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SEXUAL SELECTION AND THE EVOLUTION OF GENITAL SHAPE AND COMPLEXITY IN WATER STRIDERS

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Animal genitalia show two striking but incompletely understood evolutionary trends: a great evolutionary divergence in the shape of genitalic structures, and characteristic structural complexity. Both features are thought to result from sexual selection, but explicit comparative tests are hampered by the fact that it is difficult to quantify both morphological complexity and divergence in shape. We undertake a comparative study of multiple nongenitalic and male genital traits in a clade of 15 water strider species to quantify complexity and shape divergence. We show that genital structures are more complex and their shape more divergent among species than nongenital traits. Further, intromittent genital traits are more complex and have evolved more divergently than nonintromittent genital traits. More importantly, shape and complexity of nonintromittent genital traits show correlated evolution with indices of premating sexual selection and intromittent genital traits with postmating sexual selection, suggesting that the evolution of different components of genital morphology are shaped independently by distinct forms of sexual selection. Our quantitative results provide direct comparative support for the hypothesis that sexual selection is associated with morphological complexity in genitalic traits and highlight the importance of quantifying morphological shape and complexity, rather than size in studies of genital evolution.

KEY WORDS: Allometry, coevolution, *gerris*, morphometics, phylogenetic comparative analysis.

For centuries it has been recognized that male genitalia are among the most diverse, complex, and rapidly evolving morphological structures (Eberhard 1985). Although the basis of this observation is largely qualitative (Huber 2003), it is borne out by the fact that genitalia are key taxonomic traits and that closely related species are often identified by details of their genitalia in internally fertilizing taxa. The only study, to our knowledge, that quantitatively compared shape divergence in genitalic and nongenitalic traits found greater morphological shape divergence in genitalia (Arnqvist 1998). Much of the diversity of genitalia is characterized by differences in the shape of genitalic structures (Eberhard 1985), whereas the subset of genital variation that account for more fundamental differences in genital complexity have been suggested to be more conserved (Scudder 1971; Huber et al. 2005; Eberhard et al. 2009; Song and Bucheli 2010). There is increasing support for the hypothesis that sexual selection is driving the divergence of genital morphology (Eberhard 1985; Arnqvist 1998; Hosken and Stockley 2004; Simmons et al. 2009).

Many studies have inferred sexual selection from the static allometries of genital size (e.g., Eberhard et al. 1998; Eberhard 2009). Within species, genitalia often tend to scale to body size with slopes below unity. This negative allometry has been interpreted as evidence for stabilizing sexual selection favoring male genitalia that “fit” average-sized females in the population (e.g., Eberhard et al. 1998; Eberhard 2009). However, there is contrary evidence to this pattern (e.g., Bertin and Fairbairn 2007;

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Ramm et al. 2010) and inferring mechanisms of sexual selection based on trait allometries alone is problematic (Bonduriansky and Day 2003; Bertin and Fairbairn 2007; Bonduriansky 2007).

Fewer studies have assessed sexual selection or evolutionary divergence in genital shape. Yet, shape measures are clearly better descriptors of the diversity of genital morphology and shape metrics contain more information than do size metrics (Rohlf and Marcus 1993; Slice 2007; Shen et al. 2009). More importantly, it is diversity in shape and structure, rather than size, which has been the focus of taxonomists (Eberhard 1985; Eberhard et al. 2009; Song 2009). This implies that there is much more divergent evolution in shape than size and, in fact, it is often true that there is interspecific divergence in shape but not in size of genitalic traits (e.g., Garnier et al. 2005; Mutanen et al. 2006; McPeek et al. 2008). Both inter- and intraspecific studies of genital shape support a role for sexual selection in driving diversity in shape. For example, Arnqvist (1998) found greater divergence in genital shape in polyandrous than monogamous groups of insects, suggesting a role for sexual selection (see also Arbuthnott et al. 2010). Within species, there is now direct experimental evidence for sexual selection on genital shape in a range of taxa (Arnqvist and Danielsson 1999; Danielsson and Askenmo 1999; House and Simmons 2003; Wenninger and Averill 2006; Nessler et al. 2007; Briceno and Eberhard 2009; Hotzy and Arnqvist 2009; Simmons et al. 2009; Holwell et al. 2010; Polak and Rashed 2010).

Genitalia are generally thought to be particularly complex structures, and this feature may also be related to sexual selection (Eberhard 1985; Eberhard et al. 2009; Hosken and Stockley 2004; Song and Bucheli 2010). Yet, we are unaware of any direct quantitative tests of this widely held view. One reason for the absence of tests is that complexity is considered difficult to assess and quantify. However, a number of continuous metrics of morphological complexity are available (see McLellan and Endler 1998 for review), so the opportunity exists to conduct quantitative tests of associations between sexual selection and complexity. We are aware of only two qualitative studies that have investigated the relationship between metrics of sexual selection and genital complexity. In a novel comparative study of Nephilid spiders, Kuntner et al. (2009) assayed complexity of both male and female genitalia (counts of genital features) and aspects of the mating system. Their analyses demonstrated correlated evolution in complexity of male and female genitalia, and moreover suggested that the pattern was associated with polyandry. Tatarnic and Cassis (2010) demonstrated correlated evolution between counts of genital "features" (their measure of complexity) in males and females of a traumatically inseminating bug genus (*Coridromius* spp). If sexual selection drives both the evolution of increased complexity and divergence of genital shape, then the extent of shape divergence and the degree of complexity of genital traits should be correlated. This hypothesis has not been tested.

The spectacular structural complexity of genitalia also suggests that they may have several functions, and divergence among these structures suggests at least some evolutionary independence of their functions (Eberhard 1985). For example, genitalia often consist of external (nonintromittent) structures and those that are inserted into the female (intromittent), and these may often have different functions with different forces of selection acting upon them. In water striders, for example, the nonintromittent structures play a role in grasping and positioning the male upon the female, and these have premating sexual selection operating upon them (Arnqvist 1989, 1992; Preziosi and Fairbairn 1996, 2000; Sih et al. 2002; Bertin and Fairbairn 2005). In contrast, intromittent structures affect postmating fertilization success and have postcopulatory selection acting upon them (Arnqvist and Danielsson 1999; Danielsson and Askenmo 1999). Thus, these two sets of structures may or may not evolve independently, depending on whether pre- and postcopulatory selection covaries across species.

In addition to differing forms of selection, intromittent and nonintromittent structures may have differing constraints. For example, Eberhard (2006) has suggested that external grasping structures are functionally constrained and therefore should be relatively invariant when compared to intromittent structures. Moreover, intromittent and nonintromittent genitalia may consist of sets of structures, with different degrees of selection acting upon members of each set. For example, the nonintromittent genitalia of water striders consist of several sclerotized structures (e.g., the pygophore, proctiger, and paramere) and these appear to have differing degrees of selection acting upon them (Arnqvist and Danielsson 1999; Danielsson and Askenmo 1999; Bertin and Fairbairn 2005). Finally, each structure may have multiple features (the presence or absence of evaginations on the proctiger), and each of these may have different functions and selective effects. The combination of multiple structures and their features, with differing forms and degrees of selection acting upon them, will fuel the evolutionary diversification of both shape and complexity (Song and Bucheli 2010).

Here, we undertake a comparative analysis of the evolutionary divergence in shape and complexity of multiple genitalic structures in a well-studied clade of 15 water strider species in the genus *Gerris* (Heteroptera: Gerridae) (Arnqvist and Rowe 2002 a,b; Rowe and Arnqvist 2002). We address four main questions about genital diversity in shape and complexity, and its relationship to the forces of sexual selection. First, we determine whether these structures have diverged across the genus, and whether three different sets of traits (intromittent genitalic, nonintromittent genitalic and nongenitalic) differ in their degree of divergence. Second, we examine the complexity of these traits and ask whether complexity differs across different sets of traits and whether complexity is correlated to divergence in shape. Third, we assess patterns of correlated evolution within this clade. Finally, we use three indices of species level pre- and postcopulatory sexual selection to ask whether the shape and complexity of intromittent and nonintromittent genital structures are related to the strength and form of sexual selection.

Methods

MODEL SYSTEM AND TRAITS

We studied the genital and body morphology of 15 congeneric Holarctic and Palearctic water strider species of the genus *Gerris* (see Arnqvist and Rowe 2002a for species and collection data). This clade is uniquely suited for the current analyses: the collective weight of previous experimental and comparative work in these species provides a wealth of quantitative background information that adds depth to comparative analyses and aids in interpreting results. These species share a similar ecology: all are semiaquatic predators and/or scavengers, which inhabit water surfaces of various ponds, pools, and lakes (Andersen 1982, 1993). Moreover, all species share a basic common mating system: females and males mate multiply and the mating system is characterized by frequent male mating attempts and overt female resistance to these mating attempts, although there is substantial quantitative variation in all mating system parameters across species (Rowe and Arnqvist 2002). Previous experimental (Arnqvist 1989), observational (Arnqvist 1992; Sih et al. 2002; Fairbairn et al. 2003; Bertin and Fairbairn 2005), and comparative (Arnqvist and Rowe 2002a, b; Rowe and Arnqvist 2002) studies of water striders have shown that aspects of the male nonintromittent genitalia affect the males ability to grasp females during premating struggles and are, thus, under premating sexual selection. Previous observational studies of two species in this genus (Arnqvist and Danielsson 1999; Danielsson and Askenmo 1999) have shown that the shape of certain components of the male intromittent genitalia affects male fertilization success and is, thus, under postmating sexual selection.

We studied variation in size, two-dimensional (2D) shape and morphological complexity. There have been substantial advances in the methodologies for capturing and analyzing three-dimensional (3D) surfaces of morphological structures (see Shen et al. 2009; van der Niet et al. 2010). Although these methods allow for the capture of more information than those employed here, the small size of the structures analyzed here prohibits the use of currently widely available 3D techniques. As new methods (e.g., nanotomography) become more readily available, it will be interesting to determine the impact of this added information on the patterns we report here.

The retracted genital capsule was dissected out, macerated in 10% KOH (24 h) and placed in glycerine for examination. We studied 10 different components of genital morphology, of which five are intromittent (the dorsal, lateral and ventral sclerites of the vesica, the phallotheca and the basal apparatus [which is at least partly inserted during copulation]) and five are nonintromittent (segment 8, pygophore, proctiger in lateral view, proctiger in dorsal view and parameres) (see Fig. 1; Michel 1961; Fairbairn et al. 2003). The choice of which view (e.g., lateral or dorsal) of these structures to describe was based on our understanding of each structure's function and how it interacted with the female genitalia. In one case, we used both dorsal and ventral views (the proctiger), because there were distinct and variable features present in both views. For comparative purposes, we also studied five nongentialic traits: the distal antennal segment, the distal tarsal segment of the fore legs, the tarsal claw of the fore legs, the proximal segment of the proboscis and the body in dorsal view. We note that four of these traits are of a similar absolute size as the genitalic traits and all five relate to the species' ecology (i.e., locomotion, feeding and sensory facility). For each trait, data from two randomly chosen males per species were collected. We first captured digital images of all traits using a Lumenera® Infinity 2–2 digital camera mounted on a Leica® MZ8 dissecting microscope. We then used tpsDIG2 (Rohlf 2008) to capture 2D outlines of each of the 15 traits for each individual from these images. The dorsal and lateral sclerites of the vesica were outlined from a lateral view of the vesica and the ventral sclerite from a ventral view (Andersen 1993). All outlines were subsequently used to characterize morphological shape and complexity, using Morpheus et al. (Slice 2002). In addition, two individuals of each sex and species were dissected (by removing the sternum) and photographed in the same manner to capture outlines describing the size of sex-specific internal reproductive organs; the spermatheca and gynatrial sac in females and testes and seminal vesicles in males. The gynatrial sac is the female organ that receives the ejaculate and sperm are then stored in the spermatheca for subsequent fertilization; the testes produces sperm and possibly accessory substances and the seminal vesicle stores the ejaculates prior to ejaculation (Andersen 1982, 1993). For all traits, integrative

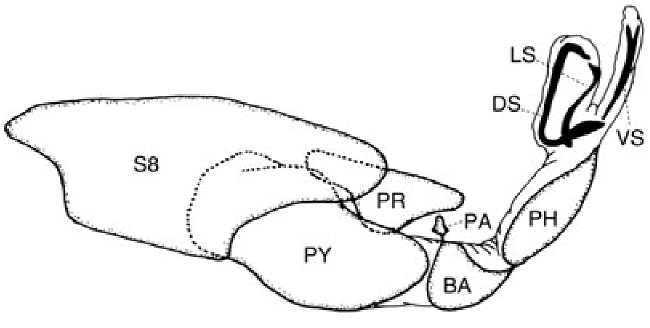

Figure 1. Schematic illustration of partially everted *Gerris* genital apparatus. Shown are the dorsal (DS), lateral (LS), and ventral (VS) sclerites of the vesica, the phallotheca (PH), the basal apparatus (BA), segment 8 (S8), pygophore (PY), proctiger (PR), and the paramere (PA). See Fairbairn et al. (2003) for an illustration of a fully everted and inflated genital apparatus.

measures of size were attained by translating areas into unidimensional sizes with square-root transformations. We used the mean value for each species and metric for all phylogenetic comparative analyses below (data shown in Appendix).

MORPHOMETRIC ANALYSES

We extracted three distinct quantitative and continuous measures of morphological complexity that are known to capture somewhat different aspects of the complexity of outline data (see McLellan and Endler 1998). First, we calculated the dissection index (DI), which simply represents the ratio of the perimeter to the square root of the area, standardized such that a circle has a value of 1.0. More complex shapes thus have larger values of DI (McLellan and Endler 1998). Second, we calculated the fractal dimension (FD) of each outline, as determined by the box-counting method employed in the program FD3 (Sarraille and Myers 1994). Third, we fitted two elliptic Fourier analysis (EFA) models to each outline: a simple model including only four harmomics and a complex model including 20 harmonics. We then used the difference in fit between these two models as our third measure of morphological complexity. This was achieved by calculating the average Euclidean distance between pairs of 100 evenly spaced out points along the reconstructed outlines of the two models (see McLellan and Endler 1998). A higher value, thus, means a greater misfit between the simple and complex model and hence signifies a more complex morphology.

Quantifying divergence in morphological shape in a set of complex traits is a nontrivial task. We employed EFA, which has been used successfully in many studies of genital evolution in the past (e.g., Liu et al. 1996; Arnqvist 1998; Arnqvist and Danielsson 1999; Zeng et al. 2000; Monti et al. 2001; Garnier et al. 2005; Rönn et al. 2007; Routto et al. 2007; Soto et al. 2007; Holwell 2008) and used a geometric approach. Briefly, this method involves describing the outline of each trait of each species with a nonlinear function, and subsequently analyzing morphological shape variation across species as variance in the parameters of the fitted functions. We used the average outline for each species and trait to first fit a common global elliptic Fourier model with 20 harmonics for all 15 traits and species. We then reduced the resulting matrix of EFA coefficients by means of a factor analysis, based on the covariance matrix, from which we retained the first 10 principal components (PCs) (collectively accounting for $> 98\%$ of total variance in shape). For each trait, we then calculated the average score across species for these 10 PCs (i.e., the centroid). Following ordination of all species and traits in one common multivariate space made up of the 10 PCs, we measured the Euclidean distance between a species' location and the centroid separately for each trait. For a given trait, thus, the mean Euclidean distance provides a measure of how divergent morphology is among species in this clade. Moreover, because divergence is measured in the same multivariate space for all 15 traits, it provides a quantitative measure of shape divergence among species which is comparable across traits (see Arnqvist 1998 for a similar analytical strategy).

To describe variation in shape among species for genital traits in phylogenetic comparative analyses, we also subjected data for each of the 10 genital traits to trait-specific EFAs, again using 20 harmonics, and retained the two first PCs from trait-specific factor analyses (see above) to characterize shape variation for each trait. These two first PCs accounted for $>81\%$ of total shape variation in all cases (data shown in appendix).

We assessed the pattern of allometric scaling between genital traits and body size, following $\log_{10}$ transformations of all measures of size. We first performed phylogenetically uncorrected analyses, aiming to estimate the slope of the evolutionary allometries rather than to test any hypothesis about correlated evolution (Klingenberg 1996), by regressing trait size on body size across species using reduced major axis regression to estimate the slope (Sawada 1999). We then also tested the evolutionary allometries using phylogenetically independent contrasts (Felsenstein 2005), employing the method detailed in Garland et al. (1992).

CORRELATED EVOLUTION

To characterize the pattern of multivariate correlated evolution of genital trait complexities, we employed phylogenetic principal components analysis (PPCA), using the MATLAB code in Revell (2009), of interspecific variation in the complexity of the 10 genital traits. The PPCA was based on the evolutionary correlation matrix.

One of the primary goals of the current study was to study correlated evolution between indices of sexual selection on one hand and the shape and complexity of intromittent/nonintromittent genital traits on the other. Drawing on previous experimental and comparative work in these species (see Arnqvist and Rowe 2002a, b; Rowe and Arnqvist 2002), we used the following three pairs of variables as indices of sexual selection. (1) Female mating rate and female mating activity (see Rowe and Arnqvist 2002 for methods). Variance in these variables presumably reflects variation in both pre- (male persistence/female resistance) and postmating (degree of polyandry) sexual selection regimes. (2) Sexual dimorphism in body morphology. This was characterized using the two multivariate axes describing sex-specific body shape variation from our earlier analyses of correlated evolution of male and female body shape (Arnqvist and Rowe 2002b). These two axes capture distinct aspects of sexual dimorphism in body morphology and collectively reflect variation primarily in premating sexual selection regimes (see Arnqvist and Rowe 2002a). These two axes do not include morphological variation in the genital traits dealt with here. (3) The relative size of male reproductive glands. Residual testes and seminal vesicle size (from regressions on body size) were used to characterize interspecific variation in postmating sexual selection regime. These organs show correlated evolution with female remating rate in several taxa (e.g., Hosken and Ward 2001; Katvala et al. 2008) and are commonly employed as indices of primarily postmating sexual selection in comparative studies (e.g., Wedell and Hosken 2010).

Our assessment of covariation between these indices of sexual selection and aspects of genital morphology involved testing for correlated evolution between several sets of variables. To test for and account for phylogenetic signal in our data while restricting the number of inferential tests, we employed phylogenetic least squares canonical correlation analyses (PCCA) using the software program PCCA (Revell and Harrison 2008). This provides both a likelihood ratio test of the null hypothesis that the multivariate phylogenetic signal $\lambda = 0$ (i.e., no phylogenetic signal) and tests for correlated evolution between two sets of variables by estimating phylogenetically adjusted canonical correlations. Our analyses were restricted to the first canonical axis and were based on the maximum likelihood estimate of multivariate λ. The phylogenetic reconstruction used in all comparative analyses was a well-supported total evidence phylogeny based on a number of molecular and morphological characters (Damgaard and Sperling 2001; Arnqvist and Rowe 2002a).

Results

EVOLUTIONARY ALLOMETRY OF GENITALIA

On average, genital traits scaled less than isometrically with body size (mean across all traits, $\beta = 0.85$; t-test of mean $\beta = 1$: $t = 3.25$, df $= 9$, $P = 0.010$). However, only two traits (one intromittent and one nonintromittent) showed an allometric slope significantly lower than 1 (phallotheca: $\beta = 0.69$, $P = 0.038$; proctiger lateral view: $\beta = 0.61$, $P = 0.007$) in phylogenetically uncorrected analyses. Further, the allometric slope did not differ significantly between intromittent and nonintromittent genital traits ($F_{1,8} = 2.47$, $P = 0.155$). Phylogenetically informed analyses yielded even lower allometric slopes (mean across all traits, $\beta = 0.43$; t-test of mean $\beta = 1$: $t = 10.85$, df $= 9$, $P < 0.001$). Here, two intromittent and two nonintromittent traits showed an evolutionary allometry significantly lower than 1 and, again, the mean allometric slope did not differ between intromittent and nonintromittent genital traits ($F_{1,8} = 0.002$, $P = 0.964$). Thus, although the size of gential traits clearly show negative evolutionary allometry overall (see Eberhard 2009 for static allometry), the two types of genital traits did not differ in their allometric scaling.

CORRELATED EVOLUTION BETWEEN INTERNAL REPRODUCTIVE ORGANS

There was a significant phylogenetic signal in internal reproductive organs ($P = 0.013$), but the size of spermatheca and gynatrial sac in females was not significantly correlated with testes and seminal vesicle size in males (PCCA: $r = 0.37$, $\chi^2_4 = 2.15$, $P = 0.708$). To validate our use of testes and seminal vesicle size in males as an index of postmating sexual selection, we assessed whether the evolution of these male traits was correlated with the evolution of female mating rate and activity. This analysis revealed a significant phylogenetic signal ($P < 0.001$) and a marginally nonsignificant canonical correlation between male reproductive organs and female mating behaviors (PCCA: $r = 0.66$, $\chi^2_4 = 8.42$, $P = 0.077$), primarily caused by a strong positive evolutionary relationship between seminal vesicle size in males and female mating rate/activity. We interpret this as providing at least partial validation of our inferential rationale.

DIVERGENT EVOLUTION OF GENITAL SHAPE

Our analyses of morphological divergence (i.e., Euclidean distances) revealed that the shape of different traits differed in their extent of divergent evolution within this clade ($F_{14,210} = 38.9$, $P < 0.001$) (see Fig. 2). Focused post-hoc contrasts revealed that nongenitalic traits showed significantly less-divergent evolution than genitalic traits ($F_{1,210} = 39.1$, $P < 0.001$). Further, intromittent genital traits have evolved more divergently than nonintromittent genital traits, whether the ventral sclerite is included in this comparison ($F_{1,140} = 12.9$, $P < 0.001$) or not ($F_{1,126} = 91.1$, $P < 0.001$).

MORPHOLOGICAL COMPLEXITY

Our metrics of complexity were repeatable. The intraclass correlation coefficients (based on values for the two replicate individuals of each species) for our measures of complexity were high and generally significant for all three measures of complexity across all 15 traits (mean repeatability = 0.61, SE = 0.07). For any specific trait, our three different measures of complexity tended to be correlated across species. However, the match was frequently less than perfect. Across all 15 traits, the mean correlations were: DI vs. EFA $r_p = 0.51$ (range: –0.19 to 0.90), DI vs. FD $r_p = 0.22$ (range: –0.43 to 0.84) and EFA vs. FD $r_p = 0.12$ (range: –0.67 to 0.79). Thus, although these three different measures of complexity showed some consistency, they also frequently captured distinct aspects of morphological complexity. The latter is especially true for FD compared to the other two measures of complexity. However, the three methods showed a correlation between the mean trait-specific complexity across all species (DI vs. EFA $r_p = 0.87$, DI vs. FD $r_p = 0.98$, EFA vs. FD $r_p = 0.86$). Hence, traits that were on average deemed more complex by one method were also on average deemed more complex by the others (Fig. 3).

Different traits showed different degrees of morphological complexity, for DI ($F_{14,210} = 78.6$, $P < 0.001$), EFA ($F_{14,210} = 31.8$, $P < 0.001$), and FD ($F_{14,210} = 64.8$, $P < 0.001$) (see Fig. 3). On average, nongenital traits were less complex than genital traits,

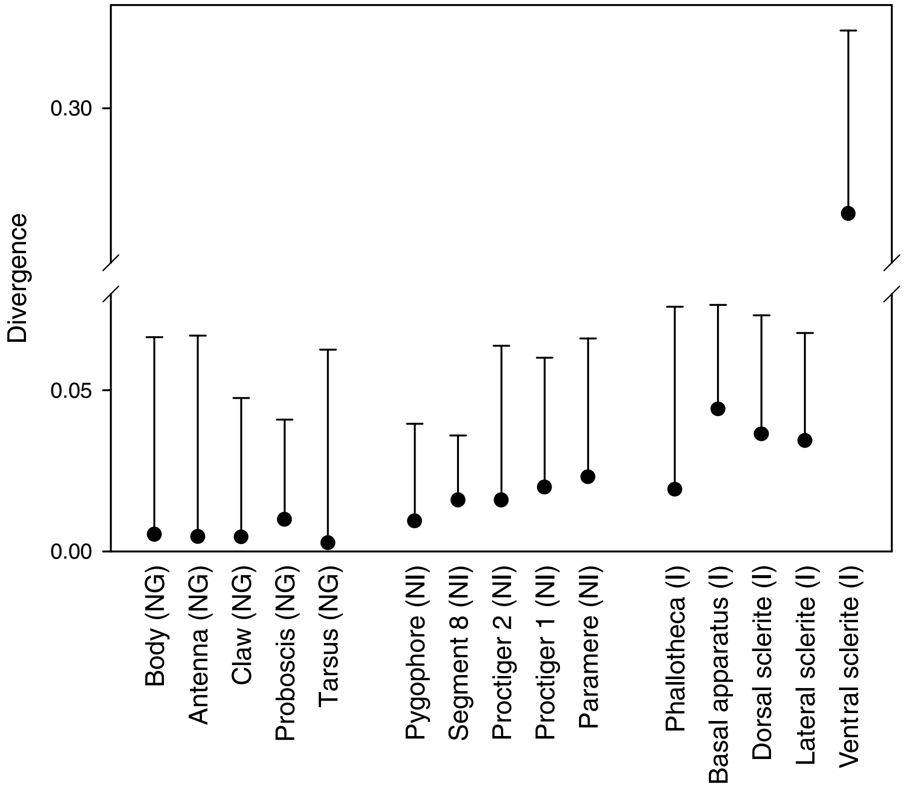

Figure 2. The amount of divergent evolution of shape of various nongenitalic (NG), nonintromittent genitalic (NI), and intromittent genitalic (I) traits within the genus *Gerris*. Error bars represents CV × 10^{-1}.

for DI ($F_{1,210} = 198.1$, $P < 0.001$), EFA ($F_{1,210} = 131.2$, $P < 0.001$), and FD ($F_{1,210} = 232.8$, $P < 0.001$). Similarly, intromittent genital traits tended to be more complex than nonintromittent genital traits, whether assessed by DI ($F_{1,140} = 189.5$, $P < 0.001$), EFA ($F_{1,140} = 23.6$, $P < 0.001$), or FD ($F_{1,140} = 134.4$, $P < 0.001$), and this effect was caused primarily by the high morphological complexity of the three intromittent vesical sclerites (Fig. 3). Further, the amount of divergent evolution within this clade was positively correlated with all three measures of morphological complexity across traits (range, $r_p = 0.63$–0.88; $r_s = 0.76$–0.87; all $P < 0.05$). In other words, traits that were deemed more complex were also more evolutionarily divergent in shape (cf. Figs. 2 and 3).

CORRELATED EVOLUTION

To restrict the number of inferential tests made, we restricted our analyses of correlated evolution of morphological complexity to a single index of complexity. We used EFA rather than the other measures, in part because it had the highest repeatability and in part because it yielded the most rational quantification of complexity (see also McLellan and Endler 1998).

If the same forces are shaping the complexity of both intromittent and nonintromittent genitalia, then one would expect their evolution to be correlated. However, although there was a significant phylogenetic signal ($P = 0.007$), in genital complexity, the evolution of morphological complexity in these two sets of genital traits was not significantly correlated (PCCA: $r = 0.68$, $\chi^2_{25} = 11.78$, $P = 0.988$). Inspections of the trait loadings on the first principal component (eigenvalue = 2.08) from the PPCA of complexity provided further insights. The loadings for intromittent traits were consistent in sign and size (mean: −0.44; range: −0.81 to −0.17) whereas those for nonintromittent traits were not (mean: 0.14; range: −0.35 to 0.71). Thus, the lack of coupling between the evolution of complexity in intromittent versus nonintromittent gential traits results from the fact that there was concerted evolution of trait complexity within the intromittent, but not the nonintromittent, set of genital traits.

PCCA analyses of correlated evolution between our indices of sexual selection and genital morphology are summarized in Table 1. We wish to emphasize three general patterns in these analyses. First, variation in genital complexity generally showed a significant phylogenetic signal whereas variation in genital shape did not. This strongly suggests that shape is evolving faster than complexity. Because morphological complexity can be seen as a subset of shape space (i.e., although shape can vary while holding complexity "constant", the opposite is not true), the fact that shape is more evolutionary labile than is complexity is reasonable.

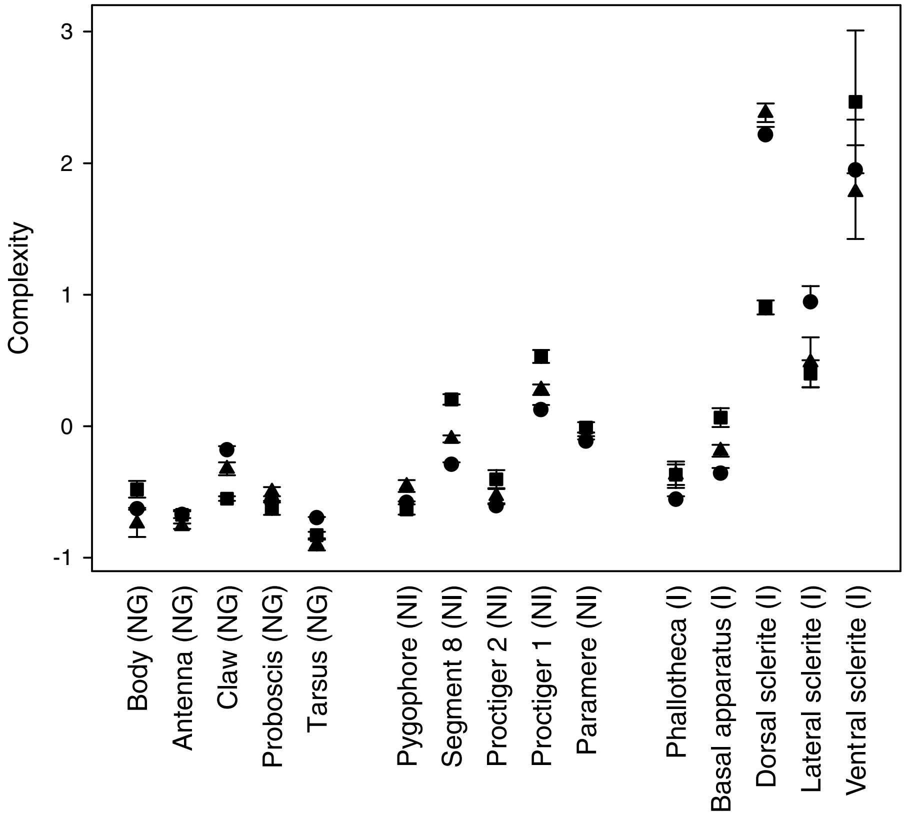

Figure 3. The average degree of morphological complexity, standardized to a mean of zero and unit variance, across nongenitalic (NG), nonintromittent genitalic (NI), and intromittent genitalic (I) traits, as quantified by DI (circles), EFA (squares), and FD (triangles). Error bars represents SE.

Second, the pattern of significant correlated evolution is remarkably consistent with the hypothesis that intromittent genitalia tends to be subject to postmating sexual selection whereas nonintromittent genitalia tends to be subject to premating sexual selection (Table 1). Female mating behavior, reflecting both forms of sexual selection, was evolutionarily correlated with the shape and complexity of nonintromittent traits and with the shape of intromittent traits. Sexual dimorphism in body shape, reflecting premating sexual selection, showed significant correlated evolution only with the shape of nonintromittent genital traits and near significance with their complexity. The internal reproductive organs of males, reflecting primarily postmating sexual selection, showed significant correlated evolution only with the shape of intromittent genital traits, and marginally nonsignificant correlated evolution with their complexity and with the shape of nonintromittent traits.

Third, although the pattern of correlated evolution between indices of sexual selection and the form of morphological shape evolution is difficult to interpret in the absence of a very detailed understanding of the functional morphology of all genital traits, the direction of evolution of complexity is in support of expectations. For example, inspection of the canonical loadings revealed that the correlated evolution between female mating behavior and the complexity of nonintromittent genitalia is caused primarily by a positive correlation between female mating activity and the complexity of the morphology of the proctiger (Fig. 4A). The latter is involved in grasping females during premating interactions. Similarly, the marginally nonsignificant correlated evolution seen between sexual dimorphism in body shape and complexity of nonintromittent genitalia is caused primarily by a positive association between sexual dimorphism and the complexity of segment 8 (Fig. 4B). Segment 8 is used by males to grasp females. In other words, these patterns support the hypothesis that stronger sexual selection is associated with a higher degree of morphological complexity. We also note that the correlated evolution seen between indices capturing variation on postmating sexual selection (female mating behavior and internal reproductive organs of males) and the shape of intromittent genital traits primarily involved shape evolution of the dorsal and ventral vesical sclerites (Fig. 4C). Notably, it is the shape of these two sclerites that are most closely associated with variation in male postmating fertilization success within species (Arnqvist and Danielsson 1999; Danielsson and Askenmo 1999). Finally, we also note that it is male seminal vesicle size (canonical loading

Table 1. The results of phylogenetic canonical correlation analyses (PCCA)

		Phylogenetic signal	Canonical correlation			
Set 1 (no. of variables)	Set 2 (no. of variables)	P (H_0: $\lambda = 0$)	r	χ^2	df	P
Female mating behavior (2)	Complexity of intromittent genital traits (5)	<0.001	0.84	13.29	4	0.208
	Complexity of nonintromittent genital traits (5)	0.002	0.91	21.34	4	0.019
	Shape of intromittent genital traits (10)	>0.9	0.99	41.56	9	0.003
	Shape of nonintromittent genital traits (10)	>0.9	0.98	35.35	9	0.018
Sexual dimorphism in body shape (2)	Complexity of intromittent genital traits (5)	<0.001	0.65	7.27	4	0.699
	Complexity of nonintromittent genital traits (5)	0.008	0.81	17.93	4	0.056
	Shape of intromittent genital traits (10)	0.429	0.95	20.69	9	0.416
	Shape of nonintromittent genital traits (10)	>0.9	0.99	42.91	9	0.002
Internal reproductive organs of males (2)	Complexity of intromittent genital traits (5)	<0.001	0.89	16.07	4	0.098
	Complexity of nonintromittent genital traits (5)	0.102	0.68	11.09	4	0.351
	Shape of intromittent genital traits (10)	>0.9	0.99	34.27	9	0.024
	Shape of nonintromittent genital traits (10)	>0.9	0.96	30.33	9	0.064

0.99), rather than testis size (canonical loading 0.07), that shows correlated evolution with the shape of intromittent genital traits in males.

Discussion

The results of our comparative analyses give quantitative support to the widely held view that genitalia are relatively complex in shape and that the shape of genitalia evolves more divergently than other traits. These analyses also revealed three main novel insights about the evolution of genital shape and complexity in this clade of water striders and its relationship to sexual selection on the genital structures. First, the shape of genital structures has diverged to varying degrees, and functional sets (intromittent and nonintromittent structures) have diverged at different rates. Second, genital structures tend to be complex and functional sets of genital traits differ in complexity, corresponding to their differences in shape divergence. Third, the evolution of shape and complexity of the genital structures is correlated with the evolution of indices of pre- and postmating sexual selection. In the following discussion we highlight these patterns and suggest new directions for comparative studies aimed at understanding the remarkable diversification of animal genitalia.

DIVERSITY, DIVERGENCE, AND COMPLEXITY OF THE GENITALIA

The analysis of genital trait shape revealed that the 10 traits differed in their degree of shape divergence (Fig. 2). There are several interesting patterns to these data. Our contrast of divergence in shape between the 10 genital traits and whole body shape supports the widely held view that genitalia are evolving faster, at least in shape, than nongenitalic structures (see also Arnqvist 1998). It is interesting to note that there was a preponderance of negative allometries in genital trait size. It is not obvious what general hypothesis could account for this pattern. However, their presence implies that lengths of genital traits are evolving slower than body size. This is the opposite pattern to our shape data. Therefore, in this dataset, length of genital segments does not capture the diversity of genitalia whereas measures of shape do. Greater interspecific diversity in shape than size of genitalic traits has been observed in other taxa (e.g., Garnier et al. 2005; Mutanen et al. 2006; McPeek et al. 2008). One important

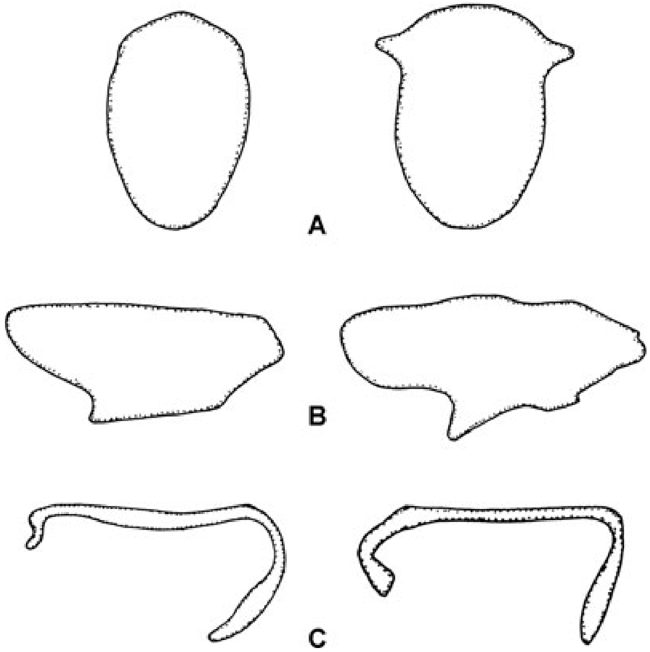

Figure 4. Examples of the qualities and directions of phenotypic evolution in the genitalic traits of *Gerris*. (A) The evolution of a more complex morphology of the proctiger (dorsal view) is associated with a higher mating activity, illustrated here with *G. gibbifer* (left; relatively low mating rate) and *G. marginatus* (right; relatively high mating rate). (B) Similarly, the degree of complexity of segment 8 shows positive correlated evolution with sexual dimorphism in body shape, here exemplified with *G. argentatus* (left; relatively low SD) and *G. buenoi* (right; relatively high SD). (C) The shape of the dorsal vesical sclerites exhibits correlated evolution with the relative size of the male seminal vesicles, such that the evolution of larger seminal vesicles is associated with the evolution of a more abrupt angle of the major leg of the sclerite. Shown here are the dorsal sclerites of *G. brasili* (left; small seminal vesicle) and *G. pingrensis* (right; large seminal vesicle).

conclusion is that studies aimed at understanding what appears to be the great diversity of genital traits, ought to focus on variation in shape rather than size to capture this diversity.

The trait or structure-specific evolutionary rates in shape divergence observed in this dataset (Fig. 2) will tend to increase interspecific diversity in the overall shape of the genital apparatus (Fig. 1), as some of its component structures change rapidly and others more slowly. This variation also clearly illustrates the fact that the genital apparatus is not a single structure, but a multistructure consisting of several subcomponents (Eberhard 1985). Finally, the independent evolutionary rates in shape change of these structures suggest that differing forms or strengths of selection may be operating on them. We found that there was more evolutionary divergence in the shape of intromittent than nonintromittent structures (Fig. 2). This suggests that the balance of selection and constraint on these sets of traits differ. Among water striders of this genus, there is premating selection on some of the nonintromittent structures (Arnqvist 1989, 1992; Preziosi and Fairbairn 1996, 2000; Sih et al. 2002; Bertin and Fairbairn 2005) and postmating selection on some intromittent structures (Arnqvist and Danielsson 1999; Danielsson and Askenmo 1999). Differences in the strength of pre- and postmating selection may account for the different rates of divergence in intromittent and nonintromittent genital structures—a point to which we will return in the next section.

Alternatively, Eberhard (2006) has argued that in cases where genital structures are used for clasping mating partners, mechanical constraints (goodness of fit) will constrain evolutionary change. Our data appear in line with this hypothesis as the nonintromittent structures are used in clasping and show lower rates of divergence than intromittent structures, which may not be used in clasping. Yet, there are a number of reasons to think that goodness of fit does not substantially constrain the evolution of clasping components of the genitalia. First, we show that these traits show a higher complexity and are evolving considerably faster than nongentialic traits (Fig. 2) and these same traits are major characters for phylogenetic reconstructions and species identification (Andersen 1993). It is interesting to note that the relative lack of divergence in the shape of nongenital traits may in part result from stabilizing natural selection that is shared among species because they tend to share a niche. Second, the logic rests on the unsupported assumptions that there is only a single efficient method to clasp a structure and that that structure itself is not evolving. Third, and perhaps most importantly, water striders themselves illustrate the potential for a remarkable diversity of genital and nongenital clasping structures in a number of genera. In *Halobates*, there is a great diversity in the shape and structure of the nonintromittent genitalia, which are used to clasp females (Andersen and Cheng 2005). The nonintromittent genitalia are used for species identification and seem at least as divergent as the intromittent genitalia in this genus (see plate 12.30–2 in Andersen and Weir 2004; Andersen 1991; Andersen and Weir 1994). Similarly, in some species of *Rheumatobates*, all male appendages (including the antennae) have been modified as grasping structures, each of which has evolved multiple times, and most of the morphological diversity in the genus is comprised of the diversity of these grasping traits (Westlake at al. 2000; Rowe et al. 2006).

COMPLEXITY AND DIVERGENCE

Genitalic traits also differed in all three measures of shape complexity and the pattern among traits was similar to that of shape divergence (Fig. 3). Genitalic traits were significantly more complex than nongenital traits and intromittent traits were significantly more complex than nonintromittent traits. We used three different measures of complexity, and these had similar overall trends (Fig. 3). Traits identified as highly complex by any one metric, tended to be indentified as complex by all metrics. However, there was some variation among the methods, which suggests that they are each capturing different aspects of complexity. Yet, the fact that intromittent traits were more complex than nonintromittent traits (Fig. 3), suggests a role for differential mechanisms of sexual selection (e.g., pre- vs. postmating selection or strengths of selection, see below).

The complexity of the genital apparatus may result from its multitasking, and therefore, multitrait nature (Eberhard 1985). In *Gerris,* there are at least nine distinct sclerotized structures and perhaps many more soft tissue structures. Each of these apparently distinct structures may, in turn, be made up of a set of substructures. Each of these structures and substructures has the potential to contribute to complexity (Fig. 2; Huber et al. 2005; Song and Wenzel 2008; Song and Bucheli 2010). Moreover, this increased dimensionality that comes with an increasing complexity may account for the observed significant positive correlation between shape divergence and the complexity of the diverging trait.

SEXUAL SELECTION AND THE CORRELATED EVOLUTION OF GENITALIA

Our results provide strong comparative support role for sexual selection in driving the evolution of shape and complexity of genital morphology in male water striders. Moreover, the patterns in our comparative results are aligned with earlier intraspecific studies in the laboratory and field (Arnqvist 1989, 1992; Arnqvist and Danielsson 1999; Danielsson and Askenmo 1999; Sih et al. 2002; Fairbairn et al. 2003; Bertin and Fairbairn 2005). There was correlated evolution between mating rate/activity and the morphology of both intromittent and nonintromittent genitalia, as would be expected if the degree of polyandry affects the opportunity for both pre- and postmating sexual selection. Further, sexual dimorphism in body shape, reflecting primarily premating sexual selection regimes in this group (Arnqvist and Rowe 2002b; Rowe and Arnqvist 2002), showed correlated evolution only with those aspects of genital morphology that are nonintromittent. Again, this is predicted as nonintromittent genital morphology in this group affects male ability to secure matings. Finally, the relative size of male internal reproductive organs (testis/seminal vesicle) showed significant correlated evolution only with intromittent genital morphology. Given that the size of these organs should reflect primarily postmating sexual selection regimes (Wedell and Hosken 2010), this is consistent with the hypothesis that the shape of intromittent genitalia affects male postmating fertilization success. We have shown that intromittent and nonintromittent traits evolve in an uncorrelated manner and the patterns of correlated evolution with indices of sexual selection (Table 1) suggest that this results from differing degrees of pre- and postmating selection on the two sets of traits. Overall, the results are consistent with the view that premating sexual selection shapes nonintromittent genital structures whereas postmating sexual selection shapes intromittent genitalia in this group of insects (Bertin and Fairbairn 2005). The fit with predictions is remarkably close given that our indices of pre- and postmating sexual selection are not completely independent, as demonstrated by a near-significant positive correlated evolution between female mating rate and male seminal vesicle size.

It is easy to understand how sexual selection could shape these genital traits, but it is not easy to understand why sexual selection leads to such remarkable patterns of divergence and complexity. There are several coevolutionary (e.g., sexually antagonistic, good genes, Fisherian; Eberhard 1985, 2010; Arnqvist 1998) and noncoevolutionary (e.g., sensory bias; Arnqvist 2006) models that have been proposed. The patterns that we have revealed here make a strong case for a role of pre- and postmating sexual selection in this evolutionary process, but patterns such as these (e.g., Eberhard 2006; Kuntner et al. 2009; Tatarnic and Cassis 2010) cannot distinguish among the various models of sexual selection that could create the pattern. More generally, there is now much comparative evidence for correlated evolution of male/female reproductive traits (e.g., Briskie et al. 1997; Presgraves et al. 1999; Arnqvist and Rowe 2002a; Brennan et al. 2007; Rönn et al. 2007; Kuntner et al. 2009; Joly and Schiffer 2010; Tatarnic and Cassis 2010), but comparative data alone cannot distinguish among alternative processes. What is required are economic studies of the coevolving traits themselves, in particular those aimed at understanding the forces of selection acting on those female traits that cause sexual selection in males (Kirkpatrick and Ryan 1991; Andersson 1994; Arnqvist and Rowe 1995; 2005; Rowe and Day 2006; Maklakov and Arnqvist 2009; Fricke et al. 2009). Such studies are very rare indeed in the study of genitalia.

A number of comparative studies have been used to reject a hypothesis of sexually antagonistic coevolution and to support cryptic female choice as a force driving the evolution of genital (cf. Eberhard 2010). However, in addition to the problem of inferring process from pattern, these are not alternative hypotheses: sexual conflict is one of several possible drivers of cryptic female choice and, in fact, sexually antagonistic coevolution includes female choice as a key component (Arnqvist and Rowe 2005). Further, the potential involvement of sexually conflict in the evolution of genitalia is in no way restricted to those cases involving “overt coercion” (cf. Eberhard and Huber 2010). Instead, it might involve all cases where there is sexually antagonistic selection on those pre- or postmating sexual interactions with which the genitalia are involved (Arnqvist and Rowe 2005). For example, the comparative data presented here suggest, and experimental data in two of these species demonstrate that there is postmating sexual selection on the vesical sclerites (Arnqvist and Danielsson 1999; Danielsson and Askenmo 1999). However, we do not know what these sclerites actually do inside the female reproductive tract and, most importantly, we do not know what, if any, forces of selection are acting on those female traits that generate the bias in fertilization success among males. Sexual conflict may or may not be involved. Unfortunately it is not possible to determine this without economic studies of the interacting male and female traits (Fricke et al. 2009).

The situation for some of the nonintromittent grasping structures is clearer. The comparative data presented here and elsewhere (Arnqvist and Rowe 2002a,b) and field studies of water striders (Arnqvist 1989, 1992; Sih et al. 2002; Fairbairn et al. 2003; Bertin and Fairbairn 2005) demonstrate sexual selection on these traits. We also know that at least some aspects of these traits function to overcome female resistance and that this comes at a cost to females through higher costly mating rates (reviews in Rowe et al. 1994; Arnqvist and Rowe 2005). Finally, coevolutionary responses in female antigrasping traits (Arnqvist and Rowe 1995; Ronkainen et al. 2005) appear to favor further modification of the male traits (Arnqvist and Rowe 2002a,b). Therefore, a model of sexually antagonistic coevolution is supported. We caution that it is the functional and economic studies, particularly in females, that support this conclusion rather than the observed coevolutionary pattern.

PHYLOGENETIC SIGNAL

Song and Bucheli (2010) asked whether the phylogenetic signal in genitalia differed from that in nongenitalic morphological traits and their analyses suggested that it was not. Our analyses suggest that the extent of similarity in phylogenetic signal may depend upon how genitalic traits are characterized. We found that genital trait complexity, reflecting presence/absence of substructures such as protrusions and invaginations, showed a strong phylogenetic signal while the shape of these genital traits did not (see also Huber et al. 2005). Because the genital apparatus is a composite character made up of a number of more or less separate structures, there are good reasons to believe that complexity (e.g., presence/absence of features) evolves slower than does more fine scaled genital shape modulations (Song and Bucheli 2010).

CONCLUSIONS

Our comparative study provides quantitative evidence of the complexity and high divergence in shape of genitalia and supports a key role for sexual selection in generating both complexity and divergence in shape. Our results imply that future studies should focus on genitalic shape and complexity rather than on genital size, and suggest promising avenues for more focused future research. For example, comparative analyses can help to identify key traits (e.g., complexity of the proctiger) and aspects of selection (e.g., premating sexual selection) that should be the focus of functional and economic studies aimed at understanding their evolution. Such functional and economic studies may also point to potentially coevolving female traits, enabling an understanding of the coevolutionary processes generating the great diversity of animal genitalia.

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Appendix

Species-level data for all variables used in phylogenetic comparative analyses.

Species	Body size	Female mating activity	Female mating rate	Body shape SD (axis 1)	Body shape SD (axis 2)	Testis size	Seminal vesicle size	Spermatophore size	Gynatrial sac size	Paramere EFA
G. lateralis	777.0	0.751	0.641	2.00	−0.508	−45.4	39.6	3.1	−51.0	0.0340
G. sphagnetorum	673.5	0.013	0.058	−0.85	0.164	−45.4	−16.6	−18.3	106.9	0.0403
G. incognitus	663.2	0.013	0.047	−2.06	0.023	40.7	34.6	61.8	14.8	0.0275
G. pingrensis	677.3	0.296	0.329	−1.12	0.049	12.3	62.5	−2.8	16.6	0.0391
G. gillettei	727.9	0.127	0.324	−0.17	0.041	−9.0	30.3	96.5	67.4	0.0360
G. argentatus	538.7	0.155	0.234	−0.58	0.415	−34.5	−24.4	−48.5	−37.4	0.0303
G. odontogaster	626.2	0.178	0.204	−1.50	−0.272	4.1	−35.5	27.6	16.5	0.0285
G. buenoi	599.1	0.265	0.303	−2.15	−0.281	25.9	−12.2	−47.1	0.2	0.0312
G. thoracicus	932.5	0.036	0.085	1.90	0.170	1.1	9.8	−6.6	58.1	0.0270
G. comatus	761.4	0.126	0.311	0.18	0.023	39.3	0.5	−28.2	−30.9	0.0291
G. marginatus	790.4	0.157	0.423	1.65	−0.045	14.6	−13.5	−21.7	−47.6	0.0298
G. incurvatus	772.8	0.079	0.147	0.64	0.123	−43.9	−33.4	−51.2	−88.2	0.0310
G. lacustris	689.9	0.425	0.657	−0.35	−0.013	34.6	−10.6	43.3	−28.1	0.0387
G. gibbifer	974.8	0.022	0.055	1.37	0.131	−3.6	−22.2	−13.6	−20.7	0.0299
G. brasili	936.2	0.099	0.202	1.04	−0.020	9.3	−8.8	5.9	23.4	0.0332

Species	Proctiger 1 EFA	Proctiger 2 EFA	Pygophore EFA	Segment 8 EFA	Phallotheca EFA	Basal apparatus EFA	Dorsal sclerite EFA	Lateral sclerite EFA	Ventral sclerite EFA	Proctiger 1 shape PC1
G. lateralis	0.0588	0.0161	0.0146	0.0376	0.0148	0.0359	0.0634	0.0300	0.0539	−0.0317
G. sphagnetorum	0.0373	0.0239	0.0176	0.0363	0.0168	0.0331	0.0555	0.0325	0.0294	0.0262
G. incognitus	0.0538	0.0314	0.0148	0.0347	0.0159	0.0322	0.0484	0.0312	0.0294	−0.0168
G. pingrensis	0.0457	0.0240	0.0168	0.0419	0.0321	0.0224	0.0528	0.0300	0.0523	−0.0549
G. gillettei	0.0474	0.0215	0.0206	0.0391	0.0282	0.0252	0.0491	0.0330	0.0247	0.0107
G. argentatus	0.0500	0.0178	0.0086	0.0343	0.0547	0.0436	0.0658	0.0460	0.1124	0.0242
G. odontogaster	0.0511	0.0119	0.0077	0.0474	0.0265	0.0462	0.0668	0.0584	0.1114	−0.0095
G. buenoi	0.0449	0.0127	0.0098	0.0473	0.0270	0.0516	0.0579	0.0439	0.1597	−0.1292
G. thoracicus	0.0468	0.0165	0.0198	0.0372	0.0130	0.0342	0.0552	0.0391	0.1070	−0.0172
G. comatus	0.0459	0.0218	0.0167	0.0346	0.0162	0.0324	0.0667	0.0526	0.0908	0.0244
G. marginatus	0.0472	0.0391	0.0219	0.0354	0.0215	0.0336	0.0558	0.0463	0.1158	0.1099
G. incurvatus	0.0410	0.0291	0.0146	0.0347	0.0116	0.0281	0.0581	0.0415	0.0894	0.0554
G. lacustris	0.0532	0.0235	0.0133	0.0378	0.0189	0.0293	0.0603	0.0647	0.1399	0.0238
G. gibbifer	0.0444	0.0150	0.0144	0.0365	0.0196	0.0331	0.0584	0.0546	0.2203	0.0035
G. brasili	0.0445	0.0158	0.0123	0.0402	0.0177	0.0360	0.0551	0.0533	0.1900	−0.0189

Continued.

Appendix. Continued.

Species	Proctiger 1 shape PC2	Proctiger 2 shape PC1	Proctiger 2 shape PC2	Pygophore shape PC1	Pygophore shape PC2	Segment 8 shape PC1	Segment 8 shape PC2	Paramere shape PC1	Paramere shape PC2	Basal apparatus shape PC1
G. lateralis	0.0729	0.0513	0.0056	−0.0256	−0.0155	0.0301	0.0112	−0.0180	−0.0128	0.2246
G. sphagnetorum	−0.0601	0.0230	0.0582	0.0043	0.0162	−0.0303	0.0112	0.0722	0.0185	−0.1339
G. incognitus	0.0431	0.0853	−0.0204	−0.0295	0.0023	0.0760	0.0188	0.0287	0.0368	−0.1473
G. pingrensis	0.0030	−0.0211	0.0114	−0.0289	0.0260	0.0086	0.0618	0.0864	−0.0061	−0.0566
G. gillettei	0.0687	−0.0121	−0.0212	−0.0251	0.0265	0.0137	0.0517	0.0501	−0.0563	−0.0882
G. argentatus	0.0018	0.0479	−0.0374	0.0235	−0.0133	−0.0444	−0.0094	−0.0119	−0.0876	0.0422
G. odontogaster	0.0484	−0.0370	−0.0338	0.0172	−0.0346	−0.0487	0.0386	0.0324	0.0185	−0.0825
G. buenoi	−0.0348	0.0488	−0.0256	−0.0063	−0.0328	−0.0727	0.0312	0.0371	−0.0137	−0.0744
G. thoracicus	0.0078	0.0183	−0.0195	0.0104	0.0193	0.0508	−0.0083	−0.1306	−0.0056	−0.1098
G. comatus	−0.0050	−0.0266	0.0055	0.0099	0.0159	0.0402	0.0026	−0.0432	0.0240	0.1716
G. marginatus	0.0062	−0.1309	−0.0254	0.0304	0.0123	0.0122	−0.0573	−0.1324	−0.0331	0.2290
G. incurvatus	−0.0340	−0.0710	0.0122	0.0319	0.0036	0.0303	−0.0352	−0.0554	0.0942	0.0126
G. lacustris	−0.0125	−0.0340	0.0475	0.0435	−0.0020	−0.0529	−0.0385	0.0257	−0.0609	−0.1527
G. gibbifer	−0.0126	0.0144	0.0112	−0.0109	−0.0035	0.0122	−0.0227	0.0337	0.0195	0.1988
G. brasili	−0.0927	0.0438	0.0318	−0.0445	−0.0206	−0.0249	−0.0557	0.0254	0.0646	−0.0334

Species	Basal apparatus shape PC2	Dorsal sclerite shape PC1	Dorsal sclerite shape PC2	Lateral sclerite shape PC1	Lateral sclerite shape PC2	Ventral sclerite shape PC1	Ventral sclerite shape PC2	Phallotheca shape PC1	Phallotheca shape PC2
G. lateralis	0.0255	0.2280	−0.0441	−0.1070	−0.0374	−0.7757	−0.0688	0.0486	0.0292
G. sphagnetorum	−0.0878	0.0537	0.1077	−0.1136	0.0197	−1.0990	0.0445	−0.0271	0.0484
G. incognitus	−0.0086	0.0509	−0.0149	−0.1408	−0.0055	−1.1018	0.0210	0.0731	−0.0017
G. pingrensis	0.0413	0.0938	−0.0359	−0.1206	0.0131	−0.9420	0.0828	−0.0620	−0.0409
G. gillettei	−0.0078	0.1200	0.0373	−0.1008	0.0074	−1.0938	0.0702	−0.0198	−0.0467
G. argentatus	−0.0569	−0.0720	−0.0071	0.0330	−0.0409	0.6324	−0.3084	0.0539	−0.0844
G. odontogaster	−0.1167	0.1859	−0.0324	0.0762	−0.0915	0.3604	−0.2460	−0.1194	−0.0004
G. buenoi	0.0463	0.0969	0.0108	−0.0553	−0.0602	0.8589	−0.6349	−0.1332	0.0226
G. thoracicus	0.0844	−0.0604	−0.0205	0.1161	−0.0010	0.3213	−0.2821	0.0731	0.0340
G. comatus	−0.0421	−0.1231	0.0566	0.2320	0.0348	0.0344	−0.2253	0.0703	0.0053
G. marginatus	0.0178	−0.0986	0.0374	0.0520	−0.0450	0.3959	−0.1714	−0.0223	0.0003
G. incurvatus	−0.1100	−0.1084	−0.0131	0.0707	−0.0892	−0.0026	−0.3500	0.0728	0.0117
G. lacustris	0.0818	−0.1601	−0.1420	0.0201	0.1209	0.6170	−0.2294	0.0142	0.0114
G. gibbifer	0.0425	−0.0876	0.0261	−0.0127	0.0913	0.6165	2.4884	0.0031	0.0174
G. brasili	0.0902	−0.1190	0.0342	0.0507	0.0834	1.1781	−0.1907	−0.0253	−0.0062