Evolutionary genetics of personality in the Trinidadian guppy II: Sexual dimorphism and genotype-by-sex interactions

Stephen John White*, Thomas Miles Houslay & Alastair James Wilson

Centre for Ecology and Conservation, University of Exeter (Penryn Campus), Cornwall, TR10 9FE, UK.

*corresponding author

s.j.white@exeter.ac.uk

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Abstract

Sexual dimorphism in behaviour and personality have been identified in a number of species, but few studies have assessed the extent of shared genetic architecture across the sexes. Under sexually antagonistic selection, mechanisms are expected to evolve that reduce evolutionary conflict, resulting in genotype-by-sex (GxS) interactions. Here, we assess the extent of sexual dimorphism in four risk-taking behaviour traits in the Trinidadian guppy, *Poecilia reticulata*, and apply a multivariate approach to test for GxS interactions. We also quantify the among-individual and genetic covariances between personality and size and growth which are known *a priori* to differ between the sexes. We found significant sexual dimorphism in three of the four behaviours, although r_{mf} between sex-specific homologous traits was significantly less than +1 for only one behaviour. Using multivariate models, we then estimated sex-specific genetic (co)variance matrices ($\mathbf{G_m}$ and $\mathbf{G_f}$) and tested for asymmetry of the cross-trait cross-sex genetic covariance structure (submatrix **B**). While $\mathbf{G_m}$ and $\mathbf{G_f}$ were not significantly different from each other overall, their respective leading eigen vectors were poorly aligned. Statistical support for asymmetry in **B** was found, but limited to a single trait pair for which the cross-sex covariances differed (i.e. $COV_{A(m,f)} \neq COV_{A(f,m)}$). Thus, while single- and multi-trait perspectives evidence some GxS, the overall picture is one of similarity between the sexes in their genetic (co)variance structures. Our results suggest behavioural traits related to risk-taking may lack the sex-specific genetic architecture for further dimorphism to evolve under what is hypothesised to be antagonistic selection.

Introduction

Traits under selection should evolve in a manner dependent on the genetic variance present, the genetic covariance structure with other traits and the strength of selection (Lande, 1979, Walsh & Blows, 2009). While homologous traits (e.g. body size) expressed in males and females can often under sexually antagonistic (SA) selection (Reeve and Fairbairn, 2001; Olsson *et al.*, 2002; Cox and Calsbeek, 2009; McPherson and Chenoweth, 2012), they are likely to share a common genetic architecture (Poissant *et al.*, 2010). Although this shared architecture can result in conflict and thus evolutionary constraint, the prevalence of sexual dimorphism across taxa and traits suggests that sexual conflict can, at least in part, be resolved (Cox and Calsbeek, 2009). Indeed, persistent SA selection is itself expected to favour mechanisms that reduce intra-locus sexual conflict, allowing the sexes to diverge towards their respective fitness optima (Lande, 1980, Rhen, 2000, Bonduriansky & Chenoweth, 2009). These mechanisms can include sex-linkage, sex-limited trait expression, sex-specific genetic modifiers and genomic imprinting (Rhen, 2000, Day & Bonduriansky, 2004, Fairbairn & Roff, 2006, Bonduriansky & Chenoweth, 2009). However, at the whole genome level, the extent to which SA selection provides scope for further dimorphism requires characterising the magnitude of genotype-by-sex interactions (GxS). In this study we investigate sexual dimorphism and GxS interactions in a suite of risk-taking behaviours in the Trinidadian guppy, *Poecilia reticulata*.

Quantitative genetics provides several tools with which to test for and estimate GxS interactions, the presence of which implies that sex-limited genetic variance may facilitate conflict resolution and allow the divergence of the sexes (Wyman *et al.*, 2013). The cross-sex genetic correlation (r_{mf}) between homologous male and female traits is most commonly used to quantify the extent of sex-specific genetic variance, where

$$r_{mf} = \frac{COV_{Amf}}{\sqrt{V_{Am}\, V_{Af}}} \quad (1)$$

79

80 V_{Am} and V_{Af} are the sex-specific (additive) genetic variances and COV_{Amf} is the cross-
81 sex genetic covariance. Typically, an r_{mf} of +1 is viewed as maximally constraining for
82 sex-specific adaptation under SA selection as any increase in fitness of one sex will
83 result in a reduction in fitness of the other sex (Bonduriansky & Chenoweth, 2009,
84 Wyman *et al.*, 2013). Note r_{mf} =+1 does not imply an absolute constraint on trait
85 evolution, as selection responses also depend on the magnitude of sex-specific additive
86 genetic variances (V_{Am}, V_{Af}) which need not be equal when r_{mf} =+1. Only in the
87 complete absence of GxS does it follow that both r_{mf} = 1 and V_{Am}=V_{Af} (Boulton *et al.*,
88 2016).

89 Assessing GxS interactions on a trait by trait basis in this manner, while
90 computationally and technically straightforward, gives a restricted view of trait
91 evolution. This is because natural selection acts on suites of traits simultaneously, and
92 many of these will be genetically correlated (Lande & Arnold, 1983, Walsh & Blows,
93 2009). Multivariate approaches that account for this among-trait genetic covariance
94 structure in the form of a **G** matrix are therefore required (Lande, 1979, Blows, 2007,
95 Walsh & Blows, 2009). In the context of understanding sexual dimorphism, one method
96 has been to estimate sex-specific **G** matrices (subsequently $\mathbf{G_f}$ and $\mathbf{G_m}$) and compare
97 them, using techniques such as eigen vector analysis. For instance, if $\mathbf{G_f}$ and $\mathbf{G_m}$ differ in
98 orientation and/or magnitude of their leading eigen vectors ($\mathbf{g_{max}}$), then continued
99 phenotypic divergence can be possible, even if homologous traits have high pairwise r_{mf}
100 (Jensen *et al.*, 2003, Campbell *et al.*, 2010, Wyman *et al.*, 2013). Conversely, if the
101 orientation of sex-specific $\mathbf{g_{max}}$ are similar, then this can constrain divergence between
102 the sexes (Leinonen *et al.*, 2011, Wyman *et al.*, 2013).

Building on this multivariate approach, it is possible to further define a block matrix, $\mathbf{G_{mf}}$ that contains $\mathbf{G_m}$ and $\mathbf{G_f}$ as well as the cross-sex, cross-trait covariance submatrix usually denoted **B**. The latter can reveal avenues for constraint or divergence between the sexes not detectable in the sex-specific **G** matrices alone (Gosden *et al.*, 2012, Wyman *et al.*, 2013). The multivariate breeder's equation can thus be modified to take into account SA selection (Lande 1980), such that

$$\begin{pmatrix} \Delta\overline{\boldsymbol{Z}}_{\boldsymbol{m}} \\ \Delta\overline{\boldsymbol{Z}}_{\boldsymbol{f}} \end{pmatrix} = \frac{1}{2} \begin{bmatrix} \boldsymbol{G_m} & \boldsymbol{B} \\ \boldsymbol{B^T} & \boldsymbol{G_f} \end{bmatrix} \begin{pmatrix} \boldsymbol{\beta_m} \\ \boldsymbol{\beta_f} \end{pmatrix} \quad (2)$$

$\Delta\overline{\boldsymbol{Z}}_{\mathbf{m}}$ and $\Delta\overline{\boldsymbol{Z}}_{\mathbf{f}}$ are the sex-specific vectors of predicted response for a set of traits and the $\boldsymbol{\beta}_{\mathbf{m}}$ and $\boldsymbol{\beta}_{\mathbf{f}}$ represent vectors of sex-specific (linear) selection gradients. The ½ coefficient accounts for both parents making equal genetic contributions to offspring of both sexes and $\mathbf{G_{mf}}$ is the block matrix (shown in square brackets in equation 2) containing submatrices $\mathbf{G_m}$, $\mathbf{G_f}$ and **B** as defined above (Lande, 1980). For the simplest case of two homologous traits (x and y) expressed in both sexes, then

$$\boldsymbol{B} = \begin{bmatrix} COV_{Amf(x)} & COV_{A(fx,my)} \\ COV_{A(mx,fy)} & COV_{Amf(y)} \end{bmatrix} \quad (3)$$

Thus, on its diagonal, **B** contains those cross-sex genetic covariances that are used to determine r_{mf} for each trait (here x and y), but also contains the between sex genetic covariances for each pair of non-homologous traits. Note that **B** may be asymmetric (i.e. the components above and below the diagonal in **B** are not equal, or $\mathbf{B} \neq \mathbf{B^T}$). In equation 3, this would be the case when the genetic covariance between male x and female y was not the same as the genetic covariance between female x and male y (i.e.

126 $COV_{Amx,fy} \neq COV_{Afx,my}$). Asymmetry in **B** leads to predictions of unequal multivariate
127 response to selection between the sexes (Steven *et al.*, 2007, Lewis *et al.*, 2011, Gosden
128 *et al.*, 2012, Berger *et al.*, 2014).

129 Despite the availability of this multivariate framework, most empirical
130 quantitative genetic studies of sexual dimorphism to date have focussed on single traits
131 (but see work on insect models by Gosden *et al.*, 2012, Reddiex *et al.*, 2013, Berger *et*
132 *al.*, 2014). Furthermore, GxS studies have been most commonly conducted on fitness
133 (Chippindale *et al.*, 2001; Brommer *et al.*, 2007; Foerster *et al.*, 2007), morphological
134 (Steven *et al.*, 2007, Leinonen *et al.*, 2011, Potti & Canal, 2011, Gosden *et al.*, 2012)
135 and life-history (Lewis *et al.*, 2011) traits. Thus while studies including average sex
136 differences in personality traits are widespread (Aragón, 2011, Gyuris *et al.*, 2011,
137 Koski, 2011, Mainwaring *et al.*, 2011), few also assess the presence of GxS interactions
138 and the potential for further dimorphism to evolve (Long & Rice, 2007, Berger *et al.*,
139 2014). This may be due, in part, to the inherent difficulty in measuring behaviour on the
140 large number of individuals required for quantitative genetic analysis.

141 Here, we aim to fill this gap by assessing the extent of GxS interactions for a
142 suite of four behaviours putatively indicative of underlying personality variation in the
143 guppy, *Poecilia reticulata*. We use a laboratory population of guppies, derived from a
144 high-predation site in the Aripo River (Trinidad) and a simple open field testing (OFT)
145 paradigm commonly used to characterise shy-bold type personality variation in fishes
146 (Burns 2008). Here we refer to the traits collectively as 'risk-taking behaviours' noting
147 that, while they should not be considered as independent, previous scrutiny of the
148 among-individual phenotypic correlation structure does not support the idea that they all
149 equivalent proxies of a simple shy-bold continuum (White *et al.*, 2016). The traits
150 included are known *a priori* to be significantly repeatable (White *et al.*, 2016) and

151 heritable in adults (White & Wilson, Submitted MS), while the genetic correlation
152 structure has not previously been investigated (within- or between sexes).

153 Although we do not estimate selection in the current study, SA selection for
154 risk-taking behaviour is expected in this species, with the degree of conflict likely to be
155 mediated by predation risk. Males can increase reproductive success by being highly
156 mobile, moving between shoals to find females (Griffiths & Magurran, 1998, Kelley *et*
157 *al.*, 1999, Croft *et al.*, 2003a, b). We therefore expect male guppies to benefit from risk-
158 taking behaviours through increased access to females. Godin and Dugatkin (1996) also
159 found evidence that females preferred to mate with bolder males (as measured by
160 approach distance to a predator). In contrast, risk-taking is expected to be selected
161 against in females. When alone and away from a shoal, predation risk is high for
162 females, with their larger size making them an energetically rewarding meal (Magurran,
163 2005). High shoal fidelity and tighter shoaling behaviour in females reduces predation
164 mortality risk and increases feeding efficiency (Griffiths & Magurran, 1998, Magurran
165 & Garcia, 2000, Magurran, 2005, Richards *et al.*, 2010).

166 The aims of this study are twofold. Firstly, we assess the extent of sexual
167 dimorphism for repeatable, risk-taking behaviours. We test the prediction that males
168 will exhibit (on average) more risk-prone or 'bold' behaviours, before testing for
169 dimorphism in the multivariate phenotypic (among-individual) covariance structure
170 itself (i.e. do males and females differ in the extent or structure of (co)variation in risk-
171 taking behaviours?). Secondly, we test for GxS interactions using both single-trait
172 analyses and the fully multivariate approach outlined above. While our principal focus
173 is on risk-taking behaviours, we also expand our analyses to include size and growth
174 traits, noting that these are known *a priori* to exhibit strong dimorphism in guppies, and
175 that shy-bold type behavioural variation has been generally linked to body size across
176 many taxa (Réale *et al.*, 2010, Wilson *et al.*, 2013).

Materials and methods

Husbandry and data collection

The data used here are derived from a larger quantitative genetics study. Most (all behavioural data, some size data) have been described elsewhere (White & Wilson Submitted MS) along with a full description of the breeding design and pedigree structure obtained from it (see supplemental Appendix 1 of White & Wilson, Submitted MS). Thus breeding design, general husbandry, and behavioural data collection are described only briefly here.

The dataset consisted of behavioural data on a total of 831 adult guppies, 616 of which were from 81 known full-sib families nested within paternal half-sibships produced between April 2013 and July 2015. To produce families, parental individuals were haphazardly sampled from a captive wild-type population (originally descended from a 2008 collection at a high-predation site in the upper Aripo river, Trinidad) at the University of Exeter, Penryn campus fish facility. After initial rearing in family groups, adult fish (average age 132 days) were tagged using visible implant elastomer (anaesthetised in buffered MS222) and put into mixed family groups of 16 (8 males, 8 females). The composition of tagged groups varied according to the availability of adult fish of suitable size for tagging, but all contained representatives of at least 4 families. Mixing individuals from different families during development reduces the risk of common environment effects biasing additive genetic (co)variance estimates but is not possible initially as the small size of juveniles precludes safe tagging for identification.

Each adult fish underwent 4 open field trials (OFTs) over the course of two weeks. Each OFT comprised transferring a fish into an empty tank filled to 5cm depth with water. Movement was tracked for 4 minutes 30 seconds (following a 30 second acclimation period) using Viewer software (www.biobserve.com) and a camera

positioned above the tank. We chose four traits for analysis, *Activity* (percent of the time the focal fish moved at a speed greater than the minimum threshold of 4cm s^{-1}), *area covered* (the total percentage of the tank explored/visited by the fish), *time in middle zone* (total time spent in the inner zone away from tank walls) and *freezings* (the total number of times movement falls below 4cm s^{-1} for more than 2 seconds). A fifth trait (track length) described in White & Wilson (Submitted MS) was omitted here for purely pragmatic reasons – it was tightly correlated with *activity* (so carried little additional information) and reducing the number of traits facilitated multivariate model fitting (see below).

The OFT testing paradigm is widely used to assay 'boldness' or risk-taking behaviour in fishes with the *a priori* expectation that risk-prone fish will be consistently more active and exploratory, freeze less often, and be less thigmotaxic (spend less time near the edges). Order of capture within each group was recorded, as was water temperature at the end of each behavioural trial (mean of 23.7°C). Water in the OFT tank was changed between groups. Standard length (henceforth *length*, measured from snout to caudal peduncle in mm) measures were taken at tagging, at each OFT, and one month after the last behavioural trial. For a subset of fish, we opportunistically collected additional size data on known age individuals at monthly intervals for up to 13 months after the last OFT. This was not possible in all cases as tanks housing groups were required for other projects in the facility. A total of 2594 behavioural trials and 4493 body size measurements were collected on 831 adults (502 females, 329 males) in a 3 generation pedigree structure.

General statistical methods

Behavioural traits *activity*, *area covered*, *time in middle zone* and *Freezings* were mean centred and rescaled into standard deviation units (using overall, rather than sex-

specific, means and standard deviations). For *time in middle zone* and *freezings* this was done after a square-root transform to reduce positive skew and increase normality of residuals. Scaling to overall standard deviation units allows better comparison of parameters among traits and facilitates convergence of multivariate mixed models while still preserving within-trait differences across sexes (in mean and/or variance). We denote traits by subscript m or f, when referring to male or female values specifically (e.g. $Activity_m$, $Activity_f$ etc).

Data were analysed using linear mixed effect models fitted by restricted maximum likelihood in ASreml version 4 (www.vsni.co.uk). Conditional F statistics were used to test for significance of fixed effects where pertinent to biological hypotheses (e.g. to test for trait dimorphism). Note, however, that in most cases fixed effects were included principally to control for potential sources of variance not directly relevant to our hypotheses. In all behavioural models, fixed effects included *temperature* (of the tank water taken following each OFT), *age* (in days), *repeat* (a 4 level factor to control for habituation to the OFT arena over the 4 repeat trials), *order caught* (the order in which fish were caught from their home tank prior to the OFT, fitted as a continuous covariate) and *generation* (a 3 level categorical effect to control for any differences in husbandry and rearing among the generations of the pedigree, see White & Wilson, Submitted MS).

Significance of random effect (co)variance components was assessed using likelihood ratio test (LRT) comparisons of nested models, with twice the difference in log-likelihoods assumed to be χ^2 distributed with degrees of freedom equal to the number of parameters being tested. We caution that all P values presented are nominal. No corrections are made for multiple testing since, by design, statistical tests are not independent (e.g. individual traits are expected to be correlated). Random effects of *group* (a 40 level categorical effect to account for environmental and social sources of

255 variation among home tanks) and *fish ID* were fitted to all traits in all models unless
256 otherwise stated. To estimate genetic (co)variance parameters we used animal models
257 (Kruuk, 2004, Wilson *et al.*, 2009) further partitioning the among-fish (co)variance into
258 additive genetic and permanent environment components. We assume an absence of
259 maternal (identity) effects, noting that our previous study (White & Wilson, Submitted
260 MS) showed maternal variance was non-significant for *activity* and bound to zero for all
261 other OFT traits in these adult fish. Although previous analyses do suggest statistically
262 significant effects of maternal weight and natal brood size on adult behavioural traits,
263 their effects sizes are low (particularly relative to impacts on juvenile behaviour) and
264 omission here has minimal impact on the sex-specific (genetic) covariance structures.
265 To model growth rate, we fitted random regressions of standard length over age
266 in mixed model and animal model formulations, resulting in estimates of among-
267 individual and additive genetic variation in both length (at average age) and growth.
268 This reaction norm approach fits a random-by-covariate effect, allowing each level of a
269 random effect to vary across a covariate and is an established technique in both
270 behavioural and life history studies (Nussey *et al.*, 2007, Dingemanse *et al.*, 2010, Roff
271 & Wilson, 2014). In all length/growth models, fixed effects of *generation* and
272 continuous effects of *age*, age^2 and age^3 were fitted, the latter to allow a curvilinear
273 average relationship between length and age.
274
275 *Sexual Dimorphism*
276 Single trait models
277 To ascertain whether our traits were dimorphic on average, we fitted univariate mixed
278 models for each behaviour and for the length/growth random regression (sexes pooled),
279 with an additional fixed effect of *sex*. A significant sex effect coefficient ($P<0.05$) was
280 considered evidence of average trait dimorphism. We refitted the behavioural models

with *length* as an additional covariate to determine whether average differences between the sexes in behaviour could, at least in principle, be explained entirely by size effects (given known sexual size dimorphism).

We then fitted a series of models to test for sexual dimorphism in the variance components of observed traits (as opposed to their means). For each trait (X), we fitted bivariate mixed models with X_m and X_f as responses in which we allowed variance components of interest to differ between males and females, and compared the model log-likelihood to the corresponding fit with homogeneous variance imposed. This was done first with no random effects (i.e. just residual variances), allowing test for heterogeneity of total phenotypic variance between sexes for behavioural traits and length. Note it is not possible to estimate the total phenotypic variance of growth from the random regression framework used here therefore this comparison was not done for growth. Models including *fish ID* and *group* as random effects were then fitted to test for differences in among-fish variance (*Group* was fitted to control for among-group variation). LRTs were used to compare the unconstrained vs constrained (homogeneous variance across sexes) models on 1 degree of freedom (DF) for the behavioural traits and 3 DF for the length random regression.

Multivariate models

We next asked whether the **ID** matrix (among-individual (co)variance matrix) of OFT behaviours differs significantly between the sexes. We fitted a multivariate model with all 8 sex-specific behaviours allowing estimation of $\mathbf{ID_m}$ and $\mathbf{ID_f}$ sub-matrices (noting that cross-sex terms are not statistically identifiable since every individual is either male or female) and compared this to a refitted model in which we imposed the condition that $\mathbf{ID_m} = \mathbf{ID_f}$. For a more qualitative comparison, eigenvector decomposition was applied to the estimates of $\mathbf{ID}_m$ and $\mathbf{ID}_f$ matrices to see if the major

axes of among-individual variation were broadly similar in males and females. More specifically, any differences in trait loadings on the first eigenvector ($\mathbf{id_{max}}$) were noted as well as the angle between $\mathbf{id_{max}}$ (the first eigen vector of **ID**) in males and females.

Among-individual association between personality and size

We sought to determine whether phenotypic associations between behaviour and size and/or growth differed between the sexes. Further expansion of the multivariate behavioural model to include male and female *length* as additional responses proved difficult, so we estimated the among-individual covariances (and corresponding correlations) with each sex specific behaviour using a series of bivariate models. Statistical inference was by LRT comparison to constrained models in which among-individual covariance between behaviour and both size (random intercept for length) and growth (random slope) were fixed to zero.

Quantitative genetic analyses

Single trait models

Previous analysis of the OFT data with univariate animal models has shown all behaviours are significantly heritable in adults (pooled sexes, see White & Wilson Submitted MS). Sex-specific parameters and genetic covariance structures (between traits and sexes) have not previously been estimated. For each trait we fitted bivariate animal models to estimate the genetic variance of the sex-specific sub-traits (V_{Am} and V_{Af}) and genetic correlation between them (r_{mf}). This was then compared to a model in which GxS interactions was assumed absent ($V_{Am} = V_{Af}$, $r_{mf} = +1$). We also compared model fits to two intermediate models, one where sex-specific V_A were constrained to be equal but r_{mf} was free to be $<+1$, and a second with r_{mf} constrained to be +1 but sex-

specific V_A free to vary. Since these intermediate models are not nested, AIC values were calculated for each model and used for additional comparison.

Multivariate models

Cross-sex multivariate animal models were fitted with the 8 sex-specific OFT sub-traits. First we compared the sex-specific **G** matrices without estimating the cross-sex, cross-trait terms (**B**), such that we estimated $\mathbf{G_{mf}}$ as:

$$\boldsymbol{G_{mf}} = \begin{bmatrix} \boldsymbol{G_m} & \mathbf{0} \\ \mathbf{0} & \boldsymbol{G_f} \end{bmatrix} \quad (3)$$

This model was compared to one in which we impose the condition that $\mathbf{G_m} = \mathbf{G_f}$ (using a likelihood ratio test on 10 df). As in our comparison of $\mathbf{ID_m}$ and $\mathbf{ID_f}$, we also subjected the sex specific-submatrices to eigenvector decomposition to facilitate a qualitative comparison of trait loadings and also the angle between $\mathbf{g_{max}}$ of males and females. We then fitted the full multivariate model including all cross-sex cross-trait terms such that

$$\boldsymbol{G_{mf}} = \begin{bmatrix} \boldsymbol{G_m} & \boldsymbol{B} \\ \boldsymbol{B^T} & \boldsymbol{G_f} \end{bmatrix} \quad (4)$$

As noted earlier, asymmetry of the upper and lower diagonals of the sub-matrix **B** can offer additional opportunities for sexual divergence under sex-specific selection as well as constraint. Ideally, we would have compared the log-likelihood of our full multivariate model to a constrained fit in which symmetry of **B** was imposed. We were, however, unable to obtain a stable model convergence with the latter constraint imposed. Therefore, to test for symmetry we calculated an estimate of $\mathbf{B} - \mathbf{B^T}$ as a square matrix, denoted as $\Delta\mathbf{B}$, noting that if **B** is symmetrical, then $\mathbf{B} - \mathbf{B^T} = \Delta\mathbf{B} = 0$. In

355 order to generate approximate 95% confidence intervals on each element of Δ**B** we
356 performed a 5000 draw parametric bootstrap on the $\mathbf{G_{mf}}$ matrix (following the general
357 approach outlined in Boulton *et al.*, 2014), implemented within the R statistical
358 environment (R core team, 2016), estimating Δ**B** for each draw. It is important to note
359 that this matrix bootstrapping procedure assumes multivariate normality.
360
361 Genetic association between personality and size
362 As we were unable to expand the multivariate animal model further to include
363 size/growth as well as the 8 behaviours, we fitted a series of bivariate animal models
364 between each sex-specific behaviour and length (again, modelled as a first order random
365 regression of age for both additive and permanent environment effects). This was to
366 determine whether behaviour-length/growth associations differed between males and
367 females at the genetic level. As with the corresponding phenotypic analysis, the
368 significance of genetic covariance with size/length was determined for each sex-specific
369 behaviour using LRT and genetic covariances were standardised to correlations for
370 easier interpretation.
371
372 **Results**
373 *Sexual dimorphism*
374 Single trait models
375 Visual inspection of raw data shows broadly overlapping distributions of male and
376 female behavioural trait observations (Figure 1). Nonetheless, univariate dimorphism
377 models indicate that, conditional on other effects, all OFT traits except *freezings*
378 differed significantly, on average, between the sexes. Females have significantly higher
379 *activity* than males, but cover less tank *area* and spend less *time in the middle* zone
380 (Table 1). As expected, sexual dimorphism is also present in *length* with females being

larger on average (Figure 1, Table 1) and showing a steeper growth trajectory than males (Figure 2). We note that with the addition of the covariate of *length* to the behavioural models, it is apparent that the dimorphism in *activity* could, at least in principle, be explained by size-dependence and coupled with the larger average size of females (Supplemental Table 1).

Bivariate mixed models indicate significantly more total phenotypic variation (conditional on fixed effects) for *time in middle* in males (χ^2_1=9.68, P=0.002) and for *length* in females (χ^2_1=1409.36, P=<0.001; figure 1 & 2). For the other behaviours we found no evidence against the null hypotheses of homogeneous phenotypic variance (*activity* χ^2_1= 1.04, P= 0.308, *area covered* χ^2_1=0.92, P= 0.337, *freezings* χ^2_1= 0.64, P= 0.424; figure 1). Partitioning sex-specific phenotypic variance into its among- and within-individual components showed there is evidence of more among-individual variance in females than males for *length*/*growth* (χ^2_3=199.2, P=<0.001), but the sex-specific estimates of V_I are very similar for each OFT trait (Supplemental Table 2) and do not differ significantly between males and females (*activity* χ^2_1= 0.254, P=0.614, *area covered* χ^2_1=1.22, P=0.269, *time in middle* χ^2_1=0.088, P=0.767, *freezings* χ^2_1= 0.16, P=0.689).

Multivariate models

Sex-specific behavioural **ID** matrices do not differ significantly from each other (χ^2_{10}= 10.62 P=0.388, supplemental Table 2). The first two eigenvectors account for 64% and 26% of the behavioural variance in males and 60% and 31% in females (Table 2a). There is little difference between the sexes in how observed behaviours load onto these first two eigenvectors. For instance, in both sexes $\mathbf{id_{max}}$ describes an axis of among-individual behavioural variation along which *activity* loads antagonistically to *time in middle* and *freezings*. The angle between sex-specific estimates of $\mathbf{id_{max}}$ is 5.70˚,

indicating very close alignment (on the scale from perfectly aligned at 0˚ to perfectly orthogonal at 90˚).

Among-individual association between personality and size

There is support for among-individual covariance between OFT behaviours and standard length (modelled as a random regression comprising size at average age and growth rate) although patterns are at least qualitatively different between the sexes. *Area covered* is the only male behaviour to significantly covary with length (Table 3, see Supplemental Table 3 for statistical inference), being negatively correlated with size at average age (weakly) and growth (moderately). In females, significant length-behaviour covariances are found for *activity*, *time in middle* and *freezings. Length at* average age and *growth* are both positively correlated with a*ctivity* and negatively so with *freezings* (Table 3). *Time in middle* was weakly correlated negatively with length at average size but more strongly positively correlated with growth.

Quantitative genetic analyses

Single trait models

Bivariate animal models of individual pairs of sex-specific homologous sub-traits provided evidence for GxS interactions for two of the five traits. The full GxS model was a significantly better fit than the constrained (no GxS) model for *Length/growth* (χ^2_7= 61.92 P= <0.001) and *time in middle* (χ^2_2=14.968, P= <0.001) but not the other behaviours (*activity* χ^2_2= 3.912 P= 0.141; *area covered* χ^2_2= 3.180, P= 0.204; *freezings* χ^2_2= 0.700 P= 0.705). However, AIC-based comparison with intermediate models in which the constraints of homogeneous V_A and r_G=+1 were relaxed separately provided a slightly more nuanced picture (Table 4). In fact, the no GxS model was only preferred (lowest AIC) for *freezings* while for *activity*, *area covered* and *time in middle* it was the

intermediate model with homogeneous V_A but r_{mf} <+1 allowed that was preferred (although we note in all behavioural traits ΔAIC to at least one other model was <2 such that there is little to choose between them). The fully unconstrained model (full GxS) is clearly the best fit for *length/growth* however, with large ΔAIC between this and all other constrained models (Table 4). Therefore, based on the combined evidence of likelihood ratio tests and AIC comparisons, we conclude there was strong support for GxS interactions for *length/growth* and *time in middle,* weak support for GxS interaction in *activity* and *area covered*, and no indication of GxS interactions in *freezings*.

Multivariate models

When modelled as sex-specific behaviours we found no evidence of overall significant differences between $\mathbf{G}_f$ and $\mathbf{G}_m$ (χ^2_{10}= 6.78 P=0.746). While reiterating the lack of significant matrix differentiation overall, visual inspection of these two sub-matrices of our $\mathbf{G}_{mf}$ estimate (Table 5) is suggestive of more additive genetic variation in male *time in middle* and a larger negative *activity-time in middle* correlation. Conversely, in females there is a larger positive *activity- area covered* correlation. Eigenvector decomposition of $\mathbf{G}_m$ and $\mathbf{G}_f$ shows that the first ($\mathbf{g}_{max}$) and second eigenvectors explain 54% and 40%, and 68% and 27% of the additive genetic variation in males and females respectively (Table 2b). In males, *area covered*, *time in middle* and *freezings* all load positively while *activity* loads negatively on $\mathbf{g}_{max}$. In females, it is *freezings* that loads antagonistically with respect to *activity*, *area covered* and *time in middle*. In addition, the angle between male and female $\mathbf{g}_{max}$ is close to being orthogonal, at 80.08˚. For comparison we also calculated the angle between leading eigen vectors of the corresponding correlation matrices as 60.74˚, indicating that the lack of alignment here arises largely from differences in among-trait genetic

459 relationships between the sexes (as opposed to differing trait-specific genetic variances
460 since these are all set to one in the correlation matrix).

461 The full estimate of $\mathbf{G_{mf}}$ also yields **B**, the cross-sex, cross-trait genetic
462 covariance matrix. Our estimate of **B** shows that the cross-sex genetic correlations are
463 all positive but low for *time in middle* (r_{mf} =0.110 (0.282)), higher for *activity* (r_{mf}
464 =0.773 (0.147)) and *area covered* (r_{mf} =0.677 (0.199)) and close to +1 for *freezings* (r_{mf}
465 =0.974 (0.124); Table 5). These effect sizes are therefore in agreement with bivariate
466 models that evidenced GxS in *time in middle* and provided some (slightly equivocal)
467 indication of $r_{mf} < +1$ in *activity* and *area covered.* Calculation of Δ**B** provided some
468 evidence for asymmetry in **B** although this is limited. Specifically, approximate 95%
469 confidence intervals span zero for all the cross-sex elements of Δ**B** except *activity-time*
470 *in middle* (95%CI = 0.005 - 0.245). The $activity_m$ - $time\ in\ middle_f$ correlation being
471 0.177 (0.285), whereas the $activity_f$-$time\ in\ middle_m$ being -0.367 (0.202) (see Table 5
472 for the full $\mathbf{G_{mf}}$ matrix and Supplemental Table 4 for the Δ**B** matrix).
473
474 Genetic associations between personality and size
475 Finally, bivariate animal models revealed no support for significant genetic correlations
476 between sex-specific behaviours and *length*/*growth* in either males or females (Table 3,
477 Supplemental Table 3).
478
479 **Discussion**
480 Here we investigated whether personality, characterised as among-individual
481 differences in risk-taking behaviours, is sexually dimorphic in a captive population of
482 guppies. We also scrutinised the relationship between behaviour and length and growth
483 – traits known to be sexually dimorphic in this species – before employing quantitative
484 genetic analyses to assess the extent of GxS. We find statistical support for sexual

485 dimorphism in behaviour and discuss this first before addressing the evidence for GxS
486 provided by both the single-trait and multivariate approaches used. In what follows, we
487 put our results into the context of the wider quantitative genetic literature and also seek
488 to highlight the benefits of taking a multivariate view of sexual dimorphism in
489 behavioural traits.
490
491 *Sexual dimorphism in the guppy*
492 Sexual dimorphism was present in OFT behaviours (except for freezing) as well as in
493 length and growth. The latter result is already well known in guppies, with female fish
494 tending to be larger, and having higher growth rates post maturity, while males
495 preferentially invest in mating opportunities over growth (Bronikowski *et al.*, 2002,
496 Miller & Brooks, 2005). Females also had significantly higher total and among-
497 individual variation in length (and growth) than males, which is not unexpected given
498 that mature fish were used and females are indeterminate growers (while males
499 effectively stop growing after maturation). Larger females are more fecund, produce
500 larger offspring (Reznick, 1983, Bronikowski *et al.*, 2002), and are preferred by males
501 (Dosen & Montgomerie, 2004, Herdman *et al.*, 2004). Males, on the other hand, are
502 selected for (relatively) fast maturation, to avoid loss of reproductive opportunities and
503 are thought to gain little from larger size. Indeed, there is some evidence that smaller
504 males are also more successful at sneak matings than their larger counterparts (Bisazza
505 and Pilastro, 1997). Thus the observed size dimorphism is thought to be adaptive in the
506 sense of reflecting divergent sex-specific optima (with larger size favoured in females).
507 Behavioural dimorphism is present, but effect sizes were more modest. For
508 example, where mean length differed by approximately 1.5 SDU (of the pooled sex
509 distribution) between males and females, for the most dimorphic behaviour (*Freezings*)
510 the difference was only 0.5 SDU. In addition, behavioural dimorphism was only

511 partially in line with our prediction that males would, on average, exhibit more risk-
512 prone or 'bold' type behaviours than females within the novel OFT environment. We
513 found that males tended to explore the tank more and spend more time in middle zone.
514 This tendency fits with previous studies, for instance, Lucon-Xiccato and Dadda (2016)
515 found that male guppies approached novel-objects and investigated more closely and
516 quickly than females. Harris *et al.* (2010) and Irving and Brown (2013) both showed
517 that male guppies emerged from the safety of a shelter more quickly than females, with
518 a similar result found in the closely related poeciliid, *Brachyraphis episcopi* (Brown,
519 Burgess, *et al.*, 2007). However, females were also more active than males and thus our
520 prediction of how traits would differ between sexes was not fully upheld.

521 Our own previous work on female guppies (males were not tested) suggests that
522 this could partially be explained by stress response. Although this interpretation is
523 tentative (and perhaps subjective), high activity sometimes occurs because individuals
524 swim rapidly and up and down one or two sides of the arena following introduction into
525 the OFT. This is probably a general escape response found in many fish, with a fast-
526 start swim profile consisting of rapid movement presumed to aid in predator escape
527 (Walker *et al.*, 2005; Marras *et al.*, 2011). This can drive a multivariate profile in which
528 high activity is coupled with relatively low exploration (area covered) and high
529 thigmotaxis (i.e., less time in middle zone - White *et al.*, 2016). We speculate that such
530 a behavioural approach to risky/novel situations may be more common in females
531 reflecting a stronger preference for finding shelter or a shoal (Griffiths & Magurran,
532 1998, Magurran & Garcia, 2000, Magurran, 2005, Richards *et al.*, 2010).

533 *Cross-sex similarity of multivariate behavioural variation*

534 Average differences in a trait are just one way that the sexes can differ. We also
535 estimated and compared sex-specific **ID** matrices to ask if the among-individual
536 variance-covariance structure of OFT traits differed. A meta-analysis conducted by

(Bell *et al.*, 2009) found that, across taxa, there were significant sex differences in the repeatabilities of a wide variety of behaviours, with males being more repeatable than females. However, this pattern was actually reversed when mate choice was excluded from the analysis. Several recent studies have, however, reached varying conclusions as to which sex, if either, exhibits more within-individual consistency (Jenkins, 2011, Hedrick & Kortet, 2012, Debeffe *et al.*, 2015).

While we found that males had higher among-individual variation in time in middle zone, there was no evidence that among-individual variation was greater in males for the other traits. Overall, trait repeatabilities were similar across sexes for homologous traits. Furthermore, multivariate analysis showed strong similarity of full **ID** matrix structure for OFT traits. Both males and females can therefore be differentiated along a similar continuum of behaviour, as shown by the low angle between male and female $\mathbf{id}_{\mathbf{max}}$, on which *activity* loads antagonistically relative to the other traits. Consequently, and in contrast to results from a similar testing paradigm applied to sheepshead swordtails (Boulton *et al.*, 2014), the structure of behavioural variation here is not really consistent with predictions under a simple shy-bold axis. Rather $\mathbf{id}_{\mathbf{max}}$ of OFT traits in guppies describes a continuum of behavioural variation ranging from 'active escape response' at one extreme to an exploratory phenotype at the other. Average differences between the sexes (as discussed above) would therefore suggest that males inhabit the more exploratory or bold end of this axis, whereas females are closer to the escape response end of this axis.

While male and female **ID** matrices were strikingly similar here, we suggest wider estimation of these structures will be generally useful to understand among-individual (co)variation and multivariate sexual dimorphism. Certainly sexes can differ greatly in selection pressure, and in the contributions of social and abiotic factors to variation among individuals at single behavioural traits (Croft *et al.*, 2006, Piyapong *et*

563 *al.*, 2010). To our knowledge, extension to multivariate phenotypes has rarely been
564 attempted. In a study of wild chacma baboons (*Papio ursinus*), Carter *et al.* (2012)
565 reported no difference between sex specific principal components of (multivariate)
566 responses to personality (boldness, novel object testing). In that case the PCA was
567 applied to observed data (rather than an **ID** matrix) and so does not explicitly separate
568 within- from among-individual covariance structure (Houslay and Wilson, 2017). In
569 contrast Fresneau *et al.* (2014) used bivariate mixed models to show that the among-
570 individual correlation between handling aggression and nest defence was significant
571 (and negative) in female blue tits *Cyanistes caeruleus*, but not in males.
572
573 *Evidence of size/growth-behaviour relationship*
574 Links between risk-taking behaviours and body size (and/or growth) have been reported
575 previously in fish (Brown and Braithwaite, 2004; Brown, Jones, *et al.*, 2007). Here our
576 univariate models indicated that while dimorphisms in (mean) area covered and time in
577 middle zone were largely size independent, higher activity in females could in principle
578 be explained by sexual size dimorphism. Thus, while we have no evidence of a causal
579 effect of body size on activity, it is possible that bigger individuals (which tend to be
580 female) exhibit more active escape responses regardless of sex when placed in the OFT
581 arena.
582 Treating standard length as response variable (rather than a 'nuisance' predictor
583 of behaviour), we found some limited support for sex differences in among-individual
584 correlations between size and behaviour. In males, individuals that cover more area in
585 the OFT are smaller and grow less. In a previous study we also detected a negative
586 correlation between area covered and growth in females from this population (White *et*
587 *al.*, 2016), but here it was not significant (though the estimate was, again, less than
588 zero). The reason for this difference is not clear. The previous study was less powerful

589 (just 32 females versus 502 here) but also used larger and thus, given indeterminate
590 growth, putatively older females. In the present case we did find that larger females tend
591 to be more active, spend less time in middle zone and freeze less. In other words, larger
592 females tended to display a more 'escape response' type behavioural profile in the OFT.
593 It is difficult to speculate further on the causes of this, or other size-behaviour
594 relationships found, beyond stating that we do not find a simple correspondence
595 between high growth rate and risk-taking or bold behaviour as has been widely
596 predicted, for example under the Pace of Life framework (Biro and Stamps, 2008; Réale
597 *et al.*, 2010).
598
599 *Evidence for genotype by sex interactions*
600 Our analysis provided strong evidence of GxS interactions for standard length
601 (modelled as *length* and *growth*) and some support for the presence of sex-specific
602 genetic variance in OFT behaviours. The former result suggests that *length* and *growth*
603 have scope for further sexual divergence if SA selection is acting, and mirrors recent
604 findings for size at maturity in another poeciliid (*Xiphophorus birchmanni*; Boulton *et*
605 *al.*, 2016). Our study does not allow us to determine the mechanism causing low r_{mf},
606 though (Postma *et al.*, 2011) found evidence of autosomal/X-linkage of body size in
607 male guppies. While it has been suggested that the X chromosome is likely to
608 accumulate sex-specific genetic variation (Gibson *et al.*, 2002), other work on closely
609 related fish have suggested that the Y chromosome could also play a role (Lampert *et*
610 *al.*, 2010; Boulton *et al.*, 2016).
611 GxS interactions on OFT behaviours were detected, notably in relation to *time in*
612 *middle*. However, across behaviours they were generally weak and less well supported
613 statistically than GxS on size. In general the literature contains sparse estimates of GxS
614 interactions for behavioural traits. However, in a study on selected lines of great tit

615 (*Parus major*), Van Oers *et al.* (2004) reported no difference in the amount of additive
616 genetic variance between sexes for either exploration or boldness. Conversely, Han &
617 Dingemanse (2017) found sex-specific genetic variances for exploration and aggression
618 in the southern field cricket (*Gryllus bimaculatus*), as well as a low value of r_{mf} for the
619 latter behaviour. While this suggest that importance of GxS interactions may vary
620 across behaviour and species, it is clearly too early to generalise and more empirical
621 studies are needed.

622 If contemporary selection favours further divergence of male and female
623 behaviour, then the cross-sex genetic architecture is likely to be largely constraining in
624 our behavioural traits. Sexual dimorphism coupled with moderate to high r_{mf} values has
625 also been observed in other species (Han & Dingemanse, 2017 Long & Rice, 2007,
626 Leinonen *et al.*, 2011, Potti & Canal, 2011) and it is important to note that the signature
627 of historical GxS need not be permanent. For instance, while SA selection should
628 favour mechanisms that allow divergence of the sexes (i.e. sources of GxS), following
629 release from genetic constraint this same selection may erode sex-specific $V_{A,}$ causing a
630 return of high values of r_{mf} (Meagher, 1992, Fairbairn & Roff, 2006, Delph *et al.*, 2011).
631 Nonetheless, across OFT traits our results are consistent with the generally negative
632 relationship between degree of dimorphism and r_{mf} (Bonduriansky & Rowe, 2005,
633 Poissant *et al.*, 2009). For instance, *Freezings* showed the least dimorphism and the
634 highest cross-sex genetic correlation (sex difference of 0.026 SDU and r_{mf} of 0.974)
635 while *time in middle* was the most dimorphic behaviour with the weakest correlation
636 estimate (sex difference of -0.507 SDU and r_{mf} of 0.110).

637 From a single trait perspective, a moderate to high r_{mf} would lead us to conclude
638 that the scope for further behavioural dimorphism to evolve under SA selection is
639 limited. However, a multivariate approach can reveal either additional avenues for the
640 sexes to diverge or additional constraints on independent evolution (Kruuk *et al.*, 2008;

641 Gosden *et al.*, 2012; Wyman *et al.*, 2013). While several studies have found differences
642 in the structure of sex-specific **G** matrices (Jensen *et al.*, 2003; Rolff *et al.*, 2005; Steven
643 *et al.*, 2007; Lewis *et al.*, 2011), our model comparisons provide no statistical support
644 for significant differentiation of $\mathbf{G_m}$ from $\mathbf{G_f}$. Nonetheless, inspection of $\mathbf{G_m}$ and $\mathbf{G_f}$
645 reveals the largest qualitative differences between elements are associated with *time in*
646 *middle* (both the additive variance, and additive covariances between *activity* and *area*
647 *covered*), the behavioural trait for which GxS was best supported in single trait models.
648 Furthermore, we also estimate a large angle between male and female $\mathbf{g_{max}}$ vectors
649 consistent with the two matrices differing in 'shape'. In fact, while $\mathbf{g_{max}}$ in males is
650 similar to $\mathbf{id_{max}}$ in both sexes (described above), in females $\mathbf{g_{max}}$ trait loadings actually
651 correspond to our *a priori* expectations for a shy-bold continuum (i.e. only freezing
652 loading antagonistically to other behaviours). Reiterating the caveat that $\mathbf{G_m}$ and $\mathbf{G_f}$ are
653 not significantly different from each other (and both estimates have high uncertainty), it
654 is interesting that **ID** is at least a qualitatively better proxy for **G** in males than in
655 females.

656 The final piece of support for multivariate GxS comes from our estimate of **B,**
657 the submatrix of $\mathbf{G_{mf}}$ that describes the cross-sex genetic covariance structure. Though
658 largely symmetrical, we found a difference in genetic association between activity_f -
659 $\textit{time in middle}_m$ (negative) and activity_m - $\textit{time in middle}_f$ (weakly-positive). Predictions
660 of (multivariate) sex-specific selection responses can be drastically altered by
661 asymmetry in **B**, though how this manifests is necessarily dependent on the relative
662 angles of SA selection (Wyman *et al.*, 2013). Here selection is not known so we cannot
663 comment directly on the consequences here. Nor are there sufficient empirical studies
664 estimating **B** where selection is known (or estimable) to generalise from the literature.
665 However, (Lewis *et al.*, 2011) initially found genetic constraints in the form of **G**
666 deflecting the angle of response away from the direction of SA selection, but by

667 including the **B** matrix these predicted responses are reversed for females and greatly
668 reduced in males, resulting in extra constraint on sexual divergence. A similarly large
669 effect was found for the cuticular hydrocarbons of *Drosophila serrata*, where
670 consideration of **B** revealed significant constraints on continued sexual divergence
671 compared to predictions from the sex-specific **G** matrices alone (Gosden *et al.*, 2012).
672
673 *Conclusions*
674 Despite strong interest in sexual dimorphism this is, to our knowledge, the first study to
675 estimate $\mathbf{G_{mf}}$ for a set of behavioural traits. We suggest that wider uptake of multivariate
676 analyses will give us a fuller picture of how behavioural dimorphism evolves (and why
677 it sometimes may not). Here we show that guppies exhibit sexual dimorphism in size
678 and growth, but also in average expression of heritable traits linked to risk-taking
679 behaviour or shy-bold type personality variation. Although the structure of among-
680 individual behavioural (co)variation (as measured by **ID**) is similar in males and
681 females, single trait and multivariate analyses also provide evidence of some GxS
682 interactions. These are detected as cross-sex genetic correlations of <1 in single trait
683 analyses. In the multivariate analyses, the covariance structure of $\mathbf{G_m}$ and $\mathbf{G_f}$ were not
684 significantly different from each other, although $\mathbf{g_{max}}$ was close to orthogonal. While
685 there was one component of **B** that was asymmetrical, it was largely symmetrical on the
686 whole. Lacking knowledge of (sex-specific) multivariate selection we cannot comment
687 directly on how these genetic covariances will shape future evolution trajectories,
688 although we broadly expect GxS to facilitate dimorphism under SA selection.
689
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696
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699
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916 **Table 1:** Estimated effect of sex on trait means. Coefficients (with standard errors in
917 parentheses) indicate the effect of being female relative to a male reference group.
918 Estimates are from pooled-sex univariate animal models with (transformed) traits in
919 standard deviation units (see main text).

Trait	effect size	df	F	P
Activity	0.249 (0.053)	1, 779.6	21.960	<0.001
Area covered	-0.189 (0.050)	1, 782.3	14.38	<0.001
Time in middle	-0.507 (0.052)	1, 802.2	94.55	<0.001
Freezings	0.026 (0.052)	1, 776.6	0.24	0.621
Length	1.527 (0.035)	1, 745.1	1934.86	<0.001

920
921 Females have significantly higher *activity* than males, but cover less tank *area* and
922 spend less *time in the middle* zone (Table 1)

923 **Table 2:** Trait loadings on the first and second eigenvectors of male and female **ID**
924 matrices (a) and **G** matrices (b).
925

	Trait	Male		Female	
		Eigen 1	Eigen 2	Eigen 1	Eigen 2
a)	*Activity*	-0.632	0.160	-0.640	0.253
	Area covered	0.102	0.813	0.193	0.779
	Time in middle	0.575	0.388	0.537	0.408
	Freezings	0.510	-0.403	0.515	-0.404
b)	*Activity*	-0.562	0.401	0.552	-0.384
	Area covered	0.320	0.644	0.584	0.377
	Time in middle	0.720	0.237	0.133	0.819
	Freezings	0.250	-0.607	-0.580	0.201

926

927 **Table 3:** Estimated sex-specific among-individual and genetic correlations between
928 each OFT trait and *length* (intercept) and *growth*. Standard errors are in parentheses and
929 bold font denotes parameters where covariance between behaviour and standard length
930 is statistically significant (see Supplemental table 3 for statistical testing).

	Trait	Male		Female	
Among-individual		Length	Growth	Length	Growth
	Activity	0.150 (0.085)	0.190 (0.130)	**0.370 (0.057)**	**0.220 (0.113)**
	Area covered	**-0.104 (0.098)**	**-0.427 (0.142)**	0.032 (0.069)	-0.348 (0.123)
	Time in middle	-0.082 (0.088)	-0.244 (0.130)	**-0.199 (0.066)**	**0.092 (0.124)**
	Freezings	0.031 (0.096)	-0.011(0.149)	**-0.205 (0.070)**	**-0.239 (0.130)**
Additive genetic	*Activity*	0.110 (0.370)	0.060 (0.304)	0.247 (0.216)	0.247 (0.242)
	Area covered	-0.205 (0.389)	-0.453 (0.307)	-0.219 (0.394)	-0.482 (0.293)
	Time in middle	-0.001 (0.387)	0.098 (0.295)	-0.123 (0.382)	0.167 (0.25)
	Freezings	-0.231 (0.375)	-0.049 (0.326)	-0.230 (0.381)	-0.055 (0.324)

931

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Table 4: Comparisons of models in which for each pair of homologous traits full GxS is allowed (unconstrained model), homogeneity of sex-specific VA is imposed ($V_{Am}=V_{Af}$), r_{mf} of +1 is imposed, or no GxS is allowed ($V_{Am}=V_{Af}$ and rmf=+1). Shading denotes the preferred model based on AIC.

Trait	Model	AIC	ΔAIC
Activity	unconstrained	1843.26	1.85
	$V_{Am}=V_{Af}$	1841.41	0
	$r_{mf} = +1$	1847.16	5.75
	No GxS	1843.18	1.77
Area covered	unconstrained	2033.90	1.91
	$V_{Am}=V_{Af}$	2031.99	0
	$R_{mf} = +1$	2036.57	4.58
	No GxS	2033.07	1.08
Time in middle	unconstrained	1915.18	0.86
	$V_{Am}=V_{Af}$	1914.32	0
	$r_{mf} = +1$	1926.53	12.21
	No GxS	1926.14	11.82
Freezings	unconstrained	2311.05	3.30
	$V_{Am}=V_{Af}$	2309.21	1.46
	$r_{mf} = +1$	2311.53	3.78
	No GxS	2307.75	0
Length	unconstrained	-7659.74	0
	$V_{Am}=V_{Af}$	-7652.49	7.25
	$r_{mf} = +1$	-7649.80	9.94
	No GxS	-7611.83	47.91

Table 5: Estimated $\mathbf{G_{mf}}$ matrix from the full multivariate model of sex-specific OFT traits with coloured blocks corresponding to $\mathbf{G_m}$ (orange), $\mathbf{G_f}$ (green) and **B** (blue). $\mathbf{G_m}$ and $\mathbf{G_f}$ are necessarily symmetric and shown with variances on the diagonal (dark shading), covariance below, and correlations above. **B** is not necessarily symmetric so covariances are scaled to cross-sex genetic correlations in the upper right block, with grey shading denoting the estimates of r_{mf} for homologous traits. Standard errors on all estimates are shown in parentheses.

	Act_m	AC_m	TIM_m	Fr_m	Act_f	AC_f	TIM_f	Fr_f
Act_m	0.275 (0.085)	0.009 (0.203)	-0.681 (0.111)	-0.772 (0.095)	0.773 (0.147)	0.598 (0.199)	0.177 (0.285)	-0.744 (0.152)
AC_m	0.002 (0.054)	0.222 (0.055)	0.639 (0.130)	-0.373 (0.197)	0.161 (0.223)	0.677 (0.199)	0.207 (0.295)	-0.492 (0.202)
TIM_m	-0.205 (0.076)	0.173 (0.043)	0.329 (0.081)	0.338 (0.177)	-0.367 (0.202)	0.130 (0.231)	0.110 (0.282)	0.209 (0.217)
Fr_m	-0.184 (0.071)	-0.080 (0.504)	0.088 (0.063)	0.207 (0.076)	-0.889 (0.145)	-0.679 (0.226)	0.138 (0.297)	0.974 (0.124)
Act_f	0.176 (0.053)	0.033 (0.046)	-0.091 (0.057)	-0.176 (0.051)	0.188 (0.057)	0.598 (0.206)	-0.237 (0.234)	-0.875 (0.064)
AC_f	0.132 (0.051)	0.135 (0.048)	0.031 (0.056)	-0.130 (0.048)	0.109 (0.040)	0.178 (0.057)	0.424 (0.208)	-0.725 (0.181)
TIM_f	0.032 (0.052)	0.034 (0.049)	0.022 (0.058)	0.022 (0.050)	-0.036 (0.043)	0.063 (0.045)	0.123 (0.054)	0.103 (0.262)
Fr_f	-0.173 (0.055)	-0.103 (0.049)	0.053 (0.058)	0.196 (0.054)	-0.168 (0.054)	-0.135 (0.043)	0.016 (0.043)	0.195 (0.062)

Titles and legends to figures

Figure 1: Boxplots of OFT raw data, comparing males (m) and females (f). Central horizontal line indicates the median, diamond indicates the mean.

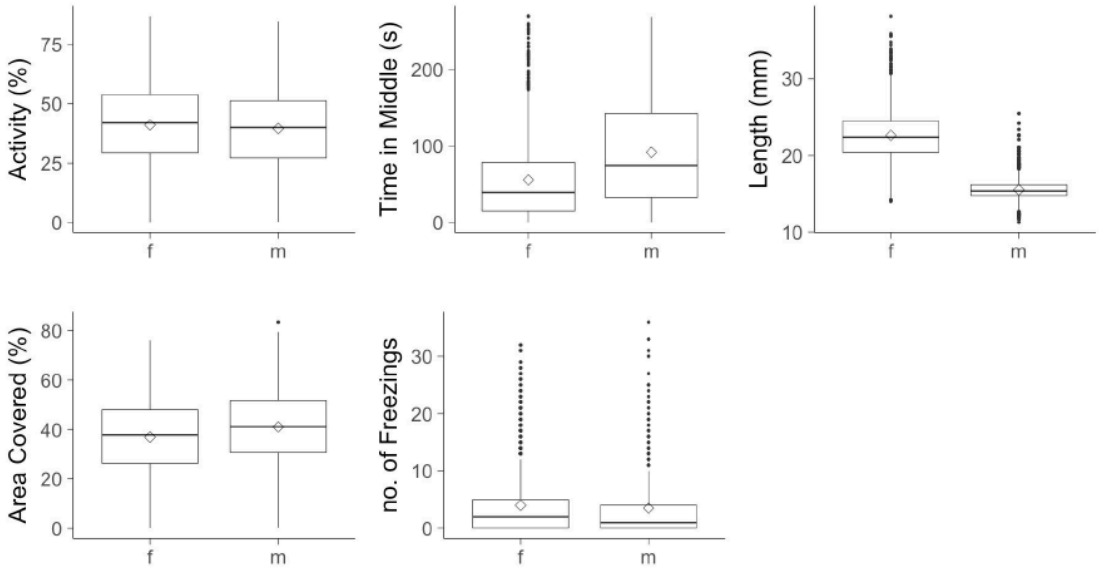
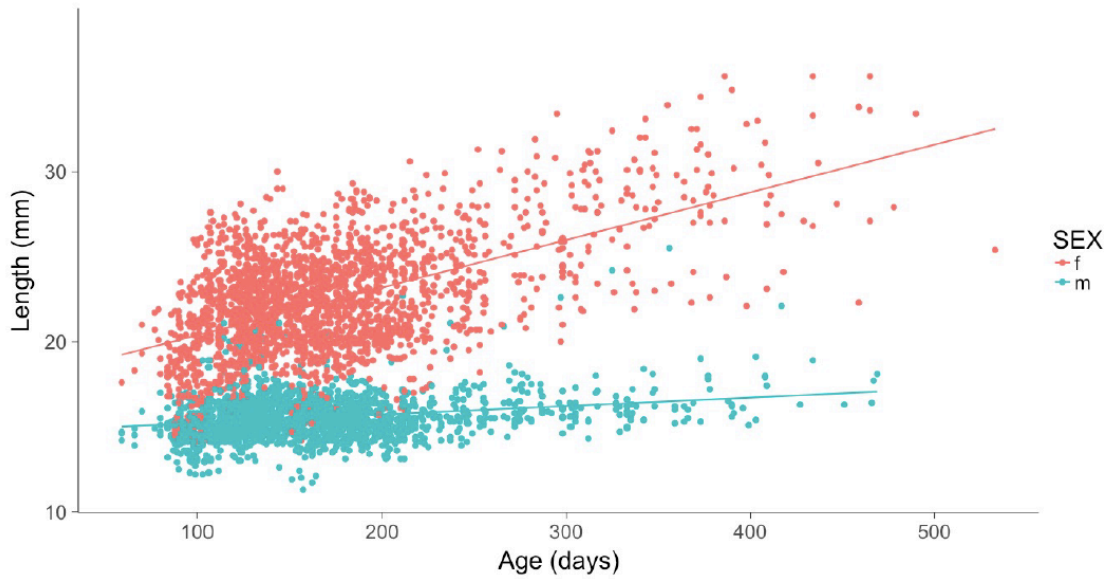


Figure 2: Scatterplot of individual length over age in males and females. Lines of best (linear) fit are shown for illustrative purposes only, noting that data points shown include multiple measures per individual and are non-independent.



Appendix 1 Breeding design and pedigree management

Breeding design

To create a pedigreed sub-population, female fish were haphazardly sampled from stock and isolated from male contact for 3 months. This was to minimise the chance of them carrying viable sperm from previous matings (see below). Following the 3-month isolation, females, along with males haphazardly taken from stock were tagged under anaesthetic (buffered MS222 solution) using visible implant elastomer (VIE) to allow individual identification. They were then assigned to breeding groups of 4 females to one male, housed in 15L breeding tanks (18.5cm x 37cm x 22cm). Females were inspected daily, and heavily gravid individuals (as determined from swollen abdomens and an enlarged 'gravid spot') were isolated in 2.8L brood tanks to give birth. Once a brood was produced, maternal standard length (measured from tip of snout to caudal peduncle, mm), weight and brood size were recorded. The female was then returned to the breeding tank (with offspring raised initially in the brood tank; see below). Any females that did not produce a brood within two weeks of being isolated were returned to their breeding tank. Any offspring born in the breeding tank were excluded from the experiment as we could not be sure of maternal identity.

The first generation of offspring produced (G1) comprised 566 individuals from 72 broods in total. These broods were produced by 54 female and 33 male individuals out of an initial 171(133 female and 38 male) sampled haphazardly from stock to represent out parental (P) generation. The G1 generation was produced in two breeding bouts, the first between April and November 2013 and the second between February and April 2014. A further offspring generation (G2) was then produced between February and July 2015, primarily using crosses between G1 fish (haphazardly sampled but ensuring no known inbreeding). Note that female G1 fish used in this way were isolated for 3 months as above. To increase the number of families we also crossed some G1 males to addition stock (P) females (again

following isolation). Thus for some G2 it is the case that paternal but not maternal grandparents are known (see Appendix 2 figure). For G2 production we also altered the housing regime slightly as each female was kept in its own 2.8L tank, with a single male moved between 3 females in the breeding group on a weekly basis. This meant it was unnecessary to isolate females to collect broods, and removed the problem of unknown maternity for broods being produced in the larger tanks. A total of 25 females and 12 males contributed 281 G2 offspring from 34 broods.

Offspring were kept initially in their brood tanks before, at an average of 56 days, being moved as families to larger "grow on" tanks (15L, 18.5cm x 37cm x 22cm). Standard length was measured on each fish on the day of birth and at ages 7, 14, 28, 42, 56, 70 and 84 days, using Vernier callipers. Note, however, that individuals cannot be identified at juvenile stage, precluding individual level analyses of repeated measures data. At an average age of 132 days (range 59-226) all G1 and G2 fish were taken from their brood groups, individually tagged using visible implant elastomer (VIE) and placed into mixed-family groups of 16 mature adults (8 males and 8 females). Tagged groups were housed in 15L tanks (with dimensions as as described above). Note, that because individuals were not tagged until adulthood we cannot link the identity of those G1 fish that became parents of G2 fish to their juvenile phenotypic records. However, the family of these fish is known, so for each we added their identity code (as a tagged G1 parent) to the set of dummy codes (for untagged individuals) corresponding to that family. This allowed us to maintain the integrity of known pedigree links between G1 and G2 generations in our animal model analyses.

Thus, in total, we collected behavioural data (as described in main text) on 847 juvenile fish (G1 and G2 generations only) contained within a pedigree structure having a maximum depth of 3 generations, and 45 sire and 79 dam individuals. Behavioural data were

collected on 841 adult fish, comprising P generation individuals (including those that did not contribute to the G1), as well as all G1 and G2 individuals that survived to maturity.

Husbandry rationale and mitigation of pedigree error risk

Female guppies can store viable sperm from previous matings for prolonged periods (up to several months). As such we acknowledge that our breeding strategy, in which females used were (almost certainly) non-virgin comes with some risk of introducing pedigree error (i.e. some paternity could come from males other than the assigned mating partner). To minimise this risk, females were isolated from males for a minimum of 3 months before use in crosses. After that time there was no offspring production and no females appearing gravid. As the gestation period for guppies is approximately 1 month, any brood produced by a female less than month after exposure to the designated male mating was discarded as an extra precaution to ensure pedigree accuracy.

Our rationale for taking this strategy here (and elsewhere, e.g., Boulton et al. 2016) was threefold. First, relative to the alternative of raising female virgins, isolating older stock females gave us faster access to; large numbers of females already held as stock; access to older, and thus larger, females expected to produce larger broods sand thus greater sample size; and, allowed us to build the multigenerational pedigree by utilising G1 females in the production of G2. Second, although sperm storage is well documented in guppies, our knowledge of the biology indicates this is unlikely to be a major source of paternity error in our experiment. Specifically, strong sperm precedence effects have been documents, even when matings are separated by an hour (rather than ≥ 3 months as here; Evan & Magurran, 2011), while storage also impairs sperm velocity (Gasparini *et al*. 2014), and, as a consequence, competitiveness (Boschetto, *et al.* 2011). Third, previous simulation studies (REFS) indicate that bias in quantitative genetic parameters caused by low levels of paternity

will generally be low (e.g., Morrissey et al 2007; Morrissey and Wilson 2010). We note in additional that the same pedigree structure is used for both juveniles and adults here, so it is also difficult to envisage how any bias in parameter estimates that does occur could compromise the main comparisons being made.

Thus, while we stress that our quantitative genetic analyses make the standard assumption that the pedigree structure is known without error, we have taken multiple husbandry steps to ensure this assumption is reasonable and note that key comparisons and conclusions are expected to be robust to minor violations.

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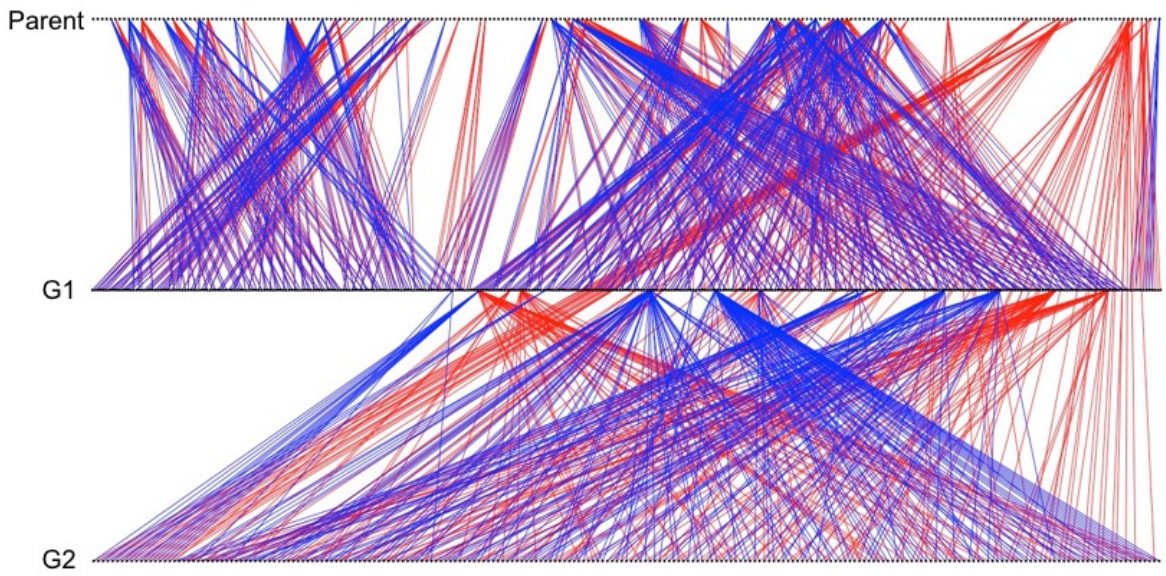
Appendix 2: Visualisation of the three generation (parental, G1 & G2) guppy pedigree structure. Black dots represent individuals, blue lines denote sire-offspring links and red lines denote dam-offspring links. Note that to G2 fish were produced by crosses between unrelated G1 fish where possible, in some cases they were between G1 males and previously unused stock (ie parental) females of unknown parentage.



Supplemental table 1: Effect size of sex (male relative to female) from univariate models with the addition of length as a fixed covariate. Effect sizes are in SDU of transformed traits and standard errors in parentheses.

Trait	Effect	Effect size	DF	F	P
Activity	sex	-0.039 (0.075)	1, 1055.3	0.28	0.596
	length	0.208 (0.039)	1, 1382.2	28.59	<0.001
Area covered	sex	-0.170 (0.073)	1, 1026.5	5.39	0.021
	length	-0.013 (0.039)	1, 1291.6	0.12	0.724
Time in middle	sex	-0.378 (0.075)	1, 1068.8	25.41	<0.001
	length	-0.093 (0.039)	1, 1370.4	5.68	0.018
Freezings	sex	0.209 (0.076)	1, 986.2	7.62	0.006
	length	-0.133 (0.040)	1, 1211.9	11.09	<0.001

Supplemental table 2: Estimated **I** matrix among OFT traits for a) males and b) females. Variances are on the diagonal (shaded), covariances on lower diagonal and correlations on upper diagonal. Standard errors in parentheses. Act= activity, AC= area covered, TIM=time in middle and Fr=freezings

a)	Act_m	AC_m	TIM_m	Fr_m	b)	Act_f	AC_f	TIM_f	Fr_f
Act_m	0.311 (0.043)	-0.058 (0.111)	-0.704 (0.050)	-0.797 (0.043)	Act_f	0.338 (0.034)	-0.061 (0.076)	-0.613 (0.047)	-0.791 (0.031)
AC_m	-0.015 (0.028)	0.207 (0.037)	0.420 (0.092)	-0.176 (0.121)	AC_f	-0.018 (0.023)	0.260 (0.030)	0.619 (0.051)	-0.128 (0.082)
TIM_m	-0.215 (0.037)	0.105 (0.031)	0.300 (0.043)	0.551 (0.080)	TIM_f	-0.190 (0.026)	0.169 (0.024)	0.285 (0.030)	0.464 (0.064)
Fr_m	-0.222 (0.039)	-0.040 (0.029)	0.151 (0.035)	0.251 (0.044)	Fr_f	-0.241 (0.030)	-0.034 (0.023)	0.130 (0.024)	0.275 (0.033)

Supplemental table 3: Likelihood ratio tests for among-individual (a) and additive genetic (b) correlations between each OFT behaviour and standard length (modelled as a first order random regression on age). See methods text for details of modelling methods and Table 3 for correlation estimates. Act= activity, AC= area covered, TIM=time in middle and Fr=freezings

a) Among individual

Behaviour	χ^2_2	P
Act_m	3.800	0.150
AC_m	6.940	0.031
TIM_m	3.340	0.188
Fr_m	3.340	0.188
Act_f	38.010	<0.001
AC_f	4.904	0.086
TIM_f	9.114	0.010
Fr_f	9.466	0.009

b) Additive genetic

Behaviour	χ^2_2	P
Act_m	0.200	0.905
AC_m	2.420	0.298
TIM_m	0.180	0.914
Fr_m	0.200	0.905
Act_f	2.264	0.322
AC_f	1.860	0.395
TIM_f	0.520	0.771
Fr_f	0.320	0.852

Supplemental table 4: Lower triangle of $\Delta\mathbf{B}$ matrix, calculated as $\mathbf{B}\text{-}\mathbf{B}^{T}$ (see main text for details). Lower and upper 95% confidence intervals from bootstrap in parentheses.

	Activity	*Area covered*	*Time in middle*
Area covered	0.099 (-0.036,0.228)		
Time in middle	0.124 (0.005,0.245)	0.003 (-0.116,0.12)	
Freezings	0.003 (-0.085,0.083)	0.028 (-0.098,0.148)	0.031 (-0.101,0.169)