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Geometric morphometrics reveals sex-differential shape allometry in a spider

Carmen Fernández-Montraveta¹ and Jesús Marugán-Lobón^{2,3}¹ Departamento de Psicología Biológica y de la Salud, Universidad Autónoma de Madrid, Madrid, Spain² Departamento de Biología, Universidad Autónoma de Madrid, Madrid, Spain³ Dinosaur Institute, Natural History Museum of Los Angeles County, Los Angeles, CA, USA

ABSTRACT

Common scientific wisdom assumes that spider sexual dimorphism (SD) mostly results from sexual selection operating on males. However, testing predictions from this hypothesis, particularly male size hyperallometry, has been restricted by methodological constraints. Here, using geometric morphometrics (GMM) we studied for the first time sex-differential shape allometry in a spider (*Donacosa merlini*, Araneae: Lycosidae) known to exhibit the reverse pattern (i.e., male-biased) of spider sexual size dimorphism. GMM reveals previously undetected sex-differential shape allometry and sex-related shape differences that are size independent (i.e., associated to the y-intercept, and not to size scaling). Sexual shape dimorphism affects both the relative carapace-to-opisthosoma size and the carapace geometry, arguably resulting from sex differences in both reproductive roles (female egg load and male competition) and life styles (wandering males and burrowing females). Our results demonstrate that body portions may vary modularly in response to different selection pressures, giving rise to sex differences in shape, which reconciles previously considered mutually exclusive interpretations about the origins of spider SD.

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Corresponding author

Carmen Fernández-Montraveta,
carmen.montraveta@uam.es

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INTRODUCTION

Sexual dimorphism (SD), defined as size or morphological differences between the sexes (*Hedrick & Temeles, 1989*) is a large source of phenotypic variation in animals. As a relevant topic in evolutionary biology ever since Darwin (*Lande, 1980; Lande & Arnold, 1985; Hedrick & Temeles, 1989; Fairbairn, Blanckenhorn & Szekely, 2007*), the evolution of SD is known to result from genetic correlations between the sexes and sex-differential selection pressures, particularly natural selection related to reproductive sex roles or life styles and sexual selection, related to mate competition (*Lande, 1980; Lande & Arnold, 1985; Hedrick & Temeles, 1989; Cox, Skelly & John-Alder, 2003; Foellmer & Fairbairn, 2005a; Fairbairn, Blanckenhorn & Szekely, 2007*). Hypotheses testing, however, remains controversial, as the aforementioned mechanisms are not necessarily mutually exclusive and several mechanisms may be operating at the same time (*Hedrick & Temeles, 1989*).

As unique systems in the analysis of the ultimate causes of SD (Foellmer & Moya-Laraño, 2007), spiders are a widely featured model system in this controversy. Overall, spiders follow the arthropod female-biased pattern of sex differences in size, but spider sexual size dimorphism (SSD) actually spans from moderately male-biased (Alderweireldt & Jocqué, 1991a; Schutz & Taborsky, 2003; Aisenberg, Viera & Costa, 2007) to the most extremely female-biased terrestrial patterns (Foellmer & Fairbairn, 2005a; Vollrath, 1998). However, the prevalent explanations about the evolutionary origins of spider SD focus on a single mechanism, namely sexual competition among males (Foellmer & Moya-Laraño, 2007). Male size usually predicts the outcome of male–male interactions (Elias et al., 2008; McGinley, Prenter & Taylor, 2015), and males are under directional selection for overall larger size even in extremely female-biased spider taxa (Foellmer & Fairbairn, 2005a). As far as interference competition relaxes when population densities are low, selection may yet favor male morphologies best advantageous at scramble competition (Foellmer & Moya-Laraño, 2007). For instance, relatively smaller males are better at climbing to reach females (Moya-Laraño, Halaj & Wise, 2002), whereas spider SSD is more accentuated when male mortality increases because of the sex-differential costs of travelling in search for females (Elgar, 1991; Vollrath & Parker, 1992; Walker & Rypstra, 2001; Moya-Laraño, Halaj & Wise, 2002; Walker & Rypstra, 2003; Brandt & Andrade, 2007). However, spider SSD fails to follow a common trend of phenotypic variation resulting from sexual selection, namely the higher variation of male size over evolutionary times. This trend would lead to a reduced SSD the larger the body size in such a female-biased taxon (i.e., Rensch's Rule (Rensch, 1959)). However, spider female size tends to vary more than male's within and across taxa (Abouheif & Fairbairn, 1997), which might be consistent with a prevalent role of fecundity selection in the evolution of spider SSD (Head, 1995; Coddington, Hormiga & Scharff, 1997).

Selection pressures acting differently on particular male and female body parts are likely to produce different patterns of allometric growth (Zelditch, Swiderski & Sheets, 2012), leading to sex differences in shape and sexual shape dimorphism (Fairbairn, 2007; Szekely, 2007). Furthermore, sexually selected traits have been long suspected to show positive static allometry, as larger individuals are expected to exhibit disproportionately larger traits (Kodric-Brown, Sibly & Brown, 2006), and sex-differential allometric variation could hence indicate a role for sexual selection in the evolution of particular traits. However, the analysis of spider shape and shape allometry has not attracted much attention, likely because of methodological constraints. For instance, as in many other groups (Stuart-Fox, 2009; Cabrera, Scrocchi & Cruz, 2013; Roitberg et al., 2015), spider SSD is usually analyzed either from single linear body measurements (Jakob, Marshall & Uetz, 1996; Aisenberg, Viera & Costa, 2007; Aisenberg et al., 2010) or from linear combinations of them obtained from multivariate statistical techniques, essentially principal components analysis (PCA) (Prenter, Montgomery & Elwood, 1995; Foellmer & Fairbairn, 2005a, 2005b), which is limited to describe shape as residual deviations from size (Mosimann, 1970). Today, landmark-based geometric morphometrics (GMM) stands among the suite of new analytical tools which have

been successfully applied to the analysis of sexual shape dimorphism (O'Higgins & Collard, 2002; Pretorius, Steyn & Scholtz, 2006; Kaliontzopoulou, Carretero & Llorente, 2007; Bonnan, Farlow & Masters, 2008; Benítez, 2013). In spite of the great potential of GMM for the evaluation of shape and sex-differential shape allometry, however, the method has not been ever applied to the study of spider sexual shape dimorphism and shape allometry, with the exception of studies on shape variation in key characters of spider taxonomy, such as genital structures (Crews, 2006; Costa-Schmidt & de Araujo, 2010).

Here, we applied GMM to the analysis of body shape allometry and sexual shape dimorphism in a wolf spider (*Donacosa merlini* Alderweireldt & Jocqué, 1991a, Araneae, Lycosidae), known to unambiguously exhibit male-biased SSD on the basis of traditional morphometrics (Alderweireldt & Jocqué, 1991b). We measured spider size and shape at several locations, covering the whole range of habitat variation in the known species distribution area. *D. merlini* shows sex differences in reproductive roles and life styles; mature males are the roving sex and search for mates, whereas females are obligate burrowers during their whole lives and remain inside their burrows for cocoon production and spiderling hatching, caring for cocoons and spiderlings before dispersal. Particular body parts may relate differentially to male and female fitness: spider opisthosoma structures relate to vegetative tasks, including reproduction (Foelix, 1996), and larger opisthosomas enable increased energy and egg storage and production. Therefore, fecundity selection should tend to favor the evolution of female-biased opisthosomas or total body length (Preziosi et al., 1996; Head, 1995). On the contrary, spider prosoma structures relate to locomotion, food intake and integrative nervous functions (Foelix, 1996), and arguably larger carapaces could improve resource holding potential, trophic efficiency or spider mobility. If natural selection drives the evolution of SD, we expected sex differences in the allometric growth of prosoma and opisthosoma structures (relatively larger female opisthosomas and more robust male prosomas), leading to size-independent sexual shape dimorphism. Moreover, if sexual selection drives the evolution of sexual shape differences, we expected sex-differential carapace shape allometry.

METHODS

The species

Donacosa merlini is a burrowing, medium-sized wolf spider the distribution of which is restricted to the sandy coastal areas surrounding Doñana in Southwestern Spain, where it inhabits a variety of habitats, ranging from grasslands to xerophytic scrublands (Fernández-Montraveta & Cuadrado, 2008). *D. merlini* is the only known worldwide representative of the genus (Alderweireldt & Jocqué, 1991a, 1991b), and exhibits singular life history traits: mating is delayed compared to closely related wolf spiders and takes place during the late autumn; males mature later than females and reach larger maturation sizes. Spiderlings hatch in spring and mature after a relatively long post-embryonic development of 15–20 months under laboratory conditions (Fernández-Montraveta, Moya-Laraño & Cuadrado, 2004). The species is semelparous,

and females exhibit maternal care; cocoon and spiderling carrying is mostly restricted to the female burrow ([Fernández-Montraveta & Cuadrado, 2008](#)).

Experimental setup

During the mating season, we captured mature spiders at four different field sites representing almost the entire range of habitats at which *D. merlini* is known to occur in the Doñana area (field project number 022/2007). At this time of the year, only mature females and juveniles are found inside burrows; we captured females by hand following burrow excavation during the day (10:00–17:00 hours) and males after the sunset (19:00–21:00 hours) during mate searching, using headlamps. We only included mature animals in the study, hence returning immature spiders back to their burrows immediately after instar determination by visual inspection. Following capture, we transported spiders to the laboratory for individual measurements. We measured spiders from dorsal pictures of living individuals (Olympus E-300 digital camera); for the pictures, we immobilized spiders inside a plastic bag placed on top of a scaled paper, to avoid any motion and prevent individual damage. To standardize the spider position, we always oriented the animal horizontally and facing to the right. We returned spiders to the field on the day following capture; to reduce predation risk, we placed females back to their reconstructed burrows and males in the vegetation. Finally, to minimize any negative effect of manipulation on populations of this singular and protected species ([Verdú & Galante, 2005](#); [Barea-Azcón, Ballesteros-Duperón & Moreno, 2008](#)), we prevented any accidental damage of burrows by marking them and removing all marks when the fieldwork was over. Overall, we captured and measured 178 spiders (97 males and 81 females).

On each spider picture, we digitized 37 bidimensional landmarks for shape analysis. The spider's anterior body portion (i.e., prosoma) is covered by two firm plates (dorsal carapace and ventral sternum) and only expands at molting, whereas the posterior opisthosoma is comparatively softer and expands as the spider stores nutrients or develops eggs ([Foelix, 1996](#)). On the spider's carapace, we digitized 17 landmarks and 20 along the outline of the opisthosoma ([Fig. 1](#)). The carapace landmarks are all optimal (Type I) ([Bookstein, 1991](#)), as their spatial positions are defined on the basis of highly repeatable and unambiguous anatomical locations (i.e., the places at which legs, pedipalps or chelicerae insert at the carapace; see [Fig. 1](#)). We spaced the points regularly along the opisthosoma outline, and therefore treated these opisthosoma coordinates as semilandmarks ([Zelditch, Swiderski & Sheets, 2012](#)).

To obtain the shape data, landmark configurations were superimposed using the generalized Procrustes method, also known as general Procrustes analysis ([Adams, Rohlf & Slice, 2004](#)), based on a generalized least-squares minimization of the distance between corresponding landmarks ([Gower, 1975](#)). Landmark configurations are compared by this superimposition, which is achieved by translating, rotating and scaling all configurations to a common reference system (the mean). Shape in this context is the residual mismatch and irreducible distance among homologous landmarks after the complete Procrustes alignment, and is thus “invariant” to (i.e., it does not possess any information about) translation, rotation and scale. To treat the opisthosoma

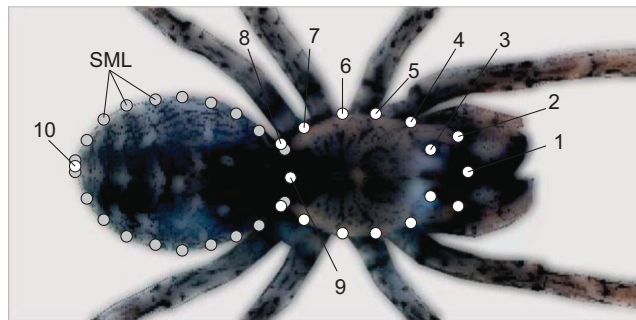


Figure 1 Landmarks (white) and semilandmarks (gray) exemplified on a spider picture.

semilandmarks, we used the minimum Procrustes distance sliding method (*Bookstein, 1997*), better suited for relatively simple outlines such as those of the opisthosoma at intraspecific levels (*Pérez, Bernal & González, 2006*). Following the Procrustes fit, we used PCA to summarize the sample shape variation into fewer components. As a proxy for size we used the centroid size of the landmark configurations, which corresponds to the squared root of the sum of the squared distances from each landmark to the centroid (*Bookstein, 1991*). We calculated two separate shape and size datasets, one including the whole set of landmarks and semilandmarks (i.e., both the carapace and the opisthosoma) which thereby accounted for overall body size and shape, and another excluding the opisthosoma semilandmarks, thereby only capturing the carapace size and shape.

We used the TPSrelw program (v.1.49; *Rohlf, 2010*) to slide the semilandmarks and MorphoJ (v. 1.6.0_27, (*Klingenberg, 2011*)) to perform all subsequent analyses, including landmark superimposition. MorphoJ allows isolating a component of shape that only accounts for symmetric variation out of a bilaterally symmetric configuration of landmarks (*Mardia, Bookstein & Moreton, 2000; Klingenberg, Barluenga & Meyer, 2002*). The method, informally called “symmetrization”, yields a component of shape variation among individuals in what might be considered a left-right averaging (*Klingenberg, Barluenga & Meyer, 2002; Savriama et al., 2012*). This helps ignoring any source of variation within the sample due to asymmetry, thus reducing the small yet potential error introduced by, for instance, measuring immobilised animals with a globular morphology and bilateral symmetry such as spiders. Shape allometry was studied using multivariate regression analyses (*Monteiro, 1999*) based on the pooled within group variance, to control for the effect of sexual size differences on shape variation. For further comparisons between the sexes, we used a discriminant function analysis. For traditional size comparisons, we applied general linear models (sex as the fixed factor) on body size and carapace size. We applied multivariate regressions of the shape variables on centroid size to analyze body and prosoma shape allometry. As a method for verification, such allometric models were compared using univariate general linear models on the obtained regression shape scores, including sex as a fixed factor and size as the covariate (significant sex×centroid size interaction would indicate that the two allometric slopes differ; *Klingenberg, 2016*). We used R (*R Core Team, 2017*) for the standard statistical analyses.

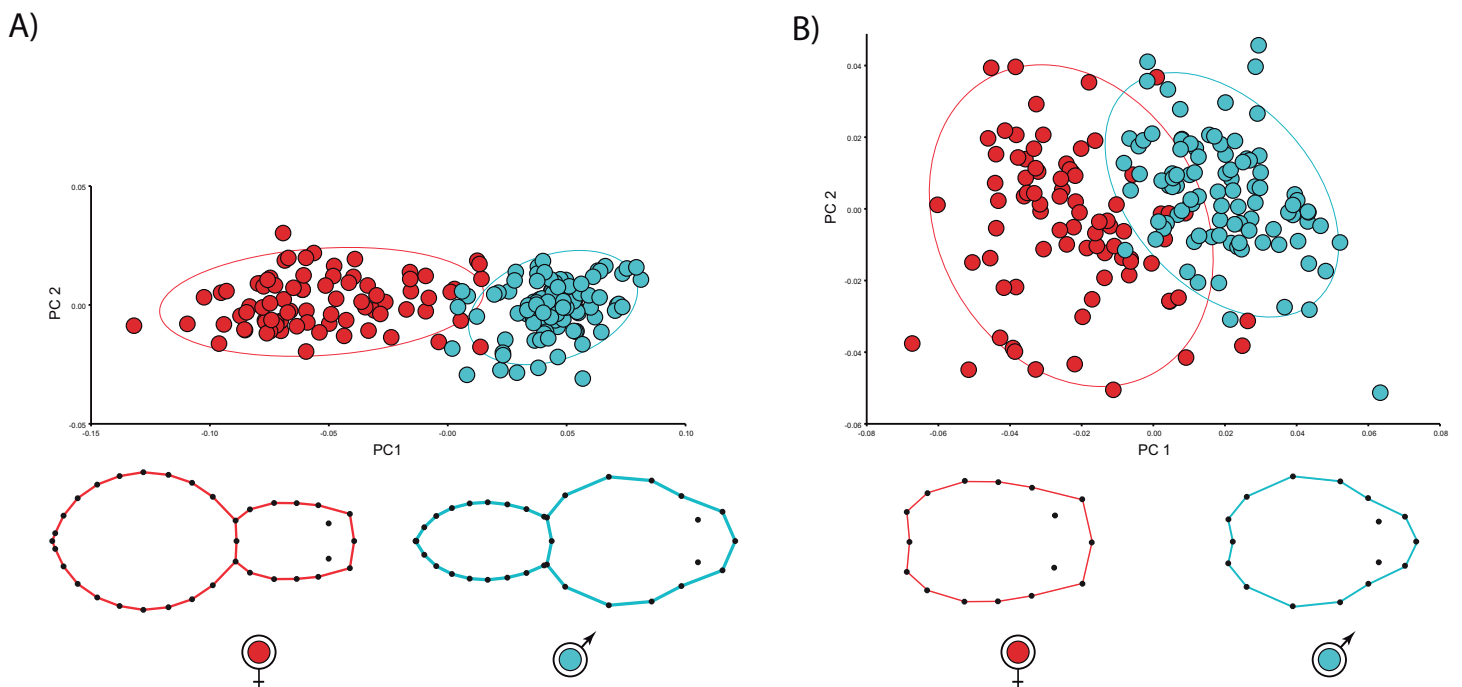


Figure 2 Principal Component Analysis of shape variation. (A) body and (B) carapace shapes. Extreme female and male shapes are polarized at the extreme of PC1 (x axis). (A) Males (blue dots) and females (red dots) clearly differ on PC1, which explains most body shape variation (85.7%). (B) Sex differences in carapace shape are also distinct.

RESULTS

Shape variation and sexual shape dimorphism

We found unequivocal sexual differences in body and carapace shapes. The first two PCs explained 89.1% body shape variance. PC1 (85.7% variance explained) is the dominant dimension of variation and unambiguously yields an ordination related to sexual shape differences (Fig. 2). Females clearly show more globular opisthosomas and laterally flattened and cephalically squared carapaces. Males exhibit much slender opisthosomas and both laterally and cephalically protruding carapaces (Fig. 2A). The ordination was far more dispersed for carapace shape, and the two first PCs only accounted for 58.4% variance. Nonetheless, PC1 (44.5% variance explained) definitely separates male and female shapes (Fig. 2B). Not surprisingly, the CVA clearly finds a statistically predictable sexual shape dimorphism both for the body ($p < 0.001$) and the carapace ($p < 0.001$).

Allometry and size-independent shape variation

The statistics of both body and carapace sizes revealed significant sex differences; females are 1.1 larger than males for body size. However, sexual size differences revert, and males turn out to be 1.1 times larger than females for carapace size (see Table 1).

The multivariate regressions indicate that spider shape variation is clearly allometric. Interestingly, the allometric slopes are not sexually dimorphic (Body shape: males 0.01,

Table 1 Summary statistics (mean \pm 1SE) of size and shape depending on the spider sex (male–female).

Trait	Males		Females	<i>F</i>	df	<i>p</i> Value
Body size	2.9 \pm 0.03		3.1 \pm 0.05	14.1	1	<0.001
Carapace size	1.3 \pm 0.02		1.2 \pm 0.02	14.3	1	<0.001
Body shape	PC1	0.04 \pm 1.7 $\times 10^{-3}$	-0.05 \pm 3.6 $^{-3}$	674.2	1	<0.001
	PC2	-0.001 \pm 1.12 $\times 10^{-3}$	0.001 \pm 1.1 $\times 10^{-3}$	2.1	1	0.15
	CV1	3.3 \pm 0.1	-4 \pm 0.1	229.8	1	<0.001
Carapace shape	PC1	0.03 \pm 1.19 $\times 10^{-3}$	-0.04 \pm 2.1 $\times 10^{-3}$	777.4	1	<0.001
	PC2	4.02 $\times 10^{-5}$ \pm 1.88 $\times 10^{-3}$	-5.2 $\times 10^{-5}$ \pm 2.2 $\times 10^{-3}$	9 $\times 10^{-4}$	1	0.98
	CV1	2.7 \pm 0.09	-3.2 \pm 0.1	154.3	1	<0.001

Note:

We estimated spider shape and size on the basis of the overall body or the carapace landmarks. Shape is described after the Canonical Variate 1 and the two first PCs from a PCA following Procrustes superimposition of individual landmark configurations. Spider size is the centroid size. Significant differences are highlighted.

Table 2 Results of regression analyses showing the allometric intercepts and slopes of regression scores on size, depending on the body portion considered (body shape, carapace shape).

Trait	Effect	df	<i>F</i>	<i>p</i> Value
Body shape	Size	1	7.5	<0.01
	Sex	1	646.3	<0.001
	Size \times sex	1	0.8	0.4
Carapace shape	Size	1	85.4	<0.001
	Sex	1	109.4	<0.001
	Size \times sex	1	0.8	0.4

Note:

Significant differences are highlighted.

females 0.01; carapace shape: males 0.04, females 0.05), whereas the intercepts (Body shape: males -0.06, females 0.007; carapace shape: males -0.04, females -0.07) are (Table 2; see Fig. 3). The sign of intercept differences revert depending on the particular body part studied (i.e., either whole body or carapace) (see Fig. 3). This notwithstanding, sexual shape dimorphism is size-independent, as sex differences remain when we statistically control for the effect of body size (Table 2). In fact, the PCA on the regression residuals extracts again two independent components, and PC1 explains 67.2% of body and 57.8% of carapace shape variance, respectively; PC1 clearly discriminates between males and females, thus corroborating the size-independent sexual shape dimorphism. This result stands either including, i.e., body shape, or excluding, i.e., carapace shape, the opisthosoma information. Again, relatively slender opisthosomas and carapaces both laterally and cephalically protruding characterize male shape. On the contrary, females exhibit relatively larger and more globular opisthosomas as well as laterally flattened and cephalically squared carapaces. Reinforcing these results, CVA unambiguously discriminates between size-independent male and female shapes (body shape: Procrustes distance 0.09, $p < 0.001$; carapace shape: Procrustes distance 0.06, $p < 0.0001$).

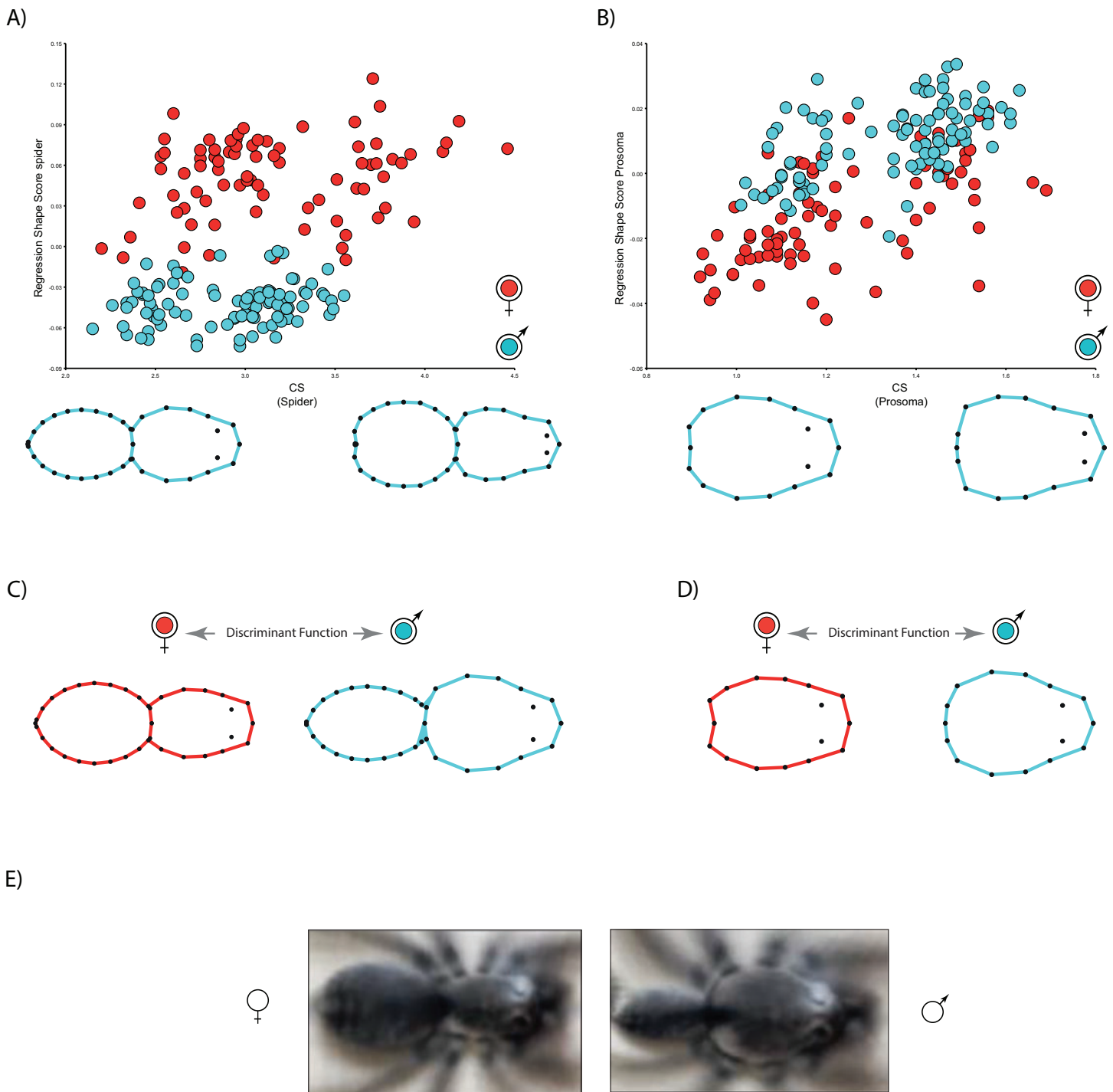


Figure 3 Allometric regressions of spider shape. (A) body and (B) carapace shapes. (C) Histogram with the scores for the leave-one-out cross-validation of Discriminant analysis in body and (D) carapace shapes. (E) Shape differences between females and males shown as image deformations (warps) of the original stock of images computed with the Image Unwarp algorithm using TPSSuper (v.1.15; Rohlf, 2013) (Marugán-Lobón & Buscalioni, 2009; Rohlf, 2002).

DISCUSSION

Our results on shape variation in *D. merlini* reveal new and previously undetected aspects of spider sexual morphological variation. Using shape analysis GMM, we have quantified for the first time that, (1) spider males and females unambiguously differ in body shape, (2) such shape differences relate to two main features: the carapace-to-opisthosoma ratio and the carapace shape, (3) a portion of spider shape variation is allometric, but sexual shape differences are completely independent on size, and (4) the shape allometric intercepts are sexually dimorphic, whereas the slopes—scaling—are not.

The evolution of spider sexual dimorphism has been widely studied (Foellmer & Moya-Laraño, 2007) on the basis of single linear measurements of size, such as the maximum carapace width or the correlated carapace body or leg lengths (Jakob, Marshall & Uetz, 1996; Aisenberg, Viera & Costa, 2007; Aisenberg et al., 2010). Thus far, such empirical evidence supports a primary role of sexual selection operating on male bodies, but hyperallometry of male size or sex-differential shape allometry had been seldom addressed. On the other hand, spider multivariate sexual shape dimorphism had been also described (Prenter, Montgomery & Elwood, 1995) using PCA based on linear combinations of linear size estimates (reviewed in Foellmer & Moya-Laraño, 2007), yielding to identify two main independent factors, one interpreted as “multidimensional size”, and another, orthogonal, interpreted as body condition or shape (Prenter, Montgomery & Elwood, 1995; Foellmer & Fairbairn, 2005a, 2005b). Not only spider shape resulting from the allometric growth of body parts had not yet been reported thus far; the fact that all such analyses were based on traditional morphometrics (i.e., linear measurements) implies that any such type of approach to spider morphological evolution would have been inevitably correlated to body mass and condition.

Patterns of shape differences in *D. merlini* suggest that sexual dimorphism results from divergent selection related to male and female reproductive roles and life styles. In our analyses, the main sex differences relate to the carapace-to-opisthosoma size, where females tend to exhibit disproportionately larger opisthosomas compared to males (Fig. 2). Additionally, sex differences in the allometric intercepts vary depending on whether we consider the body as a whole or only the carapace, and sex differences in body size and shape largely result from the relative growth of the opisthosoma. As spider opisthosoma is mostly related to egg production and storage (Foelix, 1996), fecundity selection arguably explains sex-diverging overall body morphologies in our case study species. Carapace morphologies and their sexual divergence also fit the expected considering sex-differential life-styles, particularly the different male and female activity patterns, where males are roving and females are permanent burrowers. Our results stress that male carapaces are clearly wider and more cephalically protruding, compared to those of the females. Several studies have indeed shown that mobility and competitive advantages drive the evolution of sex differences in spider carapace size (Moya-Laraño, Halaj & Wise, 2002; Foellmer & Fairbairn, 2005a, 2005b;

[Brandt & Andrade, 2007](#)); more robust male carapaces could arguably be positively selected either by scramble or by contest competition, an hypothesis that requires further testing in field experiments.

Donacosa merlini morphology scales similarly between the sexes (i.e., slopes nearly equivalent for males and females), but differ in their intercepts (i.e., sexual differences are size-independent). The lack of sex differences between allometric slopes would argue against a role for sexual selection in the evolution of the observed sexual shape differences ([Kodric-Brown, Sibly & Brown, 2006](#)). However, sexual selection has been demonstrated to not always promote an increase in the allometric slope, but to lead instead to differences in the allometric intercepts ([Bonduriansky, 2007](#); [Shingleton & Frankino, 2012](#)). On the other hand, sexually selected traits do not necessarily show allometry, whereas non-sexually selected traits often do ([Shingleton & Frankino, 2012](#)). Our results thus underscore that sexual selection might play an important role in the evolution of carapace shape differences.

These results also have important implications at the proximate level of this organism's biology. First, the lack of sex differences in the allometric slopes and the existence of differences in the intercepts suggest that shape differences originate earlier in development. Stationary anatomical spider structures such as the carapace only expand at molting ([Foelix, 1996](#)), and sex differences in *D. merlini* life styles arise following the maturation molt, when males become wandering, indicating that carapace shape differences are likely to emerge at least following the penultimate molt. During development, arthropod SSD may result from sex differences in growth rate, development duration or size-dependent survival, though they usually result from an increased number of molts in one sex ([Stillwell & Davidowitz, 2010](#)). In order to achieve their larger carapaces, males might therefore undergo either an increased number of molts or an increased growth rate (i.e., heterochrony). A recent developmental model posits that differential trait growth may arise from differential sensitivity to environmental cues (i.e., plasticity) ([Emlen et al., 2012](#)), and males of a closely related species (*Lycosa hispanica*) are indeed known to respond more than females to variation in developmental conditions ([Fernández-Montraveta & Moya-Laraño, 2007](#)). Hence, male differences in carapace shape might result from a higher degree of male morphological plasticity in response to environmental variation. The alternative hypothesis that males undergo an increased number of molts during development is yet consistent with male delayed maturation ([Fernández-Montraveta, Moya-Laraño & Cuadrado, 2004](#)), and this question requires further testing.

Our results warn about previous common scientific wisdom on the evolution of spider SSD. Previously undetected sexually dimorphic carapace traits are far less noticeable than differences in the carapace-to-opisthosoma ratio, though they are definitely statistically significant ([Fig. 2](#)). Furthermore, a portion of this shape variation is allometric, probably entailing that only relatively larger spiders tend to exhibit more pronounced traits. Thus, it is unlikely that the observed differences could be detected in samples containing only small representatives of the species or reduced ranges of

spider sizes. On the other hand, *D. merlini* could be considered either a female- or a male-biased sexually dimorphic species depending on the particular morphological trait measured: it shows the common Lycosidae pattern (F>M) on the basis of body size, yet it turns out to be male-biased and shows SSD reversal on the basis of the carapace size. This discrepancy is due to the fact that females exhibit comparatively larger opisthosomas compared to males, which show comparatively larger carapaces. Not surprisingly, the SSD pattern found when using carapace size is similar to that described on the basis of traditional metrics such as the carapace width (Foellmer & Moya-Laraño, 2007; Jakob, Marshall & Uetz, 1996). In all, SSD is a multifaceted phenomenon and our findings using shape analysis clearly warn about the limitations of single traits to make generalizations about this complex, yet crucial biological issue.

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Competing Interests

Jesús Marugán-Lobón is an Academic Editor for PeerJ. Carmen Fernández-Montraveta declares that she has no competing interests.

Author Contributions

- Carmen Fernández-Montraveta conceived and designed the experiments, performed the experiments, analyzed the data, contributed reagents/materials/analysis tools, wrote the paper, prepared figures and/or tables, reviewed drafts of the paper.
- Jesús Marugán-Lobón conceived and designed the experiments, analyzed the data, contributed reagents/materials/analysis tools, prepared figures and/or tables, reviewed drafts of the paper.

Field Study Permissions

The following information was supplied relating to field study approvals (i.e., approving body and any reference numbers):

Field experiments were approved by the Estación Biológica de Doñana (CSIC, project number 022/2007).

Data Availability

The following information was supplied regarding data availability:

The raw data have been supplied as [Supplemental Dataset Files](#).

Supplemental Information

Supplemental information for this article can be found online at <http://dx.doi.org/10.7717/peerj.3617#supplemental-information>.

REFERENCES

- Abouheif E, Fairbairn DJ. 1997.** A comparative analysis of allometry for sexual size dimorphism: assessing Rensch's rule. *American Naturalist* **149**(3):540–562 DOI [10.1086/286004](https://doi.org/10.1086/286004).
- Adams D, Rohlf F, Slice D. 2004.** Geometric morphometrics: ten years of progress following the 'revolution'. *Italian Journal of Zoology* **71**(1):5–16 DOI [10.1080/11250000409356545](https://doi.org/10.1080/11250000409356545).
- Aisenberg A, Viera C, Costa FG. 2007.** Daring females, devoted males, and reversed sexual size dimorphism in the sand-dwelling spider *Allocosa brasiliensis* (Araneae, Lycosidae). *Behavioral Ecology and Sociobiology* **62**(1):29–35 DOI [10.1007/s00265-007-0435-x](https://doi.org/10.1007/s00265-007-0435-x).
- Aisenberg A, Costa FG, Gonzalez M, Postiglioni R, Perez-Miles F. 2010.** Sexual dimorphism in chelicerae, forelegs and palpal traits in two burrowing wolf spiders (Araneae: Lycosidae) with sex-role reversal. *Journal of Natural History* **44**(19–20):1189–1202 DOI [10.1080/00222931003632716](https://doi.org/10.1080/00222931003632716).
- Alderweireldt M, Jocqué R. 1991a.** A remarkable new genus of wolf spiders from Southwestern Spain (Araneae, Lycosidae). *Bulletin de l'Institut Royal des Sciences Naturelles de Belgique, Entomologie* **61**:103–111.
- Alderweireldt M, Jocqué R. 1991b.** New data on *Donacosa merlini* (Araneae, Lycosidae), a threatened species? *Bulletin de la Société Neuchâteloise des Sciences Naturelles* **116**:19–23.
- Barea-Azcón JM, Ballesteros-Duperón E, Moreno D. 2008.** *Libro Rojo de los Invertebrados de Andalucía*. Sevilla: Consejería de Medio Ambiente, Junta de Andalucía, 1430.
- Benítez HA. 2013.** Sexual dimorphism using geometric morphometric approach. In: Moriyama H, ed. *Sexual Dimorphism*. Rijeka: InTech, 35–50.
- Bonnan MF, Farlow JO, Masters SL. 2008.** Using linear and geometric morphometrics to detect intraspecific variability and sexual dimorphism in femoral shape in *Alligator mississippiensis* and its implications for sexing fossil archosaurs. *Journal of Vertebrate Paleontology* **28**(2):422–431 DOI [10.1671/0272-4634\(2008\)28\[422:ulagmt\]2.0.co;2](https://doi.org/10.1671/0272-4634(2008)28[422:ulagmt]2.0.co;2).
- Bonduriansky R. 2007.** Sexual selection and allometry: a critical reappraisal of the evidence and ideas. *Evolution* **61**(4):838–849 DOI [10.1111/j.1558-5646.2007.00081.x](https://doi.org/10.1111/j.1558-5646.2007.00081.x).
- Bookstein F. 1991.** *Morphometric Methods for Landmark Data: Geometry and Biology*. New York: Cambridge University Press.
- Bookstein FL. 1997.** Landmark methods for forms without landmarks: morphometrics of group differences in outline shape. *Medical Image Analysis* **1**(3):225–243 DOI [10.1016/s1361-8415\(97\)85012-8](https://doi.org/10.1016/s1361-8415(97)85012-8).

- Brandt Y, Andrade MCB. 2007.** Testing the gravity hypothesis of sexual size dimorphism: are small males faster climbers? *Functional Ecology* **21**(2):379–385
DOI [10.1111/j.1365-2435.2007.01243.x](https://doi.org/10.1111/j.1365-2435.2007.01243.x).
- Cabrera MP, Scrocchi GJ, Cruz FB. 2013.** Sexual size dimorphism and allometry in *Liolaemus* of the *L. laurenti* group (Sauria: Liolaemidae): morphologic lability in a clade of lizards with different reproductive modes. *Zoologischer Anzeiger* **252**(3):299–306
DOI [10.1016/j.jcz.2012.08.003](https://doi.org/10.1016/j.jcz.2012.08.003).
- Coddington JA, Hormiga G, Scharff N. 1997.** Giant female or dwarf male spiders? *Nature* **385**(6618):687–688 DOI [10.1038/385687a0](https://doi.org/10.1038/385687a0).
- Costa-Schmidt LE, de Araujo AM. 2010.** Genitalic variation and taxonomic discrimination in the semi-aquatic spider genus *Paratrechalea* (Araneae: Trechaleidae). *Journal of Arachnology* **38**(2):242–249 DOI [10.1636/joa_a09-75.1](https://doi.org/10.1636/joa_a09-75.1).
- Cox RM, Skelly SL, John-Alder HB. 2003.** A comparative test of adaptive hypotheses for sexual size dimorphism in lizards. *Evolution* **57**(7):1653–1669
DOI [10.1111/j.0014-3820.2003.tb00371.x](https://doi.org/10.1111/j.0014-3820.2003.tb00371.x).
- Crews S. 2006.** Studies of morphological and molecular phylogenetic divergence in spiders (Araneae: Homalonychus) from the American southwest, including divergence along the Baja California Peninsula. *Molecular Phylogenetics and Evolution* **38**(2):470–487
DOI [10.1016/j.ympev.2005.11.010](https://doi.org/10.1016/j.ympev.2005.11.010).
- Elgar MA. 1991.** Sexual cannibalism, size dimorphism, and courtship behavior in orb-weaving spiders (Araneidae). *Evolution* **45**(2):444–448 DOI [10.2307/2409679](https://doi.org/10.2307/2409679).
- Elias DO, Kasumovic MM, Punzalan D, Andrade MCB, Mason AC. 2008.** Assessment during aggressive contests between male jumping spiders. *Animal Behaviour* **76**(3):901–910
DOI [10.1016/j.anbehav.2008.01.032](https://doi.org/10.1016/j.anbehav.2008.01.032).
- Emlen DJ, Warren IA, Johns A, Dworkin I, Lavine LC. 2012.** A mechanism of extreme growth and reliable signaling in sexually selected ornaments and weapons. *Science* **337**(6096):860–864
DOI [10.1126/science.1224286](https://doi.org/10.1126/science.1224286).
- Fairbairn DJ. 2007.** Introduction: the enigma of sexual size dimorphism. In: Fairbairn DJ, Blanckenhorn WU, Szekely T, eds. *Sex, Size and Gender roles. Evolutionary Studies of Sexual Size Dimorphism*. New York: Oxford University Press, 1–12.
- Fairbairn DJ, Blanckenhorn WU, Szekely T. 2007.** *Sex, Size and Gender Roles: Evolutionary Studies of Sexual Size Dimorphism*. New York: Oxford University Press.
- Fernández-Montraveta C, Cuadrado M. 2008.** Microhabitat selection in the potentially endangered wolf spider *Donacosa merlini* (Araneae, Lycosidae): implications for spider conservation. *Canadian Journal of Zoology-Revue Canadienne De Zoologie* **86**(11):1280–1288
DOI [10.1139/z08-113](https://doi.org/10.1139/z08-113).
- Fernández-Montraveta C, Moya-Laraño J. 2007.** Sex-specific plasticity of growth and maturation size in a spider: implications for sexual size dimorphism. *Journal of Evolutionary Biology* **20**(5):1689–1699 DOI [10.1111/j.1420-9101.2007.01399.x](https://doi.org/10.1111/j.1420-9101.2007.01399.x).
- Fernández-Montraveta C, Moya-Laraño J, Cuadrado M. 2004.** Life history and habitat preferences of *Donacosa merlini*, an endemic wolf spider from the area of Doñana (SW Spain). *Paper Presented at the 16th International Congress of Arachnology*. Belgium: Ghent University.
- Foelix RF. 1996.** *Biology of Spiders*. New York; Oxford; [Stuttgart]: Oxford University Press/Georg Thieme Verlag.
- Foellmer MW, Fairbairn DJ. 2005a.** Competing dwarf males: sexual selection in an orb-weaving spider. *Journal of Evolutionary Biology* **18**(3):629–641 DOI [10.1111/j.1420-9101.2005.00868.x](https://doi.org/10.1111/j.1420-9101.2005.00868.x).

- Foellmer MW, Fairbairn DJ. 2005b.** Selection on male size, leg length and condition during mate search in a sexually highly dimorphic orb-weaving spider. *Oecologia* **142**(4):653–662 DOI [10.1007/s00442-004-1756-3](https://doi.org/10.1007/s00442-004-1756-3).
- Foellmer MW, Moya-Laraño J. 2007.** Sexual size dimorphism in spiders: patterns and processes. In: Fairbairn DJ, Blanckenhorn WU, Székely T, eds. *Sex, Size and Gender Roles: Evolutionary Studies of Sexual Size Dimorphism*. New York: Oxford University Press, 71–81.
- Gower JC. 1975.** Generalized Procrustes analysis. *Psychometrika* **40**:33–51.
- Head G. 1995.** Selection on fecundity and variation in the degree of sexual size dimorphism among spider species (Class Araneae). *Evolution* **49**(4):776–781 DOI [10.2307/2410330](https://doi.org/10.2307/2410330).
- Hedrick AV, Temeles EJ. 1989.** The evolution of sexual dimorphism in animals—hypotheses and tests. *Trends in Ecology & Evolution* **4**(5):136–138 DOI [10.1016/0169-5347\(89\)90212-7](https://doi.org/10.1016/0169-5347(89)90212-7).
- Jakob EM, Marshall SD, Uetz GW. 1996.** Estimating fitness: a comparison of body condition indices. *Oikos* **77**(1):61–67 DOI [10.2307/3545585](https://doi.org/10.2307/3545585).
- Kaliontzopoulou A, Carretero MA, Llorente GA. 2007.** Multivariate and geometric morphometrics in the analysis of sexual dimorphism variation in *Podarcis* lizards. *Journal of Morphology* **268**(2):152–165 DOI [10.1002/jmor.10494](https://doi.org/10.1002/jmor.10494).
- Klingenberg CP. 2011.** MorphoJ: an integrated software package for geometric morphometrics. *Molecular Ecology Resources* **11**(2):353–357 DOI [10.1111/j.1755-0998.2010.02924.x](https://doi.org/10.1111/j.1755-0998.2010.02924.x).
- Klingenberg CP. 2016.** Size, shape and form: concepts of allometry in geometric morphometrics. *Development, Genes and Evolution* **226**(3):113–137 DOI [10.1007/s00427-016-0539-2](https://doi.org/10.1007/s00427-016-0539-2).
- Klingenberg C, Barluenga M, Meyer A. 2002.** Shape analysis of symmetric structures: quantifying variation among individuals and asymmetry. *Evolution* **56**(10):1909–1920 DOI [10.1111/j.0014-3820.2002.tb00117.x](https://doi.org/10.1111/j.0014-3820.2002.tb00117.x).
- Kodric-Brown A, Sibly RM, Brown JH. 2006.** The allometry of ornaments and weapons. *Proceedings of the National Academy of Sciences of the United States of America* **103**(23):8733–8738 DOI [10.1073/pnas.0602994103](https://doi.org/10.1073/pnas.0602994103).
- Lande R. 1980.** Sexual dimorphism, sexual selection, and adaptation in polygenic characters. *Evolution* **34**(2):292–305 DOI [10.2307/2407393](https://doi.org/10.2307/2407393).
- Lande R, Arnold SJ. 1985.** Evolution of mating preference and sexual dimorphism. *Journal of Theoretical Biology* **117**(4):651–664 DOI [10.1016/s0022-5193\(85\)80245-9](https://doi.org/10.1016/s0022-5193(85)80245-9).
- Mardia KV, Bookstein FL, Moreton IJ. 2000.** Statistical assessment of bilateral symmetry of shapes. *Biometrika* **87**(2):285–300 DOI [10.1093/biomet/87.2.285](https://doi.org/10.1093/biomet/87.2.285).
- Marugán-Lobón J, Buscalioni A. 2009.** New insight on the anatomy and architecture of the avian neurocranium. *Anatomical Record* **292**(3):364–370 DOI [10.1002/ar.20865](https://doi.org/10.1002/ar.20865).
- McGinley RH, Prenter J, Taylor PW. 2015.** Assessment strategies and decision making in male–male contests of *Servaea incana* jumping spiders. *Animal Behaviour* **101**:89–95 DOI [10.1016/j.anbehav.2014.12.014](https://doi.org/10.1016/j.anbehav.2014.12.014).
- Monteiro LR. 1999.** Multivariate regression models and geometric morphometrics: the search for causal factors in the analysis of shape. *Systematic Biology* **48**(1):192–199 DOI [10.1080/106351599260526](https://doi.org/10.1080/106351599260526).
- Mosimann JE. 1970.** Size allometry: size and shape variables with characterizations of the lognormal and generalized gamma distributions. *Journal of the American Statistical Association* **65**(330):930–945 DOI [10.2307/2284599](https://doi.org/10.2307/2284599).
- Moya-Laraño J, Halaj J, Wise DH. 2002.** Climbing to reach females: Romeo should be small. *Evolution* **56**(2):420–425 DOI [10.1554/0014-3820\(2002\)056\[0420:ctrfrs\]2.0.co;2](https://doi.org/10.1554/0014-3820(2002)056[0420:ctrfrs]2.0.co;2).

- O'Higgins P, Collard M. 2002. Sexual dimorphism and facial growth in papionin monkeys. *Journal of Zoology* 257(2):255–272 DOI 10.1017/s0952836902000857.
- Pérez SI, Bernal V, González PN. 2006. Differences between sliding semi-landmark methods in geometric morphometrics, with an application to human craniofacial and dental variation. *Journal of Anatomy* 208(6):769–784 DOI 10.1111/j.1469-7580.2006.00576.x.
- Prenter J, Montgomery WI, Elwood RW. 1995. Multivariate morphometrics and sexual dimorphism in the orb-web spider *Metellina segmentata* (Clerck, 1757) (Araneae, Metidae). *Biological Journal of the Linnean Society* 55(4):345–354 DOI 10.1111/j.1095-8312.1995.tb01070.x.
- Pretorius E, Steyn M, Scholtz Y. 2006. Investigation into the usability of geometric morphometric analysis in assessment of sexual dimorphism. *American Journal of Physical Anthropology* 129(1):64–70 DOI 10.1002/ajpa.20251.
- Preziosi RF, Fairbairn DJ, Roff DA, Brennan JM. 1996. Body size and fecundity in the waterstrider *Aquarius remigis*: a test of Darwin's fecundity advantage hypothesis. *Oecologia* 108(3):424–431 DOI 10.1007/bf00333717.
- R Core Team. 2017. *R: a language and environment for statistical computing*. Vienna, Austria: R Foundation for Statistical Computing. Available at <https://www.R-project.org/>.
- Rensch B. 1959. *Evolution Above the Species Level*. New York: Columbia University Press.
- Rohlf FJ. 2002. Geometric morphometrics in systematics. In: Macleod N, Forey P, eds. *Morphology, Shape and Phylogenetics*. London: Taylor & Francis, 175–193.
- Rohlf FJ. 2010. *TpsRelw, Relative Warps Analysis, Version 1.49*. Stony Brook: Department of Ecology and Evolution, State University of New York.
- Rohlf FJ. 2013. *TpsSuper, Superimposition and Image Averaging, Version 1.15*. Stony Brook: Department of Ecology and Evolution, State University of New York.
- Roitberg ES, Eplanova GV, Kotenko TI, Amat F, Carretero MA, Kuranova VN, Bulakhova NA, Zinenko OI, Yakovlev VA. 2015. Geographic variation of life-history traits in the sand lizard, *Lacerta agilis*: testing Darwin's fecundity-advantage hypothesis. *Journal of Evolutionary Biology* 28(3):613–629 DOI 10.1111/jeb.12594.
- Savriama Y, Maria Gomez J, Perfectti F, Klingenberg CP. 2012. Geometric morphometrics of corolla shape: dissecting components of symmetric and asymmetric variation in *Erysimum mediohispanicum* (Brassicaceae). *New Phytologist* 196(3):945–954 DOI 10.1111/j.1469-8137.2012.04312.x.
- Schutz D, Taborsky M. 2003. Adaptations to an aquatic life may be responsible for the reversed sexual size dimorphism in the water spider, *Argyroneta aquatica*. *Evolutionary Ecology Research* 5:105–117.
- Shingleton AW, Frankino WA. 2012. New perspectives on the evolution of exaggerated traits. *BioEssays* 35(2):100–107 DOI 10.1002/bies.201200139.
- Stillwell RC, Davidowitz G. 2010. A developmental perspective on the evolution of sexual size dimorphism of a moth. *Proceedings of the Royal Society B: Biological Sciences* 277(1690):2069–2074 DOI 10.1098/rspb.2009.2277.
- Stuart-Fox D. 2009. A test of Rensch's rule in dwarf chameleons (*Bradypodion* spp.), a group with female-biased sexual size dimorphism. *Evolutionary Ecology* 23(3):425–433 DOI 10.1007/s10682-008-9242-8.
- Szekely T. 2007. Introduction. In: Fairbairn DJ, Blanckenhorn WU, Szekely T, eds. *Sex, Size and Gender Roles. Evolutionary Analyses of Sexual Size Dimorphism*. New York: Oxford University Press, 13–15.

- Verdú JR, Galante E. 2005.** *Libro Rojo de los Invertebrados de España*. Madrid: Dirección General de Conservación de la Naturaleza.
- Vollrath F. 1998.** Dwarf males. *Trends in Ecology & Evolution* **13**:159–163.
- Vollrath F, Parker GA. 1992.** Sexual dimorphism and distorted sex-ratios in spiders. *Nature* **360(6400)**:156–159 DOI [10.1038/360156a0](https://doi.org/10.1038/360156a0).
- Walker SE, Rypstra AL. 2001.** Sexual dimorphism in functional response and trophic morphology in *Rabidosia rabida* (Araneae: Lycosidae). *American Midland Naturalist* **146(1)**:161–170 DOI [10.1674/0003-0031\(2001\)146\[0161:sdifra\]2.0.co;2](https://doi.org/10.1674/0003-0031(2001)146[0161:sdifra]2.0.co;2).
- Walker SE, Rypstra AL. 2003.** Sexual dimorphism and the differential mortality model: is behaviour related to survival? *Biological Journal of the Linnean Society* **78(1)**:97–103 DOI [10.1046/j.1095-8312.2003.00134.x](https://doi.org/10.1046/j.1095-8312.2003.00134.x).
- Zelditch ML, Swiderski DL, Sheets HD. 2012.** *Geometric Morphometrics for Biologists. A Primer*. New York: Academic Press.