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Genotype-by-environment interactions underlie the expression of pre- and postcopulatory sexually selected traits in guppies

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Abstract

The role that genotype-by-environment interactions (GEIs) play in sexual selection has only recently attracted the attention of evolutionary biologists. Yet GEIs can have profound evolutionary implications by compromising the honesty of sexual signals, maintaining high levels of genetic variance underlying their expression, and altering patterns of genetic covariance among fitness traits. In this study, we test for GEIs in a highly sexually dimorphic freshwater fish, the guppy *Poecilia reticulata*. We conducted an experimental quantitative genetic study in which male offspring arising from a paternal half-sibling breeding design were assigned to differing nutritional ‘environments’ (either high or low feed levels). We then determined whether the manipulation of diet quantity influenced levels of additive genetic variance and covariance for several highly variable and condition-dependent pre- and postcopulatory sexual traits. In accordance with previous work, we found that dietary limitation had strong phenotypic effects on numerous pre- and postcopulatory sexual traits. We also report evidence for significant GEI for several of these traits, which in some cases (area of iridescence and sperm velocity) reflected a change in the rank order of genotypes across different nutritional environments (i.e. ecological crossover). Furthermore, we show that genetic correlations vary significantly between nutritional environments. Notably, a highly significant negative genetic correlation between iridescent colouration and sperm viability in the high food treatment broke down under dietary restriction. Taken together, these findings are likely to have important evolutionary implications for guppies; ecological crossover may influence sexual signal reliability in unstable (nutritional) environments and contribute towards the extreme levels of polymorphism in sexual traits typically reported for this species. Furthermore, the presence of environment-specific genetic covariance suggests that trade-offs measured in one environment may not be indicative of genetic constraints in others.

Keywords: reaction norm, genetic variance, sperm quality, condition dependence

Introduction

An individual's condition can be defined as the pool of resources allocated to traits that enhance fitness (Rowe & Houle, 1996). Condition, in turn, potentially translates into fitness according to how efficiently an individual can accumulate and allocate resources to reproductive success in the light of various environmental (Bonduriansky & Rowe, 2005, Cotton *et al.*, 2004) and genetic factors (Rowe & Houle, 1996, Tomkins *et al.*, 2004). A number of studies spanning several taxonomic groups have revealed that the expression of phenotypic condition (e.g. ornamental and/or display traits that are correlated with reproductive fitness) can depend on such factors (e.g. Gienapp & Merila, 2010, Holzer *et al.*, 2003, Ingleby *et al.*, 2010), and that individuals in higher condition are better able to express elaborate phenotypic traits than those in poor condition (i.e. indicating condition dependence) (e.g. Gibson & Uetz, 2012, Holzer *et al.*, 2003). Furthermore, some genotypes are better able to maintain optimal phenotypic expression under relatively poor conditions than others, thus resulting in genotype-by-environment interactions (GEIs) (Hunt *et al.*, 2004, Rowe & Houle, 1996).

Genotype-by-environment interactions are likely to be especially relevant in the context of sexual selection, where there is increasing recognition that individual plasticity in sexual trait expression can have a genetic basis (Higginson & Reader, 2009, Hunt & Hosken, 2014, Ingleby *et al.*, 2010). In particular, evidence from numerous quantitative genetic studies have shown that traits such as courtship behaviour (Danielson-Francois *et al.*, 2009, Greenfield *et al.*, 2012, Kotiaho *et al.*, 2001, Mills *et al.*, 2007, Taylor *et al.*, 2013), ornamentation (Hughes *et al.*, 2005, Kemp & Rutowski, 2007, Lewandowski & Boughman, 2008, Miller & Brooks, 2005) and ejaculates (Engqvist, 2008, Lewis *et al.*, 2012) exhibit patterns of genetic variance that are modified by environmental effects. Furthermore, there is abundant evidence from many species that sexually selected traits can exhibit heightened condition dependence compared to other life-history traits (Cotton *et al.*, 2006), thus making these traits potentially important targets of selection for paternal 'good genes' (Kokko *et al.*, 2003). It is therefore possible that the reliability of sexual signals can be compromised when environmental conditions change (Barber *et al.*, 2001, Ingleby *et al.*, 2013). Genotype-by-environment interactions also have the potential to alter patterns of genetic covariation for multiple sexual display traits, for example where the genetic

architecture constraining evolutionary change (e.g. through trade-offs) is contingent on environmental conditions (Bussiere *et al.*, 2008, Sgro & Hoffmann, 2004). As such, the evolutionary responses of individual sexual traits may differ substantially between environments because of concomitant changes in patterns of genetic covariance with other fitness traits (Ingleby *et al.*, 2014). Despite these important evolutionary implications, there are relatively few studies that document GEIs in the context of sexual selection, particularly those revealing ecological crossovers among reaction norms (i.e. changes in the rank performance of genotypes between environments; Carreira *et al.*, 2013, Ingleby *et al.*, 2013, Lewis *et al.*, 2012) and/or changes in patterns of genetic covariance in different environments (Robinson *et al.*, 2009).

The guppy (*Poecilia reticulata*) is a highly polyandrous livebearing freshwater fish in which males exhibit complex colour patterns comprising orange (carotenoids and drosopterins), iridescent (structural) and black (melanin) spots. These highly conspicuous colour traits exhibit substantial levels of variance in size, spot number, spectral properties and position (Houde, 1997) and there is growing evidence that their expression depends on a combination of genetic (Brooks & Endler, 2001, Evans, 2010) and environmental factors (Grether, 2000, Karino & Haijima, 2004, Kodric-Brown, 1989). Males attract females by performing a ritualised courtship behaviour (termed sigmoid display) to reveal their highly polymorphic colour patterns to females (Houde, 1997). They also use forced mating attempts (termed gonopodial thrusts) to achieve copulations with sexually unreceptive (or unwilling) females (Houde, 1997). Females typically mate with multiple males during a single reproductive episode (Evans & Pilastro, 2011), and therefore sperm from different males (arising from multiple solicited or forced copulations) compete for fertilization to generate mixed paternity broods (Hain & Neff, 2007, Neff *et al.*, 2008). Postcopulatory sexual selection therefore plays an important role in the guppy's mating system, and recent studies have revealed associations between components of sperm quality (e.g. sperm number, sperm velocity and the proportion of live sperm in the ejaculate) and competitive fertilization success (Boschetto *et al.*, 2011, Fitzpatrick & Evans, 2014).

Guppies have proved to be especially useful models for studying the interplay between pre- and postcopulatory episodes of sexual selection. For example, several studies have revealed positive phenotypic associations between the extent of orange pigmentation in the male's colour patterns and sperm swimming velocity (Locatello *et al.*, 2006, Pitcher *et*

al., 2007) and ejaculate size (Pilastro *et al.*, 2002, Pitcher & Evans, 2001), and there is corresponding evidence that males with high levels of orange pigmentation are more successful than their drab counterparts during sperm competition (Evans *et al.*, 2003, Pitcher *et al.*, 2003). However, there is also evidence that some components of the male guppy's colour patterns are negatively associated with components of sperm quality. For example, Evans (2010) reported a negative genetic correlation between male iridescent colouration and sperm viability (the proportion of live sperm in the ejaculate), highlighting a possible trade-off in investment between these components of pre- and postcopulatory sexual selection. Thus, the prevailing evidence to date suggests that different components of male sexual ornamentation exhibit conflicting patterns of genetic and phenotypic covariance with traits linked to ejaculate quality; orange pigmentation is generally positively associated with sperm velocity and sperm number while iridescence is negatively associated with sperm viability. However, the extent to which the genetic integration of these pre- and postcopulatory sexual traits is contingent on environmental heterogeneity has yet to be established.

In this study we use an experimental quantitative genetic design to test for genetic (co)variance and GEIs underlying the expression of condition dependent pre- and postcopulatory traits in guppies. Recent evidence from guppies reveals that traits subject to precopulatory (colour ornamentation and behaviour) and postcopulatory sexual selection (ejaculate traits) are highly sensitive to changes in diet quantity and quality (Devigili *et al.*, 2013, Rahman *et al.*, 2013, Rahman *et al.*, 2014). Indeed, there is abundant evidence that the availability of resources such as diet, among other sources of environmental variation, influences the expression of sexually selected traits in guppies (Kolluru, 2014). Until now, however, there has been limited support for the presence of GEI through resource limitation for sexual ornamentation in guppies (Kolluru, 2014), and to our knowledge there are no tests for GEIs in any context for postcopulatory traits in guppies. In the present study we test for GEI in a range of pre- and postcopulatory traits, focusing on those known to influence mating success (body size and colouration, mating behaviour) and paternity success (the number, velocity and viability of sperm) along with other putative targets of sexual selection (where we have yet to establish relationships between trait values and fitness; e.g. sperm length). Our analyses are based on data from a paternal half-sibling breeding design from which we determine (a) whether patterns of genetic variance are

altered by diet intake; (b) the extent to which such GEIs are attributable to changes in the rank order of genotypes in different environments (i.e. ecological crossover); and (c) whether patterns of genetic covariation between sexually selected traits (particularly across the pre- and postcopulatory divide) change across nutritional environments.

Materials and methods

Study population and its maintenance

The guppies used in this experiment were reared from the descendants of fish captured in 2006 from a natural population in Queensland and kept in mixed-sex aquaria until required for this experiment. These stock tanks were lit by overhead fluorescent lamps (Philips TLD 36W) on a 12:12 light: dark cycle and maintained at $27 \pm 1^\circ\text{C}$. The stock population was fed five days per week on a diet of *Artemia* nauplii supplemented with commercial dry food one day per week.

Breeding design and rearing conditions

We established a nested half-sibling breeding design by mating 46 adult males (sires) from our stock population to three females each (dams, 138 in total). Three of the male offspring died during the experiment and some inseminations failed to generate at least two male offspring from a family. The final analysis comprised data for 31 sires, 71 dams and 408 male offspring. Matings were conducted using artificial insemination (Evans *et al.*, 2003) to minimise the likelihood that differential maternal effects (attributable to differences in the females' perception of male attractiveness) would have compromised our estimates of additive genetic variance (Kotiaho *et al.*, 2003).

The offspring produced by each dam were isolated from the mother, reared in family groups and fed a mixture of *Artemia* nauplii and dry food until they could be sexed by eye (n male offspring tested = 408; mean age in days $\pm$ SE until sexing = 70.49 ± 0.90). As male guppies exhibit minimal post-maturational growth after reaching sexual maturity, diet levels could be fixed at a standard quantity throughout the treatment phase (which commenced when males could be sexed). Upon reaching sexual maturity, males from a given full sibling family were placed individually in 2L aquaria and assigned haphazardly to one of two experimental diet treatments that were compositionally identical (see the electronic

supplementary material, Table S1) but differed in quantity ('high quantity' and 'low quantity') (diets prepared by Nutra-Kol Pty Ltd, Mullaloo, Australia). Males assigned to the high quantity diet were fed ~4% of their body weight (1.9mg) per day, while those assigned to the low quantity diet received a daily allowance of ~1% (0.5mg) of their body weight. These feed levels correspond with our previous work documenting the condition dependence of pre- and postcopulatory sexual traits in the same population (Rahman *et al.*, 2014). All males were fed once per day, six days per week for a total of four months. During the treatment phase, each focal male was exposed to visual stimuli from two adult females housed in adjacent tanks. These females were included to ensure that the focal males were able to perceive potential mating opportunities during the experimental feeding period (Bozynski & Liley, 2003, Gasparini *et al.*, 2009). Opaque paper screens were placed between adjacent male tanks to prevent visual interactions among the experimental males. In all tanks, water was treated with an anti-algal treatment (3 g l⁻¹ 2-chloro-4, 6-bis-(ethylamino)-s-triazine; Aquamaster) each week to prevent algal growth, which may otherwise provide an additional source of nutrition to the experimental fish (Grether, 2000). Male traits (see below) were measured after four months of dietary treatments (mean age in days $\pm$ SE = 190.49 $\pm$ 0.90).

Mating behaviour

Mating behaviour trials took place between 08.00 and 12.00 to correspond with the peak of sexual activity in this species (Houde, 1997). For these trials, we used an 8L observation tank for each male (28.5x14.5x19 cm, filled to 14 cm) containing aquarium gravel and artificial pondweed. In each trial, a non-virgin female arising from a mixed-sex (stock) aquarium was placed in the tank and allowed to settle overnight. Females were approximately matched for size (by eye) across trials and used only once. On the following day, a single male from one of the treatment groups was placed in the aquarium and allowed to settle for at least five minutes or until he showed interest in the female (i.e. following the female or engaging in courtship). For each 10 minute trial, we recorded male mating behaviour as the number of sigmoid displays, where males arch their body in a characteristic s-shaped posture and quiver, and gonopodial thrusts, where males attempt to forcibly inseminate females without prior courtship (Houde, 1997). After the trial, each male was returned to its individual tank and maintained on the same diet treatment for a further seven days before being assessed

for ejaculate traits and ornamentation. This period of isolation after the mating behaviour trials ensured that males would have fully replenished their sperm supplies prior to measuring sperm number and carrying out sperm analyses (see below) (Kuckuck & Greven, 1997).

Ejaculate traits

One week after the behavioural trials, each male was anesthetized and placed on a glass slide under a dissecting microscope with its gonopodium (intromittent organ) swung forward. A micropipette was used to add 500 μ l of an extender medium (207 mM NaCl, 5.4 mM KCl, 1.3 mM CaCl_2 , 0.49 mM MgCl_2 , 0.41 mM MgSO_4 , 10 mM Tris, pH 7.5) to the base of the gonopodium. The use of the extender medium ensured that sperm bundles remained intact and quiescent until they were used for the sperm velocity and viability assays (Gardiner, 1978). Light pressure was then applied to each male's abdomen to expel all strippable sperm into the extender medium (Matthews *et al.*, 1997). We then extracted subsamples of the stripped ejaculate for the various sperm assays (velocity, viability, sperm counts and sperm lengths; see below).

To measure sperm velocity, we extracted two spermatozeugmata (unencapsulated sperm bundles) from the stripped ejaculate and placed these in a single well of a 12-well multitest slide (MP Biomedicals, Aurora, OH, USA) coated with 1% polyvinyl alcohol (Sigma-Aldrich, Australia) (Wilson-Leedy & Ingermann, 2007). We then activated the sperm sample with 150 mM KCl (with 2 mg/mL bovine serum albumin) (Billard & Cosson, 1990) and used the CEROS sperm tracker (Hamilton-Thorne Research, Beverly, MA, USA) to estimate sperm velocity. Our ensuing computer-assisted sperm analysis (CASA) assays were based on an average of 38.0 ± 1.2 SE sperm tracks per sample (mean value is taken for $N = 366$ males; $N = 42$ males did not produce sperm). As the average path velocity (VAP) was highly correlated with the straight line velocity (VSL) ($r = 0.97$; $P < 0.001$) and curvilinear velocity (VCL) ($r = 0.76$; $P < 0.001$), we restricted our analysis of sperm velocity to VAP for brevity. Within-sample repeatability for VAP in this population has previously been shown to be high ($r=0.74$; see Evans, 2009). The threshold value for defining static cells was predetermined at $24.9\mu\text{m/s}$ for VAP (Evans, 2009).

For the sperm viability assays, we extracted ten spermatozeugmata and used a live/dead[®] Sperm Viability Kit (Invitrogen, Molecular Probes) to estimate the proportion of

live (with intact cell membranes) sperm in the ejaculate from 200 haphazardly chosen sperm cells per sample (Rahman *et al.*, 2013).

Sperm number was estimated for the reserved portion of each male's stripped ejaculate using an improved Neubauer haemocytometer under 40x magnification (Leica DM1000 microscope) after vortexing the sample to break down the sperm bundles. Sperm number for each sample was corrected to allow for sperm bundles that had been removed for the CASA (two bundles) and viability (10 bundles) assays (Evans, 2009). Briefly, because the number of sperm per bundle is fairly constant within and among individual spermatozeugmata (Evans *et al.*, 2003), a correction could be made for each estimate to allow for sperm that were removed prior to the sperm counts (Evans, 2009).

Following the sperm counts, the remaining ejaculate samples were preserved in a 1% formalin solution (to prevent sperm degradation) and stored at 4°C until required. To estimate total sperm length (distance in μm from the top of the sperm head to the tip of the tail) and constituent components (head, midpiece and tail), photographs of each male's sperm were obtained using a digital camera (Leica DFC320) attached to a microscope (Leica DM1000; 40x magnification). Where possible, 10 undamaged spermatozoa were measured per male (mean number of sperm cells analysed per male = 9.77 ± 0.06 SE; range = 2 – 10) using *ImageJ* software.

All ejaculate measures were performed 'blind' of experimental treatment (i.e. the observer was unaware of a sample's treatment status when conducting the various assays described above).

Male ornamentation

Following the ejaculate traits assays, males were dried with blotting paper and photographed under standard lighting (one 13W fluorescent bench lamps) against a measurement scale on a white background using a digital camera (Nikon D70s with Nikon 105mm macro lens). As with ejaculate traits, analyses of male ornamentation were performed blind of experimental treatment. *ImageJ* software was used for the measurements of body size ('standard length' = distance in mm from the snout to the tip of the caudal peduncle; hereafter SL) and colour ornamentation (the number and area of orange and iridescent spots).

Statistical analyses

All analyses were performed using 'R' version 3.1.0 (R Development Core Team, 2014). Our analysis of GEI initially involved setting up a linear mixed-effects (LME) model (with restricted maximum-likelihood methods; REML) to assess the significance of sire-by-treatment interactions for each of the measured traits, which in turn served as an approximation of GEI (Greenfield *et al.*, 2012). The LME models were performed using the lme4 package of R (Bates *et al.*, 2014) and included treatment as a fixed effect, male standard length as a fixed covariate, and sire, dam (nested within sire) and the interaction between treatment and sire as random effects. Only the data for sperm length (head, midpiece, tail, and total sperm length) were normally distributed; data for other traits were treated with following transformations: sperm viability data were subjected to arcsine square root transformation; iridescent area, sperm velocity, and both sperm number and total sperm length were subjected to cubic root, square root and $\log_{10}$ transformations, respectively; the remaining traits other than those for counts (frequency of sigmoid displays and gonopodial thrusts) were transformed using the Box-Cox method. Normality of the resultant data was confirmed from the Q-Q plots of residuals. These data were subsequently analysed using the lmer function in lme4. The behavioural data (sigmoids and gonopodial thrusts) were analysed using the glmer function with the log-normal poisson family option and the inclusion of an additional observation-level random effect to control for overdispersion (Browne *et al.*, 2005). The χ^2 and P -values for the fixed effects were obtained from the univariate Anova function of the full model, while P -values for the random effects (sire, dam and sire-by-treatment interaction) were obtained from likelihood ratio tests (which compared the full model with a reduced model in which the random factor of interest was removed). The significance of the sire-by-treatment interaction was used to test for the presence of GEI.

Genotype-by-environment interactions may arise either because the level of genetic variance differs between environments ('variance GEI'), or because of differences among families in plasticity (i.e. where there is a crossing of family reaction norms, termed 'ecological crossover') (see Conner & Hartl, 2004, Fry *et al.*, 1996, Wade, 2014). The latter (crossover) sources of GEI are of particular interest to evolutionary biologists because of their potential to influence the reliability of sexual signals and contribute towards their high levels of variance (Ingleby *et al.*, 2010). For traits revealing evidence of GEI in the above LME

models, we therefore tested for ecological crossover interactions by correlating the rank order of sire family means for each trait in the two nutritional environments (see Lewis *et al.*, 2012). We used Spearman-Rank correlations for each trait, where resultant correlation coefficients were tested against the null hypothesis that $r_s = 0$ (significant positive correlations between sire family means in each environment would indicate no significant crossover, and vice versa). We complement these analyses with a presentation of reaction norm plots for each of the traits showing evidence of GEI.

We also estimated genetic parameters (including heritabilities) through separate analyses of all traits within each treatment group (this is because GEIs were associated with the expression of several traits, and thus global estimates of these parameters were not informative). In these models, we entered each trait as response variable and included only sire and dam (nested within sire) as random effects. We used untransformed variables to analyse genetic variation (Garcia-Gonzalez *et al.*, 2012) and calculated narrow-sense heritabilities due to sires and heritabilities due to dams (Roff, 2008). We also calculated standard errors for heritabilities using the jackknife procedure (Roff, 2008). The *P*-values for sire and dam effects were obtained by likelihood ratio tests.

Finally, we used REML methods within the lme4 package of R to estimate additive genetic variances and covariances required for the calculation of pairwise genetic correlations within each nutritional environment. These analyses were restricted to traits in which the sire component was significant in at least one of the nutritional environments (using one-tailed probability of $P < 0.1$ as variances are constrained to be positive) and/or for traits in which the sire-by-environment interaction was significant (two-tailed tests; see results). In this way, we test whether patterns of genetic covariation are influenced by diet. To estimate the covariance required for each genetic correlation, we calculated variances and covariance for the pairwise combinations of traits based on separate univariate analyses of trait 1 (z_1), trait 2 (z_2) and $z_1 + z_2$ (see Evans *et al.*, 2013). Covariances for each pairwise relationship were then calculated as:

$$\text{COV}(z_1, z_2) = 0.5(\text{VAR}(z_1 + z_2) - \text{VAR}(z_1) - \text{VAR}(z_2))$$

Standard errors (SE) for each genetic correlation were calculated using the jackknife procedure (Roff, 2008), while the statistical significance of each correlation was computed

by comparing z-scores for these estimates to the corresponding two-tailed significance levels from a standard normal probability table (Åkesson *et al.*, 2008).

Results

Environmental effects and genotype-by-environment interactions

Our initial mixed-effects models revealed significant effects of dietary restriction on the majority of phenotypic traits, including body size, mating behaviour, the number and area of orange and iridescent spots, and the velocity, viability, number and length of sperm (see Table 1). By contrast, dietary restriction had no significant effect on sperm head length (Table 1). For all traits other than sperm length, males assigned to the low quantity group exhibited significantly reduced trait expression than those assigned to the high quantity group (see the electronic supplementary material, Figs S1a-l). We found that males assigned to the low quantity group had significantly longer sperm with longer tails, while those in the high quantity group had sperm with longer midpieces than their counterparts in the low quantity group (also see Table 1). Sire-by-treatment interactions (GEIs) were significant for body size, orange and iridescent area, sperm velocity, sperm tail and total sperm length (see Table 1). In the cases of iridescent area and sperm velocity, GEIs were attributable to the crossing of reaction norms (see Figs 1 and 2), where the rank orders of sire family means were not significantly correlated between nutritional environments (Table 2). By contrast, the rank orders of sire family means for orange area and total sperm length were significantly positively correlated between treatments (Table 2).

Descriptive genetic analyses

Our analyses revealed significant additive genetic variance for several male traits, although as expected in the presence of GEIs, these patterns of genetic variance were not consistent for all traits under both treatment groups (Table 3). Broadly, the patterns of GEI evident from the mixed-effects models (above) were consistent with patterns of genetic variance revealed from the descriptive analyses of each trait within each diet treatment (see Table 3).

Patterns of genetic covariation within environments

Our analyses of genetic covariance within each environment revealed differences in the magnitude, and sometimes sign, of several bivariate relationships in each diet treatment. For example, iridescent area was highly significantly negatively genetically correlated with sperm viability in the high quantity diet treatment but statistically uncorrelated in the low quantity group (Table 4). On the other hand, iridescent area was positively genetically correlated with total sperm length in the high quantity group and genetically uncorrelated in the low quantity group. These contrasting relationships between iridescent area and both sperm viability (negative) and sperm length (positive) in the high quantity group may arise because of a trade-off between total sperm length and sperm viability, although the relationship was marginally non-significant in the high quantity group ($r_G = -0.30 \pm 0.17$, $p = 0.08$; Table 4). While the relationships between iridescent colouration and ejaculate traits were contingent on environmental effects, the relationships for orange pigmentation (area and spot number) and ejaculate traits were broadly consistent between treatments. For example, both orange area and spot number were consistently positively genetically correlated with sperm viability and negatively genetically correlated with total sperm length in both nutritional environments.

Discussion

The results from this study provide further evidence that dietary restriction significantly influences sexual trait expression in male guppies (Devigili *et al.*, 2013, Gasparini *et al.*, 2013, Rahman *et al.*, 2013, Rahman *et al.*, 2014), but also extend these findings to show (a) that the phenotypic responses to changes in resource availability (i.e. phenotypic plasticity) have a genetic basis, and (b) that patterns of genetic covariation between components of pre- and postcopulatory sexual selection are strongly dependent on environmental effects. Our finding that genotype-by-environment interactions (GxE) underlie patterns of sexual trait expression is likely to have important repercussions for sexual selection in this species, which arguably provides some of the most extreme evidence for polymorphisms in male sexual trait expression in vertebrates (Brooks, 2002). In the following sections we briefly discuss the phenotypic responses to dietary restriction uncovered in our study before considering the genetic basis for these phenotypic responses and the broader evolutionary implications associated with the presence of GEs in this system.

Phenotypic responses to dietary restriction

We found that males assigned to the low quantity group exhibited significantly reduced expression of a number of traits, including body size, sigmoid displays, gonopodial thrusts, the number and area of orange and iridescent spots, sperm velocity, sperm viability, sperm number and sperm midpiece compared to those assigned to the high quantity group. Some, but not all, of these traits have been linked to reproductive fitness in guppies. For example, a number of studies have linked the expression of orange and iridescent area to mating success in guppies (reviewed by Houde, 1997). Moreover, differences in male courtship rates (Evans & Magurran, 2001) and the area of orange pigmentation (Evans *et al.*, 2003, Pitcher *et al.*, 2003) are associated with variance in male reproductive success (paternity) in guppies. Similarly, there is evidence supporting an association between paternity success and both sperm velocity (Boschetto *et al.*, 2011) and sperm viability (Fitzpatrick & Evans, 2014) in guppies, although to our knowledge no study has yet demonstrated a relationship between sperm length (e.g. total sperm length or length of constituent components) and male reproductive fitness.

Our results largely corroborate recent evidence that dietary restriction can significantly affect the expression of sexually selected traits in guppies, much of which is discussed at length in previous papers (Devigili *et al.*, 2013, Gasparini *et al.*, 2013, Rahman *et al.*, 2013, Rahman *et al.*, 2014). However, our findings for total sperm length contrasted with patterns reported previously. Specifically, we found that males assigned to the low quantity group produced marginally significantly longer sperm (see Fig. S1j), although the length of the sperm's midpiece was significantly longer in the high quantity diet (Fig. S1k). Our findings for total sperm length therefore contrast with our recent findings from two separate studies in which males fed restricted diets had significantly shorter sperm than their well fed counterparts (Rahman *et al.*, 2013, Rahman *et al.*, 2014). The basis for this difference among studies remains to be resolved.

Patterns of genotype-by-environment interaction

Our descriptive quantitative genetic analyses revealed evidence for significant additive genetic variance underlying the expression of several precopulatory (body size, orange spot number and area; iridescent spot number) and postcopulatory (sperm viability, sperm

midpiece, tail and total length) sexually selected traits (see also Evans, 2010, 2011). In contrast to Evans (2010), however, we found no evidence for significant additive genetic variance (and associated narrow sense heritabilities) in mating behaviour (sigmoid displays and gonopodial thrusts) and sperm velocity (VAP). We can only speculate on why both studies generated different results for these specific traits, although the presence of GEI for VAP and the different environmental conditions imposed between both studies (e.g. feed levels and composition [*ad libitum* and containing live prey in Evans et al.'s study] differed between studies) may be factors influencing our ability to detect genetic variance in the present study.

Two previous studies utilising full-sibling breeding designs have tested for GEI for colouration in male guppies. Miller and Brooks (2005), for example, explored the effects of age and social environment on the expression of male sexually selected traits, including orange and iridescent colouration. Although Miller and Brooks (2005) found no significant GEIs attributable to male social environment, significant age-by-treatment interactions for both traits suggested that genotypes vary in the way that colour is expressed with age. Thus, as with our study, the signalling efficacy of these two components of male sexual ornamentation (orange and iridescence) may be contingent on environmental effects (e.g. either age or diet). However, in contrast to our findings, a study by Hughes et al. (2005) based on the analysis of ($n=8$) full-sibling families from two Trinidadian guppy populations revealed no evidence for GEIs for colour traits based on food restriction. Differences in population origin, diet type, and breeding design may account for the different results between studies.

Given the presence of GEIs for some traits, patterns of genetic variance differed for individual traits expressed across both treatments. Moreover, the reaction norm plots for precopulatory (orange and iridescent area) and postcopulatory traits (sperm velocity and total sperm length) suggest that the rank order of genotypes differed to varying extents according to the nutritional environment, a finding that was supported by the crossover analysis for individual traits expressed in both environments. Specifically, we can conclude from these latter analyses that the GEIs for iridescent colouration and sperm swimming velocity were attributable, at least in part, to a change in the rank order of sire family means in both environments. This finding is likely to have important evolutionary implications

because it suggests that the success of a given genotype will be contingent on the nutritional environment it faces.

One important evolutionary implication of the presence of GEI in male iridescence is that the rank order of male sexual attractiveness, and thus sexual selection on this trait, may depend on variation in the nutritional environment. This, in turn, may help explain the extreme polymorphism for this component of male sexual ornamentation (Bussiere *et al.*, 2008, Ingleby *et al.*, 2010, Pomiankowski & Møller, 1995). Interestingly, the ranked performance of genotypes for orange area was statistically unchanged across the nutritional environments considered in this study, meaning that unlike iridescence, the reliability of this component of male sexual ornamentation is perhaps less compromised by dietary restriction (note, however, that the rank order correlation of sire family means was still well below 1; Table 2). However, it is important to note that in our study we considered just one dietary factor (feed levels), and that a change in the scale of the environmental manipulation (i.e. the boundaries of the x-axis in Figs. 1 and 2) may yet expose an ecological crossover in the reaction norms for orange area (see Ingleby *et al.*, 2010). Nevertheless, according to our results as they stand, the signal reliability of orange area, which may serve as an indicator of paternal 'good genes' (Evans *et al.*, 2004), was largely unaffected by diet restriction, as might be expected for a trait with very high (Y-linked) heritability (Brooks & Endler, 2001, Evans, 2010, Houde, 1992).

We also found that the nutritional environment modified patterns of genetic variance in postcopulatory traits, adding to just a handful of studies that have demonstrated significant GEIs for ejaculate traits in insects (Engqvist, 2008, Lewis *et al.*, 2012, Morrow *et al.*, 2008). Similar to our finding for iridescent area, our ecological crossover analysis revealed that that rank order of family means for sperm velocity – a determinant of sperm competitiveness in guppies (Boschetto *et al.*, 2011) – was uncorrelated across nutritional environments. This finding is likely to have important implications for postcopulatory sexual selection, because genotypes that convey high sperm competitiveness under certain environmental conditions (e.g. high feed levels) may be disadvantaged under less benign conditions (dietary restriction). Corresponding evidence that nutrition moderates GEIs in postcopulatory sexual selection comes from Lewis *et al.*'s (2012) recent study of flour beetles (*Tribolium castaneum*), where the rank order of family means for the male's sperm defence ability (proportion of offspring sired by the first of two males to mate with a

female; P_1) was contingent on diet quality (ratio of wheat in diets). However, as acknowledged by the authors of that study, the relatively low number of sire families ($N = 12$) used in Lewis *et al.*'s (2012) correlation (crossover) analysis may have compromised their ability to reject the null hypothesis of no correlation between genotype rankings (required to support the conclusion of ecological crossover). A similar criticism can be applied to our analysis, although our sample of $N = 31$ sire families enables us to draw firmer conclusions from the lack of statistical significance for correlations of sperm velocity ranks across treatments.

Patterns of genetic covariation across nutritional environments

Our analyses of genetic covariance in both nutritional environments revealed striking differences in the magnitude, and sometimes sign, of relationships. Here, we focus mainly on the relationships between pre- and postcopulatory sexually selected traits, given previous widespread evidence for strong phenotypic and genetic integration of traits across this selective divide and the potentially important evolutionary implications associated with such relationships (see above). Notably, the patterns of genetic covariance involving, on the one hand, the size and number of orange spots, and on the other the size and number of iridescent spots, differed markedly. In both diet treatments the relationships involving orange were consistently positive for sperm viability and sperm number, and negative for total sperm length. These findings broadly accord with prior evidence that males with relatively high levels of orange pigmentation are disproportionately more successful during sperm competition than their drab counterparts (Evans *et al.*, 2003, Pitcher *et al.*, 2003) and further supports recent (unpublished) evidence that multivariate selection drives concordant patterns of pre- and postcopulatory sexual selection in guppies (Devigili, Evans & Pilastro; unpublished data). As we note above, both sperm viability and sperm number have been linked to sperm competition success in guppies (Boschetto *et al.*, 2011, Fitzpatrick & Evans, 2014), but we currently lack evidence that links variation in sperm length to reproductive fitness in this species. Thus, the fitness implications associated with genetic correlations involving sperm length (generally negative for orange and positive for iridescence) remain to be investigated.

In contrast with the consistent (positive) patterns of genetic covariation between the extent of orange pigmentation and ejaculate quality in both treatments, the relationships

between measures of iridescence and ejaculate traits were contingent on diet treatment (see Table 4). As reported previously for this population of guppies (Evans, 2010), our analyses revealed a highly significant negative genetic correlation between iridescent area and sperm viability in the high quantity diet treatment, indicative of a trade-off between these components of pre- and postcopulatory sexual selection. However, this relationship broke down under dietary restriction, suggesting that the genetic architecture of these two traits may not be stable under temporally changing nutritional environments (see also Messina & Fry, 2003). As reported elsewhere, the dependency of genetic correlations on environmental heterogeneity is likely to have important evolutionary implications. For example, combinations of traits are often subject to multivariate sexual selection (Lande & Arnold, 1983), and patterns of selection on correlated characters may change dramatically under differing environmental conditions (Ingleby *et al.*, 2014). For example, in *Drosophila simulans*, patterns of nonlinear selection on cuticular hydrocarbons were contingent on diet composition (Ingleby *et al.*, 2014). Furthermore, the observation that negative genetic correlations can break down when environments change challenges the assumption that trade-offs represent universal constraints to evolution (reviewed by Roff, 2002). The evolutionary pathways predicted from patterns of genetic covariance measured in a single (or benign) environment therefore may not be representative of those under contrasting environmental conditions (see Robinson *et al.*, 2009).

Conclusions

In summary, we show that in guppies (a) sexual trait expression is susceptible to nutritional stress, (b) GEIs underlie such patterns of condition dependence, and (c) patterns of genetic covariance are impacted by environmental effects. As such, our findings contribute towards the growing evidence that GEIs are likely to play an important role in sexual selection, with potentially important evolutionary implications in terms of the efficacy of sexual signals to convey reliable information about male quality, the maintenance of genetic variance in sexual traits, and the stability of genetic correlations under fluctuating environmental conditions (collectively reviewed in Hunt & Hosken, 2014, Sgro & Hoffmann, 2004, Bussiere *et al.*, 2008). Our findings suggest that for guppies, the reliability of precopulatory male sexual signals (notably iridescence) may be compromised by environmental stochasticity.

Similarly, our findings for postcopulatory sexual traits (notably sperm velocity) suggest that genotypes coding for highly competitive sperm in one environment may perform quite differently under different conditions (see also Mills *et al.*, 2007, Kemp & Rutowski, 2007). Interesting directions for future work include testing whether GEIs affect sexual signalling efficacy (e.g. indirect benefits associated with mate choice), and whether environmental effects similarly moderate the rank order of female mating preferences (Engqvist, 2008, Lewis *et al.*, 2012, Morrow *et al.*, 2008) in line with expectation for sexual trait co-evolution.

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Figure Legends

Fig. 1 GEI reaction norms for (a) orange area and (b) iridescent area. Each line represents the mean score for each sire family ($N = 31$ sires). The square dark black points represent the overall mean score within each treatment across all sires

Fig. 2 GEI reaction norms for (a) sperm velocity and (b) total sperm length. Each line represents the mean score for each sire family ($N = 31$ sires). The square dark black points represent the overall mean score within each treatment across all sires

Supporting information

Table S1. Proximate composition of experimental diet

Figure S1. Effects of diet quantity on pre- and postcopulatory traits.

Table 1 Mixed-effects models for male sexual traits in guppies, including number of offspring (N), trait means and associated standard errors (SE) for each trait. Chi-squared values (χ^2) and associated P values are presented for each model (significant values are highlighted in bold font). Significant GEIs are identified when $P < 0.05$ for the Sire*Treat term.

Traits	Response variables	N	Mean $\pm$ SE	Variances	χ^2	P ($>\chi^2$)	Effect size (R^2)
Precopulatory traits	Standard length (mm)	387	13.47 $\pm$ 0.06	Treat	728.24	<0.001	0.84
				Sire	4.55	0.03	
				Dam	5.67	0.02	
				Sire*Treat	14.01	<0.001	
	Sigmoid displays (number/10min)	408	3.51 $\pm$ 0.24	Treat	110.49	<0.001	0.98
				Sire	0	1	
				Dam	0.01	0.92	
				Sire*Treat	0.12	0.73	
				SL	16.83	<0.001	
	Gonopodial thrusts (number/10min)	408	0.78 $\pm$ 0.07	Treat	10.26	0.001	0.91
				Sire	0	1	
				Dam	0	1	
				Sire*Treat	0	1	
				SL	2.82	0.09	
	Orange spots number	379	3.56 $\pm$ 0.08	Treat	40.61	<0.001	0.65
				Sire	10.76	<0.001	
				Dam	1.44	0.23	
				Sire*Treat	0.29	0.59	
				SL	16.45	<0.001	
	Orange area (mm ²)	380	5.45 $\pm$ 0.18	Treat	43.30	<0.001	0.78
				Sire	0.77	0.38	
				Dam	11.02	<0.001	
				Sire*Treat	5.94	0.01	
				SL	47.84	<0.001	
	Iridescent spots number	319	3.43 $\pm$ 0.09	Treat	36.87	<0.001	0.63
				Sire	1.79	0.18	

Postcopulatory traits	Iridescent area (mm ²)	307	2.51±0.09	Dam	0.54	0.46	0.62
				Sire*Treat	2.86	0.09	
				SL	9.52	0.002	
				Treat	28.44	<0.001	
				Sire	0.06	0.80	
				Dam	0	1	
	Sperm velocity (µm/s)	366	73.8±0.82	Sire*Treat	4.85	0.03	0.50
				SL	19.59	<0.001	
				Treat	57.83	<0.001	
				Sire	0	1	
				Dam	0.35	0.55	
				Sire*Treat	4.36	0.04	
	Sperm viability (proportion)	363	0.69±0.01	SL	0.47	0.49	0.40
				Treat	16.12	<0.001	
				Sire	1.65	0.19	
				Dam	0	1	
				Sire*Treat	2.73	0.09	
				SL	0.93	0.34	
	Sperm number (10 ⁶)	360	3.04±0.11	Treat	8.87	0.003	0.48
				Sire	2.06	0.15	
				Dam	3.13	0.08	
				Sire*Treat	1.10	0.29	
				SL	8.34	0.004	
	Sperm head (µm)	350	3.96±0.01	Treat	2.58	0.11	0.37
				Sire	0	1	
				Dam	7.41	0.006	
				Sire*Treat	0.78	0.38	
				SL	1.07	0.30	
	Sperm midpiece (µm)	350	2.67±0.02	Treat	45.53	<0.001	0.63
				Sire	0.77	0.38	
				Dam	2.71	0.09	
				Sire*Treat	1.83	0.18	
				SL	5.15	0.02	

Sperm tail (μm)	350	47.82±0.06	Treat	15.24	<0.001	0.68
			Sire	15.23	<0.001	
			Dam	13.56	<0.001	
			Sire*Treat	6.49	0.01	
			SL	0.18	0.67	
Total sperm length (μm)	350	54.45±0.06	Treat	4.48	0.03	0.65
			Sire	12.56	<0.001	
			Dam	20.98	<0.001	
			Sire*Treat	4.04	0.04	
			SL	0.24	0.62	

Table 2 Analyses of ecological crossover underlying genotype-by-environment interactions (GEI) in male body size, ornamentation, sperm velocity and total sperm length. Analyses are based on Spearman's rank correlation between sire family means for each trait in both nutritional environments. Analyses are restricted to traits in which sire-by-treatment interactions were significant (see Table 1). Significant ecological crossovers are denoted where *P* values do not significantly differ from zero (as denoted by asterisk)

Traits	Spearman <i>p</i>	<i>P</i>
Standard length (mm)	0.5286	0.0022
Orange area (mm ²)	0.3935	0.0285
Iridescent area (mm ²)	0.0028	0.9880*
Sperm velocity (μm/s)	0.0351	0.8514*
Total sperm length (μm)	0.7734	<0.0001

Table 3 Patterns of genetic variation underlying the expression of sexually selected traits in male guppies exposed to different dietary treatments. Trait means ($\pm$ SE) and variance components for sires (Var_{sire}), dams (Var_{dam}) and residual variance (Var_{res}) come from mixed-effects models. Narrow sense heritability estimates (h^2_{sire}) were calculated by dividing the estimates of additive genetic variance (V_A) by total phenotypic variance (V_P). Tests for significance for sire estimates (P_{sire}) are from likelihood-ratio tests, while mean standardised estimates of additive genetic variance (I_A) are included for completeness ($I_A = V_A/X^2$, where $V_A = 4 \times$ sire variance component and X = trait mean).

Traits	Treat	Mean $\pm$ SE	Var_{sire}	Var_{dam}	Var_{res}	V_A	V_P	h^2_{sire} (SE)	P_{sire}	CV_A	I_A
Standard length (mm)	High	14.38 $\pm$ 0.05	0.11	0.05	0.25	0.44	0.42	1.05 (0.27)	0.01	0.05	0.002
	Low	12.53 $\pm$ 0.04	0.09	0.04	0.23	0.36	0.36	1.01(0.21)	0.003	0.05	0.002
Sigmoid displays (freq.)	High	6.69 $\pm$ 0.35	1.73	0.006	23.05	6.95	24.79	0.28 (0.07)	0.27	0.39	0.16
	Low	0.24 $\pm$ 0.08	0	0	1.28	0	1.28	0 (0)	1	0	0
Gonopodial thrusts (freq.)	High	1.21 $\pm$ 0.11	0	0.08	2.34	0	2.42	0 (0.07)	1	0	0
	Low	0.34 $\pm$ 0.06	0.05	0.09	0.59	0.21	0.75	0.29 (0.09)	0.26	1.35	1.82
Orange spots number	High	4.59 $\pm$ 0.09	0.37	0.03	1.35	1.49	1.76	0.85 (0.17)	0.007	0.27	0.07
	Low	2.49 $\pm$ 0.08	0.33	0.14	0.81	1.31	1.28	1.03 (0.11)	0.001	0.46	0.21
Orange area (mm ²)	High	7.9 $\pm$ 0.21	2.49	0.19	5.76	9.99	8.45	1.18 (0.25)	<0.001	0.39	0.16
	Low	2.9 $\pm$ 0.12	0.49	0.62	1.71	1.97	2.82	0.69 (0.12)	0.05	0.49	0.24
Iridescent spots number	High	4.42 $\pm$ 0.1	0.27	0.02	1.39	1.07	1.67	0.64 (0.19)	0.13	0.23	0.05
	Low	2.36 $\pm$ 0.09	0.22	0.07	0.92	0.89	1.21	0.74 (0.08)	0.04	0.40	0.16
Iridescent area (mm ²)	High	3.5 $\pm$ 0.12	0.39	0	2.16	1.54	2.55	0.61 (0.38)	0.17	0.35	0.13
	Low	1.41 $\pm$ 0.06	0.04	0	0.48	0.18	0.53	0.34 (0.11)	0.22	0.30	0.09
Sperm velocity (VAP; $\mu\text{m/s}$)	High	82.15 $\pm$ 0.91	15.19	17.53	136.19	60.76	168.9	0.36 (0.26)	0.29	0.09	0.009
	Low	63.28 $\pm$ 0.94	13.08	0	130.70	52.32	143.8	0.36 (0.19)	0.16	0.11	0.01
Sperm viability (proportion)	High	0.75 $\pm$ 0.01	0.002	0.0005	0.009	0.008	0.011	0.73(0.27)	0.07	0.09	0.009
	Low	0.62 $\pm$ 0.01	0.002	0	0.02	0.009	0.02	0.53 (0.12)	0.02	0.12	0.01
Sperm number (10 ⁶)	High	3.79 $\pm$ 0.17	0.84	0.50	4.44	3.34	5.78	0.58 (0.29)	0.09	0.48	0.23
	Low	2.06 $\pm$ 0.09	0.19	0.01	1.19	0.75	1.39	0.54 (0.23)	0.17	0.42	0.18
Sperm head (μm)	High	3.96 $\pm$ 0.009	0.0002	0.0008	0.013	0.0009	0.014	0.07 (0.17)	0.85	0.008	<0.000

	Low	3.97±0.01	0.0007	0.006	0.02	0.003	0.03	0.10 (0.18)	0.81	0.01	0.0002
Sperm midpiece (μm)	High	2.88±0.02	0.02	<0.000	0.07	0.09	0.09	0.96 (0.28)	0.004	0.11	0.01
	Low	2.38±0.02	<0.000	0.009	0.07	0.0004	0.08	0.005 (0.25)	1	0.008	<0.000
Sperm tail (μm)	High	47.47±0.08	0.45	0.17	0.57	1.79	1.18	1.51 (0.18)	<0.001	0.03	0.0008
	Low	48.28±0.08	0.33	0.17	0.53	1.33	1.04	1.28 (0.28)	0.008	0.02	0.0006
Total sperm length (μm)	High	54.31±0.08	0.41	0.19	0.53	1.62	1.13	1.44 (0.20)	<0.001	0.02	0.0006
	Low	54.63±0.09	0.29	0.23	0.59	1.15	1.11	1.03 (0.19)	0.02	0.02	0.0004

Table 4 Genetic correlations within each nutritional environment (High quantity group = shaded rows; Low quantity = unshaded rows). Jackknife standard errors (SE) are given after each correlation coefficient (in parentheses). The significance of genetic correlations, calculated by comparing z-scores for these estimates to the corresponding two-tailed significance levels from a standard normal probability table, are denoted by asterisks (*<0.05; **<0.01; ***<0.001; significant coefficients highlighted bold). Note that for sperm velocity (VAP), some genetic correlations and/or associated SEs could not be estimated, possibly owing the structure of data which were analysed as untransformed values (*DNC* = did not calculate).

Trait	Treat	Orange spot number	Orange area	Iridescent spot number	Iridescent area	Sperm velocity	Sperm viability	Sperm number	Total sperm length
Standard length	High	0.20 (0.1)*	0.41 (0.17)*	-0.07 (0.24)	0.19 (0.30)	0.33 (0.52)	0.27 (0.21)	0.41 (0.25)	0.21 (0.15)
	Low	0.28 (0.25)	0.88 (0.07)***	0.33 (0.27)	0.20 (0.27)	-0.62 (0.16)***	0.27 (0.24)	0.46 (0.24)*	-0.97 (0.12)***
Orange spot number	High	-	0.84 (0.06)***	0.55 (0.19)**	-0.33 (0.27)	0.20 (0.52)	0.88 (0.25)***	0.25 (0.49)	-0.56 (0.15)***
	Low	-	0.62 (0.14)***	0.82 (0.12)***	0.45 (0.26)	-0.25 (0.17)	0.60 (0.14)***	0.35 (0.15)*	-0.44 (0.12)***
Orange area	High	-	-	0.24 (0.29)	-0.31 (0.32)	-0.16 (0.47)	0.85 (0.18)***	0.65 (0.46)	-0.58 (0.14)***
	Low	-	-	0.66 (0.27)*	-0.11 (0.22)	-0.29 (0.20)	0.89 (0.17)***	0.94 (0.24)***	-0.70 (0.14)***
Iridescent spot number	High	-	-	-	0.43 (0.30)	-0.37 (<i>DNC</i>)	-0.50 (0.46)	0.40 (0.45)	0.48 (0.30)
	Low	-	-	-	0.89 (0.16)***	-0.58 (0.19)**	0.70 (0.18)***	0.31 (0.24)	-0.08 (0.11)
Iridescent area	High	-	-	-	-	<i>DNC</i>	-0.81 (0.28)***	1.09 (1.24)	1.05 (0.24)***
	Low	-	-	-	-	0.34 (0.23)	0.55 (0.39)	-0.12 (0.18)	-0.04 (0.31)
Sperm velocity	High	-	-	-	-	-	0.08 (0.85)	-0.50 (0.24)*	0.73 (0.34)*
	Low	-	-	-	-	-	-0.40 (0.23)	-0.23 (0.27)	1.10 (0.16)***
Sperm viability	High	-	-	-	-	-	-	0.90 (0.32)**	-0.30 (0.17)
	Low	-	-	-	-	-	-	0.67 (0.38)	-0.25 (0.33)
Sperm number	High	-	-	-	-	-	-	-	0.90 (0.25)***
	Low	-	-	-	-	-	-	-	-0.05 (0.40)