreta twice every week for three weeks. Diet quality affected growth rate but not average activity or response to cues of predator presence, nor the correlation between the two. However, repeatability (τ) in response to cues of predator presence was reduced by 0.24 in individuals exposed to the high quality diet versus the low quality diet. Larger individuals also increased their response to predator cues when reared on a high quality diet, suggesting negative feedbacks between growth rate and antipredator behaviors. Our results also indicate that changes in the developmental environment are not sufficient to collapse behavioral syndromes, suggesting a genetic link between activity and predator cue response in house crickets, and that nutritional stress early in life can lead to more consistent behavioral responses when individuals faced predatory threats. Our results demonstrate that subtle differences in the quality of the environment experienced early in life can influence how individuals negotiate behavioral and life-history trade-offs later in life.

Key-words Activity, animal personality, antipredator response, behavioral variation, behavioral syndromes, permanent environment, developmental conditions, diet quality, house crickets

INTRODUCTION

The environment organisms experience during development can have a considerable influence on phenotypic expression. For example, individuals are primed to adjust their phenotype to developmental cues through epigenetic changes (Ledón-Rettig et al. 2012; Weaver et al. 2004) which can have long-term consequences on morphology, physiology, and behavior (Kasumovic 2013; Snell-Rood 2013; West-Eberhard 2003). Understanding the contribution of the developmental environment to phenotypic expression is therefore fundamental to understanding the generation of *stable individual differences* in behavior (i.e. animal personality and behavioral syndromes, Réale et al. 2007; Sih et al. 2004a; Sih et al. 2004b).

Personality variation arises from the combined influence of genetic and environmental factors (Dingemanse and Dochtermann 2014, Han and Dingemanse 2015) with each accounting for around 50% of observed personality variation (Dochtermann et al. 2015). A large part of personality research has focused its attention on quantifying the genetic inheritance of behavioral variation (Taylor et al. 2012), its fitness consequences (Bergeron et al. 2013), and implications for a population's evolutionary trajectories (Dochtermann and Dingemanse 2013). However, these areas of attention largely deal with ultimate causes and consequences of personality variation and behavioral syndromes and as such are fundamentally multi-generational topics. In contrast, variation in the environment an individual experiences during development—such as food abundance, food quality, conspecific density, or presence of predator cues—typically operate within the life-time of individuals.

Focusing on how within-lifetime processes affect the expression of behavioral variation is necessary because differences in early experiences may place individuals along different life-history trajectories in interaction with their behavioral types (Buss and Greiling 1999; Carere et

al. 2005). Since changes in the expression of a behavioral phenotype are often associated with costs (reviewed by Snell-Rood 2013), such changes might be restricted to sensitive periods during early development (Groothuis and Trillmich 2011). Manipulative studies have identified many of the developmental conditions that influence the expression of behaviors (diet quality: Noguera et al. 2015, immune challenge: Butler et al. 2012; predation pressure: Niemelä et al. 2012b; access to shelter: Bengston and Jandt 2014; conspecific cues: DiRienzo et al. 2012; Niemelä et al. 2012a; physical and social environment: Liedtke et al. 2015). Most of these studies, however, focus strictly on changes in the population average while effects on behavioral variation *in and of itself* remain largely ignored (but see DiRienzo et al. 2015). This is a missed opportunity because determining the effects of developmental conditions on behavioral variation can shed light on the set of factors that promote the generation of stable individual differences (Duckworth 2010; Han and Dingemanse 2015; Jandt et al. 2014; Stamps and Groothuis 2010)

The developmental environment can also create conditions by which morphological and behavioral variation dynamically interact with each other via feedback loops. For example, diet quality impacts growth rates (Hunt et al. 2005b) and resulting morphological differences can in turn affect behavioral and life history strategies (Lee et al. 2015; Metcalfe and Monaghan 2001). Feedback loops connecting behavior with an individual's morphology (e.g. condition or other state variables, *sensu* Houston and McNamara 1999) can then affect the expression of behavioral variation within a population. Under a negative feedback scenario, if individuals with access to higher quality or more food are larger or have positive energy balances, they are expected to engage in asset protection (Clark 1994; Clark and Mangel 2000) and limit the amount of risk-taking behaviors over time. This behavioral change is then expected to reduce the amount of personality variation observed over time (Dall et al. 2004; Luttbeg and Sih 2010). In a positive

feedback scenario, such as state-dependent safety (Luttbeg and Sih 2010), individuals with higher body-sizes or more positive energy balance are less likely to be captured by predators and can increase their foraging effort under predation risk, in turn increasing their state value. Such a feedback loop is again expected to increase the amount of among-individual variation present within a population and lead to stable behavioral differences (Luttbeg and Sih 2010). While state-dependent models of personality have been extensively investigated through theoretical and conceptual models (Dall et al. 2004; Luttbeg and Sih 2010; Montiglio and Royauté 2014; Sih et al. 2015; Wolf et al. 2007), they remain poorly studied empirically (but see Mathot et al. 2011). In the absence of direct tests, state-dependent feedback can be indicated by among-individual state-based differences in behavior (Sih et al. 2015).

State-dependent feedback loops and their effects on behavioral variation are further complicated by the fact that behavioral responses are frequently integrated within behavioral syndromes (Sih et al. 2004 a,b). Because they are generated in part through genetic correlations (Dochtermann and Roff 2010; Dochtermann 2011; Han and Dingemanse 2015), behavioral syndromes may constrain evolutionary responses (Dochtermann and Dingemanse 2013) and may prevent populations from reaching adaptive peaks if behavioral syndromes are robust to environmental variability. There is, however, considerable variation among organisms and within populations in the behaviors associated within a syndrome, often depending on environmental conditions (Bell 2005; Bell and Sih 2007; Dingemanse et al. 2007; Royauté et al. 2014, 2015a). Unfortunately, despite the stability of behavioral syndromes over multiple life-stages having been well studied (Brodin 2009; Sinn et al. 2008; Wilson and Krause 2012), the developmental characteristics that shape the emergence of syndromes are poorly understood (Han and Dingemanse 2015).

Here we investigated how variation in a single component of the developmental environment, diet quality, influenced subsequent variation in and correlations among body mass, activity, and response to cues of predator presence in juvenile house crickets (*Acheta domesticus*). Specifically, we aimed to answer the following questions: (1) Does variation in diet quality generate different developmental trajectories in growth rates, activity, and response to cues of predator presence? (2) Does diet quality increase or decrease body mass and behavioral variation? (3) Does diet quality affect the strength of correlations between morphological and behavioral traits? At the population average level, we expected a low quality diet to decrease growth rate and, as a result, increase individual's propensity to exhibit risky behaviors in order to meet energetic demands (state-dependent safety and starvation avoidance principles, Luttbeg and Sih 2010; Figure 1a). Here, this would translate into higher activity levels and a higher propensity to ignore the presence of predatory cues. In contrast, we expected individuals fed a high quality diet to increase their growth rate and avoid risky situations through the asset protection principle (Clark 1994; Luttbeg and Sih 2010; Figure 1b). For effects on trait covariance, we predicted a negative correlation between activity and antipredator response in both treatments since the patterns of effect of growth rates on these traits are opposite and symmetrical across diet quality treatments (Figure 1). We also predicted that due to the cost of plasticity, individuals fed a low quality diet would decrease the amount of behavioral plasticity (measured here as the residual within-individual variance V_R—*sensu* Westneat et al. 2015—although here this measure conflates measurement error, organismal error, and plasticity). Finally, since individuals would be more constrained in terms of energy allocation we also expected a "ceiling effect" on among-individual variation (V_{ID}) such that V_{ID} in body mass would be lower in the low quality diet treatment.

MATERIALS AND METHODS

House cricket rearing and diet preparation

We purchased one-week old *Acheta domesticus* from Fluker's Farm (Port Allen, LA, USA). Crickets were housed individually in 0.71L containers and provided with *ad libitum* food and water, as well as pieces of egg carton for shelter. All individuals were maintained under a 12:12 light/dark photoperiod. Individuals were fed a standard laboratory diet and allowed to habituate to their containers for a minimum of seven days before being randomly assigned to either a high or low quality diet (Table 1) and beginning behavioral trials.

We varied diet quality by producing food pellets baked from high protein fish flakes (high quality diet, TetraMin Plus) and cellulose mixed with fish flakes (low quality diet, two parts cellulose to one part fish flakes). Thus the nutritive quality of the high quality diet was 3 times higher than that of the low quality diet (Table 1). The use of non-nutritive cellulose also imposed gut limitations on intake, preventing individuals in the low quality diet treatment from circumventing diet quality differences. Food pellets were produced by mixing ground fish flakes with water or cellulose and water and cooking the mixture in a Plexiglas mold at 60°C for 12h following Hunt et al. (2005). A preliminary study completed on a subset of 40 crickets (20 low quality, 20 high quality) showed substantial differences in growth rates between the two diets after 10 days of treatment (diet × day interaction, $P < 0.05$) (Figure S1). Because these crickets originate from captive populations, a direct comparison with their wild diet is difficult. However, the low quality diet treatment provided considerably lower energy than our standard laboratory diet (ground Purina chick starter chow, 2.88 cal/g, Purina Mills®, St Louis, MO, U.S.A.; Table 1).

Behavioral tests

To test whether diet quality affected patterns of behavioral variance and covariance, we recorded individuals' activity levels in a modified open-field arena (obstacle course assay) and response to diluted gecko excreta (response to a cue of predator response assay). Behavioral testing began within one week of arrival and testing occurred between 1 October 2014 and 3 March 2015. Due to logistical constraints, crickets were assayed in batches of 20 individuals with 10 individuals randomly assigned to either the high or low diet quality treatments. Five separate batches were sequentially reared and assayed and "batch" was included in all analyses (see below) to statistically account for any potential effects on average behavior. Behavioral trials for batches 1 – 5 began on 1 October 2014, 11 November 2014, 12 January 2015, 27 January 2015, and 6 February 2015 respectively. In order to minimize potential carry-over effects from exposure to cues of predator presence, the obstacle course assay was always conducted first followed by testing an individual's antipredator response. We thoroughly cleaned each arena in between trials with 70% ethanol wipes to avoid chemical trace of conspecifics from influencing the behavior of subsequently tested individuals. We recorded mass to the nearest mg immediately after the antipredator assay. This procedure was repeated twice every week over a three week period for a maximum of six behavioral measures per individual per assay. In total we were able to record the behavioral responses of 98 individuals (low quality diet: n = 45, high quality diet: n = 53) with a total of 446 individual observations (low quality diet: n = 198, high quality diet: n = 248; Table S1). Due to mortality during the course of development, particularly in the low quality diet, only 35% of individuals survived until sexual characteristics were noticeable. We thus did not include sex in analyses.

175

176 *Obstacle course activity*

177 Activity was measured in a modified open field test, in which individuals had to navigate around
178 multiple obstacles to explore the entire arena. The arena was 60 cm × 60 cm × 15 cm high,
179 constructed of sealed plastic, and had a Plexiglas lid. This obstacle course behavioral protocol
180 has previously been used with *A. domesticus* to evaluate exploratory behavior (Dochtermann and
181 Nelson 2014) and behavior-physiology correlations (Royauté et al. 2015b). Individuals were left
182 to rest for 30s in a 5 cm diameter container introduced into the lower right section of the arena
183 (Z1, Figure S2a). We then allowed the cricket to move freely through the arena for 220 seconds.
184 We calculated the total distance travelled through the arena (in cm) with Ethovision X (Noldus
185 Information Technology).

186

187 *Response to cues of predator presence*

188 Considerable research with a variety of cricket species has examined latency to emerge from
189 shelter following disturbance under the assumption that this latency relates to anti-predator
190 behavior (e.g. Hedrick 2000; Hedrick and Kortet 2012; Kortet et al. 2007; Niemelä et al. 2015).
191 Such latencies are ecologically reasonable measures of anti-predator behavior, particularly for
192 males, but are not the only means by which crickets assess or respond to potential predator
193 presence. For example, over the last decade it has been repeatedly demonstrated that Gryllidae
194 crickets respond to chemical cues of predators via either escape or avoidance behaviors (Kortet
195 and Hedrick 2004; Storm and Lima 2008; Storm and Lima 2010). Response to chemical cues has
196 likewise been used to measure response to potential predation threat in a variety of invertebrates

197 and vertebrates (e.g. Herman and Valone 2000; Dochtermann et al. 2012; Nelson et al. 2013;
198 Patterson et al. 2013).

199 That crickets respond to chemical cues of predator presence should not be surprising as
200 chemosensory cues are known to be important and often sufficient and/or necessary to illicit a
201 variety of behavioral responses. The importance of chemical cues to crickets has been
202 demonstrated, for example, in male-male agonistic interactions (e.g. Iwasaki and Katagiri 2008),
203 in female assessment of male quality (e.g. Kortet and Hedrick 2005), self-reference to prevent
204 repeated matings with the same individuals (e.g. Weddle et al. 2013), and, as mentioned above,
205 in eliciting response to potential predator presence.

206 To measure response to cues of potential predator presence here we collected excreta
207 from two subadult and one adult leopard gecko (*Eublepharis macularius*) that were fed a mixed
208 diet of crickets (*A. domesticus*) and mealworms (*Tenebrio molitor*). Leopard geckos were housed
209 according to North Dakota State University IACUC standards (Protocol number: A14006).
210 Collected excreta was ground weekly and diluted with deionized water (1 ml H_2O : 5 mg
211 excreta). This solution was then applied to 15 cm diameter Whatman filter paper discs (Figure
212 S2b) with a 5 cm diameter central cutout that allowed crickets to be left to rest unexposed to the
213 predatory cue. Each predatory disc was left to dry for a minimum of 2 h and was stored at 4 °C
214 before trials. We inserted the predatory cue disc at the bottom of a 15cm diameter arena and left
215 the cricket to rest for 30 s under a 5 cm diameter cup in the non-treated central cutout. We then
216 allowed the cricket to move freely for 220 s and estimated the distance travelled (in cm) through
217 Ethovision. Previous experiments with this protocol showed that crickets had heightened activity
218 levels in presence of diluted gecko excreta compared to a water control (t_{48} = 2.05, p = 0.046;

Figure S3), thus greater activity during anti-predator trials was interpreted as greater responsiveness to predator cues.

Data analysis

All analyses were conducted in R 3.2.2 (R Development Core Team 2013) with the package MCMCglmm for Bayesian mixed models (Hadfield 2010).

Effect of diet quality on average trait value and trait repeatability

We estimated the effects of diet type and development on activity, response to cues of predator presence, and mass using Bayesian univariate mixed models. Mass was $\log_{10}$-transformed and all response variables were expressed as standard deviation units. The third batch of crickets had stronger mortality due to winter shipment and we included a three-way interaction for diet type, day (centered) since placed on either the low or high quality diet, and batch number. Additional fixed effects included condition (injured or not), time of testing, temperature (expressed as among and within individual values; van de Pol and Wright 2009), and whether the cricket crawled under the filter paper during the antipredator response assay to control for potential confounds and "pseudo-repeatability" (Nakagawa and Schielzeth 2010; Westneat et al. 2011). Individual identity was included as a random effect and variance components were estimated separately by diet treatment to allow comparison of trait repeatabilities (Royauté et al. 2015a). We specified a Markov chain Monte Carlo (MCMC) chain with 1.3×10^6 iterations, 300000 burn-in period and a thinning interval of 1000. We tested the influence of prior type on our results by comparing results from an inverse-Wishart prior to those estimated with maximum

likelihood. Prior type had very little influence on our estimates and we present the results based on the inverse-Wishart prior.

MCMC approaches to fitting mixed models, like used above, do not allow classic tests of "significance" Thus, to test for the "significance" of the diet × day × batch effects on body size and behaviors we employed two approaches. First, we specified seven *a priori* models (Table S3) of different ways in which our predictor variables might have affected body size or behaviors. The models we evaluated differed in the complexity of interactions included, ranging from a full model including the three-way interaction of diet × day × batch, all two-way interactions, and all main effects to reduced models that included single main effects. For these different models, we then compared their "Deviance Information Criterion values" (DIC). Models with lower DIC have better support and models with $\Delta DIC < 4$ were considered as statistically equivalent. This procedure is analogous to the Likelihood Ratio tests for fixed effects performed with frequentist mixed models (Zuur 2009). Second, to assess the "significance" of particular terms we examined whether individual model terms as estimated had 95% credibility intervals that overlapped zero.

We calculated repeatability (τ) as the posterior mode of $\tau = V_{ID} / (V_{ID} + V_R)$ with V_{ID} being the among-individual variance and V_R the within-individual (or residual) variance. We also calculated the MCMC posterior distribution of differences in repeatability between the diets ($\Delta\tau = \tau_{HighQual} - \tau_{LowQual}$). This metric provides an estimation of the effect size for the difference in repeatability (Royauté et al. 2014, 2015a). Here positive values of $\Delta\tau$ indicate greater repeatability in the high quality diet treatment. "Significance" and inference of this difference was based on the proportion of posterior estimates that excluded zero. We repeated this procedure on each variance component (V_{ID} and V_R) to determine whether changes in

repeatability were linked to changes in any specific variance component. Importantly estimates of values like $\Delta\tau$ may differ from a comparison of $\tau_{HighQual} - \tau_{LowQual}$ because the former is calculated directly from each MCMC iteration.

Effect of diet quality on behavioral correlations

We tested whether diet quality affected correlations among activity, antipredator response, and mass by specifying a multivariate mixed model which was fit separately for each diet type. We included all three traits as response variables and used individuals as random effects. All fixed effects and model conditions were as above. This procedure allowed us to estimate and compare among- (r_{ID}) and within-individual (r_{WI}) correlation matrices between diet types (following Dingemanse and Dochtermann 2013). As above, we compared the magnitude of the difference in posterior estimates of correlation coefficients between the treatments ($\Delta r = r_{HighQual} - r_{LowQual}$) and base our inferences on the proportion of estimates excluding zero.

RESULTS

Effect of diet quality on average trait value and trait variation

Diet quality positively affected growth rate (diet × day interaction, estimate ± CI; $\beta = 0.04 \pm$ [0.03:0.05]) but had no effect on average activity ($\beta = 0.00 \pm$ [-0.05:0.05] or response to cues of predator presence ($\beta = 0.02 \pm$ [-0.03:0.08]) (Figure 2, Table S2). These inferences are consistent with our model comparison results which indicated similar patterns as none of the best models had substantially higher support than the null model for behavioral data (ΔDIC < 4, Table S3). We did, however, detect a significant three-way interaction between diet, day, and batch number

on body mass, indicating that the positive effect of the high quality diet on growth rate varied among batches (Table S3, Figure S4).

The repeatability of response to cues of predator presence was lower in the high quality diet (low quality: $\tau = 0.36 \pm [0.21; 0.53]$, high quality: $\tau = 0.16 \pm [0.07; 0.27]$, $\Delta\tau = -0.24$, $P = 0.01$; Figure 3), but no significant differences were detected in repeatability for activity or mass (activity: $\Delta\tau = -0.02$, $P = 0.43$; mass: $\Delta\tau = 0.03$, $P = 0.27$; Figure 3, Table S2). The observed changes in the repeatability of antipredator response were more strongly influenced by an increase in within-individual variation in the high quality diet rather than a decrease in among-individual variance (among-individual variance: $\Delta V_{ID} = -0.18$, $P = 0.07$; within-individual variance: $\Delta V_R = 0.27$, $P = 0.008$; Figure 3 and Figure S5). This suggests that individuals provided with a high quality diet were more inconsistent across repeated testing in their response to predator cues while individuals provided with a poor diet quality remained relatively consistent over the duration of the experiment.

Effect of diet quality on behavioral correlations

We found no evidence for a change in behavioral syndrome structure mediated by diet quality. Instead, activity and antipredator response were positively correlated with similar effect sizes in both treatments (low quality: $r_{ID} = 0.39 \pm [0.05; 0.71]$, $r_{WI} = 0.31 \pm [0.18; 0.47]$; high quality: $r_{ID} = 0.31 \pm [0.02; 0.64]$, $r_{WI} = 0.40 \pm [0.27; 0.51]$, Figure 4). In the high quality diet, larger individuals had a greater antipredator response while this association was not detected in the low quality diet (i.e. body mass × antipredator correlation: low quality: $r_{ID} = 0.01 \pm [-0.41; 0.33]$; high quality: $r_{ID} = 0.39 \pm [0.02; 0.62]$; $\Delta r_{ID} = 0.38$, $P = 0.04$) (Figure 4, Table S4).

DISCUSSION

Our aim here was to test whether the developmental environment experienced by individuals affects behavioral means, behavioral variation, and the covariance between activity and response to cues of predator response. We did find evidence of diet manipulation affecting multiple levels of trait variation however those patterns often contradicted our theoretical predictions (Figure 1). Besides greater growth (and thus mass) for the high versus low quality diet, we predicted decreased activity and increased response to predator cues in the high versus low quality diets. We also had initially predicted that among-individual variation in mass would be lower in the low quality diet versus the high quality diet and that within-individual variation in behaviors would be higher in the high quality diet than the low, resulting in greater behavioral repeatability in the low quality diet. Although a higher diet quality increased cricket growth rate, we found surprisingly little effect on either average activity or response to predator cues. Further, we found decreased within-individual variation—evidence of lower behavioral plasticity in the low quality diet—but only for the response to cues of predator presence. Finally, the activity-predator cue response behavioral syndrome was not affected by diet quality but the sign of the correlation was opposite to that expected. This suggests that the developmental diet, as manipulated here, can have non-intuitive consequences on trait expression when hierarchical patterns of variation are taken into account.

As expected, increasing diet quality resulted in faster growth rates and thus greater body mass. However, this change in population growth rate did not have consequences on population average behaviors. This led us to generally reject our prediction that individuals in a low quality diet would follow the state-dependent safety principle while individuals in the high quality diet would follow an asset protection model (but see below). Instead, the influence of diet quality had

stronger consequences on trait repeatability and covariance. That diet affected the magnitude of behavioral variation in the absence of a population-level behavioral change is particularly intriguing. Because individuals were maintained in their treatments for only 30 days, it is possible that the effects of diet quality manifest themselves quickly on individual variation but require longer exposure before population shifts are detected. In such a case, maintaining individuals on the different diet regimes over the entirety of development and maturity would be necessary to determine the long-term consequences of diet type on activity and response to cues of predator presence.

Crickets reared on a lower quality diet demonstrated a higher repeatability of their response to cues of predator presence but not their activity. As expected, plasticity in responses to cues of predator presence were favored only when individuals had access to sufficient nutritive resources (i.e. the high quality diet) and individuals under nutritive stress exhibited higher individual consistency in their responses to cues of predator presence, causing higher repeatability. In addition, the largest crickets from the high quality diet treatment reduced risk-taking by expressing a stronger response to cues of predator presence (Figure 4). This indicates partial support for the asset protection principle—wherein individuals that accumulate more assets over time (e.g. increase in body mass) preserve assets by reducing risky behaviors—despite a lack of general support for the principle herein.

These observations lead to two important insights on the influence of diet quality on behavioral variation: First, a higher diet quality and higher caloric content diet may alleviate the costs to behavioral plasticity by attenuating individual trade-offs. In contrast, individuals experiencing nutritive stress may face stronger allocation trade-offs due to limits on energy acquisition and a higher cost of switching behavioral responses. Second, changes in diet quality

may have the potential to change the magnitude of state-dependent feedback loops (e.g. in this case the correlation between body mass and response to cues of predator presence between the two treatments), which in turn can have a profound influence on the maintenance of among-individual variation. Unfortunately our experimental design only allowed us to test for the direct effect of growth rate on behavior through diet manipulation and not for recursive effects of behavior on growth rates. Recursive effects are expected to reduce the amount of personality differences over time, because individual protecting assets would acquire resources at a lower rate (Luttbeg and Sih 2010; Montiglio and Royauté 2014).

According to our predictions, we expected a negative among-individual correlation between activity and response to cues of predator presence. However, the traits were positively correlated. Because activity and response to cues of predator presence were both measured as distance moved through an arena, it is possible that a positive structural correlation will be present between measurements, over-riding more biologically relevant and interesting relationships. However, we view this explanation as insufficient given past experiments with this species. For example, movement rates of *A. domesticus* measured in different arenas can be uncorrelated even if each behavior demonstrates repeatability (Dochtermann and Nelson 2014). Specifically, Dochtermann and Nelson (2014) found that activity (distance moved) measured in an obstacle course arena and activity (distance moved) in maze exploration trials were not correlated. A more plausible explanation for our results therefore is that the activity-predator cue response syndrome is robust to changes in the permanent environment as the among-individual correlations did not vary between low and high quality diet treatments (Figure 4). This general inference holds even if we were assaying activity in different contexts (e.g. open field trials: "exploration of a control environment", predator cue trials: "exploration of an altered

environment"; *sensu* Dingemanse et al. 2007). That is, even if considering activity in the presence and absence of predators as separate contexts, we still demonstrated that the among-individual correlation was robust to permanent environmental differences.

Among-individual correlations, i.e. behavioral syndromes (Dingemanse et al. 2012), are generated by both additive genetic correlations and permanent environment correlations (Dingemanse and Dochtermann 2013; Dingemanse and Dochtermann 2014). In our study, additive genetic correlations should have been the same between the two treatments as individuals were randomly assigned to the two diet types. Any differences in among-individual correlations between the two treatments are therefore attributable to changes in permanent environmental correlations or **G** × **E** effects (Han and Dingemanse 2015). That the activity-predator cue response syndrome was unchanged suggests two alternative explanations: First, this syndrome might be primarily underpinned by genetic correlations. Second, diet quality may have little contribution to the permanent environment correlation and other environmental cues (e.g., temperature, predator presence, maternal effects) could be more influential. We view this latter explanation as less likely because of the strong influence of diet quality on life-history trajectories (Houslay et al. 2015; Tatar and Carey 1995), morphological (e.g. Braendle et al. 2006), physiological (e.g. Cruz et al. 2004), and behavioral traits (Akman et al. 2004; Tremmel and Muller 2013; Wilder and Rypstra 2008) that has been demonstrated in a considerable number of species, including crickets (Hunt et al. 2005a; Hunt et al. 2004; Hunt et al. 2006; Zajitschek et al. 2009; Zajitschek et al. 2012).

As mentioned, there has been extensive research demonstrating the effects of diet and condition on behavior in crickets (see above). Moreover, beyond just general condition or energy availability, attention to particular stoichiometric relationships has highlighted subtle differences

401 in the effects of diet on behavior (e.g. Bertram et al. 2009; Han et al. 2016; reviewed by Han and
402 Dingemanse 2015). Our results are particularly interesting even with this existing literature for
403 several reasons. For example, despite a major difference in dietary value (Table 1), no mean
404 effects on behavior were statistically detectable. This lack of detectable effects is surprising not
405 only because of the existing literature but also given the clear predictions available from
406 conceptual and theoretical frameworks (e.g. the asset protection principle; Luttbeg and Sih
407 2010). Moreover, our approach of partitioning variation and explicitly estimating among- versus
408 within-individual variation and correlations revealed that, despite a lack of mean effects, within-
409 individual variation in response to cues of predator presence differed between the two diet
410 treatments. Most previous manipulations of diet, condition, and state have largely failed to
411 explicitly estimate these types of effects and thus have failed to detect the effect noted here (but
412 see Han et al. 2016 for a notable exception). More generally, our study shows that the outcomes
413 of state manipulation on behavioral variation and behavioral syndromes are not obvious and may
414 fail to confirm theoretical predictions. Indeed, while we found partial support for the asset
415 protection principle regarding antipredator response in the high quality diet, the changes in
416 behavioral variance were more due to an increase in behavioral plasticity than to a decrease in
417 among-individual variance contrary to our predictions. It remains unclear whether manipulating
418 state generally has stronger effects on behavioral averages, behavioral variances, or on
419 behavioral syndromes.
420

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Data Accessibility

Analyses reported in this article can be reproduced using the data provided by Royauté and Dochtermann (2016).

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Figures

Figure 1. Expected effects generated by changes in diet quality on growth rate, activity/exploration and antipredator response. (a) A low quality diet is expected to reduce growth rate and thus increase risk taking in order to meet energy demands. This should lead to an increase activity/exploration levels as well as a decrease in sensitivity to predator cue (i.e. antipredator response). (b) With a high quality diet, individuals are expected to protect their assets and avoid risky situations. As a result, individuals should decrease their activity levels and increase their antipredator response. At the trait variance level, we expect a low quality diet to reduce among-individual variance (V_{ID}) and repeatability (τ) in body mass due to constraints on energy allocation patterns. We also expect that because plasticity is costly, individuals in the low quality diet should have lower within-individual variation in behavior (V_R) and higher repeatability (τ) as a result. Note that in both diet treatments, these patterns of effects are expected to generate a negative correlation between activity and antipredator response.

Figure 2. Effect of diet quality on behaviors and mass. Diet quality affected growth rate but not behavioral response. Values are presented on their original scales, mass data were fitted with loess smoothed curves. Black squares, dashed line: low quality diet; white circles, solid line: high quality diet.

Figure 3. Effect of diet quality on trait repeatability (τ), among-individual (V_{ID}) and residual (V_R) variance components. Inference is based on the magnitude of the change in repeatability and variance component, $\Delta\tau$ and ΔV, and the probability of observing significant difference, P. Only antipredator response showed significant differences in repeatability.

Figure 4. Effect of diet quality on among (r_{ID}) and within-individual (r_{WI}) correlations. The shape of the ellipse indicates the strength of the correlation, positive correlations are indicated in blue, negative correlations are indicated in red. Bold values indicate significant correlations based on overlap of 95 % CIs with zero.

Tables

Table 1. Nutritional composition of the low and high quality diets.

Nutrient content	Low quality diet	High quality diet	Standard laboratory diet
Total energy (cal/g)	1.12	3.35	2.88
Protein (%)	15.33	45.00	18.00
Lipid (%)	3.66	11.00	3.00
Carbohydrate (%)	7.66	23.00	56.90
Non-nutritive fiber (%)	65.00	3.00	5.50