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Do structures with sexual contact functions evolve negative static allometries? A case study with the harvestman *Leiobunum vittatum* (Opiliones Sclerosomatidae)

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Morphological traits vary in their scaling relative to body size. One of the most general scaling patterns has been found for male genitalia: across many animal groups, differences in body size are associated with small differences in genital size (i.e., genitalia exhibit shallow scaling on body size, or negative static allometry). One potential explanation for this pervasive pattern is that genitalia are selected to scale shallowly for reasons related to the contact nature of the functions they perform during copulation. This hypothesis predicts that non-genitalic structures that touch mates during copulation (to stimulate or grasp the mating partner; "contact structures") will also scale shallowly with body size. We tested this prediction by comparing the static allometric slopes of non-genitalic contact and non-contact structures in *Leiobunum vittatum* harvestmen. We found that contact structures had shallower allometric slopes than non-contact structures, in support of the contact-function hypothesis. However, we also found that the distalmost segments of appendages had lower slopes than non-distal segments, regardless of their sexual function. Because all of *L. vittatum*'s contact structures are distal, we were unable to isolate the effects of a structure having a contact function from having a distal position. We recommend further tests to tease apart the effects of these variables.

KEY WORDS: allometry, evolution of genitalia, sexual selection, contact structures.

INTRODUCTION

Genitalia tend to show low variation in size across a range of adult body sizes within species (Eberhard et al. 1998; Eberhard 2009). That is, instead of scaling in a one-to-one relationship with body size (isometry), genitalia scale shallowly such that

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large differences in body size are associated with small differences in genital size among conspecifics. This shallow scaling of structure size relative to body size is termed negative static allometry, and it is found in genitalia across many taxa, including insects, arachnids, mollusks and at least some vertebrates (Eberhard et al. 1998; Eberhard 2009; Rodríguez et al. 2015). The pattern of shallow genitalic scaling is maintained in spite of marked divergence in genital shape across species (Eberhard et al. 2009), and it is shared by the many non-homologous structures that function as genitalia in different animal groups, like insect aedeagi and spider pedipalps (Eberhard et al. 1998), suggesting that shallow scaling is important for one or more of the functions that genitalia perform.

One possible explanation for the shallow allometry of genitalia is offered by the "contact-function hypothesis". This hypothesis states that genitalia evolve shallow scaling because their functions involve direct contact with mates during copulation (Eberhard 2002, 2009). The rationale for this hypothesis is as follows. Success in mating may depend on the fit between male and female genitalia. Fit can be important either for direct mechanical coupling or for any stimulation that requires proper alignment to a mate's sensory receptors (Eberhard et al. 1998; Eberhard 2009). Given that individuals cannot adjust the size of their genitalia to fit the mates that they encounter (especially when genitalia are sclerotized, as they often are in arthropods), selection may favor genitalia that fit with mates of common or intermediate sizes (Eberhard et al. 1998; Eberhard 2009). Thus, the contact functions that genitalia perform could select for a restricted size range in genitalia (i.e., negative allometry).

The contact function hypothesis makes the prediction that structures other than genitalia that have similar copulatory contact functions (henceforth, "contact structures") should also exhibit similarly shallow allometries (Eberhard 2002, 2009). The rationale for this prediction is that (just as for genitalia) fitting common or intermediate mates may be advantageous for non-genitalic contact structures that deliver external tactile stimulation or that grasp the mate during mating. Thus, this prediction isolates the copulatory contact nature of a structure's function. Previous work on sepsid flies has found initial support for this prediction; male sepsid flies have sternal brushes that contact and stimulate females during copulation, and that scale shallowly, just like genitalia (Eberhard 2002).

We tested this prediction with a suite of morphological structures in the harvestman *Leiobunum vittatum* (Opiliones Sclerosomatidae). A strict test of this prediction would focus on contact structures for which there is evidence that they have been modified by selection to perform their contact function (e.g., that are sexually dimorphic and rapidly evolving, or species-specific; Eberhard 2002). In our data set, this would restrict our sample to a single contact structure (see below). We therefore conduct a qualitative comparison applying this strict criterion, but we also examine structures that are not sexually dimorphic and lack species-specificity but that do contact the mating partner during mating.

One potential confounding variable in our study is the fact that structures with contact functions may often have distal positions on their appendages (as is the case in our data set). We were concerned that distal positioning might offer another reason why structures might exhibit shallow allometries (e.g., if precise positioning of distal structures aids in efficient locomotion or in sensing the environment). We attempted to account for this potential confounding effect by including a sample of distal non-contact male and female structures in our analyses.

We found that non-genitalic structures with copulatory contact functions had shallower allometric slopes than non-contact structures, comparable to those of

genitalia. However, this effect may be confounded by contact structures having distal positions on appendages, as all distal structures scaled shallowly regardless of their sexual function. While contact functions could explain the pervasive scaling patterns of genitalia, we encourage further research to tease apart the effects of having a sexual contact function vs those of having a distal position.

METHODS

We chose structures to measure in *L. vittatum* on the basis of a description of the mating behavior for our local population (Fowler-Finn et al. 2014). We used this description to categorize structures as having or not having contact functions during mating (Table 1). We selected three male contact structures: the tarsi of the pedipalps, of legs II and of legs III. Males use their pedipalp tarsi to grab hold of the female's prosoma, and they use their leg III tarsi, and sometimes leg II tarsi, to wrap around the female's legs II (Fowler-Finn et al. 2014; Fig. 1). Note that because these structures are used for grasping rather than stroking or tapping, we are unable to distinguish between the effects of having grasping vs stimulatory functions. In females, we measured a single structure with a contact function: the pedipalp tarsus, which females use to tap the male and help guide the penis into place for mating (Edgar 1971; Fowler-Finn et al. 2014). Note that this female structure performs a different role from male contact structures, as it both stimulates and grasps. We also measured a sample of non-contact structures (18 in males, 20 in females) on the main body, the pedipalps and the legs (Table 1). Some of these structures were distal while others were not. Of the structures we measured, segments of the pedipalps are the most likely to have been shaped by selection to perform their copulatory functions, as evidenced by their sexual size dimorphism (Fig. 2) and species specificity (K.D. Fowler-Finn pers. comm.).

Measurements

We collected 46 male and 22 female *L. vittatum* harvestmen in the summer of 2010 in Milwaukee, Wisconsin, and we preserved them in 75% ethanol. These individuals belonged to the same population as those from the mating descriptions cited above. We photographed prosomas, pedipalps, penes, legs II, legs III and legs IV using a digital camera (Moticam 2500, Motic, Causeway Bay, Hong Kong) connected to a stereo microscope (SZ61, Olympus Corporation, Tokyo, Japan). For each photograph, we aligned structures to be parallel to the objective lens of the microscope in order to minimize measurement error caused by positioning imprecision. We also made sure to straighten out all leg tarsi (which tend to curl) in order to improve the accuracy of our measurements.

From each photograph, we measured structures using ImageJ version 1.47a (US National Institutes of Health, Bethesda, MD). For both sexes, we took the following measurements: carapace length and width; the length of pedipalp femurs, tibiae, patellae and tarsi; and the lengths of femurs, patellae, tibiae, metatarsi and tarsi of legs II, III and IV (Fig. 1). We also measured penis length to use as a reference for shallow genital scaling. For all female structures and non-leg male structures, we took multiple photographs and measurements so that we could estimate measurement error. All photographs and measurements were made by the same experienced measurer (J.T. Kilmer).

Calculating allometric slopes

In studies of allometry, structures are typically regressed against a single measure of body size. We initially regressed all structures against the size of the carapace on the prosoma, and we found that very few structures correlated with the main body at all (average correlation between

Table 1.

Structures measured for allometric slope analysis in *Leiobunum vittatum*. To generate allometric slopes, we regressed leg and pedipalp segments against their respective femurs (see text). For each structure used in the analysis, we indicate whether it is used by males or females as a copulatory contact structure. We also indicate which structures are distal on each appendage. Data are shown as slope $\pm$ 1 SE followed by the corresponding r^2 value in parentheses. Note that we measured the penis solely for qualitative comparisons, and we did not include it in statistical analyses.

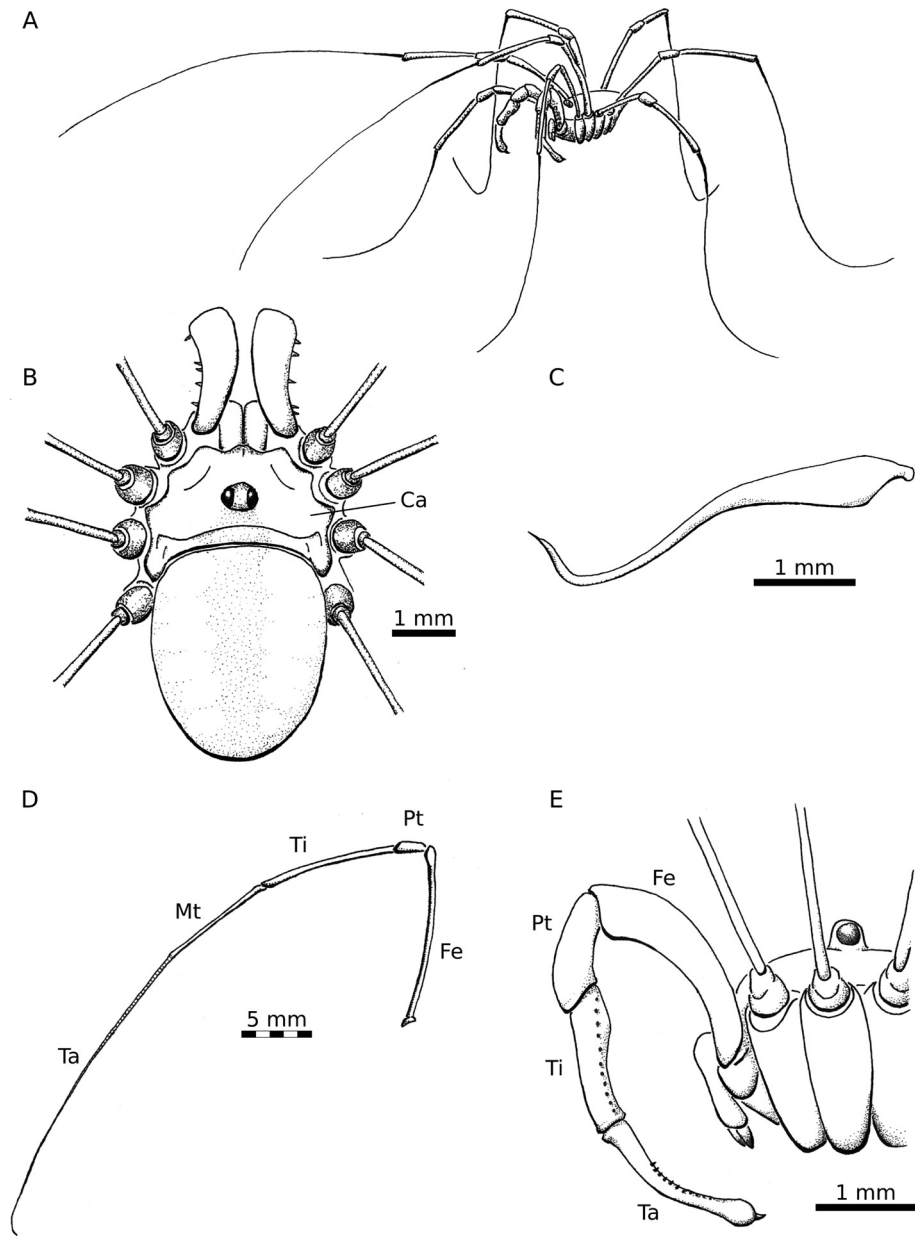| Structure	… Regressed against	Contact?	Distal?	Male slope	Female slope
Carapace width					
Carapace length	Carapace width	No	No	0.591 ± 0.140 (0.30)	0.869 ± 0.087 (0.89)
Penis	Carapace width		No	0.399 ± 0.188 (0.13)	
Pedipalp femur					
Pedipalp patella	Pedipalp femur	No	No	1.022 ± 0.418 (0.12)	1.095 ± 0.092 (0.92)
Pedipalp tibia	Pedipalp femur	No	No	1.046 ± 0.130 (0.61)	0.979 ± 0.97 (0.89)
Pedipalp tarsus	Pedipalp femur	Male, female	Yes	0.533 ± 0.140 (0.26)	0.899 ± 0.93 (0.88)
Leg II femur					
Leg II patella	Leg II femur	No	No	0.480 ± 0.144 (0.28)	0.679 ± 0.354 (0.22)
Leg II tibia	Leg II femur	No	No	0.806 ± 0.077 (0.80)	0.886 ± 0.12 (0.81)
Leg II metatarsus	Leg II femur	No	No	0.542 ± 0.136 (0.37)	0.767 ± 0.171 (0.61)
Leg II tarsus	Leg II femur	Male	Yes	0.437 ± 0.182 (0.22)	0.309 ± 0.131 (0.30)
Leg III femur					
Leg III patella	Leg III femur	No	No	0.288 ± 0.327 (0.05)	0.867 ± 0.346 (0.33)
Leg III tibia	Leg III femur	No	No	0.747 ± 0.115 (0.74)	0.684 ± 0.163 (0.58)
Leg III metatarsus	Leg III femur	No	No	0.539 ± 0.144 (0.48)	0.881 ± 0.151 (0.72)
Leg III tarsus	Leg III femur	Male	Yes	0.206 ± 0.210 (0.07)	0.196 ± 0.211 (0.06)
Leg IV femur					
Leg IV patella	Leg IV femur	No	No	0.482 ± 0.422 (0.09)	0.411 ± 0.346 (0.10)
Leg IV tibia	Leg IV femur	No	No	0.541 ± 0.165 (0.45)	0.763 ± 0.101 (0.81)
Leg IV metatarsus	Leg IV femur	No	No	0.524 ± 0.185 (0.38)	0.845 ± 0.154 (0.70)
Leg IV tarsus	Leg IV femur	No	Yes	0.168 ± 0.215 (0.05)	0.205 ± 0.187 (0.08)

Fig. 1. — Morphology of a male *Leiobunum vittatum*. A: Full body sketch for anatomical context; B: dorsal view of the body, including the carapace (Ca); C: side view of the penis, which is typically kept completely inside the male body; D: a leg II, which is composed of a femur (Fe), patella (Pt), tibia (Ti), metatarsus (Mt) and tarsus (Ta); E: side view of the prosoma and a pedipalp, which also has a femur, patella, tibia and tarsus.
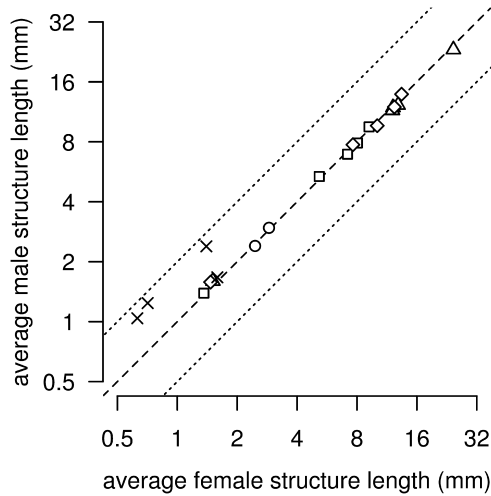
Fig. 2. — A check for sexual size dimorphism of different structures in *Leiobunum vittatum*. We plotted male structure size against corresponding female structure size. The dashed line has a slope of 1, and represents equal structure size between males and females. The dotted lines have slopes of 2 and 0.5; they represent one sex having a structure twice as large or half as large as the other sex, respectively. Note: The lines appear to have equal slopes because the axes are on a log scale. Structures plotted here include measures of the carapace (circles), segments of the pedipalps (×), legs II (triangles), legs III (squares) and legs IV (diamonds). The three pedipalp segments that stray from sexual monomorphism are the femur, patella and tibia.

structure size and carapace width ± SD was $r = 0.09 ± 0.18$ in males, and $r = 0.41 ± 0.42$ in females). We suspect that this is related to the large difference in scale between body size and structure size. These harvestmen have tremendously long legs in relation to their bodies; e.g., the legs II of *L. vittatum* often measure over 20 body widths long (Fig. 1). Because of this huge difference in scale, harvestman legs are not necessarily expected to show isometric relationships with body size. Therefore we described scaling patterns in ways that made sense given the biology of the study species; instead of regressing all structures against the carapace, we used the femur lengths of each leg and pedipalp as baseline structures (henceforth "*x*-axis structures") against which all other structures in the corresponding appendage were regressed. For example, the pedipalp femur served as the *x*-axis structure when determining allometric slopes for the pedipalp patella, tibia and tarsus. So instead of asking whether structure allometry deviates from the baseline programs governing the overall body, our slopes ask whether structures differ from baseline programs governing the size of the appendage on which they are located. Table 1 gives a complete summary of the structures measured and the allometric comparisons we made. Note that the sample size of allometric slopes was lower than the number of structures we measured, because the slopes are estimated regressing some structures against others.

We calculated allometric slopes by running log–log ordinary least squares (OLS) regressions on the structures of interest versus their respective *x*-axis structures. Some authors discourage the use of OLS regression in studies of allometry, citing a predicted underestimation of slope when measurement error is present in the *x*-axis (McArdle 1988; Fuller 2006). However, this underestimation is negligible as long as measurement error is low and measurement repeatability is high (McArdle 2003; Al-Wathiqui & Rodríguez 2011; J.T. Kilmer & R.L. Rodríguez in prep.), which was the case for our data: measurement repeatability estimated for a subset of the structures we measured was high across all structures (mean ± SD repeatability = 0.95 ± 0.06; median = 0.97; $n = 28$) and also among *x*-axis structures (mean ± SD

repeatability = 0.95 ± 0.08; median = 0.99; n = 7). Therefore, we expect bias in our allometric slope estimates to be negligible. Additionally, OLS gives better descriptions of scaling patterns than its common alternative, the reduced major axis (RMA) slope estimator. This is because OLS slopes are calculated with the covariance between x and y, so they take the relationship between the two variables into account, whereas RMA slopes are simply a ratio of standard deviations of the two variables, and so they confuse dispersion of data with steepness of slope (Al-Wathiqui & Rodríguez 2011; Voje & Hansen 2013; Pélabon et al. 2014).

Data analysis

To test the prediction that non-genitalic contact structures scale more shallowly than non-contact structures (and similarly to genitalia), we first looked only at the scaling of a contact structure on a sexually dimorphic appendage that shows species-specific morphology (the pedipalps). Because this only afforded one contact structure, this comparison was qualitative. We then expanded our analysis to include sexually monomorphic structures with little species specificity that nevertheless have copulatory contact functions for a quantitative test of the prediction of the contact-function hypothesis. We ran a linear mixed model (using the restricted maximum likelihood [REML] method) in JMP 10.0.0 (SAS Institute, Cary, NC). Our dependent variable was the set of allometric slopes of non-genitalic structures from both sexes. We included appendage (pedipalp, leg II, leg III, leg IV and body) as a random term to account for the use of multiple correlated structures (average correlation ± SD within appendages was r = 0.45 ± 0.27 for males, and r = 0.56 ± 0.30 for females; average correlation ± SD across appendages was r = 0.29 ± 0.28 for males and r = 0.40 ± 0.37 for females). The fixed effects of our model were sex and a categorical trait-type grouping variable with three levels segregating non-distal structures (none of which were contact structures), contact structures (all of which were distal) and non-contact distal structures. We performed a Tukey honestly significant difference (HSD) post hoc test to check for differences between these three grouping levels. Because the prediction of the hypothesis pertains specifically to non-genitalic structures, we excluded penis slope from our model and used it only as a reference to help interpret the pattern for non-genitalic contact structures. Note that this makes our test more conservative; including the penis would risk biasing downwards the mean allometric slope for the contact structures.

RESULTS

When focusing on the sexually dimorphic body part with species-specific morphology (the pedipalp), we found that non-contact structures had allometric slopes close to 1 in both sexes. The male palp tarsus (a contact structure) had a shallow slope, close to that of the penis (Fig. 3). By contrast, the female palp tarsus, which both taps and grasps males during copulation, showed scaling close to isometry (Fig. 3).

In the broader sample of traits that included all non-genitalic structures regardless of whether or not they were sexually dimorphic or showed species-specific morphology, we found that contact structures scaled significantly more shallowly than non-contact, non-distal structures (Fig. 4; Table 2). However, there was no significant difference in scaling between contact structures and distal non-contact structures, suggesting a potential confounding effect of having a distal position (Fig. 4; Table 2). Additionally, male structures had significantly shallower allometric slopes than female structures (Fig. 3; Table 2). Because females use their contact structure differently from how males use theirs (see above), we repeated this analysis excluding the female palp tarsus; this had very little effect on the
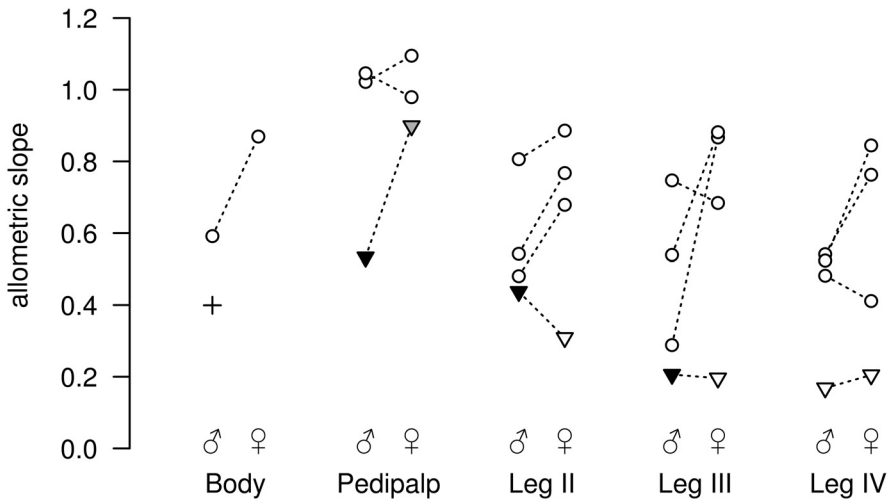
Fig. 3. — Allometric slopes for each structure measured in *Leiobunum vittatum*, organized by sex and body part. Dotted lines connect corresponding structures across the sexes. Filled symbols are non-genitalic structures that contact mates during copulation, while empty symbols are non-contact structures. The female pedipalp tarsus is filled with gray to denote that it is a contact structure that has both stimulatory and grasping functions. Triangles represent the structures found most distally on each appendage, while circles represent non-distal structures. The slope for the penis is indicated by a plus sign, and serves as a point of comparison for other slopes. Standard errors of slope estimates can be found in Table 1.
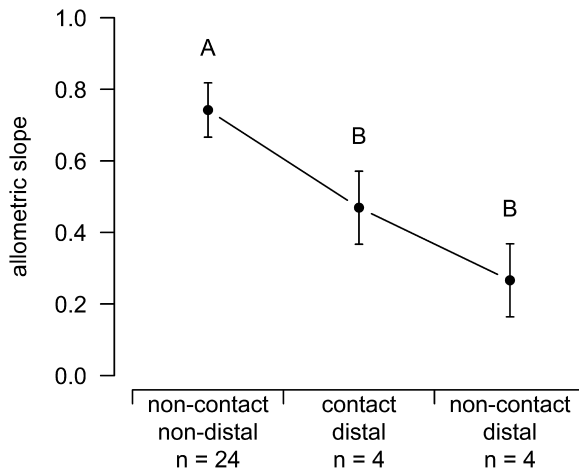

Fig. 4. — Mean allometric slope (least square means ± SE) for different structure types in *Leiobunum vittatum*. Structures were grouped based on whether they were the distalmost structure in an appendage (distal vs non-distal) and whether they contact mates during copulation or not (contact vs non-contact). Note that *L. vittatum* has no non-distal contact structures. Different letters indicate significant differences between groups with a Tukey honestly significant difference (HSD) post-hoc test.

Table 2.

Test for differences in allometric slopes among a sample of structures in male and female *Leiobunum vittatum*. We included a total of 16 structures for each sex. Structures were grouped by trait type based on whether they are used to contact mates during copulation and whether they are the distalmost segment of their respective appendage. We also included appendage (pedipalp, leg II, leg III, leg IV or body) as a random effect. Bold text indicates significant terms.

Fixed effects	*F-ratio* $_{df\ num,\ df\ den}$	*P*
Trait type	$24.59_{2,\ 24.67}$	**< 0.0001**
Sex	$12.34_{1,\ 24.18}$	**0.002**
Random effect	Variance component	95% CI
Appendage	0.024 (57.2% of total)	– 0.014 0.063

CI: confidence interval.

outcome of our model for both the sex effect ($F_{1,\ 23.12} = 9.19$, $P = 0.006$) and the trait type effect ($F_{2,\ 23.46} = 24.32$, $P < 0.0001$).

DISCUSSION

We calculated static allometric slopes for 16 non-genitalic structures in *Leiobunum vittatum* harvestmen that had either contact or non-contact copulatory functions. We used these data to test the prediction that structures that contact mates during copulation are selected to have shallow allometries, in a way that could explain the shallow allometric scaling of genitalia. We first focused on the pedipalp, which is sexually dimorphic and species-specific in form, and thus shows evidence of having been modified by selection to perform its copulatory contact function. A qualitative comparison of pedipalp segments found that the contact structure (male tarsus) scaled more shallowly than all non-contact structures, a finding that supports the prediction of the hypothesis. We then included all slopes in a quantitative analysis, regardless of whether or not they came from structures that were sexually dimorphic or species-specific. We again found that contact structures scaled more shallowly than non-contact, non-distal ones. However, all distal segments, whether contact or non-contact, also scaled shallowly with body size. Given that *L. vittatum* lacks non-distal contact structures (other than the penis), we were unable to disentangle the effect of having a distal position from the effect of having a sexual contact function. Thus, our results provide only tentative support for the contact-function hypothesis. However, they are consistent with previous findings that support the hypothesis (Eberhard 2002).

We also found that male structures had overall shallower allometries than female structures, regardless of whether or not they had contact functions or were located distally. This indicates pervasive sexual dimorphism, not in the size of structures (like we found in the pedipalps), but in their scaling patterns.

In conclusion, we find support for the hypothesis that structures with copulatory contact functions (whether genitalic or non-genitalic) are selected to have shallow allometric scaling (Eberhard 2002, 2009). These results have the potential to explain the widespread pattern of shallowly scaling genitalia across taxa. However, we also identify an additional variable that may confound tests of this hypothesis: non-genitalic structures with copulatory

contact functions may often have distal positions on appendages, which may also select for shallow scaling if precise placement on substrates (for grip or sensing) is an important aspect of their function. We encourage further research to determine how broadly applicable these findings are. Ideal tests of the contact-function hypothesis would involve non-distal structures with copulatory contact functions (e.g., specialized spurs or brushes on the body or non-distal segments; cf. Eberhard 2002), as well as distinguish between the contributions of having stimulatory versus grasping functions, and focusing on sexually dimorphic structures with species-specific morphology or size. Comparative research focusing on such structures will be highly illuminative.

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