# Protein deprivation facilitates the independent evolution of behavior and morphology

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**Data archival statement**

We confirm that the data supporting our results will be archived in an appropriate public repository such as Dryad and the data DOI will be included at the end of the article when our manuscript is accepted.

**Author Contributions**

CH and ND conceived the study. CH collected data. CH and TG carried out statistical analyses; all authors substantially contributed to drafting the manuscript, and gave final approval for publication.

**Conflict of Interest Statement**

All authors gave final approval for publication.

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**Abstract**

Ecological conditions such as nutrition can change genetic covariances between traits and accelerate or slow down trait evolution. Since adaptive trait correlations can become maladaptive following rapid environmental change, poor or stressful environments are expected to weaken genetic covariances, thereby increasing the opportunity for independent evolution of traits. Here, we demonstrate the differences in genetic covariance among multiple behavioral and morphological traits (exploration, aggression and body weight) between southern field crickets (*Gryllus bimaculatus*) raised in favorable (free-choice) versus stressful (protein-deprived) nutritional environments. We also quantify the extent to which differences in genetic covariance structures contribute to the potential for the independent evolution of these traits. We demonstrate that protein-deprived environments tend to increase the potential for traits to evolve independently, which is caused by genetic covariances that are significantly weaker for crickets raised on protein-deprived versus free-choice diets. The weakening effects of stressful environments on genetic covariances tended to be stronger in males than in females. The weakening of the genetic covariance between traits under stressful nutritional environments was expected to facilitate the opportunity for adaptive evolution across generations. Therefore, the multivariate gene-by-environment interactions revealed here may facilitate behavioral and morphological adaptations to rapid environmental change.

Keywords: genetic covariance, evolvability, genotype-by-environment interaction, nutrition, autonomy



## Introduction

Genetic covariances among phenotypic traits often differ between populations of the same species (reviewed in Wood and Brodie 2015). Population differences in genetic covariance structures may reflect the existence of multivariate gene-by-environment (G×E) interactions that allow rapid changes in genetic covariance structures in response to environmental change. Various environmental factors, such as temperature (Begin and Roff 2001; Bégin et al. 2004; Garant et al. 2008; Ingleby et al. 2014), diet (Delcourt and Rundle 2011; Ingleby et al. 2014), or predation risk (Kraft et al. 2006), have been identified as key factors causing multivariate G×E. The resulting variation in the strength of genetic covariance has important consequences for how suites of correlated traits evolve (Lande 1979; Lande and Arnold 1983; Cheverud 1984; Phillips and Arnold 1989; Blows and Hoffmann 2005; Walsh and Blows 2009).

Since genetic covariances among phenotypic traits are caused by two nonexclusive evolutionary processes - (correlational) selection and pleiotropy, environmental specificity of the two processes may generate a change in genetic covariances between environments. When spatial variation in multivariate selection is relatively greater than temporal variation in selection over generations, it can drive differential linkage disequilibrium between different genes and result in environment-specific genetic covariance structures (Sinervo and Svensson

2002). Because linkage disequilibrium by selection can change rapidly when selection pressures change with the environment, maladaptive trait covariation may quickly erode in a new environment. As a result, a change in genetic covariances can be maladaptive, neutral or adaptive. In contrast, trait covariance can be maintained via pleiotropy and can persist even when it is maladaptive in a new environment. However, despite strong persistence of trait covariance via pleiotropy, pleiotropic genes can also have different effects on traits depending on environments through the environmental sensitivity of pleiotropic genes. Environment-dependent allele-specific differential expression within a generation (Saltz et al. 2017) or mutations in pleiotropic genes over generations (Camara and Pigliucci 1999; Estes et al. 2005; Estes and Phillips 2006; Houle and Fierst 2013; McGuigan et al. 2014; McGuigan and Aw 2017) can lead to a change in genetic covariances among traits.

Despite evidence for environmental effects on genetic covariances among traits, it is unclear whether environmental stress strengthens or weakens genetic covariances. Some studies have demonstrated that stressful environments increase the strength of genetic covariances among traits (Robinson et al. 2009; Ingleby et al. 2014), possibly because genetic (co)variation not expressed in the original favorable environment (i.e., cryptic genetic (co)variation) is released in stressful environments (McGuigan and Sgro 2009; Paaby and Rockman 2014). In contrast to this prediction, unfavorable environments may weaken genetic covariances, thereby facilitating the independent evolution of previously correlated traits. Strong genetic covariances in a favorable environment can maintain trait covariances and impede the independent evolution of associated traits via pleiotropic effects. However,

covariances in a favorable environment can be deleterious in a stressful environment because of the constraints they impose on independent trait evolution and selection favoring different trait combinations (Pavličev and Cheverud 2015; Saltz et al. 2017). Thus sudden environmental stress resulting from rapid environmental changes will not only elevate the mutation rates of genes including pleiotropic loci over long timescales (i.e., changes in allele frequency) (Hoffmann and Parsons 1991) but also weaken trait covariances in a single generation via differential expression of genetic covariance to facilitate independent trait evolution. Although there has been no empirical evidence supporting this mechanism, it is suggested to facilitate rapid shifts to new trait optima over short evolutionary timescales. A recent meta-analysis failed to identify the overall direction by which environmental factors affect genetic covariances (Wood and Brodie 2015), which might be due to these mixed predictions on the effect of environmental stress on the strength of genetic covariances.

Furthermore, multivariate G×E is predicted to be a function of sex. Males and females of the same species share a common genetic underpinning but often differ in the expression of homologous phenotypes. This sexual antagonism can be resolved by sex differences in multivariate additive genetic structure generated by selection acting in opposing way between sexes (i.e., sexually antagonistic selection) (Lande 1980; Meagher 1999; Bonduriansky and Chenoweth 2009; Cox and Calsbeek 2009; Connallon and Clark 2010; Connallon et al. 2010; Poissant et al. 2010; Connallon and Clark 2011; Wyman et al. 2013). When genes are differently expressed between males and females, the reduced genetic dependency between the sexes can facilitate the evolution of sexual dimorphism (reviewed in

Poissant et al. 2010). Moreover, sex differences in genetic structures also depend on environmental conditions. For example, a stressful environment is known to suppress sex specificity of genetic variation in life history traits and can lead to strengthened genetic covariances between the sexes (Long et al. 2012; Reddiex et al. 2013; Berger et al. 2014; Han and Dingemanse 2017a). This indicates that the genetic components of male and female life history traits are less antagonistic when males and females are exposed to poor environmental conditions. This further implies that the pattern of multivariate G×E may differ between males and females (e.g., multivariate G×E may only be significant in one sex). To date, few studies have experimentally tested the notion that the level of environmental specificity of the genetic covariance structure varies as a function of sex.

Here, we focus on the nutritional environment as a major ecological factor shaping the expression of genetic covariance, hence the potential for traits to evolve independently. We used wild-caught southern field crickets (*Gryllus bimaculatus*) bred using standard breeding designs to assess how nutritional environments alter the genetic covariance between traits, and consequently, each trait's potential to evolve independently from other traits. Nutritional factors, such as the macronutrient composition (e.g., carbohydrate:protein ratio), determine the energy intake and balance of nutrient intake in animals and provide cues for the optimal expression level of multiple phenotypes (Simpson and Raubenheimer 2012). In particular, protein-deficiency is an important nutritional stressor for field crickets because food sources rich in protein are limited in wild cricket populations (Gangwere 1961; Gwynne 1984), and animals often prioritize satisfying requirements for proteins over those for other

macronutrients (Simpson and Raubenheimer 2005). Despite the positive effects of low levels of protein in diets on cricket lifespan (Hunt et al. 2004; Maklakov et al. 2008; Harrison et al. 2014), crickets suffer high mortality when their diets contain extremely low levels or a complete lack of protein (Piper et al. 2014; Han and Dingemanse 2017a). Under protein deprived conditions, they may not only experience protein deficiency but also need to consume excess carbohydrates to meet protein requirements. Thus, compared to a diet environment where crickets can choose multiple different food sources freely (i.e., nutritionally complementary food) and regulate nutrient intake to reach nutritional balance, a diet environment where crickets are restricted to a protein-deprived single food source is stressful for crickets.

We subjected male and female crickets to one of two nutritional treatments, a stressful protein-deprived diet (less than 2% protein) versus a free-choice diet, and measured three traits (exploration, aggression and body weight) repeatedly for the same set of individuals. We previously analyzed this dataset through a univariate perspective where each phenotypic trait was considered in isolation (Han and Dingemanse 2017b); in contrast, a multivariate perspective was applied here to study how genetic covariances among those traits differed between nutritional treatments. We also tested how the effect of nutritional stress on genetic covariances differed between males and females. We expected stronger genetic covariances among traits in the 'favorable' free-choice environment than in the 'stressful' protein-deprived environment. In the favorable free-choice nutritional environment, body weight and behavioral traits are expected to be strongly correlated at the

genetic level because larger and heavier crickets should be less willing to be explorative in order to protect their reproductive assets (Clark 1994), and these crickets are expected to be more aggressive because body size is a strong determinant of aggression in crickets (Simmons 1986). A poor nutritional environment, such as one deprived of protein, is predicted to cause most individuals to become asset-poor and be more explorative and aggressive to obtain resources regardless of their state (e.g., body weight) (Han and Dingemanse 2017b), leading to a decrease in the genetic covariances between body weight and behavior. Consequently, multivariate G×E would thereby facilitate the opportunity for a more rapid independent evolution of traits to new optima when faced with protein-deprived nutritional conditions. Moreover, as our previous univariate analyses showed that *G. bimaculatus* males were more vulnerable to protein deprivation than females (Han and Dingemanse 2017a), multivariate G×E was also expected to be sex-specific.

We examined how our nutritional treatment affected the potential for multivariate genetic covariance to constrain the independent evolution of associated traits by calculating trait-specific evolvability independent of pleiotropy (Houle 1992; Hansen and Houle 2008; Hansen et al. 2011). Trait-specific evolvability relative to overall evolvability, referred to as autonomy (*a*) (Hansen and Houle 2008), represents the impact of genetic covariance on the potential of the independent evolution of genetically associated traits. Additionally, we used a geometric approach (Krzanowski 1979) to test the differences in the properties of the genetic variance–covariance matrix (**G**-matrix) between crickets in favorable (free-choice) and stressful (protein-deprived) nutritional environments. This approach enabled us to estimate

the effect of nutritional stress on the genetic covariances, which possibly results in environment-specific autonomies. Furthermore, we evaluated whether the effects of nutritional stress on the genetic covariances and their potential role in evolution were sex-specific by partitioning the **G**-matrix into sex-specific **G**-matrices and analyzing them.

## Methods

### *Breeding design and nutritional treatment*

We collected adult southern field crickets (150 males and 150 females) from Tuscany (Italy) in July 2014, and transported them to the Ludwig Maximilians University of Munich. In the laboratory, we housed them (and their offspring, described below and in Supplementary material S1) at 26 °C with 40% relative humidity under a 14L:10D photoperiod. We created breeding pairs using wild-caught adults, collected the offspring from each pair and then used them as breeders (a parental generation in the breeding design) once all offspring had eclosed into adults. The procedure used to generate a parental generation from wild-caught individuals is detailed in Supplementary material S1. When all offspring had eclosed into adults, we selected a random sample of individuals to become breeders (detailed below). Laboratory-bred (rather than wild-caught) individuals were used as the parental generation because using grand-offspring of wild-caught parents alleviates the influence of maternal effects (Wolf and Wade 2009; Matos 2012).

We implemented a nested half-sib/full-sib breeding design (Falconer and Mackay 1996) using virgin offspring from wild-caught parents, where each of 45 parental males

(sires) mated with 2 unrelated females (dams) with the aim of producing 90 full-sib families. Because of some mating failures, the design finally yielded 79 full-sib families nested within 41 paternal half-sib families, where 3 of the full-sib families did not include paternal half-sibs (38 pairs of half-sib families and 3 full-sib families without paternal half-sibs). Within each full-sib family, emerging full-sib nymphs were split into four groups, placed into containers ($20 \times 30 \times 20$ $cm^3$; each housing up to 20 nymphs), and provided with dry bird food (Aleckwa Delikat, Germany) and water *ad libitum*. The design produced 1397 offspring (744 males and 653 females). When nymphs developed into adults, adults were subsequently randomly assigned to a 'protein-deprived' (366 males and 325 females) or 'free-choice' (378 males and 328 females) nutritional environment. Two different artificial diets (high-protein and high-carbohydrate) were made according to an established protocol (detailed in Simpson and Abisgold 1985)) and the experimental protocol has been detailed fully elsewhere (Han and Dingemanse 2017b). The protein-deprived treatment group was provided with only the high-carbohydrate diet (98% carbohydrate, 2% protein, ~500 mg), whereas the free-choice treatment group was provided with both the high-carbohydrate (98% carbohydrate, 2% protein, ~400 mg) and high-protein (2% carbohydrate, 98% protein, ~100 mg) diets, which were offered in two separate dishes and presented simultaneously. Adults were individually placed in plastic home containers ($10 \times 10 \times 9$ $cm^3$) with a piece of egg carton for shelter, a plastic water bottle plugged with cotton wool, and two dishes containing the artificial diets. Every three days, the containers were cleaned, and food and water were refreshed.

*Behavioral assays and body weight measurements*

After individuals had received three weeks of nutritional treatment, we performed a set of behavioral assays to measure exploratory activity and aggression. Prior to the initiation of behavioral assays, each individual was marked for identification with a small dot of paint (Testors enamel paint) on its pronotum. Exploration and aggression were measured in a fixed order on the same day because fixed order assays ensured that all individuals experienced the exact same treatments. Each individual was assayed 4 times for each of 2 behaviors, with a 2-day interval between tests. All behavioral assays were recorded with a digital camcorder and analyzed with tracking software, Noldus Ethovision XT 10 (Noldus Information Technology).

We present the details of the behavioral assays in Supplementary material S1. To summarize, in the exploration assays, the tracking software measured each individual's total distance moved in the compartment (15 × 15 × 10 cm) for 10 minutes (Santostefano et al. 2016; Han and Dingemanse 2017c, b). After the exploration assay, we put one same-sex opponent (an individual from the stock population) into the compartment and measured the amount of time (duration) that the focal individual chased the opponent over 10 minutes (aggression assay). At the end of the second and the fourth set of behavioral assays, we weighed each individual to the nearest 0.001 g.

*Statistical procedures*

We used a two-step approach to analyze our data. Our first analysis focused on testing how nutritional treatment altered the effect of genetic covariance on the potential for the independent evolution of traits. This calculation was based on the treatment-specific genetic variance–covariance matrix (**G**-matrix) for six traits (three male traits and three female traits), for which we fitted multivariate mixed-effects animal models using mean-standardized data. As genetic variances for some traits were sex-specific (Han and Dingemanse 2017b), we built the **G**-matrix by combining sex-specific genetic variance-covariance matrices and a between-sex covariance matrix (**B**-matrix). Thus, to calculate a treatment-specific **G**-matrix for sexually homologous multiple traits, we fitted two multivariate animal models with six response variables each (i.e., 3 traits × 2 sexes) (Figure S2). To estimate the **G**-matrix, we partitioned the phenotypic variance–covariance matrix (**P**-matrix) into additive genetic (**G**-matrix), permanent environment (**PE**-matrix) and within-individual residual (**R**-matrix) variance–covariance matrices using pedigree information (Wilson et al. 2010) (Figure S3). The **PE**-matrix is a variance–covariance matrix that is not due to additive genetic effects but is caused by other non-additive genetic (or environmental) effects that are conserved across repeated measures in the same individual. The models to partition the **P**-matrix included the testing order, which was fitted as a fixed covariate.

Our second analysis focused on testing the effect of nutritional treatment on the following two properties of the **G**-matrix: 1) the amount of genetic variance and 2) differences in the direction of the vectors along which most of the genetic (co)variance was found (i.e., the orientation of genetic (co)variance) (detailed below). This calculation was based on the treatment-specific **G**-matrix, for which we fitted multivariate mixed-effects animal models using z-transformed (mean=0, standard deviation=1) data (Supplementary material S3) (Kruuk 2004; Wilson et al. 2010). All traits were square-root transformed (which resulted in normally distributed residuals) prior to further transformation (z-transformation or mean standardization).

We fitted the model within a Bayesian framework using the MCMCglmm package (Hadfield 2010) in R (version 3.2.0). To minimize autocorrelation among the samples, 53,000,000 Markov Chain Monte Carlo (MCMC) iterations were performed, which were sampled at 50,000-iteration intervals after an initial burn-in period of 3,000,000 iterations, using Gamma priors. This resulted in a total of 1000 samples from the posterior distribution. Convergence was attained by visual inspection of output plots and by assuring that the autocorrelation between consecutive samples did not exceed 0.1 (Hadfield 2010).

*Effect of diet on autonomy* To estimate how the nutritional treatments altered the effects of genetic covariance on a trait's independent potential to evolve, we measured each trait's unconditional evolvability (*e*), conditional evolvability (*c*) and autonomy (*a*). Unconditional evolvability (*e*) is defined as trait evolvability not considering covariations

with other traits (Houle 1992; Hansen and Houle 2008; Hansen et al. 2011), which is identical to the mean-standardized genetic variance (Houle 1992; Hansen and Houle 2008; Hansen et al. 2011) and indicates the potential for evolutionary changes in a trait mean in response to the directional selection of a unit of strength (Houle 1992; Hansen and Houle 2008; Hansen et al. 2011). In contrast, conditional evolvability (*c*) is the focal trait's evolvability when other traits were not allowed to change due to the strong stabilizing selection on them (Houle 1992; Hansen and Houle 2008; Hansen et al. 2011). The role of genetic covariance in altering the evolutionary response of a trait can thus be quantified by the ratio between the conditional and unconditional evolvability, which is referred to as autonomy (*a*) (Hansen and Houle 2008). Autonomy shows how much the focal trait's potential to evolve is affected by genetic covariance with other traits. Autonomy measurements have been used to test how trait evolution is affected by genetic covariance among similar types of traits, such as multiple cuticular hydrocarbons (CHCs)/wing traits of *Drosophila bunnanda* (McGuigan and Blows 2010), or pollination/bract traits of the Neotropical vine *Dalechampia scandens* (Bolstad et al. 2014). Conditional evolvability is much lower than unconditional evolvability when the genetic covariance is stronger. Thus, an autonomy of 0 versus 1 indicates that the focal trait's potential to evolve is completely dependent ($a$=0) versus independent ($a$=1) of other traits, respectively. We evaluated the degree of genetic constraint on the independent evolution of traits by testing whether treatment-specific autonomy differed from null expectations of maximal independence (i.e., $a$=1) (Simonsen and Stinchcombe 2010; Stinchcombe et al. 2010; Teplitsky et al. 2011).

To estimate the uncertainty associated with evolvability and autonomy, we used posterior distributions (1000 samples) of **G**-matrices from the MCMC iterations calculated using data standardized through division by sex-specific, treatment-specific means (mean-standardization). The evolvability of these parameters were calculated using the R package *evolvability* (Bolstad et al. 2014).

*Effect of diet on **G**-matrix properties* We also used a Bayesian approach to calculate a diet-specific **G**-matrix for multiple sexually homologous traits (Supplementary material S3). We used the posterior distributions of the genetic (co)variance components in the **G**-matrix and assessed how diet contributed to differences in the following two matrix properties: 1) the amount of genetic variance and 2) the orientation of the vectors along which most of the genetic variance was found. First, to test for differences in the amount of additive genetic variance between treatments, we estimated the trace (sum of variances along the diagonal) of each treatment-specific **G**-matrix. We then compared the posterior estimate of the magnitude and its 95% highest posterior density (HPD) interval with the values calculated by generating an empirical random null matrix (null **G**-matrix) from our observed data (as described in (Aguirre et al. 2014)). This null **G**-matrix was obtained by randomly generating 1000 variance–covariance matrices (6×6) by randomizing the pedigree from a multivariate normal distribution (mean=0, variance=observed variance) and back-solving for the **G**-matrix.

Next, to compare orientations among the **G**-matrices, we applied Krzanowski subspace comparison (Krzanowski 1979), measuring the overall similarity in the subspace

orientation between two different matrices (detailed in Blows et al. 2004; McGuigan and Blows 2007). For this comparison, we focused on the first 3 primary eigenvectors for both matrices **X** (e.g., free-choice diet) and **Y** (e.g., protein-deprived diet). We chose to use a subset of 3 primary eigenvectors because it is within these dimensions that most of the genetic variance in each **G**-matrix was found, and importantly, if more than half of the principal components from any matrix decomposition are included, the analysis will be forced into recovering shared dimensions (Blows et al. 2004). We then defined matrix **S** as follows: $\mathbf{S}=\mathbf{X}^{T}\mathbf{Y}\mathbf{Y}^{T}\mathbf{X}$. The similarity of the two subspaces was subsequently assessed as the sum of the eigenvalues of matrix **S** (Blows et al. 2004). This similarity measure can have a value between 0 (orthogonal) and 3 (identical) when comparing **G**-matrices between treatments. We estimated **S** for each of the posterior estimates and assessed the overlap by comparing their 95% HPD intervals with those estimated from our random sampling (null **G**-matrix).

*Effects of diet treatment on sex-specific **G**-matrix properties* To compare properties of sex-specific **G**-matrices, we split the full **G**-matrix into the following diet-specific, sex-specific submatrices: multivariate male **G**-matrix ($\mathbf{G_m}$), multivariate female **G**-matrix ($\mathbf{G_f}$) and **B**-matrix (**B**, cross-sex, cross-traits genetic variance–covariance matrix) (Figure S2). We then investigated the effects of protein deprivation on 1) the amount of genetic variance and 2) the orientation of the sex-specific **G**-matrices ($\mathbf{G_m}$ or $\mathbf{G_f}$). Additionally, we also compared whether components in the intersexual genetic covariance matrix (**B** matrix) differed between treatments.

To calculate the **B** matrix (the cross-trait, cross-sex genetic covariances) in the full **G**-matrix (Figure S2, S3), the cross-trait, cross-sex genetic covariances in the residual and permanent-environment matrices must be constrained to zero because those components could not be measured (Figure S3). Although the MCMCglmm package is unable to constrain those components, the estimated cross-trait, cross-sex genetic covariances in the residual and permanent-environment matrices calculated using the MCMCglmm package were close to zero.

**Results**

*Effects of diet treatment on autonomy*

For all three traits, autonomy (i.e., the ratio between the conditional and unconditional evolvability, see details in the Materials and Methods, and Supplementary material S2) was estimated to be significantly less than one when crickets were fed a free-choice diet (Figure 1, Figure S1), indicating a low degree of independent evolutionary potential of traits in a favorable nutritional environment. However, when crickets were reared on the protein-deprived diet, autonomy estimates, of all traits other than female aggression, were not different from one (Figure 1, Table S1). Although the 95% HPD intervals of the autonomy estimates overlapped between the two treatments, altogether, our results implied, as predicted, that traits had the potential to evolve more independently under stressful protein-deprived nutritional conditions.

*Effects of diet treatment on **G**-matrix properties*

Tests for differences in the properties of the overall genetic covariance structures between nutritional treatments revealed that the total amount of genetic variance (i.e., the trace of **G**) in the **G**-matrix did not significantly differ between the treatments (due to large overlap in the 95% highest posterior density intervals (HPDIs) for variance; free-choice diet: 1.96 (95% HPDI: 1.47, 2.53); protein-deprived diet: 2.02 (95% HPDI: 1.31, 2.65); Figure 2). However, the Krzanowski subspace analysis showed that the principal vectors in the subspaces of the **G**-matrix were not aligned (Figure 2): the difference was significantly greater than the null expectation. The sum of the eigenvalues of **S**, 2.06 (95% HPDIs: 1.66, 2.56), was smaller than that of 1,000 randomized **G**-matrices (S=2.98, 95% HPDIs: 2.95, 3.00; Figure 2). This finding indicated that genetic covariance structures were significantly different between the nutritional treatments. Specifically, when crickets were fed the protein-deprived diet, significant genetic correlations between traits were not observed in either sex (Figure 3). In contrast, when crickets consumed the free-choice diet, a significantly negative genetic correlation was observed between body weight and exploration, although only in males, whereas a positive genetic correlation between body weight and aggression was found for both sexes (Figure 3).

*Effects of diet treatment on sex-specific **G**-matrix properties*

When the overall matrix ($\mathbf{G_{mf}}$) was decomposed into the submatrices, namely a male **G**-matrix ($\mathbf{G_m}$) and female **G**-matrix ($\mathbf{G_f}$) (Figure S2), there was also no difference in the amount of genetic variances across the sexes or diet treatments ($\mathbf{G_m}$, free-choice diet: 0.95 (95% HPDI: 0.67, 1.29); $\mathbf{G_f}$, free-choice diet: 1.01 (0.66, 1.40); $\mathbf{G_m}$, protein-deprived diet: 1.21 (0.78, 1.72); $\mathbf{G_f}$, protein-deprived diet: 0.80 (0.42, 1.21); Figure 4a). However, protein deficiency changed the orientation of both $\mathbf{G_m}$ and $\mathbf{G_f}$ (Figure 4b). The eigenvalue of 0.25 (95% HPDIs: 0.00, 0.72) in $\mathbf{G_m}$ and 0.72 (95% HPDIs: 0.10, 1.00) in $\mathbf{G_f}$ tended to be smaller than the values from 1,000 of the randomized **G**-matrices (male: 0.97 (95% HPDIs: 0.88, 1.00); female: 1.00 (95% HPDIs: 0.98, 1.00)) (Figure 4b).

Cross-sex, cross-traits genetic variance–covariance components (i.e., **B**-matrices) did not differ between the treatments, though diet stress tended to weaken the genetic covariance between female body weight and male aggression. This lack of environment specificity for **B**-matrices indicates a non-significant G×E×SEX interaction.

**Discussion**

Protein deprivation decreased the strength of the genetic covariance among behavioral and morphological traits and, further, tended to increase the potential for the independent evolution of traits. Aggression, exploration, and body weight were more strongly correlated when crickets were raised on a free-choice diet. By contrast, protein-deprivation reduced the strength of the genetic covariances. Although we did not measure selection, or how it might

differ between environments, strong genetic covariance between traits in this favorable environment decreased the potential of these traits to evolve independently, whereas the stressful environment allowed the traits to increase the potential to evolve more independently. These weakening effects of protein deprivation on the strength of genetic covariances also tended to be stronger in males than in females.

From an adaptive viewpoint, the weakening effects of stressful environments on genetic covariance structures among traits may facilitate adaptive evolution by increasing the opportunity for rapid trait evolution. For example, the positive genetic covariance between body weight and aggression in a rich nutritional environment may be present because body size is a strong determinant of aggression in crickets (Dixon and Cade 1986; Simmons 1986). In contrast, protein requirements in a stressful protein-deprived environment weakened of the genetic covariance between aggression and body weight in both males and females. That is, trait covariances that are adaptive in one environment may become maladaptive in another environment (Saltz et al. 2017). However, it is unlikely that rapid changes in the frequencies of pleiotropic loci will produce new adaptive covariances in a stressful environment (Pavličev and Cheverud 2015; Saltz et al. 2017). It appears impossible that mutations at pleiotropic loci contributing to new adaptive trait covariances in a stressful environment arise in the short term (Pavličev and Cheverud 2015). In our previous analyses on the studied traits, additive genetic variances did not differ between the treatments (Han and Dingemanse 2017b), suggesting that novel mutations were not responsible for a within-generation change in multivariate genetic structures between environments in our study. It is also unlikely that

simultaneous mutations in all correlated traits will result in adaptive changes (Blows and Hoffmann 2005). Instead, environment-specific differential expression of pleiotropic loci (i.e., multivariate G×E) achieved within a single generation would potentially reduce the constraining effects of genetic covariance over short timescales. For example, different alleles in pleiotropic loci are differentially sensitive to stress and, therefore, differentially expressed. Such changes may facilitate the evolution of new genetic covariances among traits in a stressful environment without needing to evoke novel mutations. Although we provide evidence that weak genetic covariances are likely to allow certain traits to increase the potential to evolve more independently in populations that are exposed to stressful environments, selection experiments are now required to verify this interpretation. The role of genetic covariances in trait evolution measured without selection experiments might not reflect realistic conditions because trait evolution depends on the strength and direction of selection on traits in addition to trait genetic structures (Lande 1979; Lande and Arnold 1983; Hansen and Houle 2008). Based on the assumption that a focal trait is under directional selection while other traits are under stabilizing selection, autonomy measures provide only suggestive predictions of how genetic covariances affect trait evolution (Hansen and Houle 2008). Thus, in the future, it will be necessary to explore how genetic covariances change over generations in stressful environments and how multiple non-exclusive mechanisms (e.g., changes in allele frequency, differential expression of genetic covariance, or selection) contribute to these changes.

Moreover, the weakening effects of stressful, protein-deprived, environments on genetic covariance structures tended to be stronger in males than in females, though this tendency should be viewed with caution because of the large error associated with our estimates. This finding is nevertheless in line with our previous result that *G. bimaculatus* males suffered higher mortality under protein-deprived conditions than females (Han and Dingemanse 2017a). Assuming that the effect of protein deficiency on mortality implied nutritional stress, protein-deprived conditions appear to cause more stress for males, which leads to stronger weakening effects on genetic covariance structures in males. Genetic correlations between exploration and other traits (aggression or body weight) in males showed opposite signs when comparing the two dietary treatments, whereas the genetic correlations for females showed different magnitudes but the same sign between the two diet treatments. This finding indicates that the signs of genetic covariances between exploration and other traits are responsible for the tendency of sex differences in multivariate G×E. Thus we suggest that male behavioral and morphological traits tend to be more condition-dependent than female traits.

The asset protection principle can explain the opposite signs for the male genetic correlations between nutritional environments. This principle implies that individuals with fewer assets (e.g., weight and mating opportunities) tend to behave less cautiously to increase their assets (Clark 1994). In a rich nutritional environment that provides balanced access to nutrients, heavier individuals (asset-rich individuals) should be less willing to take risks (decreased exploration) (Clark 1994). In contrast, a stressful nutritional environment (e.g.,

protein-deprived diet) should cause most individuals to become asset-poor and, as a direct result, to become more explorative to increase their resources (Han and Dingemanse 2017b). This results in a weakening of the negative genetic covariance between body weight and exploration. However, it seems that the asset protection principle does not apply to female crickets. In female crickets, the tendency to take risks for resources such as proteins might depend on mating history rather than on body weight because mating experience is known to increase protein intake of females for egg production (Wheeler 1996).

Sex differences in the response of **G**-matrices to environmental stress can also be reflected in the environmental specificity of cross-sex components of **G**-matrices, which also possibly drive strong environmental effects on the genetic covariance structures. However, the roles of cross-sex components of **G**-matrices in shaping environmental effects on the genetic covariance structures were limited in our results. First, our results show that within-trait (exploration or weight) cross-sex genetic covariances are strongly positive and not different from unity in either diet treatment, though dietary stress tends to increase the strength of cross-sex genetic covariances for aggression. Additionally, the cross-trait cross-sex genetic covariances found in the **B**-matrix can also have a role in constraining or facilitating a sex-biased change in the phenotype (Lewis et al. 2011; Gosden et al. 2012; Gosden and Chenoweth 2014; White et al. 2019) and their environmental specificity could lead to different responses of **G**-matrices to environmental stress. Despite this possibility, cross-trait cross-sex genetic covariances also did not differ between the diet treatments due to the large overlap in credible intervals. Altogether, we suggest that the contribution of changes

in cross-sex components of **G** between environments is not strong enough to drive the changes in the orientation of **G**-matrices between environments.

In the literature, there are mixed patterns of the effect of environmental stress on the direction of genetic covariances. In contrast to our results, previous research has suggested that stressful environments strengthen genetic covariances (Robinson et al. 2009; Ingleby et al. 2014). In *Drosophila simulans*, the genetic covariance among CHCs in males is stronger and is more likely to act as a constraint on the independent evolution of individual CHCs under stressful environments (low temperature) (Ingleby et al. 2014). In a wild population of Soay sheep (*Ovis aries*), the genetic covariances between morphological traits (body weight and horn length) were found to be stronger under poorer overwintering conditions (higher density and poorer weather) (Robinson et al. 2009). An increase in the strength of additive genetic (co)variance in poorer environments might occur because stressful environments induce effects of alleles that are suppressed under normal conditions and increase the expression of cryptic genetic (co)variance (McGuigan and Sgro 2009; Paaby and Rockman 2014). Our results, which show opposite trends to those found in previous research, might also imply that the effect of stress on genetic covariances varies as a function of the trait type (e.g., Rowiński and Rogell 2017). As discussed above, we suggest that the strength of genetic covariances among traits subject to the asset protection principle (Clark 1994) can be stronger in a favorable environment. In addition, the maintenance and persistence of genetic covariances across generations in response to environmental changes may depend on the mechanism underlying genetic covariances. Compared with genetic covariances shaped by

pleiotropy, genetic covariances shaped by linkage disequilibrium can be easily disrupted by changes in selection. As a result, in the case of genetic covariances caused by linkage disequilibrium, maladaptive trait covariation may quickly erode in a stressful environment, while maintaining strong genetic covariance might require strong selection. In contrast, genetic covariances maintained by pleiotropy could persist in response to environmental stress. Therefore, given the contrasting evidence for the effects of environmental stress on the direction of genetic covariance, future research is required to investigate a wider range of organisms and traits to determine this relationship.

A recent meta-analysis showed that behavioral traits tend to have stronger genetic covariances than life history traits, resulting in stronger evolutionary constraints on their evolutionary responses (i.e., lower autonomy) (Dochtermann and Dingemanse 2013). However, environmental factors have short-term effects on the plastic expression of behaviors within individuals, as well as long-term effects on the development of behaviors, suggesting a significant contribution of the environment to plasticity in the strength of the genetic covariance of behavioral traits. Given that the evolvability of behavioral traits is higher than that of many other phenotypes ((Hansen et al. 2011); this study), behavioral traits and their covariances with other traits (e.g., body weight) are predicted to have a high potential for rapid evolution and to be able to change more flexibly in response to changing environments and selection. However, since we still lack an understanding of the ecological and evolutionary implications of the genetic covariance among behavioral traits (Dochtermann and Roff 2010; Dochtermann and Dingemanse 2013; Killen et al. 2013;

Brommer 2014; Dingemanse and Dochtermann 2014), it will be necessary to investigate how the genetic covariance among behavioral traits responds to various types of environmental stresses.

In conclusion, we demonstrate that the genetic architecture of multiple morphological and behavioral traits varies as a function of the nutritional environment. Our findings support the prediction that strong environmental stressors (e.g., protein deficiency) weaken genetic covariances between traits (Killen et al. 2013; Han and Dingemanse 2015). Our study also implies that a weakened genetic covariance is likely to lead to increased evolutionary autonomy, thereby facilitating the independent evolution of traits. Hence, the flexible expression of genetic covariance for multiple traits may play an important role in rapidly adapting to a stressful environment. Furthermore, fluctuations in nutritional environments, such as changes in protein availability, are suggested to be an important ecological phenomenon that alters the genetic architecture and evolutionary trajectories of traits. This indicates that ecology can drive evolution on both short-term and long-term evolutionary time scales (Lande 1979; Lande and Arnold 1983; Via and Lande 1985).

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**Figure legends**

**Figure 1.** Autonomy estimates for exploration (E, squares), aggression (A, triangles) and body weight (W, circles) in males (closed symbols) and females (open symbols) exposed to the free-choice diet and protein-deprived diet. Autonomy estimates range from the case that trait evolution is completely dependent ($a$=0) of genetic correlations to the case that trait evolution is completely independent ($a$=1) of genetic correlations. Symbols indicate posterior medians, and error bars indicate the 95% HPDI.

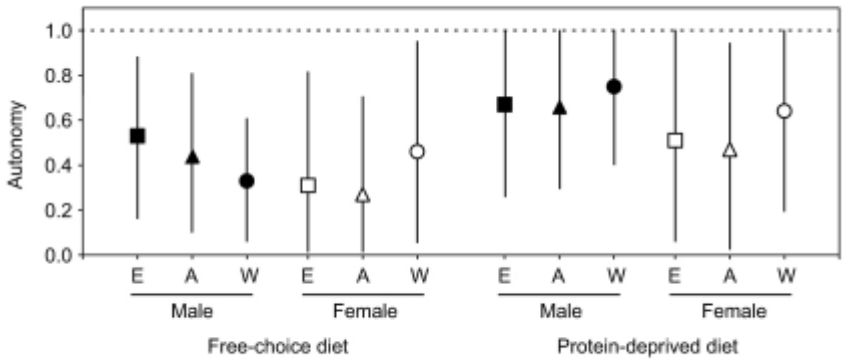

**Figure 2.** Comparison between the two treatments regarding (a) the total amount of genetic variance and (b) the similarity of the orientation of the main dimensions for the diet-specific overall **G**-matrices (including both sex-specific **G**-matrices and the cross-sex **B**-matrix). The total genetic variance (i.e., trace) is the sum of the genetic variances along the diagonal of the **G**-matrix. We used Krzanowski's subspace comparison to estimate the overall similarity of our observed **G**-matrix and compared it to that calculated from random sampling (null **G**-matrix). Error bars indicate the 95% highest posterior density intervals (HPDIs) around the point estimates.

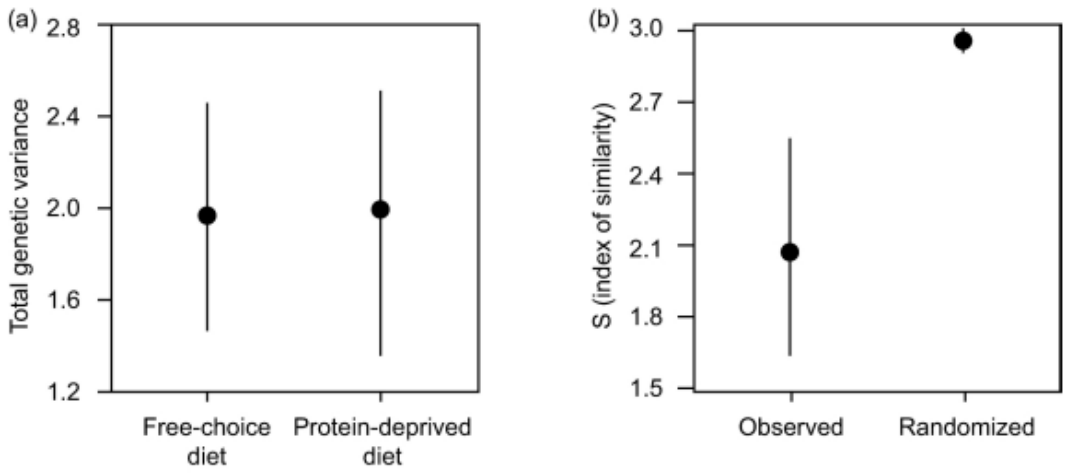

**Figure 3.** Genetic correlation structures for the free-choice (below the diagonal) and protein-deprived diet (above the diagonal) treatments. The 95% HPDIs around the point estimates are provided in parentheses.

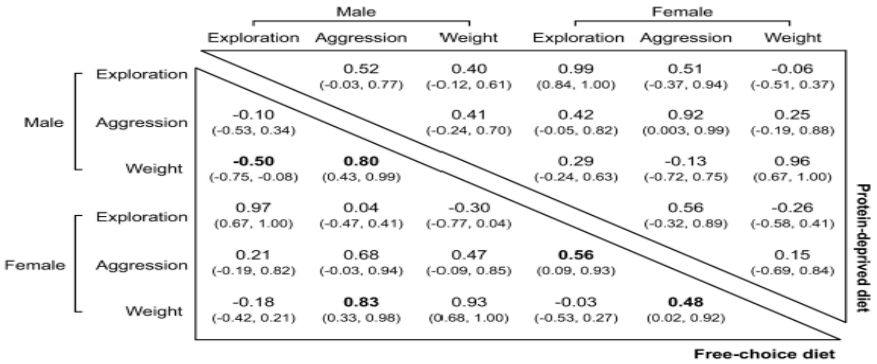


**Figure 4.** Comparison of (a) the amount of total genetic variance and (b) the orientations between the sex-specific and diet-specific **G** matrices. Using Krzanowski's method, we also defined S as an index of similarity varying from 0 (orthogonal) and 1 (identical). We compared the S calculated from the observed **G** to the one calculated from random sampling (null **G**) generated by randomizing the pedigree data. Error bars indicate 95% HPDIs around the point estimates.