

HAVE MALE AND FEMALE GENITALIA COEVOLVED? A PHYLOGENETIC ANALYSIS OF GENITALIC MORPHOLOGY AND SEXUAL SIZE DIMORPHISM IN WEB-BUILDING SPIDERS (ARANEAE: ARANEOIDEA)

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Abstract.—Sexual size dimorphism (SSD) can strongly influence the evolution of reproductive strategies and life history. If SSD is extreme, and other characters (e.g., genitalic size) also increase with size, then functional conflicts may arise between the sexes. Spiders offer an excellent opportunity to investigate this issue because of their wide range of SSD. By using modern phylogenetic methods with 16 species of orb-weaving spiders, we provide strong evidence for the “positive genitalic divergence” model, implying that sexual genitalic dimorphism (SGD) increases as SSD increases. This pattern is supported by an evolutionary mismatch between the absolute sizes of male and female genitalia across species. Indeed, our findings reveal a dramatic reversal from *male* genitalia that are up to 87× larger than female genitalia in size-monomorphic species to *female* genitalia that are up to 2.8× larger in extremely size-dimorphic species. We infer that divergence in SGD could limit SSD both in spiders, and potentially in other taxa as well. Further, male and female body size, as well as male and female genitalia size, are decoupled evolutionarily. Finally, we show a negative scaling (hypoallometry) of male and female genitalic morphology within sexes. Evolutionary forces specific to each sex, such as larger female size (increased fecundity) or smaller male size (enhanced mate-searching ability), may be balanced by stabilizing selection on relative genitalic size.

Key words.—Genitalic evolution, Orbiculariae, scaling, sexual size dimorphism, spiders.

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Sexual size dimorphism (SSD) is a widespread phenomenon that can profoundly affect the evolution of reproductive strategies and life history (Andersson 1994; Hartcourt et al. 1995a; Fairbairn 1997). Many researchers have debated the underlying causes for the evolution of SSD and the resulting consequences for individual fitness (e.g., Darwin 1871; Shine 1989; Kraushaar and Blanckenhorn 2002; Cox et al. 2003). Because most morphological characters increase with size, species that exhibit extreme SSD may face a functional conflict because of the different sizes of the reproductive organs of males and females. Consequently, one would predict co-evolution of male and female genitalia size as the sexes diverge in size to ensure successful coupling of genitalia. While some studies have examined the scaling of reproductive characters within females or males of the same species (e.g., Hartcourt et al. 1995a; Eberhard et al. 1998; Schmitz et al. 2000; Uhl and Vollrath 2000; Bernstein and Bernstein 2002), we are aware of no studies that have examined the interspecific scaling of both female and male genitalia across different species. Here, we examine the interspecific relationship of female to male genitalic size (sexual genitalic dimorphism or SGD) in relation to SSD using spiders as a model system. The models we test can be applied to many other animal groups, therefore, this study has broad implications for understanding the evolution of SSD and life-history characteristics.

Three possible evolutionary models exist to explain how SSD and SGD are related among species. The “constant genitalic dimorphism” model posits that SGD remains constant as SSD increases. In this case, natural selection favors a constant ratio of female to male genitalic size as body sizes

diverge. If so, as females become larger than males (SSD increases), male genitalic size will increase at a much faster rate than for females (higher slope). This model predicts a positive matching (slope of 1) between the absolute sizes of genitalia of males and females across species across species diverging in SSD. The “positive genitalic divergence” model posits that SGD increases with SSD. As females become progressively larger than males (SSD increase), female genitalic size increases relatively more than in males (SGD increase). A prediction of this model is a *mismatch* (lack of correlation) between the absolute sizes of the genitalia of males and females across species differing greatly in SSD. Further, if male and female genitalia scale isometrically (i.e., proportionally the same, slope = 1), or even hypoallometrically (i.e., slope < 1), then “positive genitalic divergence” will occur simply because the size of one sex (e.g., females) changes faster than the size of the other (e.g., males). The third, an unlikely possibility, is “negative genitalic divergence”: SGD decreases evolutionarily as SSD increases. This model predicts a negative relationship between the absolute sizes of the genitalia of males and females across species differing greatly in SSD. Genitalic size would scale negatively or hypoallometrically with female size but positively in males, however to our knowledge such a phenomenon is yet unknown. We tested these three models by examining SSD and SGD in a clade of 16 spider species.

Web-building spiders are an excellent model system to investigate SSD because the range from monomorphism to dimorphism is dramatic (e.g., Hormiga et al. 2000; Fig. 1). Phylogenetic analysis implies that extreme SSD has arisen independently in various instances through several different evolutionary pathways, including female size increase and male size decrease (Hormiga et al. 2000). Large size may

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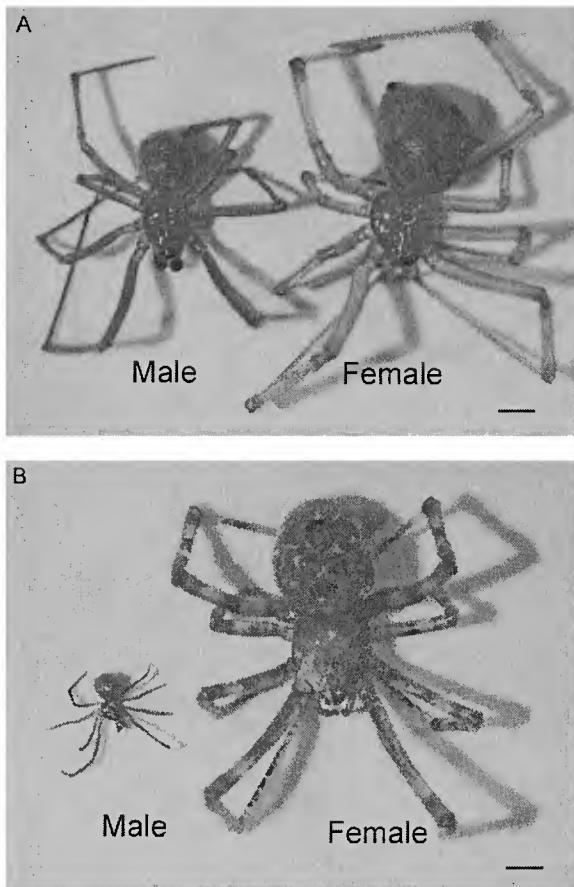


FIG. 1. Male and female (A) *Achaearanea tepidariorum*, a monomorphic species, and (B) *Tidarren sisyphoides*, a dimorphic species. Both females are similar in body size (4.8 vs 5.2 mm in body length, respectively), but the dimorphic *T. sisyphoides* male is substantially smaller compared to the monomorphic *A. tepidariorum* male (1.3 mm vs. 3.5 mm in body length, respectively). Scale 1 mm.

favor females by enhancing fecundity (Head 1995; Coddington et al. 1997; Prenter et al. 1998; Hormiga et al. 2000), and small size may favor males by enhancing their ability to climb while searching for sedentary females (Moya-Laraño et al. 2002), or by accelerating maturation (Ghiselin 1974; Vollrath and Parker 1992). Selection for effective functional coupling should be especially strong in animals whose genitalia are sclerotized and hard, like spiders, because their copulatory organs cannot mold to each other during copulation. Spider sex organs are complex (Foelix 1996) and precisely fit and orient to each other during copulation (Kraus 1984). Long and sometimes convoluted female uptake ducts precede the paired sperm storage sacs, and the male embolus (tip of the sperm-transferring blind duct) must fit snugly to transfer sperm efficiently (Foelix 1996).

We examined aspects of genitalic morphology for 16 araneoid spider species to test the three above models for how genitalia size changes for males and females in relation to SSD. We employed three primary analyses: (1) we tested whether SSD and SGD showed a general pattern of increase with one another and if this relationship was different between monomorphic and dimorphic species; (2) we tested how the absolute value of male and female body size and

genitalia size relate between the sexes across species; (3) we examined the scaling of genitalic morphology within males and within females. Finally, we conduct two other analyses to understand (4) generally the evolution of genitalic shape (as opposed to size) and (5) also to understand how genitalic morphology differs between dimorphic and monomorphic spiders.

MATERIALS AND METHODS

Species examined

We choose 16 araneoid spider species (*Steatoda grossa*, *Latrodectus mactans*, *Achaearanea tepidariorum*, and *Tidarren sisyphoides* [all Theridiidae], *Meta menardi* and *Nephila clavipes* [Tetragnathidae], *Mecynogea lemniscata*, *Cyrtophora moluccensis*, *Gea heptagon*, *Argiope aurantia*, *Mastophora cornigera*, *Hypognatha mozamba*, *Chaetacis cornuta*, *Xylethrus scrupus*, *Metepeira labyrinthica*, and *Kaira aliventer* [all Araneidae]) based on (1) their availability in museum collections (the National Museum of Natural History, Washington, DC, and the American Museum of Natural History, New York), (2) local availability (New Orleans area), and (3) a wide range in SSD (Fig. 2). Each relatively closely related pair of genera includes one dimorphic and one monomorphic taxon, as per Hormiga et al. (2000), and each represents an independent evolutionary event of extreme SSD. Therefore, although the number of species sampled is not large (16), as noted by other authors (e.g., Irschick and Losos 1999), the statistical power for any comparative study is the number of independent evolutionary events, as opposed to the number of taxa sampled. Therefore, our sample should be viewed as a first attempt to understand the broad macroevolutionary relationships between SSD (sexual size dimorphism) and SGD (sexual genitalic dimorphism), and further studies will hopefully reveal the evolutionary relationships between these aspects within more closely related groups (e.g., within spider families). Where available, we measured five to six adult males and females of each species ($n = 138$ total, Table 1), but for some species, fewer specimens were available in museum collections.

Measurement of somatic and genitalic characters

We used maximum cephalothorax width to standardize body size rather than total body length because the latter varies with feeding and reproductive status (Prenter et al. 1999). Nevertheless our primary results hold even if body length is used. We quantified three aspects of the female genitalic structure: epigynal area, epigastric furrow length, and spermatheca volume (see below for definitions). In males, we measured pedipalpal volume (modified copulatory organ) because it most clearly expresses genitalic size.

We photographed dorsal and lateral views of either the right or left pedipalp ($n = 65$), and modeled the bulb as an ellipse to estimate overall pedipalp volume (Fig. 3B). We also added the volume (as cones or boxes, Fig. 3B) of any significantly large structure beyond the bulb itself (e.g. the *N. clavipes* conductor, the *T. sisyphoides* theridiid tegular apophysis, and the median apophyses of *C. cornuta* and *A.*

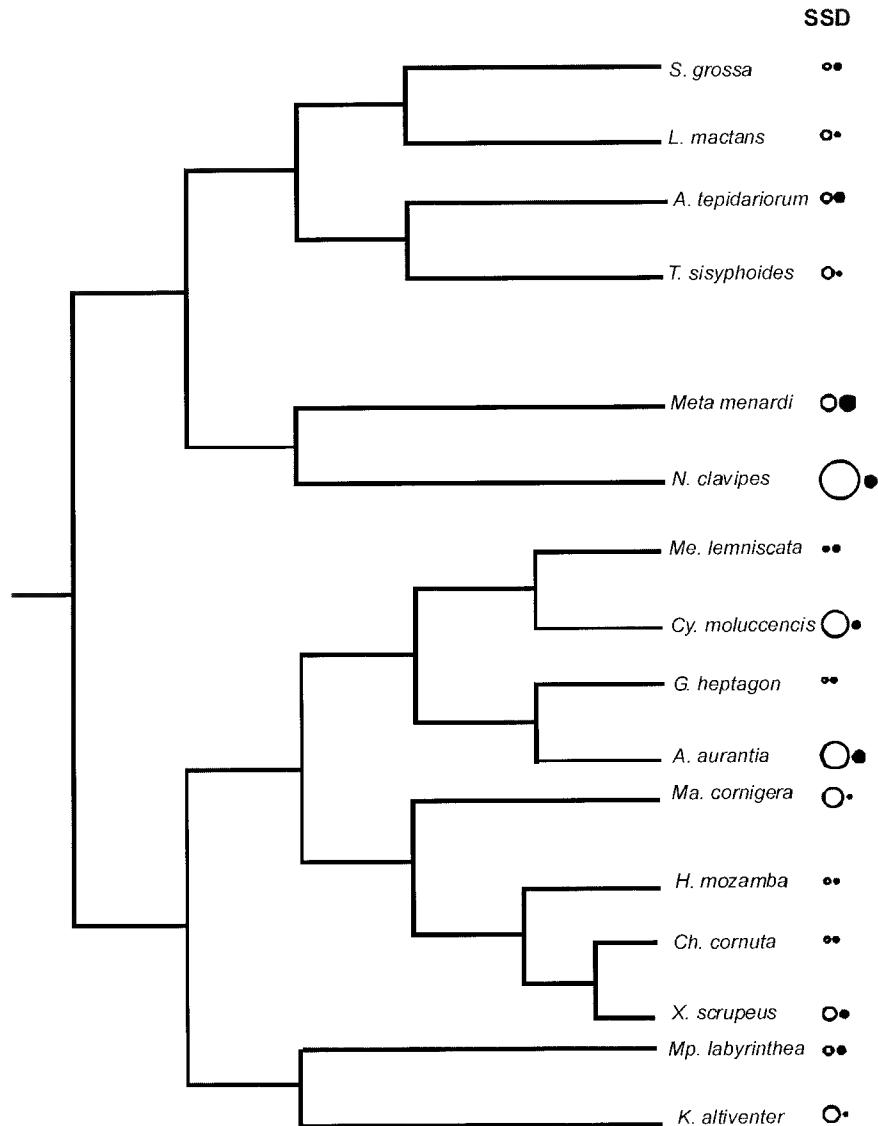


FIG. 2. Phylogeny for the species studied (Hormiga et al. 2000; Arnedo et al. 2004; Agnarsson 2005). Circle diameters are proportional to actual relative cephalothorax size for females (open) and males (filled) in each species based on Table 1.

aurantia) in order to estimate the overall volume of the organ as accurately as possible.

We photographed the female venter ($n = 73$), removed and cleared the epigynum (sclerotized plate modified as an external copulatory organ) with NaOH, and photographed the lateral and dorsal aspects. We then calculated the epigynal area (which contains two uptake tracts to the two sperm storage sacs or spermathecae), epigastric furrow length (which contains the two genital openings and uterus externus or fertilization site, but excludes the length of the lung slits), and the volume of one spermatheca as either a sphere or ellipsoid (Fig. 3C). We treated *L. mactans* as having a cylinder capped by two ellipsoids. We used either a Nikon digital camera DXM 1200 for specimens measured at the National Museum of Natural History, or a QImaging Micropublisher camera (Opelco Elements Corp., Dulles, VA; for specimens measured at the American Museum of Natural History) mounted on a stereoscope. Two to six pictures of each view at different

focal planes were assembled with Auto-montage version 4.01.0034 (Beta Synoptics 2002) to enhance dept of field, and the resulting image was digitized with tpsdig (vers. 1.31).

Phylogenetic analyses

Because taxa are phylogenetically related, one cannot assume statistical independence among data points (Felsenstein 1985). We used independent contrasts as implemented in the phenotypic diversity analysis program (PDAP) (Garland et al. 1999; Garland and Ives 2000) to correct for purely phylogenetic effects on scaling and multivariate relationships between male and female genitalic size and shape. Neglecting phylogeny in interspecific scaling analyses can over- or underestimate scaling coefficients (e.g., Harvey and Pagel 1991; Irschick et al. 1996). The phylogenetic hypothesis used here is identical to, but pruned from, that presented Hormiga et al. (2000). The theridiid nodes *Latrodectus*-*Steatoda*, and

TABLE I. Summary of morphological measurements for species studied. Sister taxa are paired, with the monomorphic species first and the dimorphic species second.

Species	Pair	Cephalothorax width (mm)		Genitalic volume (mm ³)		Epigynal area (mm ²)	Epigastic furrow length (mm)	SSD ^b	SGD ^c vol	
		N ^a	Male	Female	Pedipalp					
<i>S. grossa</i>	1	5/5	1.19 ± 0.04	1.39 ± 0.04	0.0182 ± 0.0016	0.0022 ± 0.0001	0.746 ± 0.058	1.17	0.12	
<i>L. mactans</i>	1	5/5	0.89 ± 0.10	2.04 ± 0.12	0.0531 ± 0.0066	0.0109 ± 0.0008	3.131 ± 0.086	2.30	0.20	
<i>A. tepidariorum</i>	2	5/5	1.92 ± 0.11	1.98 ± 0.06	0.0167 ± 0.0014	0.0085 ± 0.0008	1.612 ± 0.033	1.03	0.51	
<i>T. sisyphoides</i>	2	3/3	0.54 ± 0.00	2.40 ± 0.17	0.0089 ± 0.0009	0.0041 ± 0.0005	1.141 ± 0.004	0.897 ± 0.073	4.43	0.46
<i>Meta menardi</i>	3	5/5	3.07 ± 0.48	3.21 ± 0.43	0.1334 ± 0.0383	0.0020 ± 0.0003	0.698 ± 0.064	2.826 ± 0.138	1.04	0.02
<i>N. clavipes</i>	3	4/6	2.35 ± 0.10	8.31 ± 1.24	0.0636 ± 0.0050	0.0032 ± 0.0025	0.711 ± 0.387	4.594 ± 0.262	3.54	0.51
<i>Me. lemniscata</i>	4	5/5	1.28 ± 0.04	1.09 ± 0.06	0.0780 ± 0.0065	0.0009 ± 0.0001	0.313 ± 0.055	1.677 ± 0.092	0.85	0.01
<i>Cy. moluccensis</i>	4	5/4	1.44 ± 0.14	5.58 ± 1.11	0.1096 ± 0.0775	0.0070 ± 0.0014	0.762 ± 0.112	5.478 ± 0.842	3.87	0.06
<i>G. heptagon</i>	5	5/5	1.00 ± 0.07	1.07 ± 0.04	0.0406 ± 0.0075	0.0033 ± 0.0006	0.164 ± 0.021	1.304 ± 0.050	1.07	0.08
<i>A. aurantiu</i>	5	6/5	2.56 ± 0.09	5.83 ± 0.40	0.0581 ± 0.0067	0.0037 ± 0.0002	2.774 ± 0.170	5.729 ± 0.270	2.28	0.06
<i>H. mozamba</i>	6	5/2	0.88 ± 0.08	1.19 ± 0.02	0.0093 ± 0.0000	0.0001 ± 0.0000	0.097 ± 0.007	1.033 ± 0.038	1.35	0.01
<i>Mu. cornigeru</i>	6	5/5	0.69 ± 0.08	4.23 ± 0.29	0.0029 ± 0.0002	0.0082 ± 0.0009	0.111 ± 0.009	3.711 ± 0.143	6.16	2.80
<i>Ch. cornuta</i>	7	5/2	0.93 ± 0.03	1.13 ± 0.18	0.0076 ± 0.0002	0.0024 ± 0.0002	0.1465 ± 0.005	1.465 ± 0.450	1.21	0.31
<i>X. scripeus</i>	7	1/1	1.30 ± 0.00	2.80 ± 0.00	0.0185 ± 0.0000	0.0262 ± 0.0000	0.103 ± 0.000	9.936 ± 0.000	2.15	1.41
<i>Mp. labyrinthica</i>	8	5/5	1.53 ± 0.16	1.85 ± 0.12	0.0118 ± 0.0019	0.0004 ± 0.0001	0.058 ± 0.007	1.598 ± 0.074	1.21	0.04
<i>K. atliventer</i>	8	4/2	0.62 ± 0.18	3.44 ± 0.72	0.0062 ± 0.0014	0.0098 ± 0.0021	0.088 ± 0.026	2.336 ± 0.345	5.54	1.58

^a Number of individuals measured, females/males.^b Sexual size dimorphism (female cephalothorax width/male cephalothorax width).^c Sexual genitalic dimorphism (spermathecal volume/male pedipalp volume).

Achaearanea-Tidaren were added from Arnedo et al. (2004) and Agnarsson (2005). Both nodes and the monophyly of Theridiidae were strongly supported by both publications. Quantitative support values for various branches in Figure 2 are therefore not provided, but Hormiga et al. (2000) provide such statistics and discuss in detail the relative strengths and weaknesses of the various parts of the tree. The data used to construct the original trees were based on morphological and behavioral characters (except in the case of Arnedo et al. 2004), and therefore, real-number branch lengths are inapplicable. Therefore, we set all branch lengths to 1 in the independent contrasts analyses, which does not unduly affect correlations (Martins and Garland 1991).

Mathematical transformations

For the following analyses, female epigynal area was square-root transformed and male pedipalp volume and female spermatheca volume were each cube-root transformed prior to the implementation of independent contrasts. Therefore both somatic and genital variables are on the same biological scale. In addition, all data were \log_{10} transformed prior to analyses, which is standard in scaling studies.

Statistical analysis

Previous authors have discussed various means of presenting SSD (for a recent review, see Smith 1999). We define SSD as mean female to male cephalothorax width ratio, and SGD as mean female to male genitalic volume ratio (mean spermatheca volume divided by mean pedipalp volume). These simple ratios are straightforward mathematically, and Smith (1999) showed that they behave equivalently to more complex measures (e.g., $\log [SSD]$). However, we recognize that statistical analysis of such ratios is problematic (for a review of this issue, see Smith 1999), and hence only provide values of SSD and SGD, but conduct several other analyses to understand the evolutionary relationships between male and female body size, genitalia size, and genitalia shape, and more generally, test the models outlined in the introduction. All statistical analyses were conducted in SYSTAT (vers. 10, SPSS 2000).

We conducted five primary analyses:

Analysis 1: Evolutionary shifts in SSD and SGD.—We provide values of both SSD and SGD (see below), and also plot these variables against one another without conducting statistical analyses to detect if a general pattern of increase or decrease exists among these variables. In addition, we used a sign ranked test (Møller and Birkhead 1992) to determine whether SSD and SGD change to the same degree in dimorphic species when compared to monomorphic species.

Analysis 2: Evolutionary matching of male and female body size and genitalia size.—Following previous studies that have examined the evolution of size in the context of Rensch's rule (e.g., Fairbairn 1997), we examined both whether male and female body size (cephalothorax width) have co-evolved, and also whether the *absolute size* (as opposed to shape examined in analysis 4) of male and female genitalia have co-evolved. For both comparisons, we regressed male characters (dependent variable) on female characters (independent variable) using major axis regression.

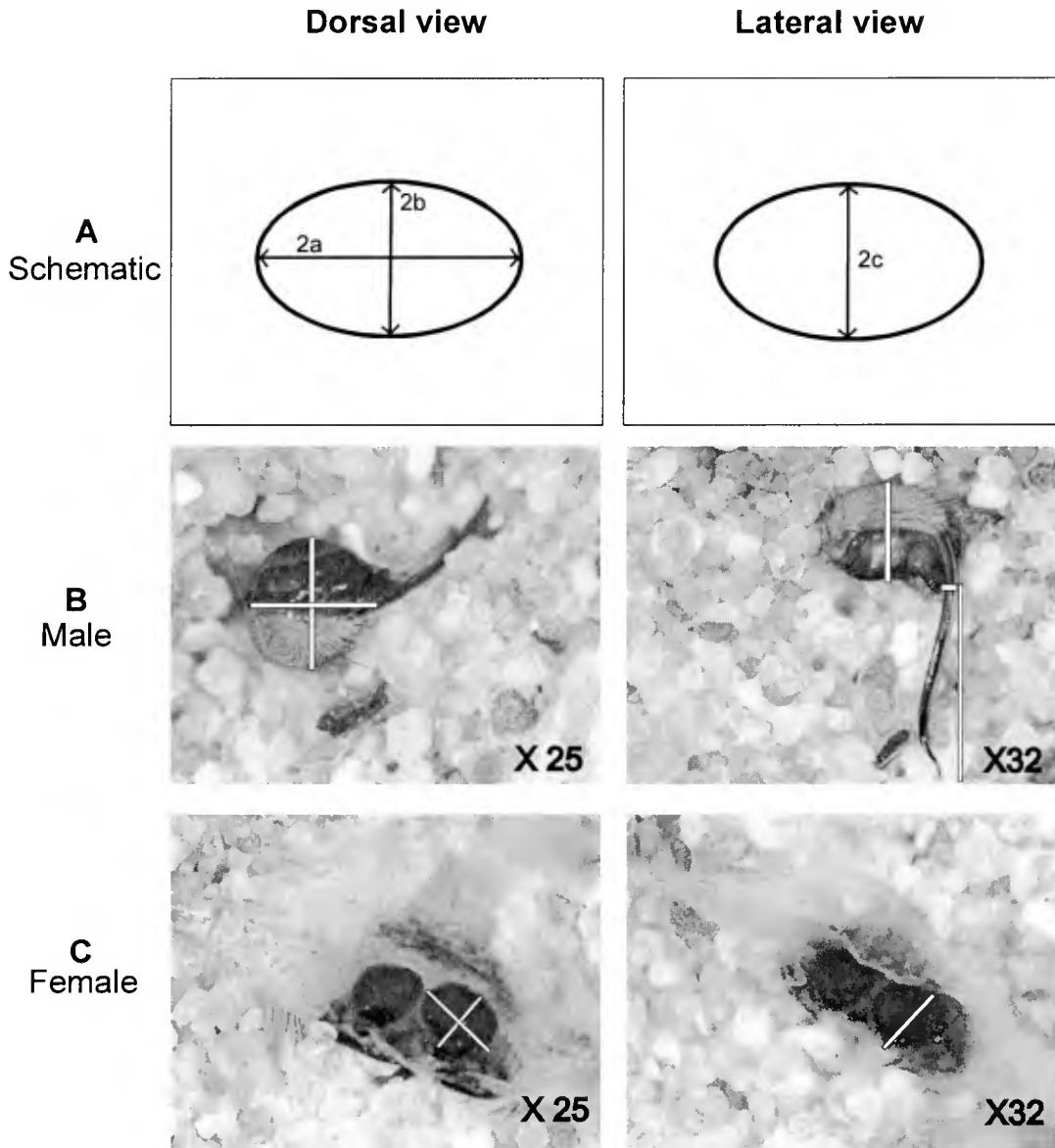


FIG. 3. Orientation and measurements of genital structures. (A) Schematic diagram of dorsal and lateral orientation of the structures (total volume = $4/3\pi$). (B) Pedipalp and (C) spermatheca for male and female *N. clavipes* from dorsal and lateral orientation for measurement. The volume of the long conductor in this species was measured separately from the bulb as a cone (see lateral orientation of pedipalp for measurement, volume = $\pi r^2 \times \text{length}$).

Analysis 3: Scaling of genitalia within males and within females.—We examined the scaling of genitalia size *within* males and *within* females across all species using reduced major axis regression, and tested whether the resulting slopes differed significantly from isometry. Analysis 3 is important in that analysis 2 focuses on the co-evolution of male and female body size and genitalia size, whereas this analysis focuses on how genitalia size changes within males and within females. The expected slope for volume (cube-root transformed), area (square-root transformed), and linear dimensions is one when plotted against the linear dimension of cephalothorax width. We tested isometric versus observed slopes with *t*-tests.

Analysis 4: Multivariate analysis of genitalic characteris-

tics.—We conducted a principal components analysis (PCA) of size-adjusted measures of male and female genitalic morphology to examine how various aspects of genitalic *shape* have co-evolved among species. To remove size effects, we first obtained the independent contrasts of log-transformed values of the four genitalic variables and then residuals by regressing (linear least-squares) the contrasts of each genitalic variable (dependent) against log-transformed cephalothorax width (independent). All regressions using independent contrasts were forced through the origin (Garland et al. 1992). We used these phylogeny and size-free genitalic variables in the PCA. We used only PCs with eigenvalues >1.0 as an approximate criterion of statistical significance (Jackson 1993). Because the PCA used independent contrasts, cor-

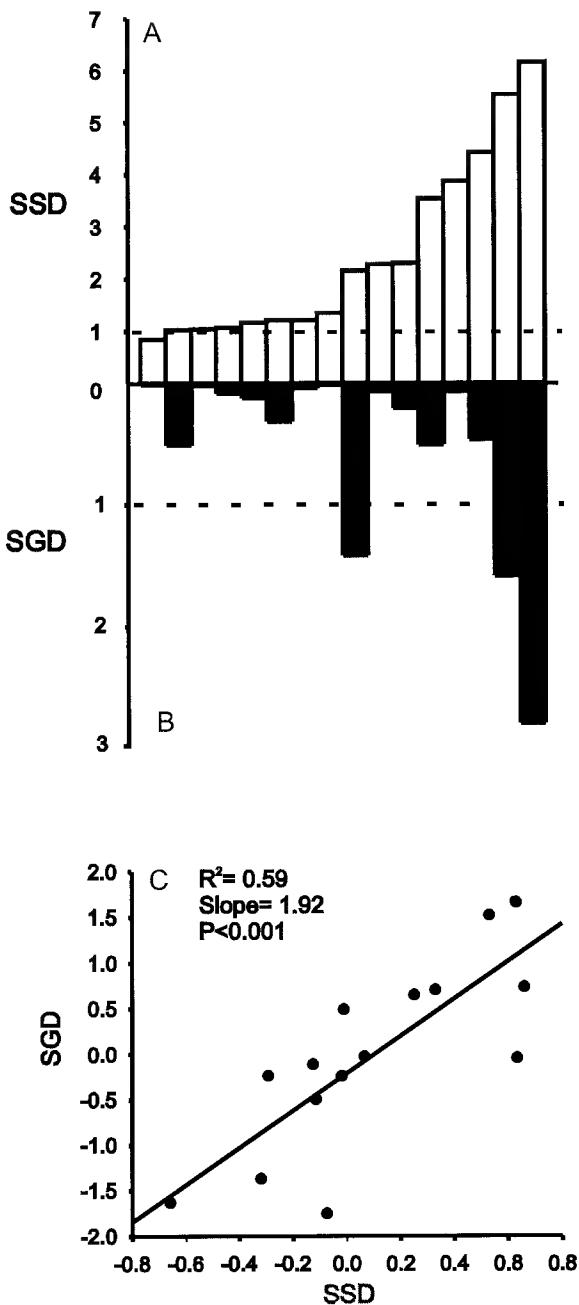


FIG. 4. (A) Plot of SSD values (Table 1) from low (left) to high ratios (right). (B) Plot of SGD (spermathecal volume/pedipalp volume) for each of the 16 species in plot A. (C) SGD generally increases with SSD. SGD reverses from much less than 1.0 (see dotted line) in monomorphic species to almost 3 in highly dimorphic species.

relations among variables are interpretable as evolutionary change.

Analysis 5.—Comparison of genitalic morphology between monomorphic and dimorphic species. We conducted *t*-tests comparing monomorphic and dimorphic pairs for all genitalic characters measured (female spermatheca volume, female epigynal area, female epigastric furrow length, and male pedipalp volume).

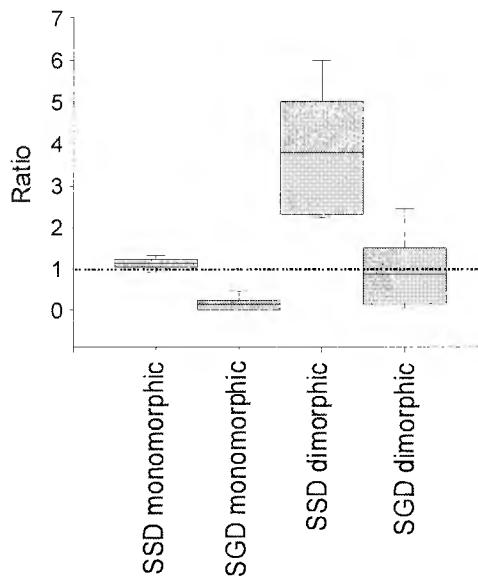


FIG. 5. A box plot comparing the relative changes in SSD and SGD in monomorphic ($n = 8$) versus dimorphic ($n = 8$) species. Note the much larger increase in SSD than in SGD in the dimorphic group. The higher variance in the dimorphic species corresponds to a wider range in body sizes in this group. The bottom of the box represents the 25th percentile, the solid line represents the mean, the top of the box the 75th percentile, and the whiskers the 5th and 95th percentile. The dotted line represents no dimorphism.

RESULTS

Analysis 1.—By design, the 16 spider species varied dramatically in SSD (sexual size dimorphism) (Table 1, Fig. 2), but also in both measures of SGD (sexual genitalic dimorphism) (Table 1). SGD generally increases with SSD across species (see Fig. 4), and therefore the relative proportions of male to female genitalia dramatically reverse across the x-axis (see Fig. 4). In monomorphic species (e.g., *Me. lemniscata*) male genitalia are relatively large compared to females (either as epigynum area or spermatheca volume), whereas in strongly size-dimorphic species (e.g., *Ma. cornigera*), female genitalia are relatively larger than males (Table 1). Male *Me. lemniscata* are actually slightly larger than females (SSD = 0.85), with genitalic volumes about 87 times greater than females (SGD = 0.01). At the other extreme, *Ma. cornigera* is strongly size dimorphic (SSD = 6.16), and each female spermatheca is about three times the volume of a male pedipalp (SGD = 2.80). In addition, the difference in SSD between monomorphic and dimorphic species in our sample is much larger than their differences in SGD ($P < 0.05$) (Fig. 5), suggesting that SSD increases at a faster rate than SGD.

Analysis 2.—Across all species, male and female size were not significantly correlated, regardless of the regression technique used ($n = 15$ contrasts, MA regression, slope = 1.45 ± 0.40 [SE], $P > 0.25$; linear least-squares regression, $r^2 = 0.07$, $P > 0.25$, Fig. 5). Similarly, male and female genitalia size were not significantly related among species regardless of the regression technique used (MA regression, slope = 1.98 ± 0.20 , $P > 0.25$; linear least-square regression, $r^2 = 0.04$, $P > 0.40$, Fig. 6).

Analysis 3.—The scaling analyses showed several trends.

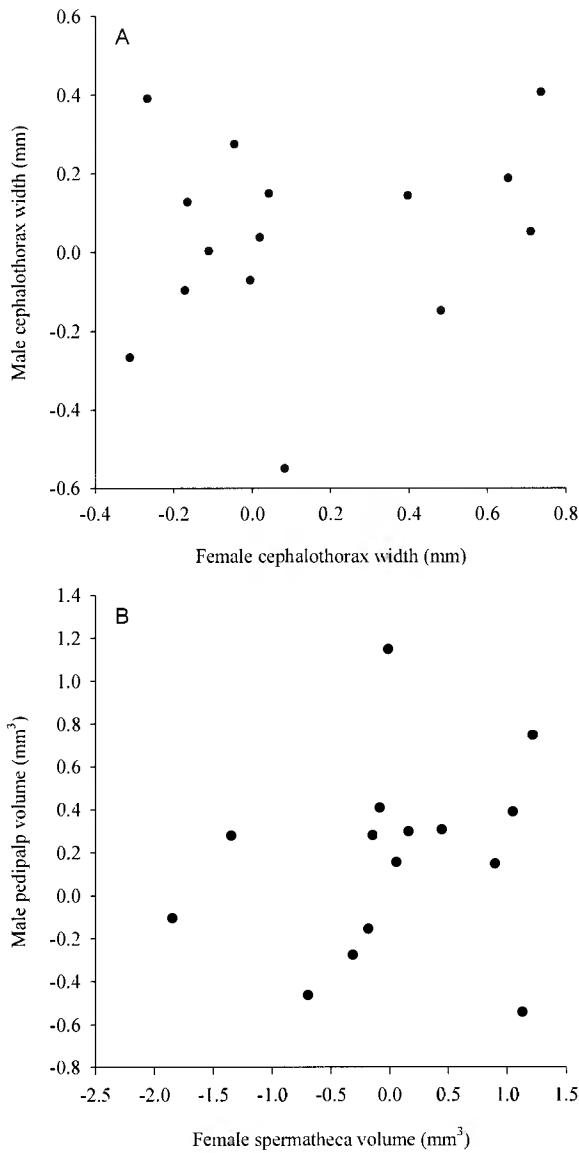


FIG. 6. Scatterplots of (A) independent contrasts of female versus male cephalothorax width, and (B) independent contrasts of female versus male genitalia size (spermathecal volume [females], and pedipalp volume [males], respectively). Both relationships are nonsignificant (see text for statistics), and indicate an evolutionary mismatch between the absolute sizes of the genitalia of female and male spiders, supporting the "positive genitalic divergence model."

First, male (slope = 1.10) and female (slope = 0.81) body length scaled approximately isometrically with cephalothorax width and neither slope differed significantly from the expected value of 1.0 (Table 2). Second, all genitalic characters measured correlated positively and significantly with cephalothorax width, although all with slopes significantly lower than isometry (Table 2): 0.52 for male pedipalpal volume, 0.46 for female spermathecal volume, and 0.52 for female epigastric furrow length. In both sexes, genitalic features scale with pronounced hypoallometry.

Analysis 4.—Principal components analysis of the size-adjusted genitalic variables revealed two statistically significant PCs explaining 44.8% and 32.0% of interspecific var-

TABLE 2. Scaling of genitalic for both males and females (based on cephalothorax width as the independent variable) using independent contrasts ($n = 15$ for all comparisons). All regressions (MA) were forced through the origin. Expected slopes are 1 for each test.

Dependent variable	Observed slope	SE	r^2	T ¹
Body length (males)	1.10	0.16	0.77***	0.56 ns
Body length (females)	0.81	0.11	0.79***	1.80 ns
Pedipalp volume	0.52	0.14	0.49*	3.43**
Spermathecal volume	0.46	0.16	0.38*	3.38**
Epigynal area	0.52	0.22	0.28*	2.18*
Epigastric furrow length	0.69	0.09	0.82***	3.44**

¹ t-test comparing observed versus expected slope.

* $P < 0.05$, ** $P < 0.005$, *** $P < 0.001$.

iation, respectively (Table 3). PC 1 shows that female epigynal area positively correlates with both female epigastric furrow length and male pedipalp volume, but female spermathecal volume showed a low loading (0.206). Thus, although epigynal area and epigastric furrow length are highly correlated in female spiders, large values of these variables do not necessarily imply large spermathecal volume. Further, spider species with large female epigynal area and long female epigastric furrow length tend to have relatively large male pedipalps. The second PC showed high, negative loadings for female spermathecal volume and male pedipalp volume (Table 3). Thus, species with large female spermathecal volume tend to have large male pedipalpal volume. Thus, female variables are decoupled evolutionarily (epigynum area/furrow length vs. spermathecal volume), but both positively correlate with male pedipalp volume.

Analysis 5.—Monomorphic and dimorphic pairs differed significantly in two aspects of genitalic morphology (female spermatheca volume, t -value = 5.49, $df = 7$, $P < 0.0025$; epigynal area, t -value = 2.93, $P < 0.025$), and did not differ significantly in two other aspects (female epigastric furrow length, t -value = 1.09, $df = 7$, $P > 0.25$; pedipalp volume, t -value = 0.81, $df = 7$, $P > 0.40$). Both spermatheca volume and epigynal area in females were generally larger in dimorphic compared to monomorphic species.

DISCUSSION

Based on a small but broad sample of araneoid spider species varying greatly in SSD, we have shown several striking trends. (1) SGD showed a general pattern of increase with SSD among species, although these two variables do not change at the same rate (Figs. 4, 5, 7). (2) Male and female body size, as well as male and female genitalia size, are

TABLE 3. Loadings from a principal components analysis of four size-adjusted morphological genitalic variables based on independent contrasts for 16 species of web-weaving spiders.

Variable	PC 1	PC 2
Epigynal area	0.913	0.264
Epigastric furrow length	0.706	0.445
Female spermatheca volume	0.206	-0.806
Male pedipalp volume	0.647	-0.602
Eigenvalue	1.79	1.28

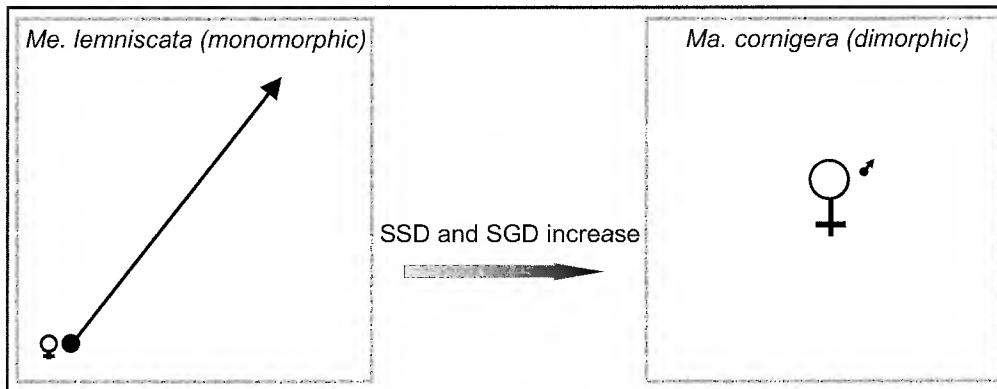


FIG. 7. A proposed evolutionary model for how SGD increases as SSD increases. The length of the relevant lines in the female and male symbols represents the relative volume of genitalia (scale to $\times 500$) at the extremes of the range of SSD (based on the species *Me. lemniscata* [monomorphic] and *Ma. cornigera* [dimorphic]). Similarly, the relative sizes of the circles in each of the male and female symbols represents the relative actual body sizes of males and females in these two species. However, the relative size of the lines in the female and male symbols (representing genitalic sizes) are not in proportion to the sizes of the circles (representing body sizes). Note how SGD reverses from male genitalia being larger than female genitalia in the monomorphic species to female genitalia being larger in the dimorphic species.

decoupled evolutionarily (Fig. 6, 7, Table 3). This latter finding is particularly striking: In other words, the absolute size of female genitalia for a given species is not a significant predictor of the size of male genitalia for that same species. (3) Within males and within females, genitalic morphology scales negatively; three of the four genitalic variables (male pedipalp volume, female spermathecal volume, and female epigastric furrow length) scale substantially lower than expected isometric slopes (Table 2). (4) For genitalic shape (i.e., size-adjusted analysis), we found that female genitalic characteristics are evolutionarily decoupled from one another, but show an evolutionary correlation with male genitalic volume (Table 3). (5) Both female spermatheca volume and female epigynal area were generally larger in dimorphic compared to monomorphic species (Figs. 5, 7, Table 1).

Our findings support the “positive genitalic divergence” model which posits that as females and males diverge in size, they will also diverge in the absolute dimensions of their genitalia, resulting in a “mismatch” of male and female genitalia size. Thus, our results suggest that extreme size divergence in genitalia could impair effective mating, especially in species with hard or sclerotized genitalia. The “positive genitalic divergence” model generally implies that SGD can constrain SSD in animal species (see Fig. 7).

In our sample, female and male body size appear to be decoupled in evolutionary time. We suggest that the lack of co-evolution of male and female size is a consequence of our broad macroevolutionary comparisons of spider species varying dramatically in SSD, in contrast to previous studies (e.g., Prenter et al. 1998) which show co-evolution between male and female body size among spider species that diverge less in SSD. By contrast, there is little correspondence between male and female body size within highly dimorphic species (e.g., Head 1995). The nonsignificant correlation between the absolute sizes and male and female genitalia also suggests a general evolutionary mismatch between male and female spiders varying greatly in SSD, thus supporting the “positive genitalic divergence” model.

Some key size components of the female genitalic struc-

tures appear to be largely decoupled in evolutionary time. Although relative epigynum area and epigastric furrow length were correlated with each other, neither variable was correlated with spermathecal volume. Epigynal area and epigastric furrow length (external female genitalia), which may be characteristics related to genital coupling or stimulation, respond to different selective pressures than spermatheca volume (internal female genitalia), which strictly concerns sperm storage capacity. As female spiders appear efficient in the activation and use of sperm for fertilization (Berendonck and Greven 2002) and may not be sperm limited (Snow and Andrade 2005), spermathecal volumes need not be large. Selection might act to limit the size of organs such that they show a functional fit. Large female epigyna relative to small female spermathecae may reflect stimulatory interactions between males and females during copulation. In contrast, male genitalic shape (relative pedipalp volume) tracks both female epigynum area/epigastric furrow length and female spermatheca volume. Large male pedipalps are probably more efficient at engaging and/or interacting with large female epigyna, and filling large female spermathecae.

Rapid and divergent evolution of genitalia is widespread among animals but its causes remain poorly understood (Eberhard 1985; Arnqvist 1998; Arnqvist and Danielsson 1999; Hosken and Stockley 2004). The “lock-and-key” hypothesis is controversial (Eberhard 1985; Ware and Opell 1989), particularly for explaining interspecific evolutionary relationships, but may still hold value for understanding trends within species. Leading alternative hypotheses include-cryptic female choice (Eberhard 1985, 1996) and sexual conflict (for a recent review, see Hosken and Stockley 2004). In spiders, cryptic female choice might be particularly important. Copulation is complex both behaviorally and mechanically, and release of sperm is not necessarily linear in relation to copulatory duration (Bukowski and Christenson 1997; Snow and Andrade 2005). Copulation prior to insemination can be lengthy (van Helsdingen 1965; Elgar 1998) and stimulation during copulation influences uptake of sperm (Bukowski and Christenson 1997). We agree with Eberhard

(2004) that sexual conflict related to natural selection and female fecundity should be rare in spiders, other things being equal, because the large size and cannibalistic tendencies of the female should overrule the effects of male manipulation. We note that the large size of females would not necessarily preclude chemical manipulations by males, however. The species most prone to sexual conflict would be those in which the female has a much larger genitalic volume than a single male can fill. Females would therefore benefit from multiple copulations, whereas the male would always try to monopolize the female. However, according to our data, these are the species with greatest SSD and in which the size advantages of the female are most pronounced.

Intraspecific genitalic size generally scales with negative allometry (also termed hypoallometry) in insects and spiders (e.g., Cohn 1990; Eberhard et al. 1998; Schmitz et al. 2000, but see Pérez-Miles 1988; Green 1999). We found the same pattern interspecifically (Table 2), implying a lower evolutionary size increase in genitalic dimensions than isometry would predict. Considering the high variability observed in the genitalic structure in both males and females, even in closely related species (Eberhard 1985; Pitnick et al. 1999; Hosken and Stockley 2004), it is remarkable to detect decreased variability in genitalic size. The most obvious hypothesis to explain this hypoallometry is that isometry would result in functional inefficiencies during mating. If true, stabilizing selection may even level or equate genitalia size within a lineage.

Despite this hypoallometry within each sex, it is intriguing that SGD nevertheless changes so dramatically across species (Figs. 4, 6), and that male and female genitalia are partly decoupled. We suggest the underlying reason for these patterns is that female spiders more in body size than males on a broad macroevolutionary scale. For example, in the sample of 536 species from Hormiga et al. (2000), female body length varied from 1.6 to 45 mm (28 \times), whereas males ranged from 1.4 to 17.5 mm (13 \times). Our smaller (and less random) sample shows about 25% greater variation in female size compared to males. Spiders are an exception to Rensch's rule (i.e., SSD increases with body size in species in which males are larger, and decreases with body size in species in which females are larger) as female body size diverges more over evolutionary time than male body size, and SSD increases with female body size (Head 1995; Prenter et al. 1995, 1997, 1998; but see Prenter et al. 1999). This often occurs in species in which the female is the larger sex (Fairbairn 1997). All genitalic variables increase significantly with size (Table 3), and as female size varies much more than in males, female genitalic size also outpaces that of males, but not as much as isometry would predict. Evolutionary forces that stabilize genitalic size may balance sex-specific size-determining factors, such as increased fecundity or enhanced mate-searching ability (e.g., Vollrath 1980; Head 1995; Prenter et al. 1998; Moya-Laraño et al. 2002). Although we did not observe any evidence for the constant genitalic ratio model, such a ratio might apply to the components of male and female genitalia that interact during mating, thus scaling differently than gross genitalic volume. Individual species could be observed during courtship and copulation to identify the appropriate genitalic structures.

The dramatic shift in SGD with SSD increase may influence sperm competition in all animals where SSD is extreme. Previous work on sperm competition has focused on the size of male testes in relation to sperm competition, or the likelihood that females will repeatedly mate (Short 1979; Birkhead and Möller 1992; Hartcourt et al. 1995b; Stockley et al. 1997). In male spiders, the gonads (abdominal) are disconnected from the two copulatory organs or pedipalps (modified appendages). Sperm must be transferred to the exterior of the body and then picked up with the pedipalps for successful insemination. Thus, the amount of sperm a pedipalp contains at any given time is limited. If one assumes that sperm storage capacity correlates with male pedipalpal volume, then the relatively large pedipalpal volumes in monomorphic species may signal stronger sperm competition. This assumption might not be entirely valid, because in some species there is not a 1:1 correlation between pedipalp volume and male sperm storage capacity. Several authors have suggested that there may be preconditions which intensify sperm competition in spiders (Austad 1984; Elgar 1998), such as polyandry, and time delays between copulation and fertilization. Our data suggest that the relative volumes of male and female reproductive organs may also influence sperm competition. In monomorphic species, one male may fill a female's spermathecae, whereas in dimorphic species one male would be incapable of filling her spermathecae during a single mating. If the positive genitalic divergence model is correct, females of dimorphic species in general should mate with more males than females of monomorphic species. This prediction suggests that evolutionary changes in SGD as a consequence of SSD may help to explain variation in animal social and mating systems.

Finally, evolutionary changes in the relationship between SGD and SSD clearly has functional consequences. The spider *Tidarren sisypoides* is highly size dimorphic (the male is 1% of the total female body weight) and male palps are large enough (about 10% of their total body weight) that they substantially hinder locomotion (Ramos et al. 2004). Such large pedipalps may be maintained because they ensure a fit to the much larger female copulatory apparatus (Vollrath 1998). If so, these spiders appear to have resolved the conflict by self-amputating one of their two overly large pedipalps, and thus considerably enhancing both maximum speed and endurance (Ramos et al. 2004). In sum, different and even opposing scaling trends in sexual genitalic and size dimorphism could have profound consequences for reproduction and life history of spiders, and perhaps other taxa as well. Further research that examines whether genitalic size changes in a similar manner for males and females in other taxa with high SSD might shed light on the generality of the positive genitalic divergence model. Finally, we note that further comparisons of both the body sizes and genitalia sizes of spider species would also be informative for testing Rensch's rule (Fairbairn 1997), which we were unable to consider because of our relatively small sample of spiders.

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