Journal of Animal Ecology

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Article type : Research Article

Section: Evolutionary ecology

Handling Editor: Dr David Berger

Article submission

Environmental effects on the genetic architecture of fitness components in a simultaneous hermaphrodite

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This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the Version of Record. Please cite this article as doi: 10.1111/1365-2656.13607

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Total word count: 6639 (excluding references, tables and figures)

Page count: 51 in total
23 text pages (without references)
4 figures (plus 3 figures in the supplementary material)
4 tables (plus 5 table in the supplementary material)

Running head: Stability of G-matrix in a hermaphrodite

Keywords: environmental stress; sexual selection; sexual conflict; G-matrix; opportunity for selection; condition-dependence; genetics

Abstract

1. Understanding how environmental change affects genetic variances and covariances of reproductive traits is key to formulate firm predictions on evolutionary responses. This is particularly true for sex-specific variance in reproductive success, which has been argued to affect how populations can adapt to environmental change.
2. Our current knowledge on the impact of environmental stress on sex-specific genetic architecture of fitness components is still limited and restricted to separate-sexed organisms. However, hermaphroditism is widespread across animals and may entail interesting peculiarities with respect to genetic constraints imposed on the evolution of male and female reproduction.
3. We explored how food restriction affects the genetic variance-covariance (G) matrix of body size and reproductive success of the simultaneously hermaphroditic freshwater snail *Physa acuta*.
4. Our results provide strong evidence that the imposed environmental stress elevated the opportunity for selection in both sex functions. However, the G matrix remained largely stable across the tested food treatments. Importantly, our results provide no support for cross-sex genetic correlations suggesting no strong evolutionary coupling of male and female reproductive traits.
5. We discuss potential implications for the adaptation to changing environments and highlight the need for more quantitative genetic studies on male and female fitness components in simultaneous hermaphrodites.

Introduction

Understanding how populations cope with and adapt to changing environments is at the very core of ecology and evolutionary biology. Classic theory posits that firm predictions on evolutionary responses require detailed knowledge, not only on the strength of selection, but also on the material that selection is acting on – that is the genetic variance (Lande & Arnold 1983; Chevin *et al.* 2010). Importantly, environmental change itself may not only alter the strength, form and direction of selection but also genetic variances and covariances, which often makes it difficult to formulate predictions on evolutionary trajectories. While there is solid evidence that novel environments often impose changing selection pressures on morphological, behavioural, and life-history traits (Agrawal & Whitlock 2010; Caruso *et al.* 2017; Siepielski *et al.* 2017), our knowledge on how environmental conditions affect the genetic architecture is still very limited (Wood & Brodie 2015).

Partly motivated by global change concerns over the last two decades, there has been an increased interest in deciphering the immediate effects of environmental stress on genetic variation (Hoffmann & Merila 1999; Charmantier & Garant 2005). Yet, the theoretical framework allowing precise predictions of how stressful conditions affect genetic variation of a given population is still limited and controversial. Several hypotheses have been developed postulating contrasting effects of stress on genetic variation depending on the source of genetic variation (i.e., standing genetic variation *versus* de novo mutations) and on the type of trait studied (i.e., phenotypic *versus* fitness-related traits) (Hoffmann & Merila 1999; Agrawal & Whitlock 2010; Berger *et al.* 2021). For standing genetic variation of fitness-related traits we may predict that environmental stress (defined as conditions that impose a reduction in fitness relative to the absolute fitness in another reference context) inflates genetic variation. This is because effects of deleterious alleles can be environment-specific and novel environmental conditions may unmask cryptic genetic variation (Paaby & Rockman 2014). Specifically, selection under benign conditions is expected to reduce the frequency of alleles that are particularly deleterious under benign conditions but not of those that are mainly deleterious in a stressful environment. Importantly, stressful conditions often represent novel environments so that alleles that are deleterious in the benign (ancestral) environment might have been largely eliminated whereas selection could not

act long enough to remove alleles that are deleterious in the stressful (novel) environment (Hoffmann & Merila 1999; Hermisson & Wagner 2004). Consequently, we may expect larger genetic variation in a stressful environment. In a recent meta-analytic attempt to synthesise our current knowledge, such a positive effect of high stress levels on genetic variation has been found for life-history traits but not for morphological traits (Rowinski & Rogell 2017).

The most crucial trait in the context of adaptation is fitness. Fisher's fundamental theorem of selection asserts that genetic variation of fitness equals the population's rate of increase in mean fitness that results from selection (Fisher 1930) and may therefore serve as proxy for the total net strength of natural selection against deleterious alleles (Whitlock & Agrawal 2009). Importantly, genetic variation of sexually reproducing organisms can be sex-specific. Sexual selection (i.e., selection arising from competition for mating partners and/or their gametes) is often considered to be typically stronger on the male sex (Darwin 1871; Bateman 1948; Schärer *et al.* 2012) – a view that is supported by meta-analytical (Janicke *et al.* 2016a) and theoretical work (Lehtonen *et al.* 2016). This sex difference in sexual selection is predicted to translate into stronger net selection (i.e. higher genetic variation in reproductive success) in males, which has been argued to allow populations to purge their genetic load at a low demographic cost and thereby facilitate the adaptation to novel environments (Whitlock & Agrawal 2009; Martínez-Ruiz & Knell 2017; Martinossi-Allibert *et al.* 2019).

The vast majority of theoretical and empirical work exploring environment- and sex-specific effects on the genetic architecture of fitness-related traits focuses on separate-sexed species. This is unfortunate because hermaphroditism is widespread across the tree of life, occurring in 70% of animal phyla (Jarne & Auld 2006) and >90% of angiosperm genera (Renner & Ricklefs 1995). Studying the genetic architecture of male and female fitness components in hermaphrodites is therefore essential to obtain a more general perspective of how animals can adapt to environmental change. In simultaneous hermaphrodites both sex functions are expressed in the same individual, which may lead to certain peculiarities regarding the genetics of male and female fitness compared to gonochorists (Abbott 2011; Schärer *et al.* 2015). Specifically, simultaneous hermaphrodites can invest reproductive resources in both sex functions and sex allocation theory assumes a trade-off in the relative investment of resources towards the male versus the female sex (Schärer 2009). If such a trade-off holds and if sex allocation has a genetic basis, every allele

that affects the individual's sex allocation is likely to have a sexually antagonistic effect. Therefore, hermaphroditic organisms are potentially more prone to exert sexually antagonistic genetic variation of fitness components compared to separate-sexed organisms (Abbott 2011; Schärer *et al.* 2015) and such larger genetic constrains may impede their potential to adapt to novel environments. One the other hand, hermaphroditism may also promote positive genetic correlation between male and female fitness components simply because all genes are expressed in one and the same individual also meaning that every allele that promotes resource acquisition and/or survival is likely to be beneficial for both sex functions (Bonel *et al.* 2018; Noel *et al.* 2019). Both assertions are very speculative simply because we still know almost nothing about the genetic architecture of male and female reproduction in simultaneous hermaphrodites.

We used a full-sib breeding design with inbred lines to fill this gap by studying the quantitative genetics of sex-specific reproduction of the hermaphroditic freshwater snail *Physa acuta* under experimentally induced food stress. Food availability is a major ecological determinant of an individual's condition and is therefore expected to have a strong effect on reproductive performance in *P. acuta* (Janicke & Chapuis 2016) but its effect on the genetic architecture of male and female fitness components has not been studied before. Our objective was to address three main questions. First: Does food restriction affect the opportunity for selection for male and female reproductive success and phenotypic variance of body weight? Second: How does food restriction affect the genetic variances and co-variances between traits? Third: Does genetic variance in reproductive success differ between sex functions and how are male and female reproductive success genetically correlated? For reasons outlined above, we predicted that food restriction reveals larger phenotypic and genetic variation of reproductive success and body weight compared to *ad libitum* food conditions. Moreover, we expected to observe larger genetic variation of reproductive success in the male relative to the female sex function as a consequence of the previously documented male-biased sexual selection in *P. acuta* (Pélissié *et al.* 2012). Understanding these aspects is essential in order to predict how environment- and sex-specific effects may alter the adaptation to novel environments in simultaneous hermaphrodites.

Methods

Model organism

Physa acuta is a simultaneously hermaphroditic freshwater snail, which reproduces bi-parentally through cross-fertilization in the presence of mating partners but is capable of self-fertilisation in isolation (Jarne *et al.* 2000). Selfing rates in natural population have been found to be very low (i.e., not differing from zero (David *et al.* 2007)). Sexual selection has been demonstrated to be stronger in the male sex function as inferred from Bateman's metrics (Pélissié *et al.* 2012) and operates primarily at post-copulatory episodes (Pélissié *et al.* 2014). Food limitation has previously been shown to impair primarily female fitness components (Janicke & Chapuis 2016) and to intensify the relative importance of post-copulatory sexual selection (Janicke *et al.* 2015). In mating trials (see below) we used an albinotic lab strain as potential competitors and mating partners. The albinotic marker is recessive so that all wild-type offspring produced by an albinotic sperm recipient must have been sired by a wild-type focal individual whereas all albinotic offspring have been sired by albinotic competitor snails. This allowed us to assess male reproductive success in a competitive context.

In the laboratory, snails are maintained at 25°C with a 12h : 12h light : dark cycle and fed with boiled lettuce. Under these conditions, snails mature within 6 – 8 weeks and adults lay a gelatinous egg capsule every 1 – 2 days containing several tens of eggs.

Experimental design

Field sampling and breeding design

The experiment was designed to study the effect of food availability on the genetic architecture of male and female fitness components under conditions that allow for sexual selection to operate (Figure S1). This was achieved by (*i*) sampling snails from the field, (*ii*) generating inbred lines, (*iii*) applying a full-sib breeding design, (*iv*) splitting each family among two different environments and (*v*) performing mating trials in which focal snails compete against a standard competitor.

Adult snails were sampled from a wild population of the Lez River (43°43'47.0"N 3°49'50.4"E), located close to the village of Les Matelles, 15 km north of Montpellier (France) on 23 October

2013. Individuals were brought to the lab where they were kept in isolation and fed *ad libitum* for egg laying. The resulting offspring were raised in isolation and constitute the starting stock of individuals (G_0) to generate inbred lines. Each G_0 individual was then kept in isolation to obtain self-fertilised offspring for the next generation of inbreeding. This protocol was repeated for three generations of self-fertilization resulting in G_3 individuals with an inbreeding coefficient of 7/8. In total we raised 44 of these inbred lines.

In July 2014, we paired two unmated individuals from two randomly selected inbred lines to form full-sib families. Specifically, we allowed pairs to interact for 48 hours in small 200 mL plastic boxes and then isolated both partners to let them lay eggs for 72 hours in 100 mL plastic boxes. The resulting juveniles, which are the focal individuals of this experiment, were fed *ad libitum* until an age of 31 days and then the full-siblings of each mother were split into two food treatments: 'High-Food' (HF) and 'Low-Food' (LF). In the HF treatment, focal individuals were fed *ad libitum* until the mating trials. In the LF treatment, focal individuals were exposed to a feeding regime in which food was provided *ad libitum* for 2 days followed by 2 days of food deprivation. These food treatments lasted two weeks until an age of 45 days (after eggs have been laid) assuring that all individuals reached maturity in both sex functions.

In parallel to breeding focal wild-type individuals, we raised albinotic snails, which served as mating partners and competitors in the mating trials. Albinotic snails were raised in isolation until maturity and fed *ad libitum* throughout life. Two days before the mating trials, all unmated albinotic snails were pooled in one plastic tank and allowed to copulate. After 24 hours, all albinotic individuals were put back in isolation in small plastic boxes, which we checked for clutches after one day. Only individuals that produced clutches after the mating trials were considered to be mated (i.e., having sperm from other albinotic donors in storage) and were used as potential partners in subsequent mating trials.

For logistic reasons the entire experiment was split into three blocks (including the full-sib breeding, albinotic snail breeding, and the mating trials), which were separated by seven days each.

Mating trials and fitness assay

Mating trials were performed in 200 mL plastic boxes by pairing one focal wild-type individual (aged 46 days after egg laying) with one randomly selected, already mated albinotic individual for 72 hours. Therefore, mating trials did not allow for pre-copulatory male-male competition because sperm competitors could not interact directly. However, given that we only used mated albinotic mating partners, there was scope for intense post-copulatory sexual selection in terms of sperm competition and cryptic female choice to operate, which has been found to be the predominant form of sexual selection in *P. acuta* with evidence for first-mate sperm precedence (Janicke *et al.* 2013; Pélissié *et al.* 2014). Moreover, there was potential for pre-copulatory mate choice by albinotic snails because they had the possibility to reject mating with focal snails. During the mating trials food was provided *ad libitum*.

One day prior to the mating trials, we assessed body weight (to the nearest mg) as a morphological trait that is shared by both sex functions. After the mating trials both focal and albinotic snails were isolated in 100 mL plastic boxes for egg laying under *ad libitum* food conditions for 96 hours. Ten days later, we counted all hatched offspring to obtain estimates of reproductive success. Male reproductive success of a given focal individual was assessed as the proportion of wild-type juveniles produced by albinotic mating partners. Female reproductive success was defined as the number of juveniles produced by the focal individual (all wild-type). All measurements were taken blind with respect to the food treatment and family identity.

Statistical analysis

In total, the experiment included 22 full-sib families with a total number of 768 focal individuals resulting in 23'962 offspring. Thus, each family comprised on average 34.9 (range: 11 – 55) focal individuals from both mothers, with slightly more focal individuals in the High-Food treatment (mean = 18.2; range: 6 – 32) than in the Low-Food treatment (mean = 16.7; range: 5 – 26) due to an apparent lower survival under restricted food conditions.

Statistical analyses included three steps. First, we tested whether the food treatment was effective in imposing a difference in body weight and reproductive performance. Second, we quantified the phenotypic and genetic variances together with the co-variances of the three traits

and tested for differences between food treatments and sex functions. And third, we explored differences of the variance co-variance (G) matrix between food treatments.

In a first series of analyses using a maximum likelihood approach, we tested for an effect of food treatment and genotype on the three measured traits. Specifically, we ran univariate Linear Mixed-Effects Models (LMMs) using the lmer function implemented in the lme4 R package version 1.1-23 (Bates *et al.* 2015) with body weight, male reproductive success or female reproductive success defined as response variable and food treatment as fixed effect together with family identifier, mother identifier (i.e., the female parent of the focal) and block as random terms. The family term accounts for the effect of the genotype and the mother terms accounts for maternal effects, where the latter is often interpreted as pure maternal effects though they may also include paternal effects. In all models, we included the family by treatment interaction term (as a random effect) in order to test for genotype by environment interactions. For simplicity and better interpretation of results, we assumed Gaussian error distributions when modelling all target traits. Visual inspection of model residuals suggested that assumption of normality was met. We also performed Generalized Linear-Mixed-Effects Models with male reproductive success (binomial errors) or female reproductive success (Poisson errors) using the glmer function lme4 R package in which we added observation identity as an additional random term to account for overdispersion. These alternative analyses provided qualitatively similar results (Table S1).

In a second set of analyses we estimated phenotypic variances, genetic variances and genetic covariances using multi-response Generalised Linear Mixed Models (GLMMs) in a Bayesian framework by applying the MCMCglmm function of the R package 'MCMCglmm' version 2.29 (Hadfield 2010). We used the MCMC approach because it provides more flexibility and allowed us to compute confidence limits of the estimated genetic variances and covariances. Specifically, we ran a single GLMM with relativized body weight, relativized male reproductive success and relativized female reproductive success obtained from both food treatments as response variables using the cbind function. In all GLMMs we included family, mother and block as random terms using uninformative priors (V = 0.02, nu = 10) and settings aiming at an effective sample size of 10'000 (number of iterations: 5'100'000, burnin: 100'000, thinning interval: 500). We also tested alternative priors, which revealed very similar results. All models were run multiple times to verify convergence and we checked for autocorrelation in the chains. We used a quasi-clonal breeding

design in which each family is the product of a cross between two inbred lines. Hence, we estimated the genetic variance (V_G) as the variance explained by family and computed broad sense heritability (H^2) as the proportion of V_G over the sum of the family, maternal, block and residual variance. Note that due to our hermaphrodite-specific breeding design (i.e., having offspring from two mothers of the same family) our estimate of genetic variation only encompasses variance associated with additive, dominant and epistatic effects but excludes environmental variance arising from maternal effects or a shared environment. Therefore, our estimate of V_G approximates the additive genetic variance better than estimates obtained from typical full-sib breeding designs applied in studies of separate-sexed organisms. Similar to V_G and H^2, we estimated the maternal variance (V_m) as the variance explained by the mother term and computed m^2 as the proportion of V_m over the sum of the family, maternal, block and residual variance. Phenotypic variance (V_P) was quantified as the sum of V_G, V_m and the residual variance. All analyses were run on relativised data so that that phenotypic variances in male and female reproductive success correspond to the opportunity for selection (I) (Crow 1958) of the male and female sex function, respectively. For all estimates we extracted highest posterior density (HPD) intervals and assessed the mean difference with its HPD credible intervals (food treatment: HF – LF; sex difference: male – female) so that positive values arbitrarily indicate higher variance in the High-Food treatment and in males. We obtained genetic correlations between the three traits within food treatments from GLMMs using the 'us' variance structure. For completeness, we also report phenotypic correlations inferred from Spearman's rank correlation coefficient *rho*.

In a final set of analyses we compared the genetic variance-covariance matrix (**G**) between the two food treatments. First this purpose, we first ran two GLMMs to estimate G separately for both treatments, which then allowed us to follow the guidelines provided by Aguirre *et al.* (2014). We note that phenotypic and genetic (co)variance obtained from the two separate models yielded very similar estimates compared to those obtained from a single model combining all data measured in both environments (see above). The comparison of **G** was based on three summary statistics as proposed in Hansen and Houle (2008) and Kirkpatrick (2009). First, we assessed the *effective number of dimensions* (n_D), which is defined as the sum of the eigenvalues divided by the largest eigenvalue and provides information on the shape of **G**. Second, we computed the *maximum evolvability* (e_{max}), which is the square root of the eigenvalue of the first eigenvector

(g_{max}) and constitutes the genetic coefficient of variation for the combination of traits with the highest genetic variation. Third, we compared the *total genetic variance* (ν_T), which is the sum of the eigenvalues of **G**. For all summary statistics we computed posterior modes and inferred differences between the two **G** matrices from the overlap of the Highest Posterior Density (HPD) intervals.

In addition to the above-mentioned summary statistics, we used two complementary methods to formally compare **G** between the two food treatments: (*i*) random skewers method (Cheverud & Marroig 2007) and (*ii*) the genetic covariance tensor (Hine *et al.* 2009). We capitalized on the Bayesian approach to apply these methods directly on the posterior distributions of **G**. Here, we only outline the principles of these methods but for more details on the calculations and tutorials to perform these analyses, we refer to Aguirre *et al.* (2014). First, the random skewers method is based on the projection of random vectors through the different **G** matrices to evaluate the quantity of genetic variance in the direction of each vector. An eigenanalysis of the vectors that signify significant differences between the **G** matrices allows then to determine which part of the space shows differences in genetic variance. We did so by projecting 1,000 random vectors on each **G** matrix and compared the 95% HPD intervals of genetic variance between the two treatments. Second, the genetic covariance tensor method is a multilinear algebra approach that can be used to describe variation between matrices. The eigenanalysis of a covariance tensor estimated on multiple **G** matrices returns second-order eigentensors and eigenvalues that can then be interpreted to determine which aspects of the original matrices differed. Eigentensors correspond to the higher-level equivalent of eigenvectors for matrices and describe independent aspects of variation between **G** matrices. Here, in a two matrices case, the number of eigentensors with non-zero eigenvalues is bounded to 1 (see Aguirre *et al.* (2014)). Following Aguirre *et al.* (2014), we applied the covariance tensor method on the posterior distributions of the two G-matrices. In order to test for differences, we compared the 95% HPD intervals of their positions along the eigenvectors explaining most of the variation in the first eigentensor. All analyses were carried out in the R environment for statistical computing (R Core Team 2020) version 4.0.3. We interpret HPD intervals of summary statistics that show no overlap with zero as statistically significant.

Results

The applied food treatment was effective in reducing resources available for growth and reproduction as indicated by a reduction of body weight, male reproductive success and female reproductive success in the low food treatment (Table 1; Figure 1). Body weight, male and female reproductive success were reduced by 48.3 %, 20.6 % and 47.1 %, respectively, in the Low-Food treatment relative to the High-Food treatment suggesting that the female sex function was impacted more strongly by food limitation than the male sex function. Moreover, we detected statistically significant effects of family and mother in all measured traits implying pervasive genetic variation and maternal effects on the three measured traits (Table 1). Finally, there were significant family by treatment interactions for body weight and reproductive success in both sex functions. Graphical inspection of the family means (breeding values) suggests that these genotype by environment interactions were primarily driven by moderate cross-over effects and slightly larger genetic variances under low food conditions (Figure S2).

We found that V_P of relativized body weight and relativized reproductive success in both sex functions was higher under food restriction (Table 2; Figure 2). However, this effect of the food treatment was only significant for relativized male and female reproductive success, which increased by 39.7 % and 66.0 %, respectively. We also observed a higher V_P in male reproductive success compared to female reproductive success but this difference was only statistically significant in the High-Food treatment (Table 2).

Despite the consistent effects of food restriction on phenotypic variances, we did not detect significant differences in V_G between treatments or sexes (Table 2; Figure 2). Only male reproductive success showed a relatively strong but statistically non-significant tendency for an increase of V_G in the Low-Food environment (Table 2; Figure 2). In addition, although estimates of V_G were about twice as high for male than for female reproductive success in both food treatments, the values of V_G were so small that their imprecise estimation may have prevented sex-differences from reaching the significance threshold (Table 2; Figure 3). Similar to these findings on genetic variances, we also found no evidence for a significant influence of food restriction on maternal effects (Table S3).

We found no clear support for genetic correlations between the three measured traits (Table 3; Figure 4). Specifically, we observed positive but statistically non-significant positive correlations of breeding values between body weight and male reproductive success in the High-Food treatment (Figure 4G) and between body weight and female reproductive success in the Low-Food treatment (Figure 4B). Importantly, there was no indication for a genetic correlation between male and female reproductive success neither in the High- nor in the Low-Food treatment (Figure 4F and H). This was also the case for phenotypic correlations in both food treatments (Table S2). For both sex functions, we found significant positive genetic correlations of reproductive success between the High- and Low-Food treatment (modes with 95% HPD intervals; male reproductive success: 0.831, 0.429 – 0.949; female reproductive success: 0.628, 0.012 – 0.869) and a tendency for a positive relationship for body weight (mode with 95% HPD intervals; 0.354, -0.171 – 0.721) indicating that genotypes performed consistently across environments (Figure 4A, E, I).

In correspondence to the relatively minor differences in genetic variances and genetic correlations between environments, we also did not detect significant changes in **G**. Comparison of the dimensionality of **G** indicates that the vast majority of all genetic variation could be explained along g_{max} in High- and Low-Food conditions suggesting a cigar-shaped **G** in both environments (Table 4; Table S4). Maximum evolvability and total genetic variance tended to be higher under Low-Food conditions, which was probably driven by the increase in genetic variance in male reproductive success (Table S4). However, HPD intervals of all summary statistics showed considerable overlap suggesting that the observed differences were not statistically significant (Table 4).

In accordance to these findings on the summary statistics, we found no evidence for differentiation of **G** based on the two matrix comparison methods. In the random skewers analysis, none of the vectors showed differences between the two **G** matrices as the HPD intervals overlapped for all vectors. In the genetic covariance tensor analysis, we compared the position of the two **G** matrices along the first two eigenvectors of the first eigentensor since they accounted for most of the variance (74.9 % and 23.2%, respectively; Table S5). The HPD intervals of the two eigenvectors overlapped largely suggesting no significant differences between the two G matrices (Figure S3).

Discussion

This study provides, to our knowledge, the first experimental test of how an environmental factor affects the opportunity for selection (*I*) and genetic variances of reproductive success in both sex functions of a simultaneous hermaphrodite. We found that food restriction had a proportionally stronger effect on female reproductive success confirming earlier findings on sex-specific condition-dependence in this species (Janicke & Chapuis 2016). Importantly, food restriction increased the opportunity for selection in both sex functions, which, however, did not translate into a detectable higher genetic variance under food limitation. Moreover, our results show that the opportunity for selection was stronger in the male function with a particularly strong and significant sex-difference in the High-Food treatment. We also observed a trend towards higher evolvability of the male sex function in the Low-Food treatment, but this was not found to be statistically significant potentially due to a lack of statistical power. Finally, we found no support for phenotypic and genetic correlations between male and female reproductive success. In the following we discuss these main findings one after the other with emphasis on their implications for the adaptation to novel environments.

Environmental effect on the phenotypic and genetic opportunity for selection

Environmental stress in terms of demographic change or resource limitation has repeatedly been shown to change *I* in various systems (Wacker *et al.* 2013; Morimoto *et al.* 2016; Cattelan *et al.* 2020). Food restriction has already been tested for affecting *I* and the strength of sexual selection measured in terms of the opportunity for sexual selection (i.e., variance in relativized mating success; I_s) and the Bateman gradient (i.e., selection differential of mating success) in *P. acuta* (Janicke *et al.* 2015). In this previous study, we detected an increase in male I_s and a decrease in the Bateman gradient but no clear change in *I* under food restriction. By contrast, in this study we observed a clear increase in *I* of male and female reproductive success. This discrepancy with the previous findings may stem from a more severe food limitation (i.e., alternating food restriction over 2 weeks in this study versus food restriction for only 4 days prior to mating trials in the previous study), a different experimental setup of the mating trials and/or a considerably higher sample size in the present study. Our present study suggests that phenotypic

selection via the male and female function is elevated under a more stressful environment such as lowered food-availability.

The crucial question in evolutionary terms is, however, whether these changes in phenotypic variance have a genetic basis. Overall, we detected a significant genetic signal for the three measured traits as indicated by moderate heritability for body weight and lower heritabilities for reproductive success in both sex functions, which is predicted for fitness related traits (Mousseau & Roff 1987). Importantly, the observed effect of food restriction on phenotypic variances did not translate into larger genetic variances under food stress. Point estimates of genetic variance of male reproductive success showed a 75.9% increase under food restriction but this effect was statistically non-significant because posterior distributions largely overlapped resulting in considerable uncertainty in the observed difference. Previous studies on separate-sexed organisms testing for responses to maladapted food environments also found evidence for an increase in genetic variances (Holman & Jacomb 2017; Martinossi-Allibert *et al.* 2017; Martinossi-Allibert *et al.* 2018) but like in our study the observed differences are mostly statistically non-significant due to low statistical power.

It is important to notice that despite significant genotype-by-environment interactions, and potential changes in genetic variances with the environment, the genetic correlations between the different environments are strongly positive for male and female reproductive success, meaning that the rankings of the different genotypes are largely preserved. Food stress may therefore increase variances (especially in male fitness) but doing so it amplifies (rather than overturns) genetic differences expressed in optimal conditions. Form a methodological point of view, this finding also suggests that our breeding design granted sufficient statistical power to detect at least strong genetic correlations.

Our findings of genetic variances and genetic correlations together with the analysis of key summary statistics of **G** and the two matrix comparison methods (i.e., random skewers and genetic covariance tensor) are suggestive of a conserved genetic architecture under the tested stressor. However, given the relatively low statistical power, we may not have been able to detect small differences in genetic variances and to detect weak genetic correlations. Having this limitation in mind, our study supports the view that G-matrices are largely stable across environments (Arnold *et al.* 2008). Nevertheless, the observed changes in maximum evolvability

and total genetic variance agree, at least qualitatively, with the prediction that genetic variances increase with environmental stress (Rowinski & Rogell 2017), although the confidence intervals were large and overlapping.

Sex-specific opportunity for selection and cross-sex genetic correlations

Theory predicts that sexual selection is typically stronger on males (Schärer *et al.* 2012; Parker & Birkhead 2013; Parker 2014; Lehtonen *et al.* 2016), which is expected to translate into a higher opportunity for selection in males (Bateman 1948; Arnold 1994; Shuster & Wade 2003) and potentially into a higher additive genetic variance of reproductive success in the male sex. While there is solid comparative evidence for the former prediction (Janicke *et al.* 2016a), the empirical support for the latter is limited and rather mixed (Hendry *et al.* 2018; Winkler *et al.* 2021). In this study we found a male bias of I in both food treatments, which also translated into a higher genetic variance in male reproductive success, though the latter effect was weak and posterior intervals of the sex-difference largely overlapped with zero.

To our knowledge, our study provides the first formal test for a cross-sex genetic correlation of reproductive success in a simultaneously hermaphroditic animal. We did not detect any sign for a genetic correlation between male and female reproductive success, though both traits tend to be positively correlated with body weight in one or the other environment (High-Food for male reproductive success, Low-Food for female reproductive success). In simultaneous hermaphrodites every allele affecting sex allocation is predicted to manifest in a sexually antagonistic effect on reproductive success (Schärer *et al.* 2015) so that negative cross-sex genetic correlations of reproductive success might be particularly widespread (Abbott 2011; Schärer *et al.* 2015). Our results do not support this hypothesis. Theory also predicts that a higher contribution of sexually antagonistic loci to variance in reproductive success in environments to which populations are well-adapted, because directional selection is expected to deplete variation at loci with concordant effects in males and females (Connallon & Hall 2016). Experimental quantitative genetic studies testing for environmental effects on cross-sex genetic correlations are constrained to insect model systems and provided rather mixed results. For example, heat stress has been shown to impose a shift from a negative towards a positive cross-sex genetic correlation in seed beetles *Callosobruchus maculatus* (Berger *et al.* 2014). By contrast, exposure to a novel food

source has been found to lead to a more negative cross-sex genetic correlation of fitness in *Drosophila serrata* (Delcourt *et al.* 2009). Interestingly, another study quantifying cross-sex genetic correlations in *D. serrata* in three novel food environments suggests no consistent changes illustrating their unpredictable nature (Punzalan *et al.* 2014). Importantly, genetic correlations are often estimated with very low precision meaning that comparisons across treatments have usually very limited statistically power and in this respect our study is no exception.

The here documented absence of a negative cross-sex genetic correlation may, of course, reflect limitations of our experimental design (see below) but could also arise from the genetic architecture of sex allocation and its link to reproductive success. First, sex allocation in *P. acuta* may show only little genetic variation but exhibit high levels of phenotypic plasticity, which has been documented for many simultaneously hermaphroditic species (Janicke *et al.* 2016b). Second, alleles affecting the allocation into the male or female sex function are only predicted to induce sexually antagonistic effects of reproductive success if there is a sex allocation trade-off. However, there is surprisingly little empirical evidence for such a trade-off between both sex functions in simultaneous hermaphrodites (Schärer *et al.* 2005; Schärer 2009) potentially because individual differences in the total budget of reproductive resources may mask the predicted negative correlation between male and female reproductive investment (Van Noordwijk & De Jong 1986). We are aware of only one empirical study testing for a genetic correlation between allocation into the male and female sex function in a simultaneous hermaphrodite. Specifically, Yund *et al.* (1997) found evidence for a negative genetic correlation between testis area and egg number in the colonial ascidian *Botryllus schlosseri*. Despite potential implications of our results for the genetic architecture of sex allocation, we note that investment into male *versus* female reproduction may not necessarily translate directly into sex differences in reproductive success. Notably, simultaneous hermaphrodites are predicted to have a female biased sex allocation but given the Fisher's condition mean reproductive success of both sex functions must be identical (Schärer 2009). Hence, the fitness return per investment may often differ between the male and female sex function, which potentially relaxes the link between allocation and reproductive success.

Implications and limitations

Sexual selection is expected to facilitate genome-wide purging of deleterious alleles and thereby promote the adaptation to novel and often stressful environments (Candolin & Heuschele 2008; Holman & Kokko 2013; Cally *et al.* 2019) and may even explain the evolution and maintenance of sexual reproduction (Agrawal 2001; Siller 2001). This concept hinges critically on two key assumptions: (*i*) net selection is stronger on the male sex and (*ii*) selection on males also favours female fitness as indicated by a positive cross-sex genetic correlation (Whitlock & Agrawal 2009; Martínez-Ruiz & Knell 2017). A previous experimental study on *P. acuta* revealed that selection against deleterious alleles measured in terms of inbreeding depression of reproductive success can be stronger in the male compared to the female sex function (Janicke *et al.* 2013). This result is in line with our finding of a trend for a higher genetic variance in male reproductive success. Moreover, in an experimental evolution experiment of *P. acuta*, Bonel *et al.* (2018) found that sexual selection on males prevents the accumulation of male-detrimental alleles but has no effect on female reproductive success, which corresponds to the lack of strong, either positive or negative, cross-sex genetic correlation found in our study. However, reproductive success measures only one component of overall fitness. Male and female fitness may still be genetically correlated if reproductive success of both sexes shows genetic linkage or pleiotropy with the other major fitness component, which is survival. In simultaneous hermaphrodites, survival is a shared trait and cannot be expressed in a sex-specific way as it is the case for many separate-sexed organisms (Lemaitre *et al.* 2020). This has important implications because genetic variants favouring pre-reproductive survival will automatically have identical positive effects on lifetime male and female reproductive success, and the same is expected to happen to variants affecting survival after the onset of reproduction assuming that senescence is similar in both sex functions. Therefore, male and female lifetime reproductive success of simultaneous hermaphrodites might be inherently positively linked despite constraints arising from sex allocation loci, which are likely to show sexually antagonistic effects. Interestingly, the above-mentioned experimental evolution study in *P. acuta* found that lines evolving in the presence of selection on male reproductive success showed a higher juvenile survival compared to lines where this selection had been suppressed, leaving only the female function under selection (Bonel *et al.* 2018; Noel *et al.* 2019). In the present study we did not detect any significant genetic variation in juvenile survival (data

not shown) and it was therefore excluded from the presented analyses. Presumably, long-term experimental evolution studies in which small effects accumulate over tens of generations, can capture sources of genetic variance and covariance that are more difficult to detect in assays encompassing only a single generation, such as this study.

Further quantitative genetic studies are clearly needed to elucidate the genetic architecture under environmental change in simultaneous hermaphrodites. This includes more complete measurements of male and female reproductive success than the ones used in this study. Specifically, our mating trials allowed for intense post-copulatory sexual selection, which is favourable compared to assays preventing any form of sexual competition (Holman & Jacomb 2017). However, our setup only allowed for limited pre-copulatory sexual selection, which is an important though not necessarily the predominant component of selection in *P. acuta* (Pélissié *et al.* 2014; Janicke *et al.* 2015). Moreover, just as many other experimental studies with a similar angle, our measures of reproductive success provide only snapshots of reproductive performance rather than an estimate of lifetime reproductive success, which limits our inferences on the genetic architecture of male and female fitness. Furthermore, the breeding design of this study, including only 22 families, was clearly a limitation in terms of statistical power required to estimate genetic variances and correlations. Higher sample sizes might be needed to detect low levels of genetic variance and weak genetic correlations. Finally, the use of inbred lines may have allowed to quantify only a fraction of the standing genetic variation of the sampled population because deleterious alleles have been purged during inbreeding. More powerful breeding designs together with fitness assays tracking lifetime reproductive success of both sex functions provide a promising avenue to a more complete understanding of the genetic architecture of fitness components in simultaneous hermaphrodites.

Conclusions

Taken together, this study provides compelling evidence that environmental stress in terms of food restriction leads to an increase in opportunity for selection of both sex functions but is not accompanied by a similar change in phenotypic variance in body weight in a simultaneous hermaphrodite. These effects were paralleled by a non-significant increase in genetic variance of male reproductive success, and no detectable trend in the two other traits. Having in mind the

limited statistical power of our study, we did not observe significant changes in the G-matrix with respect to the tested environmental stress and genotype ranks tended to be preserved across environments. We also did not detect any genetic correlation between male and female reproductive success suggesting that fitness-related traits of both sex functions are not tightly coupled in their evolution. Further empirical tests of the genetic architecture of fitness components in simultaneous hermaphrodites are required to better understand the genetic implications and peculiarities of having both sex functions expressed in one and the same individual.

Acknowledgements

We are very grateful to the late Violette Sarda for so much more than technical support. We also thank Maria Moiron and Céline Teplitsky for statistical advice as well as Lennart Winkler, David Berger, Ivain Martinossi-Allibert and one anonymous reviewer for very constructive comments on an earlier draft. TJ was funded by grants from the Swiss National Science foundation (SNSF grant number: PA00P3-145375/1), the German Research Foundation (DFG grant number: JA 2653/2-1) and the CNRS.

Conflict of interest

The authors declare to have no conflicts of interest.

Statement of authorship

TJ conceived the study. TJ, EC and PD designed the experiment. EC, SM and TJ collected the data with help of NB. TJ, BD and EC performed the statistical analyses. TJ wrote the paper with the help of EC, SM, BD, NB and PD.

Data Availability Statement

All data can be retrieved from the Dryad Digital Repository: https://doi.org/10.5061/dryad.fj6q573w3 (Janicke *et al.* 2021).

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Figure Legends

Figure 1. Comparison of (A) body weight, (B) male reproductive success and (C) female reproductive success between High-Food (green) and Low-Food (yellow) treatments. Boxplots show the median (horizontal line), 25% and 75% percentiles (box), minimum and maximum (whiskers, extending to 1.5 times the interquartile range) and outliers (black filled circles). Jittered points show the distribution of raw data.

Figure 2. Effect of food restriction on phenotypic (V_P; A-C) and genetic variances (V_G; D-F) in body weight (A, D), male reproductive success (B, E) and female reproductive success (C, F) – all traits relativized by their mean in the corresponding food treatment. Density plots of show frequency distribution of bootstrapped samples for V_P and Posterior distributions obtained from GLMMs for V_G. High-Food treatment in green, Low-Food treatment in yellow. See Method section for details.

Figure 3. Sex differences in phenotypic (V_P; A, B) and genetic variances (V_G; C, D) in male (blue) and female (red) reproductive success under High-Food (A, C) and Low-Food (B, D) conditions. Density plots of show frequency distribution of bootstrapped samples for V_P and Posterior distributions obtained from GLMMs for V_G. See Method section for details.

Figure 4. Genetic correlations among the three studied traits within (green circles: High-Food; yellow: Low-Food) and across food treatments (black circles). Data points represent residual family means after accounting for maternal and block effects. See Method section for details.

Tables

Table 1. Results of Linear Mixed-Effects Models (LMMs) testing the effect of the food-treatment, block, parent, family and the treatment by family interaction. In all models only food-treatment was defined as fixed effect whereas all other traits were specified as random terms. All analyses ran on raw values (not relativized data) are shown.

Trait	Food-treatment		Block		Parent		Family		Treatment x Family	
	χ^2	*P*-value	χ^2	*P*-value	χ^2	*P*-value	χ^2	*P*-value	χ^2	*P*-value
Body weight	211.960	< 0.001	72.399	< 0.001	80.135	< 0.001	82.402	< 0.001	71.828	< 0.001
Male reproductive success	7.172	0.007	38.213	< 0.001	9.484	0.009	19.023	< 0.001	7.138	0.008
Female reproductive success	77.334	< 0.001	20.143	< 0.001	11.663	0.003	10.683	0.005	5.410	0.020

Table 2. Summary of phenotypic variances (V_P), genetic variances (V_G) and broad sense heritabilities (H^2) shown for both food treatments together with the food-treatment difference. Sex differences are given with positive values indicating a male-bias. Estimates of V_P, V_G and H^2 are modes of posterior distributions obtained from GLMMs and are shown together with 95% HPD intervals. Note that variances were computed from relativized data so that V_P and V_G correspond to the phenotypic opportunity for selection (I) and the squared evolvability (Houle 1992), respectively. Estimated differences between sexes and treatments for which HPD intervals do not overlap with zero in bold.

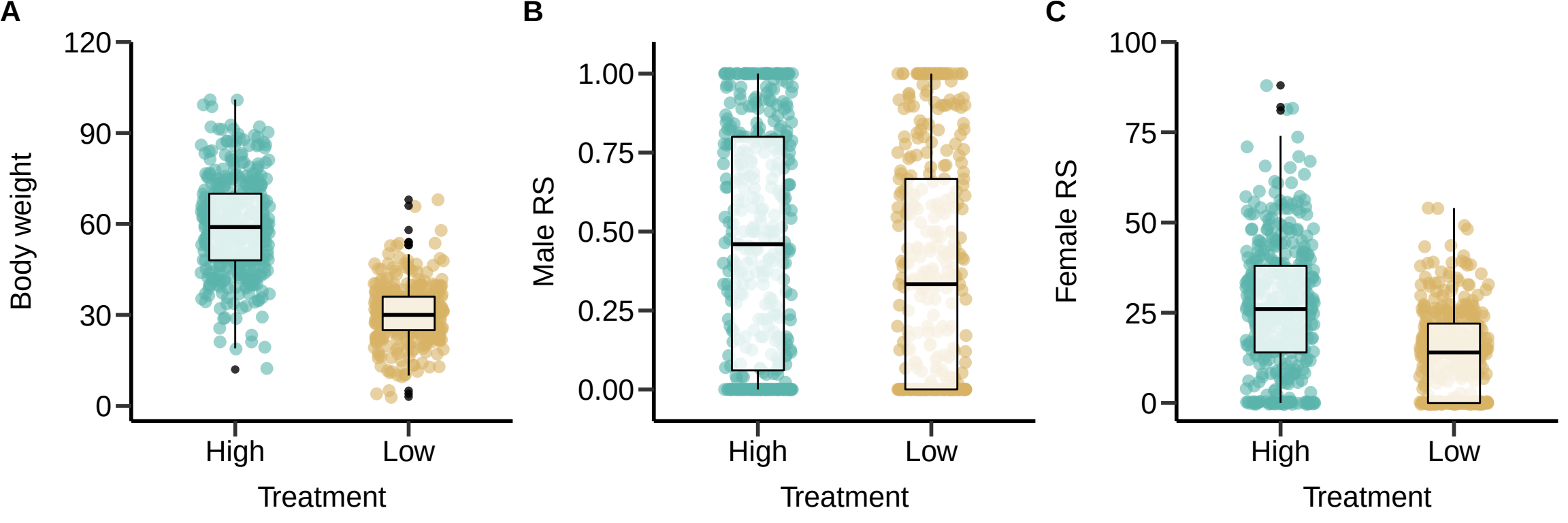
Estimate	Trait	High-Food		Low-Food		Treatment difference	
		Mode	(l 95%, u 95%)	Mode	(l 95%, u 95%)	Mode	(l 95%, u 95%)
V_P	Body weight	0.084	(0.065, 0.110)	0.099	(0.079, 0.131)	-0.018	(-0.053, 0.016)
	Male reproductive success	0.584	(0.506, 0.713)	0.816	(0.711, 1.019)	**-0.261**	**(-0.423, -0.089)**
	Female reproductive success	0.412	(0.361, 0.494)	0.684	(0.575, 0.801)	**-0.249**	**(-0.385, -0.132)**
	Sex difference	**0.157**	**(0.060, 0.308)**	0.166	(-0.016, 0.368)		
V_G	Body weight	0.021	(0.011, 0.050)	0.022	(0.009, 0.049)	-0.001	(-0.027, 0.032)
	Male reproductive success	0.071	(0.030, 0.189)	0.124	(0.039, 0.284)	-0.034	(-0.181, 0.073)
	Female reproductive success	0.033	(0.011, 0.087)	0.048	(0.012, 0.152)	-0.024	(-0.112, 0.045)
	Sex difference	0.032	(-0.033, 0.152)	0.068	(-0.066, 0.248)		
H^2	Body weight	0.192	(0.095, 0.396)	0.183	(0.074, 0.345)	0.031	(-0.143, 0.243)
	Male reproductive success	0.123	(0.050, 0.265)	0.146	(0.046, 0.279)	0.017	(-0.148, 0.118)
	Female reproductive success	0.065	(0.023, 0.174)	0.065	(0.018, 0.203)	-0.014	(-0.122, 0.102)
	Sex difference	0.060	(-0.077, 0.195)	0.062	(-0.094, 0.224)		

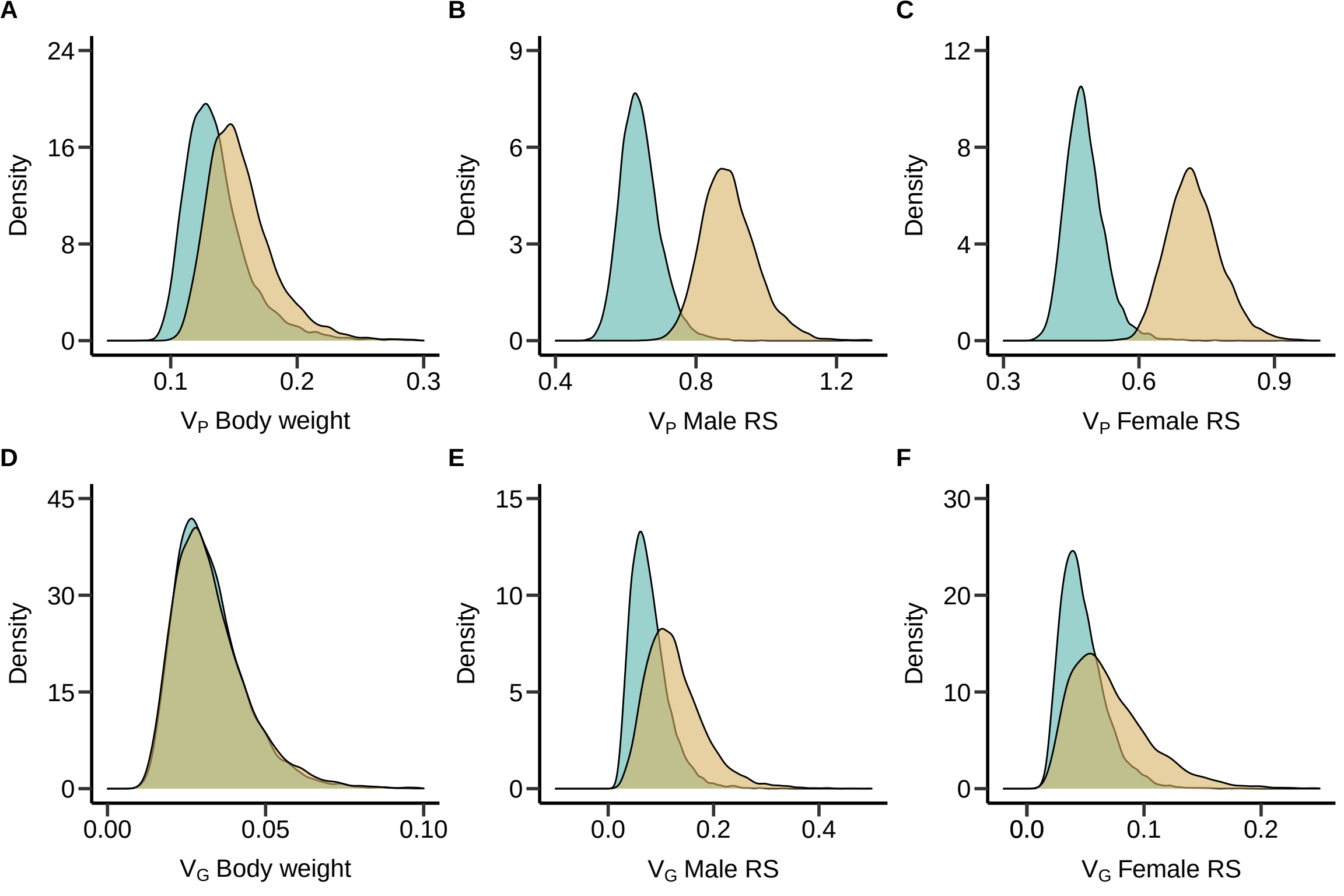
Table 3. Summary of genetic correlations between body weight, male reproductive success and female reproductive success shown for both food treatments together with the food-treatment difference. Modes and 95% HPD intervals were obtained from GLMMs.

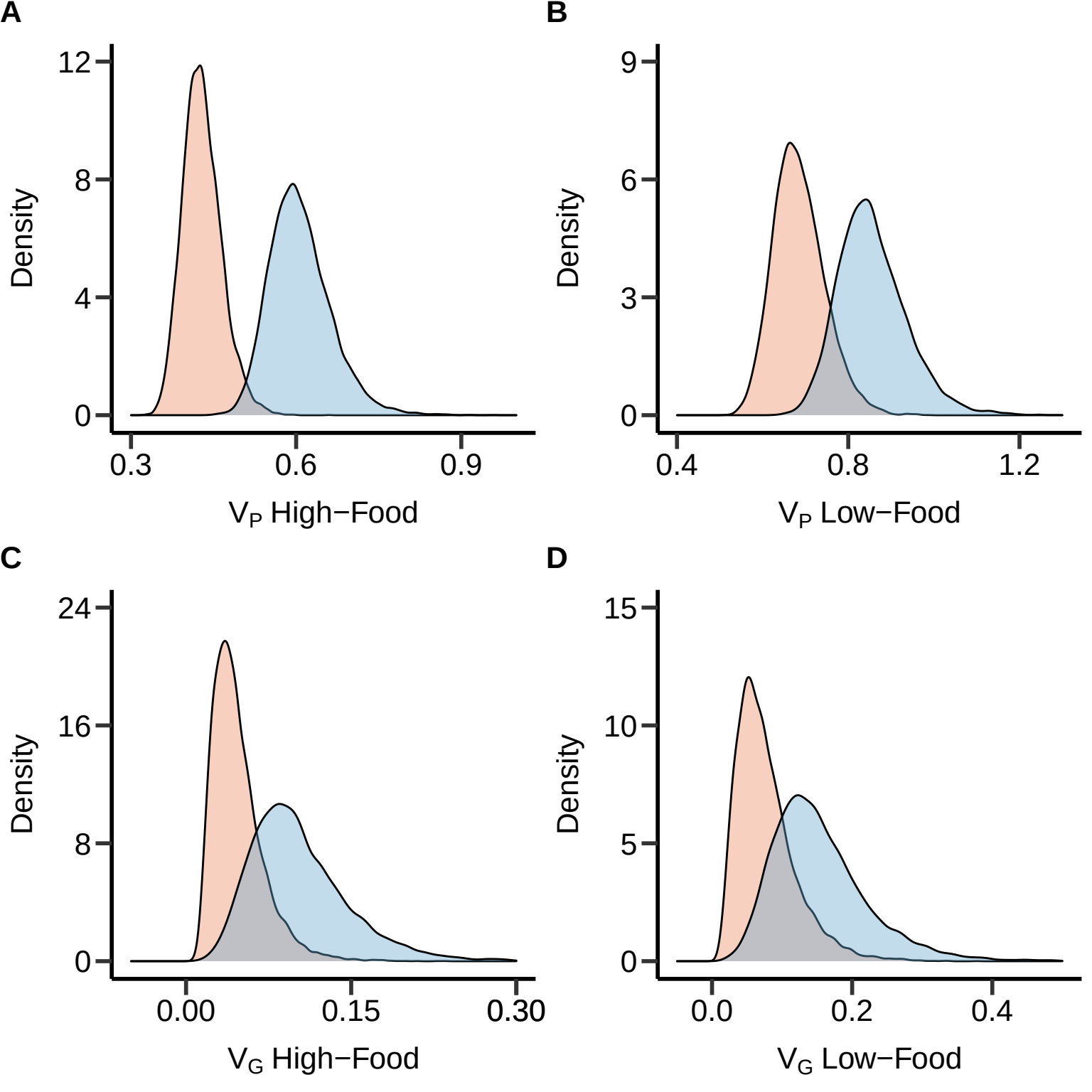
Correlation	High-Food			Low-Food			Treatment difference		
	Mode	(l 95%,	u 95%)	Mode	(l 95%,	u 95%)	Mode	(l 95%,	u 95%)
Body weight and male reproductive success	0.493	(-0.003,	0.817)	0.242	(-0.381,	0.689)	0.183	(-0.358,	0.872)
Body weight and female reproductive success	0.094	(-0.431,	0.641)	0.551	(-0.197,	0.814)	-0.241	(-0.925,	0.463)
Male and female reproductive success (r_{MF})	0.197	(-0.506,	0.704)	-0.217	(-0.822,	0.463)	0.178	(-0.450,	1.075)

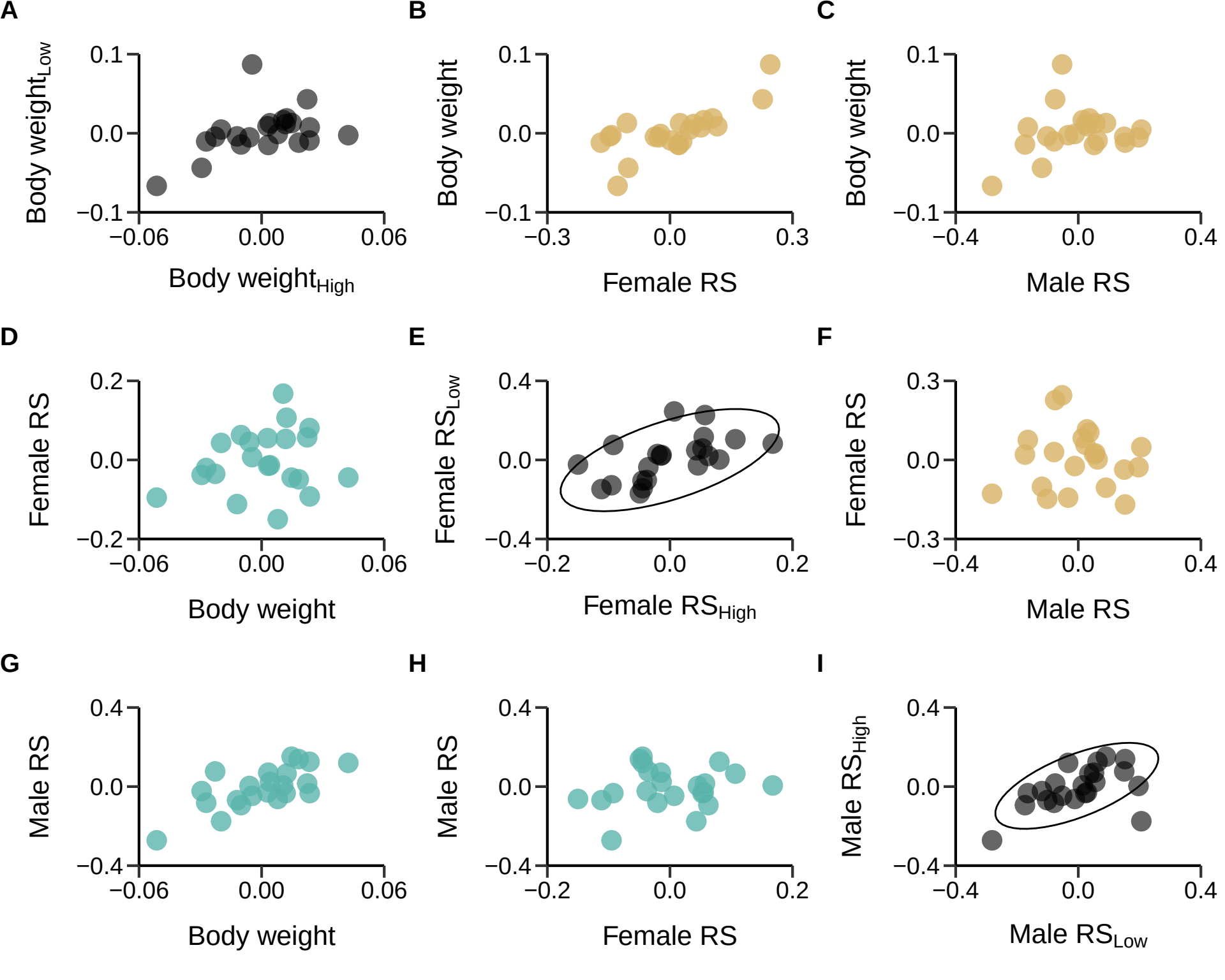
Table 4. Comparison of **G** matrices between High- and Low-Food treatment based on summary statistics proposed by Kirkpatrick (2009). Food-treatment difference is calculated by subtracting estimates of Low-Food from High-Food treatment. Posterior modes and 95% HPD intervals were obtained from GLMMs.

Summary statistic	High-Food			Low-Food			Food-treatment difference		
	Mode	(l 95%,	u 95%)	Mode	(l 95%,	u 95%)	Mode	(l 95%,	u 95%)
Effective number of dimensions (n_D)	1.524	(1.245,	2.216)	1.452	(1.192,	2.051)	0.174	(-0.606,	0.763)
Maximum evolvability (e_{max})	0.304	(0.198,	0.426)	0.366	(0.251,	0.528)	-0.069	(-0.252,	0.114)
Total genetic variance (ν_T)	0.155	(0.082,	0.249)	0.198	(0.108,	0.368)	-0.078	(-0.246,	0.086)


A
Body weight
120
90
60
30
0
High
Low
Treatment
B
Male RS
1.00
0.75
0.50
0.25
0.00
High
Low
Treatment
C
Female RS
100
75
50
25
0
High
Low
Treatment


A
B
C
D
E
F
Density
24
16
8
0
0.1
0.2
0.3
V_P Body weight
9
6
3
0.4
0.8
1.2
V_P Male RS
12
4
0.3
0.6
0.9
V_P Female RS
45
30
15
0.00
0.05
0.10
V_G Body weight
5
0.0
0.2
0.4
V_G Male RS
20
0.1
V_G Female RS


A
B
C
D
Density
12
8
4
0
0.3
0.6
0.9
V_P High−Food
9
6
3
0.4
0.8
1.2
V_P Low−Food
24
16
0.00
0.15
0.30
V_G High−Food
15
10
5
0.0
0.2
0.4
V_G Low−Food


A
Body weight$_{Low}$
0.1
0.0
−0.1
−0.06
0.00
0.06
Body weight$_{High}$
B
Body weight
0.1
0.0
−0.1
−0.3
0.0
0.3
Female RS
C
Body weight
0.1
0.0
−0.1
−0.4
0.0
0.4
Male RS
D
Female RS
0.2
0.0
−0.2
−0.06
0.00
0.06
Body weight
E
Female RS$_{Low}$
0.4
0.0
−0.4
−0.2
0.0
0.2
Female RS$_{High}$
F
Female RS
0.3
0.0
−0.3
−0.4
0.0
0.4
Male RS
G
Male RS
0.4
0.0
−0.4
−0.06
0.00
0.06
Body weight
H
Male RS
0.4
0.0
−0.4
−0.2
0.0
0.2
Female RS
I
Male RS$_{High}$
0.4
0.0
−0.4
−0.4
0.0
0.4
Male RS$_{Low}$