Quantitative genetic insights into the coevolutionary dynamics of male and female genitalia

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The spectacular variability that typically characterizes male genital traits has largely been attributed to the role of sexual selection. Among the evolutionary mechanisms proposed to account for this diversity, two processes in particular have generated considerable interest. On the one hand, females may exploit postcopulatory mechanisms of selection to favour males with preferred genital traits (cryptic female choice; CFC), while on the other hand females may evolve structures or behaviours that mitigate the direct costs imposed by male genitalia (sexual conflict; SC). A critical but rarely explored assumption underlying both processes is that male and female reproductive traits coevolve, either via the classic Fisherian model of preference-trait coevolution (CFC) or through sexually antagonistic selection (SC). Here, we provide evidence for this prediction in the guppy (Poecilia reticulata), a polyandrous livebearing fish in which males transfer sperm internally to females via consensual and forced matings. Our results from a paternal half-sibling breeding design reveal substantial levels of additive genetic variation underlying male genital size and morphology—two traits known to predict mating success during non-consensual matings. Our subsequent finding that physically interacting female genital traits exhibit corresponding levels of genetic (co)variation reveals the potential intersexual coevolutionary dynamics of male and female genitalia, thereby fulfilling a fundamental assumption underlying CFC and SC theory.

1. Introduction

Animal genitalia provide striking examples of rapid and divergent morphological trait evolution [1], and there is now widespread evidence that sexual selection has generated much of this diversity [2]. This evidence comes from comparative studies revealing evolutionary associations between variation in male genitalia and the strength of sexual selection acting on these traits [3,4], and direct evidence linking their expression to male reproductive success [5–8]. Because of the ubiquity of polyandry (i.e. multi-male mating within a single reproductive episode [9]), and the increasing evidence that male genitalia play a role in mediating the ensuing competition between ejaculates [10–14], postcopulatory mechanisms of sexual selection have been implicated as major drivers shaping the evolution of genitalia [2].

Three postcopulatory sexually selected processes have been invoked to account for the evolution of male genital traits. First, these structures can play a direct role in mediating sperm competition—the contest between ejaculates from two or more males for the fertilization of a female’s eggs [15]. For example, the observation that male damselflies use their aedeagus (intromittent organ) to physically remove ejaculates deposited by prior males [16] provides an iconic example of the role of male genitalia in sperm competition (reviewed in [17]). Second, females themselves may generate fertilization biases in favour of males whose genitalia stimulate them to differentially use their sperm. For example, the cryptic female choice (CFC) hypothesis [18] predicts that females exercise postcopulatory choice in favour of males possessing ‘preferred’ genital
traits [19]. Third, because the reproductive interests of males and females often differ, selection can favour genital adaptations that benefit males but are costly to females (i.e. sexual conflict; SC), resulting in a cycle of sexually antagonistic selection on (male) genitalia and (female) modifications that counteract the harm imposed by these structures [20]. For example, in seed beetles (Callosobruchus maculatus), males possess sclerotized genital spines that damage females and reduce the likelihood that they will mate with subsequent males [21], resulting in immediate reproductive payoffs for males [14] but fitness costs for females [11]. For their part, female seed beetles exhibit morphological counter-adaptations that are better developed in species characterized by highly damaging male genital spines [22].

While evidence that male genitalia play a direct role in sperm competition is reasonably widespread, particularly in insects [2,17], explicit support for the CFC and SC hypotheses is more limited and still subject to considerable controversy [23,24]. In particular, two critical assumptions underpin both hypotheses. First, genital traits should exhibit sufficient levels of additive genetic variation to facilitate evolutionary responses to selection [25]. While it is clear that selection has the potential to erode genetic variation [26], continued evolutionary change critically depends on the heritability of selected traits [27]. Studies characterizing the genetic architecture of male genital traits [28,29], significant additive genetic variation underlying their expression [8,30–32] and evolutionary responses to selection [8,33] have fulfilled this basic assumption in a range of insect taxa. However, a second but rarely evaluated premise underlying the CFC and SC models is the expectation of corresponding levels of additive genetic (co)variation in female reproductive traits that either mediate sexual selection for different genital traits (CFC) or mitigate the costs imposed by them (SC). Both the CFC and SC models predict the coevolution of male and female traits [34,35], although patterns of genetic covariance can vary according to the underlying coevolutionary dynamics (conflict versus preference) and the nature of the traits involved [36]. SC theory, for example, predicts that when there is conflict over an interaction between males and females (e.g. mating rate), selection will favour sexually antagonistic adaptations that function to bias the outcome of the interaction towards the evolutionary interests of their bearers (theory and evidence reviewed in [20]).

The ensuing ‘sexual arms race’ is expected to result in genetic covariance in traits involved in this battle of the sexes [36]. While there is evidence from phenotypic studies of insects [37–41] and fish [42,43] that female genital traits can exhibit either intra- or interspecific covariation with male genital size and shape, the evidence to support an explicit quantitative genetic association between male and female genital traits is limited to a single study of dung beetles (Onthophilus taurus) in which the size and shape of the male’s intromittent organ (aedeagus) is genetically correlated with interacting structures in the female’s internal reproductive anatomy [32].

In this paper, we seek evidence for a genetic correlation between male and female genital traits in the guppy (Poecilia reticulata), a livebearing fish that has served as an important vertebrate model in sexual selection [44,45]. Guppies are freshwater fish exhibiting internal fertilization and a highly polyandrous mating system, which is characterized by some of the highest recorded rates of multiple paternity in any vertebrate [46]. For their part, males employ a combination of courtship and forced matings to obtain copulations [45], and recent work on natural populations has revealed that both the size and shape of the male’s intromittent organ (gonopodium) are associated with the success of forced matings [42]. Specifically, males with relatively longer gonopodia are more successful at achieving unsolicited (forced) copulations, while the shape of the gonopodium’s distal tip predicts how many sperm are transferred during these coercive mating attempts [42]. By contrast, neither the size nor the shape of the male’s gonopodium plays a direct role in sperm competition when females mate cooperatively with two successive males [47]. Given the prevalence of forced copulations in natural populations [48], and the known costs incurred by females from male sexual harassment [49,50], we have speculated previously on the potential role that SC plays in fuelling the evolution of male genitalia [42,47]. Indeed, evidence to support SC as a driver of genital evolution in guppies comes from the observation that male and female genital traits exhibit marked patterns of phenotypic covariation across natural populations, and that these patterns reflect differences in the level of sexual harassment endured by females in these different locations [42]. While such patterns are consistent with a history of sexually antagonistic selection, the evidence for underlying genetic covariation between the sexes for these traits that is required to support such a mechanism is so far lacking.

We conducted a standard paternal half-sibling design to explore patterns of genetic variation and covariation in male and female genital traits in guppies. For males, we focused primarily on the length of the gonopodium and the shape of its distal tip, given the importance of both traits in predicting copulation success during forced matings [42]. In females, we focused on the length and width of the gonoduct, as this is the area of the females’ anatomy that comes into direct physical contact with the tip of the gonopodium during copulation [51]. Importantly, for both sexes, we employed precisely the same linear and geometric landmark approaches for trait measurements as those used in a previous study revealing the phenotypic (co)variation between these male and female traits among natural populations [42].

2. Material and methods

(a) Breeding design and rearing conditions

The fish used as the parental generation for the breeding design were approximately third- to fourth-generation descendants of wild-caught guppies captured from a feral population in Queensland, Australia [52]. We established a nested paternal half-sibling design by mating each of 40 sires to five dams (i.e. 200 dams in total). Matings were conducted using artificial insemination (see [53] for details) to minimize possible differential maternal effects that may arise due to the female’s perception of male sexual attractiveness. Such effects can inflate estimates of additive genetic variance [54]. The conditions experienced by males during the rearing phases are described in detail by Evans [55]. Briefly, all males were reared individually in 21 containers from the onset of sexual maturity. Females, on the other hand, were reared in sibling pairs, but only one female from each pair was used for subsequent genetic analyses. Thus, all fish were reared independently of all other family members for which phenotypic traits were measured (and in single sex groups), thus minimizing the possibility that shared common environmental effects would have inflated the estimates of dam variance (which are not

Downloaded from http://rspb.royalsocietypublishing.org/ on June 26, 2017
interpreted in this study; see also [55]). Male offspring were killed and tested at approximately seven months of age, whereas females were killed when they were 10 months old to allow for behavioural assays that were the focus of a different study [56]. Both sexes were preserved in Dietrich's fixative (58% DI H2O, 30% ethanol, 10% formalin, 2% glacial acetic acid) until required for genital trait measures (see below). The final dataset comprised 752 offspring coming from 30 sires and 90 dams (note that traits for male offspring come from n = 29 sire families, as offspring from one set of sire families comprised only females). Male traits (body length, gonopodium length and gonopodium shape) were estimated for all available offspring (n = 499 males), while for logistical reasons, owing to the amount of time required for dissections, female traits (body length, gonoduct length and gonoduct width) were restricted to three randomly selected individuals (where available) from each full-sibling family (final sample size: n = 253 females).

(b) Female genital tract measures

Preserved females were photographed and measured for standard length (SL; distance from snout to the tip of the caudal peduncle). Dissections and subsequent linear measures of gonoduct length and width were performed on these specimens using the methods described by Evans et al. [42]. Briefly, measurements of each female's reproductive tract were obtained from digital photographs collected during dissection. Each female's body was positioned on a polystyrene base with the ventral side facing upwards. Once the gonoduct and the ovary were exposed a digital photograph was taken at 2.5× magnification using a stereomicroscope (Leica MZ75). From these images we measured gonoduct length (distance in micrometres from the gonopore to the connection with the ovary sac) and width (measured halfway along the length of the gonoduct) to within 0.1 μm.

(c) Male genital length and shape

The left side of the male’s intromittent organ (gonopodium) was photographed under a stereomicroscope (Leica MZ75) fitted with a digital camera (Leica DFC320). Two photographs were taken of each sample: one at 2.5× magnification (including the whole gonopodium; figure 1a) and a second at 5.0× magnification (gonopodial tip; figure 1b). The first photograph was used to measure total gonopodium length (±0.01 mm) with IMAGEJ software and the second was used for geometric morphometric analysis of shape [57], including only the portion of the male’s gonopodium that physically penetrates the female’s genital pore [51]. For the latter shape analyses, we used TPS software (http://life.bio.sunysb.edu/morph/) to superimpose eight fixed homologous landmarks (figure 1b) using precisely the same coordinates as those used by Evans et al. [42] in their interpopulation study. A subsequent screening of data using TPSRELW was performed to check for digitization errors. Relative warps (RWs), which describe variation in shape, were generated using the program TPSRELW [58]. From our samples the software generated 12 RWs, with the first four accounting for 82 per cent of the total variation in male genital shape (see the electronic supplementary material, table S1). Our subsequent quantitative genetic analyses focused on these first four RWs. Graphical visualizations of these RWs (see figure 1c,d; electronic supplementary material, figure S1) were obtained using MORPH [59] software (http://www.flywings.org.uk/Morphol_page.htm).

(d) Quantitative genetic analyses

The heritability of individual traits and genetic correlations between them were estimated using restricted maximum-likelihood procedures in the lme4 package of R v. 2.11.1 [59]. We fitted standard nested models for half-sib designs that included sire and dam (nested within sire) as random effects. In univariate tests, significance levels for the sire (additive) genetic variance components were determined using likelihood-ratio tests, where twice the difference in log-likelihoods between hierarchically structured models was tested against a χ2 distribution with 1 d.f. [60]. Narrow-sense heritabilities (h2) were estimated from the ratio of additive genetic to total phenotypic variance, while standard errors around these estimates were calculated by jack-knifing across sire families [61].

Restricted maximum-likelihood methods were also used to estimate additive genetic covariances required for the estimation of within- and between-sex genetic correlations [27]. Genetic correlations (rxy) from these models were calculated only for pairs of traits that exhibited a significant sire effect in the univariate analyses, as genetic covariances are theoretically undefined for traits exhibiting no underlying additive genetic variance [60]. To estimate the covariances required for the calculations of rxy we randomly assigned individual males to a full-sibling female within each family (i.e. method 3 in [62]). For each randomly assembled dataset, we calculated variances and covariances for specific pairs of traits based on separate univariate analyses of trait 1 (z1), trait 2 (z2) and z1 + z2. Covariances for each pairwise relationship were then calculated as Cov(z1,z2) = 0.5×[VAR(z1 + z2) − VAR(z1) − VAR(z2)]. This procedure was repeated 1000 times, every time on a resampled dataset (i.e. with random combinations of male and female offspring in each full sibling family), to generate mean estimates of rxy and corresponding 95% CIs (see the electronic supplementary material for full details of analysis and resampling procedures). The significance of intersex genetic covariances was estimated by comparing z-scores for these estimates to the corresponding two-tailed significance levels from a standard normal probability table [63]. Standard errors for rxy estimates were calculated by jack-knifing across (half-sibling) sire families [66].

As we report below, our analyses revealed a significant positive genetic correlation between male and female genital length, but also a positive genetic correlation between male and female body size, and positive phenotypic and genetic correlations between body size and genital length within the sexes (see §3). Although these correlations were generally weak and non-significant, they may nevertheless contribute towards the intersex genetic covariation in body size if not controlled statistically. Thus, we carried out an additional partial correlation analysis [65] for male and female genital length based on the analysis of n = 29 sire family means while controlling for the effects of body size.

Finally, for completeness we calculated estimates of evolvability using mean-standardized additive genetic variances (I sav), which estimate the expected percentage change in a trait under a unit strength of selection [66]. Estimates of I sav were calculated using the formula I sav = V g / V x (V g = 4 × sire variance component; V x = trait mean), while standard errors around these estimates were estimated by jack-knifing across sire families [67].

3. Results

Our univariate analyses revealed significant levels of additive genetic variance in both sexes (table 1). In females, SL and gonoduct length exhibited significant additive genetic variance (table 1a), whereas in males body length, gonopodium length and the two principal sources of variance in the shape of the gonopodial tip (RW1 and RW2; figure 1) exhibited highly significant levels of additive genetic variation and correspondingly high heritabilities (table 1b). The first relative warp (RW1) described differences in the thickness of the gonopodium’s tip (figure 1c), while the second (RW2) was mostly associated with the variation in the angle of the tip’s ‘hook’ (figure 1d).
Our subsequent bivariate analyses revealed that the length of the female’s gonoduct was significantly and positively genetically correlated with male gonopodium length (z-score and corresponding two-tailed significance level: \( z = 2.24, p = 0.025 \); table 2 and figure 2). Importantly, this relationship between male and female genital length remained significant when controlling for within- and between-sex (co)variation in body size. Specifically, our analyses revealed a positive intersex genetic correlation for body size (table 2) and positive phenotypic and genetic correlations between body size and genital length in both sexes (table 3), which may contribute towards the intersex genetic covariation in genital size if uncorrected in bivariate tests. Thus, in an additional test we estimated the partial genetic correlation coefficients for male and female genitalia when holding male and female body size constant. This analysis, based on sire family means for each of the traits, supported the results presented in table 2 by revealing significant positive genetic covariance between the sexes that was independent of body size (partial correlation coefficient \( r_p = 0.59, \text{ d.f.} = 25, p = 0.001 \); table 4).

Figure 1. The male’s intromittent organ (gonopodium) in the guppy Poecilia reticulata. (a,b) Digital image of (a) the full gonopodium and (b) the location of landmarks used to characterize the shape of the gonopodium’s distal tip. (c,d) Visualizations of the principal components of shape variation in male genital morphology, described by (c) RW1 and (d) RW2. In both panels, the variation in the shape of gonopodial tip is depicted by a solid line (from the most extreme negative to positive values), while the average (consensus) morphologies for each RW are depicted by dashed lines.
Table 1. Number of offspring (n), trait means with associated standard errors (s.e.), and variance components for sires (sire variance component; $V_s$), dams (dam variance component; $V_d$) and total phenotypic variation ($V_T$). Narrow-sense heritabilities are presented separately for sex (h²) and dam (h²d) traits. Mean-standardized estimates of additive genetic variance (A) were calculated using the formula $A = \frac{\text{meantrait}}{2 \text{Trait mean}}$, while standard errors around these estimates (in parentheses) were estimated by jack-knifing across sire families. Significance levels (p values) for the sire variance components were determined using likelihood-ratio tests (see main text). Significant p values are shown in italics. Note that for some measures photographs were unusable, hence sample sizes vary among traits.

<table>
<thead>
<tr>
<th>Trait</th>
<th>n</th>
<th>mean (s.e.)</th>
<th>$V_s$ (s.e.)</th>
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<tbody>
<tr>
<td>(a) female traits</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>standard length (mm)</td>
<td>253</td>
<td>21.1 (0.09)</td>
<td>0.32</td>
</tr>
<tr>
<td>gonoduct length (mm)</td>
<td>254</td>
<td>18.11 (1.87)</td>
<td>0.30</td>
</tr>
<tr>
<td>gonoduct width (µm)</td>
<td>271</td>
<td>324.8 (52.59)</td>
<td>0.15</td>
</tr>
<tr>
<td>(b) male traits</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>standard length (mm)</td>
<td>499</td>
<td>14.82 (0.08)</td>
<td>0.15</td>
</tr>
<tr>
<td>gonoduct length (mm)</td>
<td>497</td>
<td>3.69 (0.08)</td>
<td>0.0056</td>
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<tr>
<td>gonoduct shape RW1</td>
<td>497</td>
<td>—</td>
<td>—</td>
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<td>gonoduct shape RW2</td>
<td>497</td>
<td>—</td>
<td>—</td>
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<tr>
<td>gonoduct shape RW3</td>
<td>497</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>gonoduct shape RW4</td>
<td>497</td>
<td>—</td>
<td>—</td>
</tr>
</tbody>
</table>

4. Discussion

Our study reveals significant levels of additive genetic variance and covariance underlying a number of male and female genital traits in guppies, and to our knowledge is the first to explore the intersex genetic correlation for these traits in any vertebrate. Our finding that the length of the male's gonopodium is positively genetically correlated with the length of the female's reproductive tract, when controlling for (co)variation in body size, provides insights into the possible coevolutionary dynamics of these physically interacting genital traits. Although intersex genetic correlations may arise as a by-product of male and female traits sharing a common genetic basis (i.e. through pleiotropy [68]), our previous finding that variation in male genital length is associated with the outcome of an antagonistic mating interaction [42], and the fact that the traits considered in the present study are interacting and morphologically distinct, lead us to suspect that these patterns of genetic covariation are signatures of sexual selection on both sexes.

Clearly, the ability to distinguish between CFC and SC models depends on an understanding of (i) the functional basis for variation in male and female genital traits and (ii) the balance between costs and benefits of (forced) matings. Our study, in conjunction with previous work, enables us to move closer towards addressing both questions. From the male’s perspective, previous work on Trinidadian guppies indicates that the length of the gonopodium predicts the likelihood of attaining successful genital contact during forced mating attempts [42]. By contrast, male genital length has no influence on precopulatory female mating preferences in our study population [47]. Together, these findings suggest that SC may fuel selection on male genital length, particularly in the light of the known costs of male sexual harassment endured by females [49,50]. Further evidence to support this conclusion comes from two studies that collectively span 14 natural guppy populations and show that gonopodium length is relatively longer in guppy populations characterized by high levels of male sexual harassment [42,69]. These within-species patterns mirror comparative work documenting macroevolutionary patterns of selection on gonopodium length across poeciliid lineages that differ in the level of male sexual harassment [70]. Taken together, this body of work provides strong evidence that the level of engagement in alternative mating tactics fuels selection on male genital length, thus potentially fuelling counter-adaptations in females to mitigate the costs of sexual harassment.

Our finding that gonoduct length exhibited corresponding patterns of genetic variation and covariation with male genital length leads us to speculate about the possible coevolutionary dynamics underlying female genitalia. As our conclusions for male genitalia centre around the role of SC, we naturally speculate that corresponding covariation in the length of the female’s internal reproductive anatomy is a hallmark of sexually antagonistic selection (i.e. the co-evolving female ‘resistance’ trait underlying SC models [20]). Clearly, distinguishing between alternative evolutionary
Table 2. Intersex genetic correlations for male and female reproductive traits and their associated jack-knife standard errors (in parentheses). Note that estimates of intersex genetic correlations and s.e. are mean values from 1000 randomly resampled datasets in which male and female offspring from each full-sib family were reorganized into random pairings (see the electronic supplementary material). The 95% confidence intervals (CIs) for these estimates are based on these random assignments of offspring and therefore quantify stochasticity in $r_A$ estimates attributable to the specific combination of males and females within each family. Note that significance testing for estimates of $r_A$ comes from tests of the covariance across paternal half-siblings (value in italics indicates statistically significant genetic covariance—see main text for details).

<table>
<thead>
<tr>
<th>male traits</th>
<th>standard length</th>
<th>gonoduct length</th>
</tr>
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<tbody>
<tr>
<td>standard length</td>
<td>mean $r_A$ (s.e.)</td>
<td>0.26 (0.26)</td>
</tr>
<tr>
<td>gonopodium length</td>
<td>0.05 (0.34)</td>
<td>$-0.01$ to $0.12$</td>
</tr>
<tr>
<td>gonopodium shape (RW1)</td>
<td>$-0.01$ (0.38)</td>
<td>$-0.12$ to $0.09$</td>
</tr>
<tr>
<td>gonopodium shape (RW2)</td>
<td>$0.23$ (0.36)</td>
<td>$0.15$ to $0.32$</td>
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</table>

Figure 2. The genetic correlation between male and female genital length. The relationship is illustrated by a plot of sire family means (± s.e.) for gonopodium length against sire family means (± s.e.) for gonoduct length. The relationship remains significant after controlling for covariance between body size and genital length in both sexes (see main text). Note that the genetic correlation remains significant when the sire families with large variance in male and female traits (bottom left point) are removed from the analysis.

analyses did not reveal significant variation in the relative length of the gonopodium’s tip. Instead, the principal source of variance in male gonopodium shape was explained by variance in the depth of the gonopodium’s tip (figure 1c), which in turn exhibited no significant genetic covariation with either measure of female genital size. Our analyses therefore suggest that in the population studied here, sexual selection may act on different genital traits than those revealed by the previous study. However, Evans et al. [42] also reported significant interpopulation variation in the angle of gonopodium’s distal ‘hook’, which in turn predicted the success of forced matings during mating trials on a single population. In the present study, our RW analyses also revealed variation in the angle of the genital hook (i.e. RW2), which closely matches the patterns reported previously [42]. Importantly, our subsequent quantitative genetic analysis revealed highly significant levels of additive genetic variation in RW2, and a highly significant positive genetic correlation between this component of genital shape and gonopodium length. We have not yet conducted behavioural trials to verify whether either component of gonopodial shape and length is associated with the success of forced matings in the focal population, but in the light of previous work revealing such associations in guppies [42,71] we suspect that sexual selection plays an important role in fuelling the evolution of these traits in the focal population.

Heritability values for both female and male genital length were high ($h^2 = 0.61$ and $h^2 = 0.90$, respectively), which, although unexpected for fitness traits under directional or stabilizing selection [72], is not without precedent in studies of genital traits [73] and other fitness traits [60]. These values may reflect high additive genetic variance ($V_A$) and/or correspondingly low residual variance in these traits [74], or violations of the assumption of autosomal inheritance, which would have inflated our estimates of $V_A$ [27,60]. Alternatively, high levels of $V_A$ may be maintained through sexually antagonistic selection, where the optimal values of coevolving traits in both sexes are continually shifting [75].

Finally, it is possible that our failure to establish patterns of genetic covariation involving male genital shape and female gonoduct measures was due to our reliance on linear measures for females rather than morphological variation. Clearly, there is a need to develop preservation and geometric morphometric scenarios (i.e. CFC and SC) to explain patterns of covariation in male and female reproductive traits is challenging (see also [32]). In particular, we require knowledge of the function of coevolving traits to support either scenario, which for female genital traits is currently lacking. In the meantime, our study reveals a putative candidate trait on which to focus in future studies of sexual selection on female genitalia.

The patterns of genetic covariance for male and female genital traits uncovered in this study differ from the phenotypic patterns of covariance uncovered in a previous study of natural populations in Trinidad [42]. Specifically, Evans et al. [42] found that the relative length of the male’s gonopodial tip was negatively phenotypically correlated with the width of the female’s oviduct when tested across 10 natural populations. In the present study, we found no corresponding evidence for a genetic correlation between these traits, and indeed our RW
Table 3. Within-sex genetic correlations (above diagonal) and phenotypic correlations (below diagonal) for (a) female and (b) male traits. The value in italics indicates statistical significant covariance between traits.

<table>
<thead>
<tr>
<th></th>
<th>standard length</th>
<th>gonoduct length</th>
<th>gonopodium shape (RW1)</th>
<th>gonopodium shape (RW2)</th>
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<tr>
<td>(a) females</td>
<td></td>
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<tr>
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<td>—</td>
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<td>gonoduct length</td>
<td>0.17</td>
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<td></td>
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<tr>
<td>(b) males</td>
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<td></td>
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<td>0.40 (0.29)</td>
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<td>gonopodium shape (RW2)</td>
<td>0.05</td>
<td>0.19</td>
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Table 4. Partial correlation coefficients revealing the intersex genetic correlation for genital length in guppies when controlling for (co)variation in body size. Note that partial coefficients are based on the correlation of sire family means for each trait. Standard length is abbreviated to MSL and FSL for males and females, respectively. Significant correlations for d.f. = 25 (n − 4) are highlighted with asterisks, where *<0.05 and **<0.01.

<table>
<thead>
<tr>
<th>trait</th>
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<th>FSL</th>
<th>gonoduct length</th>
<th>gonopodium length</th>
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<td>—</td>
<td>0.44*</td>
<td>0.64**</td>
<td>−0.34</td>
</tr>
<tr>
<td>FSL</td>
<td>—</td>
<td>—</td>
<td>−0.31</td>
<td>0.14</td>
</tr>
<tr>
<td>gonopodium length</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>0.59***</td>
</tr>
<tr>
<td>gonoduct length</td>
<td>—</td>
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</table>

methods that will enable us to characterize the shape of the female’s reproductive tract in guppies and other internal fertilizers. With the recent exploitation of three-dimensional geometric morphometric approaches in studies of evolution and ecology [76], we anticipate that future studies will have increasing power to uncover intersex phenotypic and genetic correlations for morphometric traits in these systems.

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