






Intraspecific mating system evolution and its effect on complex male secondary sexual traits: Does male–male competition increase selection on size or shape?

Julian Baur^{1,2}  | Jeannine Roy¹ | Martin A. Schäfer¹  | Nalini Puniamoorthy³  |
Wolf U. Blanckenhorn¹  | Patrick T. Rohner^{1,4} 

¹Department of Evolutionary Biology and Environmental Studies, University of Zurich, Zurich, Switzerland

²Uppsala Universitet, Institute for Ecology and Genetics; Animal Ecology, Uppsala, Sweden

³Department of Biological Science, National University of Singapore, Singapore City, Singapore

⁴Department of Biology, Indiana University, Bloomington, IN, USA

Correspondence

Patrick T. Rohner, Department of Biology, Indiana University, 915 East Third Street, 102 Myers Hall, Bloomington, IN, 47405, United States.
Email: prohner@iu.edu

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Abstract

Sexual selection is generally held responsible for the exceptional diversity in secondary sexual traits in animals. Mating system evolution is therefore expected to profoundly affect the covariation between secondary sexual traits and mating success. Whereas there is such evidence at the interspecific level, data within species remain scarce. We here investigate sexual selection acting on the exaggerated male foreleg and the male wing in the common and widespread dung flies *Sepsis punctum* and *S. neocynipsea* (Diptera: Sepsidae). Both species exhibit intraspecific differences in mating systems and variation in sexual size dimorphism (SSD) across continents that correlates with the extent of male–male competition. We predicted that populations subject to increased male–male competition will experience stronger directional selection on the sexually dimorphic male foreleg. Our results suggest that foreleg size, width and shape were indeed positively associated with mating success in populations with male-biased SSD in both species, which was not evident in conspecific populations with female-biased SSD. However, this was also the case for wing size and shape, a trait often assumed to be primarily under natural selection. After correcting for selection on overall body size by accounting for allometric scaling, we found little evidence for independent selection on any of these size or shape traits in legs or wings, irrespective of the mating system. Sexual dimorphism and (foreleg) trait exaggeration is therefore unlikely to be driven by direct precopulatory sexual selection, but more so by selection on overall size or possibly selection on allometric scaling.

KEYWORDS

allometric scaling, body shape, body size, Diptera, geometric morphometrics, mating system, secondary sexual traits, Sepsidae, sexual selection

1 | INTRODUCTION

Sexually dimorphic trait expression, be it in physiology, morphology or behaviour, represents a hallmark of gonochoristic species and therefore contributes greatly to the phenotypic diversity across the tree of life. Although sexual dimorphism can be driven by niche differentiation between sexes via ecological character displacement (Hedrick & Temeles, 1989; Shine, 1989; Temeles, Pan, Brennan, & Horwitt, 2000), its evolution has classically been attributed mostly to variation in the intensity of sexual selection acting on males and females (Andersson, 1994; Darwin, 1872; Fairbairn, Blanckenhorn, & Székely, 2007). Correspondingly, mating system evolution is often identified as a major covariate of sexual dimorphism (Shine & Fitzgerald, 1995; Székely, Reynolds, & Figuerola, 2000; Webster, 1992; Weckerly, 1998).

However, it is now clear that the expression of sexual traits is shaped by more than sex-specific patterns of sexual selection. General life history, behaviour and ecology can all interact with, bias or modulate the evolution of sexual traits (Blanckenhorn, 2005; Emlen, 2001, 2006; Gomez & Perfectti, 2010; McCullough, Tobalske, & Emlen, 2014). In addition, variation in the underlying genetic and developmental mechanisms, as well as their dependence on environmental variation, can give rise to sex-specific trait expression (Badyaev, 2002; Chenoweth, Rundle, & Blows, 2008; Lande, 1980) to further influence the establishment of sexual dimorphisms (Allen, Somjee, & Miller, 2015; Bonduriansky, 2007a; Ledón-Rettig & Moczek, 2016; Rohner & Blanckenhorn, 2018; Zinna et al., 2018). Hence, whereas sexual selection is often responsible for the establishment of sexual dimorphisms, it does so in interaction with other evolutionary forces and hence necessitates studying the mechanism in action in detail. This particularly applies to traits that act as armaments and/or ornaments. Such traits are frequently structurally and developmentally complex, and due to their intrinsically high dimensionality give rise to multivariate fitness landscapes. Examples include the chemical bouquets used in mate attraction (Chenoweth & Blows, 2005), acoustic signalling (Bentsen, Hunt, Jennions, & Brooks, 2006), elaborate visual displays used in courtship (Katayama, Abbott, Kjærandsen, Takahashi, & Svensson, 2014), or the evolution of elaborate sexual morphology of primary (Arnqvist, 1998; Simmons, House, Hunt, & García-González, 2009) and secondary sexual traits (Emlen, Marangelo, Ball, & Cunningham, 2005; O'Brien, Katsuki, & Emlen, 2017).

Another common and frequently highlighted feature of secondary sexual trait expression is its dependence on overall size. Allometric scaling is the norm rather than the exception in general, but exaggerated structures are often considered to show particularly steep allometric slopes. Whereas this is sometimes seen as evidence for sexual selection acting on a trait, this is not necessarily expected (Bonduriansky, 2007b; Cotton, Fowler, & Pomiankowski, 2004; Eberhard et al., 2018; Fairbairn, 2005). Nevertheless, selection on size is very common in the animal kingdom (Blanckenhorn, 2000), and therefore, variation in overall body size can confound

selection on specific body parts, making it important to account for body size variation when investigating the selective forces driving secondary sexual morphology.

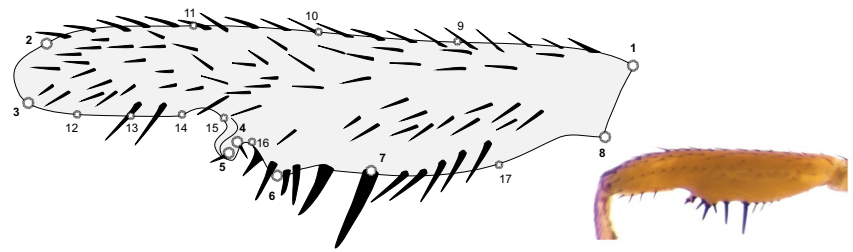
To better understand and characterize the role of sexual selection and allometry in the diversification of complex morphological traits, we here investigate the covariation between mating success and morphology in two male traits in sepsid flies (Diptera: Sepsidae): the sexually dimorphic and exaggerated fore femur as well as the unexaggerated wing. Several comparative studies document that even closely related species of sepsids can differ substantially in sexual morphology (e.g. abdominal sternite brushes, pronounced fore leg modifications: Eberhard, 2001a, 2001b, 2005) and mating behaviour (Puniamoorthy, 2014; Puniamoorthy, Su, & Meier, 2008). Such differentiation is however not limited to the species level but can also be found within species (Baur, Giesen, Rohner, Blanckenhorn, & Schäfer, 2019). Populations of some widespread species exhibit clear differences in mating systems. For instance, in the Holarctic *Sepsis punctum*, European populations are characterized by strong male–male competition with male-biased sexual size dimorphism (SSD) and the absence of precopulatory behavioural displays. In contrast, North American conspecifics exhibit female-biased SSD, and males perform elaborate copulatory courtship in order to secure copulations with choosier females (Dmitriew & Blanckenhorn, 2012, 2014; Puniamoorthy, Blanckenhorn & Schäfer, 2012a; Puniamoorthy, Schäfer & Blanckenhorn, 2012b; Schulz, 1999). A similar but geographically reversed situation is evident in the closely related *Sepsis neocynipsea*. In this species, North American populations exhibit male-biased SSD, male–male competition for access to females, and consequently experience stronger selection on male body size, whereas European populations of the same species show female-biased SSD and weaker selection on male size (Rohner, Blanckenhorn, & Puniamoorthy, 2016; Rohner, Teder, Esperk, Lüpold, & Blanckenhorn, 2018). Because female-biased SSD is the ancestral state in the genus *Sepsis* (as in insects in general: Blanckenhorn, Meier, & Teder, 2007), male-biased SSD evolved rather recently and independently in *S. punctum* and *S. neocynipsea* (Rohner et al., 2016). The cross-continental populations of either species are able to mate under laboratory conditions, produce viable offspring and genetically cluster as the same species based on mitochondrial barcodes.

As is typical for the genus *Sepsis*, males of both species have pronounced modifications of the fore femur (Figure 1). Since males use their femur to hold on to the female's wing base during copulation, these structures appear crucial for mating (Blanckenhorn, Kraushaar, Teuschl, & Reim, 2004; Ang, Puniamoorthy, & Meier, 2008; Puniamoorthy et al., 2008; Puniamoorthy, Ismail, Tan, & Meier, 2009; Eberhard, 2001a). Their precise function, however, remains unclear beyond the femur and tibia not merely forming a simple lock-and-key mechanism around the wing base (Eberhard, 2001a, 2005). Previous studies suggest that the replicated mating system shifts in sepsids (including continental variation in *S. punctum* and *S. neocynipsea*) coincide with an increase in the relative size and sex-specific condition dependence of the fore femur, which are presumably simultaneously driven by strong directional sexual selection on fore

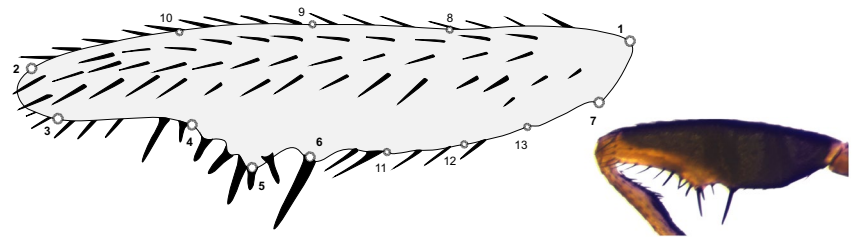
FIGURE 1 Modifications on the male fore femur in *Sepsis punctum* (a; redrawn from Hennig, 1949) and *S. neocynipsea* (b; redrawn from Duda, 1926). Wing morphology (c) is not exaggerated. The wing spot is present in both sexes and represents a synapomorphy of the *Sepsis* clade that includes *S. punctum* and *S. neocynipsea*. Landmarks used in this study are indicated with open circles

Femur morphology

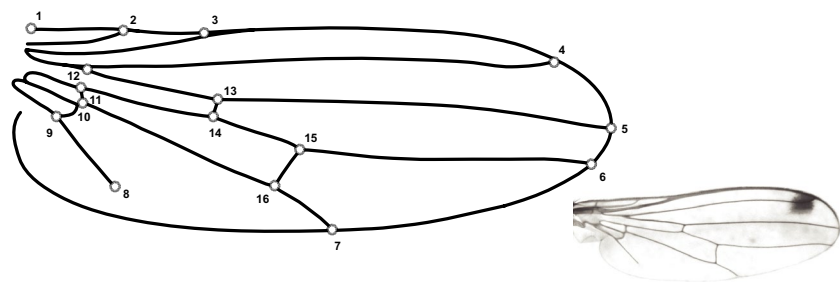
(a) *Sepsis punctum*



(b) *Sepsis neocynipsea*



Wing morphology



femur length and its relative width (Rohner & Blanckenhorn, 2018). Wings, on the other hand, are typically primarily under stabilizing ecological selection (Dudley, 2002; Gilchrist, Azevedo, Partridge, & O'Higgins, 2000) and are not involved in sexual courtship in *S. punctum* and *S. neocynipsea*.

Although a number of comparative studies have explored how mating system evolution influences (complex) morphological traits (Bro-Jørgensen, 2007; Simmons & Tomkins, 1996), few studies investigate this intraspecifically (e.g. Shine & Fitzgerald, 1995). Studying the function of complex morphological traits in cross-continental populations of both *S. punctum* and *S. neocynipsea* hence offers a unique opportunity to investigate how the evolution of increased male–male competition influences the role of male secondary sexual morphology for male mating success. Based on increased condition dependence and stronger sexual dimorphism in populations with male-biased SSD, we expected fore femur size and shape to be subject to directional precopulatory sexual selection, the intensity of which should be greater in populations with male-biased SSD. In contrast, we expected little sexual selection on wing size and shape, as wings are primarily thought to be under natural selection in these species. Because both structures show allometric scaling and size is known to covary with reproductive success, we additionally test for the effect of static allometry on selection on trait size and shape.

2 | MATERIALS AND METHODS

2.1 | Mating trials

We here revisit two studies of *S. punctum* by Puniamoorthy, Schafer, et al. (2012b) and *S. neocynipsea* by Rohner et al. (2016). Both studies investigated sexual selection on male body size in multiple North American and European populations in the laboratory. In both studies, test animals were acquired by rearing the offspring of multiple laboratory iso-female lines per population under variable food quantities, resulting in extensive adult size variation. Immediately upon eclosion, males and females were separated and kept in single-sex containers for 4–5 days. To assess selection on male morphology, mating trials were conducted in groups of different operational sex ratios (OSR) in 1L plastic containers equipped with water, sugar and cow dung ad libitum. These competitive mating experiments were conducted across multiple European and North American populations of both species (for details, see Puniamoorthy, Schäfer, et al., 2012b and Rohner et al., 2016, Table 1). Copulating pairs were removed from the arena, and depending on the number of copulations per replicate, mating trials were terminated after 2–4 hr. All mated and unmated individuals were subsequently frozen and stored in 70% EtOH until recently dissected for morphometric analysis. Although the two

Species	Continent	Population	OSR2	OSR4
<i>S. neocynipsea</i>	N. America	Kentucky, USA	4	3
<i>S. neocynipsea</i>	N. America	Ramona, USA	3	3
<i>S. neocynipsea</i>	N. America	Syracuse, USA	4	4
<i>S. neocynipsea</i>	N. America	Yellowstone, USA	3	3
<i>S. neocynipsea</i>	Europe	Sörenberg, Switzerland	4	4
<i>S. neocynipsea</i>	Europe	Bignasco, Switzerland	2	2
<i>S. neocynipsea</i>	Europe	Oberwald, Switzerland	4	5
<i>S. neocynipsea</i>	Europe	Zürich, Switzerland	3	3
<i>S. punctum</i>	N. America	Davis, CA, USA	4	4
<i>S. punctum</i>	N. America	Athens, GA, USA	4	4
<i>S. punctum</i>	N. America	New York (Manhattan), NY, USA	4	4
<i>S. punctum</i>	Europe	Wien, Austria	4	4
<i>S. punctum</i>	Europe	Zürich, Switzerland	4	4
<i>S. punctum</i>	Europe	Berlin, Switzerland	4	4
<i>S. punctum</i>	Europe	Nyköping, Sweden	4	4

TABLE 1 Number of mating container replicates per species, continent, population and operational sex ratio (OSR)

original studies included three OSR treatments (OSR1: 5 males vs. 5 females, OSR2: 10 male vs. 5 females, OSR4: 20 males vs. 5 females), we here restricted our analysis to the 2:1 and 4:1 sex ratios because neither study found significant selection under the most unnatural OSR1 condition (Blanckenhorn et al., 2004; Kraushaar & Blanckenhorn, 2002), rendering this treatment least informative in the context here.

2.2 | Morphometric measurements

Male forelegs and wings were removed from the adult thorax and dried at room temperature to allow evaporation of the ethanol. They were then embedded in Euparal (Carl Roth GmbH) on a glass slide. Slides were placed on a 35°C heating plate for five minutes to liquefy the Euparal resin and subsequently dried at room temperature. Wings and legs were photographed using a LeicaDFC490 camera mounted on a Leica MZ12 microscope, and tpsDig2 (Rohlf, 2009) was used to acquire landmark coordinates.

In both species, 16 homologous landmarks were chosen to describe wing morphology, marking vein-node positions covering the wing base and the blade following previously published methods (Puniamoorthy, 2014; Rohner, Roy, Schäfer, Blanckenhorn, & Berger, 2019) (Figure 1). Because fore femur morphology differs strongly between *S. punctum* and *S. neocynipsea* (Figure 1), we could not use the same landmarks in both species. Note that while this hampers species comparison, it still permits assessing (inter-continental) intraspecific variation, as landmarks are homologous among populations within species. For *S. neocynipsea*, we used 7 landmarks to describe shape variation of the male fore femur, marking distinct features. In addition, three sliding semi-landmarks were placed between landmarks 1 and 2 and between landmarks 6 and 7 to measure the curvature of the leg between the

fixed landmarks (Figure 1). In *S. punctum*, we used 8 full landmarks and 8 semi-landmarks. Individuals that had damaged appendages and/or missing wing veins were removed from the analyses. We performed a separate Procrustes transformation for each species and trait, and calculated fore femur and wing centroid size as an estimate of the overall structural size.

2.3 | Sexual selection on trait size

To test whether hind tibia length (a proxy for body size; cf. Rohner & Blanckenhorn, 2018), wing centroid size, fore femur size and fore femur width are subject to precopulatory sexual selection, we followed standard logistic regression approaches following Janzen and Stern (1998). We first z-scored all trait values by subtracting the replicate mean from each individual's trait value and dividing this centred score by the corresponding standard deviation ($z_i = (x_i - \bar{x}_i) / SD_x$). A binomial generalized mixed model with mating success (0 or 1) as dependent variable was then performed to test for the overall effect of standardized selection on trait size and for differences in selection strength between continents and OSRs. To accommodate the experimental setting, replicate mating trials nested within populations were entered as random effect. Standardized univariate selection gradients (β_1) were calculated by multiplying the coefficients of the binomial mixed model α by the constant $W_i(1 - W_i)/W_i$, where W_i indicates the absolute fitness value for each individual (Janzen & Stern, 1998). Because the traits investigated here are strongly correlated, it is difficult to distinguish between direct selection on trait size and indirect selection via selection on overall size. We therefore accounted for selection on overall size by calculating bivariate selection estimates (β_2), using standardized hind tibia length (HTL) as covariate and again testing for an effect of trait size on fitness. This procedure produced one

bivariate selection coefficient for the corresponding trait of interest (β_2) plus one for hind tibia length (β_{2HTL}).

2.4 | Sexual selection on shape

To test for sexual selection on shape of the fore femur and wings, we used a pooled within-group two-block partial least squares analysis (PLS). Two-block PLS quantifies the association between two data matrices and can therefore be used to quantify the covariation between phenotypes (first block) and fitness (second block), rendering a scaled version of the selection gradient (Klingenberg & Monteiro, 2005; Rohlf & Corti, 2000). For each species, continent and OSR, we tested for a relationship between Procrustes-transformed coordinates and reproductive success. Due to our hierarchical experimental design, individuals within a mating container represent the unit of observation and hence the appropriate level of analysis. We therefore used a pooled within-group PLS. We first calculated the deviation of each individual to its group (= mating container) mean shape, using these in one block and mating success (0 or 1) in the other block. This procedure removes variation in shape due to genetic population differentiation as well as random variation in male morphology between mating containers. The direction of the first PLS vector then represents the direction of selection within mating containers irrespective of population. Significance of selection was tested by means of randomization (10,000 random samples). As a quantitative measure of the shape difference associated with reproductive success, we computed the Procrustes distance between the mean shape of all individuals (mean phenotype before selection) and the mean shape of all successful males (mean phenotype after selection). In addition, we calculated selection differential (S) following Klingenberg and Monteiro (2005). We first calculated a score of predicted fitness by multiplying an individual's shape by the selection gradient (i.e. the vector of multiple regression coefficients of fitness on shape). Regressing shape onto this score using a multivariate regression then results in a scaled version of the selection differential. Dividing this vector by the variance of the expected fitness results in the unscaled selection differential (S) that has the same direction as the PLS vectors but has an appropriate magnitude.

To test to which extent the combined effects of sexual selection on overall size and allometric scaling of shape influence selection on shape, we assessed the similarity between the selection vectors and the effect of allometry. To this end, we used multivariate regression of shape against body size and extracted the vector of allometric shape change (A). To quantify the similarity between allometry and selection, we computed the vector correlation between A and S as

$$r_{SA} = \frac{|S \cdot A|}{\|S\| \times \|A\|}.$$

That is, we scaled the dot product of A and S by their norm (cf. Claude, 2008; Pitchers, Pool, & Dworkin, 2013; Schäfer et al., 2018). To test for selection on nonallometric shape effects (i.e. to correct for selection that is merely due to selection on overall size), we again used a PLS as above, now using the residuals of a multivariate regression of shape on trait size as shape variables.

3 | RESULTS

3.1 | Sexual selection on absolute and relative femur and wing size

Univariate sexual selection gradients (β_1) were generally positive across species, continents and traits except for European *S. neocynipsea* (Table 2), indicating that large males generally have an advantage in precopulatory sexual selection, in line with findings from the earlier studies. In *S. punctum*, the intensity of sexual selection on hind tibia length, femur size and wing size was stronger in the (European) populations with male-biased SSD (continent \times trait interaction: femur: $\chi_1^2 = 5.49$, $p = .019$; wing: $\chi_1^2 = 5.74$, $p = .017$, hind tibia length: $\chi_1^2 = 10.33$, $p = .001$). This was also the case in (North American) *S. neocynipsea* for wing size (continent \times trait interaction: $\chi_1^2 = 4.19$, $p = .041$), whereas fore femur size showed a marginally nonsignificant interaction ($\chi_1^2 = 3.57$, $p = .059$), and selection on hind tibia length did not vary across continents ($\chi_1^2 = 1.43$, $p = .232$). However, in contrast to the two previous studies, the trait \times OSR \times continent interactions were all nonsignificant. This difference is likely caused by neglecting mating trials with equal sex ratios (OSR1) in this study, leading to less overall variation and diminishing power to detect an interaction.

When controlling for selection on overall body size by adding standardized HTL as covariate, we very often found the bivariate selection gradient opposing the corresponding effect of body size (β_{2HTL}). However, due to the strong correlation among explanatory variables (average correlation $r = .91$), these results must be interpreted with caution. Strong covariance among predictor variables is known to be problematic, such that the resulting partial selection coefficients are difficult to interpret (Lande & Arnold, 1983; Mitchellolds & Shaw, 1987). As an alternative approach, we computed residual trait size from a regression of log trait size on log HTL for each population and used a binomial generalized mixed model with residual trait size, continent, OSR plus any appropriate interactions to test for selection on relative trait size. The residual trait size and continent \times residual trait size terms in this analysis were all statistically unrelated to mating success (see Tables S1 and S2). There were however weak tendencies for potentially differential selection on residual trait size between continents in *S. punctum* (femur and wing size, Table S1) and also on femur width in *S. neocynipsea* (Table S2), yet compared to the main effects of relative trait size, these effects are, if at all, of minor importance. We thus conclude that precopulatory sexual selection acts

TABLE 2 Univariate and bivariate selection gradients with their corresponding standard error based on binomial generalized mixed models following Janzen and Stern (1998)

Trait	Species	SSD	Continent	OSR	$\beta_1 (\pm SE)$	$\beta_2 (\pm SE)$	$\beta_{2HTL} (\pm SE)$
(a) Hind tibia length	<i>S. neocynipsea</i>	Female-biased	Europe	2	0.04 (\pm 0.10)		
	<i>S. neocynipsea</i>	Female-biased	Europe	4	0.02 (\pm 0.10)		
	<i>S. punctum</i>	Female-biased	N. America	2	0.11 (\pm 0.13)		
	<i>S. punctum</i>	Female-biased	N. America	4	0.19 (\pm 0.16)		
	<i>S. neocynipsea</i>	Male-biased	N. America	2	0.16 (\pm 0.08)		
	<i>S. neocynipsea</i>	Male-biased	N. America	4	0.35 (\pm 0.20)		
	<i>S. punctum</i>	Male-biased	Europe	2	0.35 (\pm 0.08)		
	<i>S. punctum</i>	Male-biased	Europe	4	0.54 (\pm 0.11)		
(b) Femur size	<i>S. neocynipsea</i>	Female-biased	Europe	2	0.02 (\pm 0.10)	-0.5 (\pm 0.53)	0.53 (\pm 0.53)
	<i>S. neocynipsea</i>	Female-biased	Europe	4	-0.02 (\pm 0.10)	-0.58 (\pm 0.38)	0.58 (\pm 0.38)
	<i>S. punctum</i>	Female-biased	N. America	2	0.19 (\pm 0.14)	0.46 (\pm 0.30)	-0.30 (\pm 0.30)
	<i>S. punctum</i>	Female-biased	N. America	4	0.23 (\pm 0.15)	0.44 (\pm 0.42)	-0.22 (\pm 0.43)
	<i>S. neocynipsea</i>	Male-biased	N. America	2	0.20 (\pm 0.08)	1.05 (\pm 0.40)	-0.87 (\pm 0.40)
	<i>S. neocynipsea</i>	Male-biased	N. America	4	0.57 (\pm 0.23)	1.89 (\pm 0.67)	-1.37 (\pm 0.63)
	<i>S. punctum</i>	Male-biased	Europe	2	0.33 (\pm 0.08)	-0.13 (\pm 0.25)	0.48 (\pm 0.26)
	<i>S. punctum</i>	Male-biased	Europe	4	0.50 (\pm 0.11)	-0.31 (\pm 0.33)	0.84 (\pm 0.34)
(c) Femur width	<i>S. neocynipsea</i>	Female-biased	Europe	2	-0.02 (\pm 0.20)	-1.16 (\pm 0.61)	1.20 (\pm 0.60)
	<i>S. neocynipsea</i>	Female-biased	Europe	4	-0.01 (\pm 0.19)	-0.30 (\pm 0.48)	0.32 (\pm 0.47)
	<i>S. punctum</i>	Female-biased	N. America	2	0.02 (\pm 0.13)	-0.23 (\pm 0.24)	0.3 (\pm 0.24)
	<i>S. punctum</i>	Female-biased	N. America	4	0.28 (\pm 0.16)	0.37 (\pm 0.27)	-0.11 (\pm 0.27)
	<i>S. neocynipsea</i>	Male-biased	N. America	2	0.15 (\pm 0.16)	-0.06 (\pm 0.33)	0.24 (\pm 0.34)
	<i>S. neocynipsea</i>	Male-biased	N. America	4	0.38 (\pm 0.20)	0.28 (\pm 0.34)	0.12 (\pm 0.34)
	<i>S. punctum</i>	Male-biased	Europe	2	0.37 (\pm 0.08)	0.26 (\pm 0.19)	0.11 (\pm 0.18)
	<i>S. punctum</i>	Male-biased	Europe	4	0.48 (\pm 0.11)	0.63 (\pm 0.24)	-0.10 (\pm 0.23)
(d) Wing size	<i>S. neocynipsea</i>	Female-biased	Europe	2	0.07 (\pm 0.10)	0.42 (\pm 0.36)	-0.37 (\pm 0.36)
	<i>S. neocynipsea</i>	Female-biased	Europe	4	-0.08 (\pm 0.10)	-1.11 (\pm 0.36)	1.08 (\pm 0.36)
	<i>S. punctum</i>	Female-biased	N. America	2	0.18 (\pm 0.14)	0.29 (\pm 0.24)	-0.12 (\pm 0.24)
	<i>S. punctum</i>	Female-biased	N. America	4	0.14 (\pm 0.15)	-0.10 (\pm 0.30)	0.28 (\pm 0.30)
	<i>S. neocynipsea</i>	Male-biased	N. America	2	0.20 (\pm 0.08)	0.53 (\pm 0.25)	-0.34 (\pm 0.25)
	<i>S. neocynipsea</i>	Male-biased	N. America	4	0.61 (\pm 0.24)	1.39 (\pm 0.50)	-0.85 (\pm 0.45)
	<i>S. punctum</i>	Male-biased	Europe	2	0.32 (\pm 0.08)	-0.09 (\pm 0.21)	0.44 (\pm 0.21)
	<i>S. punctum</i>	Male-biased	Europe	4	0.44 (\pm 0.11)	-0.58 (\pm 0.28)	1.09 (\pm 0.29)

Note: Univariate selection gradients (β_1) were calculated by regressing mating success against the corresponding standardized trait as the only predictor variable. To account for selection on overall size, we used standardized hind tibia length as covariate and calculated bivariate selection estimates (producing one bivariate selection coefficient for the corresponding trait of interest (β_2) plus one for hind tibia length [β_{2HTL}]). Significant coefficients are bold.

mostly indirectly via body size and not directly on relative trait size (Lynch & Walsh, 1998).

3.2 | Sexual selection on femur and wing shape

Selection on femur morphology was only significant in populations with male-biased SSD (Table 3). However, fore femur morphology also showed allometric variation (all $p < .001$) that closely aligned with the selection vector in populations where selection on shape

was significant (see Figure 2). This association was so strong that after correcting shape for allometric scaling there was no evidence for a statistical association between residual shape and mating success throughout (irrespective of species, SSD or OSR).

Similar to fore femur morphology, wing shape co-varied significantly with mating success in populations of both species with male-biased SSD (Figure 3; Table 4). This association was, however, again mainly due to allometric variation (with the exception of North American *S. neocynipsea* showing a significant association at OSR4).

TABLE 3 Procrustes distance between mean shape before and after selection, the norm of the selection differential ($\|S\|$), as well as the significance of the two-block PLS analyses for femur morphology by species, direction of SSD and operational sex ratio (OSR).

Fore femur	SSD	Continent	OSR	Procrustes distance $\times 10^3$	$\ S\ \times 10^2$	P_{PLS} full shape	P_{PLS} allometry-corrected	$r_{S,A}$
<i>S. neocynipsea</i>	Female-biased	Europe	2	6.89	1.61	0.470	0.204	0.21 [0.01, 0.63]
	Female-biased	Europe	4	7.05	0.51	0.272	0.078	0.09 [0.01, 0.24]
	Male-biased	N. America	2	7.6	0.06	<0.001	0.180	0.82 [0.15, 0.97]
	Male-biased	N. America	4	16.03	0.22	0.005	0.124	0.94 [0.73, 0.99]
<i>S. punctum</i>	Female-biased	N. America	2	5.83	0.53	0.090	0.061	0.37 [0.01, 0.84]
	Female-biased	N. America	4	10.5	0.1	0.091	0.219	0.68 [0.17, 0.93]
	Male-biased	Europe	2	9.88	2.19	0.010	0.665	0.97 [0.94, 0.99]
	Male-biased	Europe	4	19.64	11.09	<0.001	0.783	0.98 [0.93, 0.99]

Note: Sexual selection on shape was only significant in populations with male-biased SSD, yet the shape difference between successful and unsuccessful males was strongly correlated with the allometric shape change ($r_{S,A}$). After accounting for allometry, there was no statistical support for selection on any aspects of shape in any population or species. Significant values in bold ($P < 0.05$). The correlations between the vectors of allometry (A) and the shape changes associated to selection (S) are given with corresponding 95% confidence intervals.

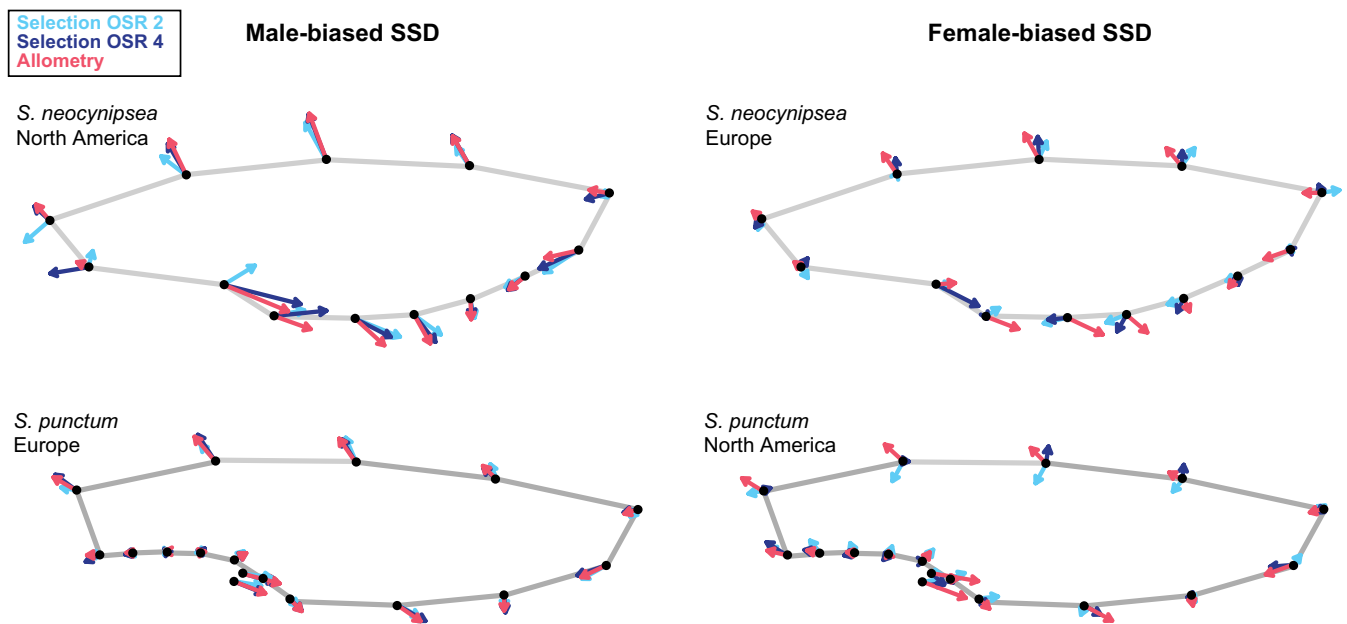


FIGURE 2 Fore femur shape deformations associated with mating success under different sex ratios (OSR2: light blue; OSR4: dark blue). Red arrows indicate the shape change associated with an increase in body size. See Table 3 for estimates of the magnitude of the selection differentials

4 | DISCUSSION

Conspicuous exaggerated male secondary sexual traits are often perceived to be subject to directional sexual selection. However, the evolution of sexually dimorphic trait expression can be influenced by a plethora of ultimate as well as proximate mechanisms, requiring detailed assessment of the evolutionary role of any given trait. Comparing two species that display intraspecific, continental variation in mating systems, we predicted the evolution of increased male–male competition for access to females in populations with male-biased SSD to have profound effects on directional selection

on male secondary morphology. Fore femur size, width and shape were indeed associated with mating success in populations with male-biased SSD in both species, but not consistently so in conspecific populations with female-biased SSD. Surprisingly, however, this was also the case for wing size and shape, a trait assumed to be primarily under natural selection (e.g. in drosophilids: Gilchrist et al., 2000, but also in sepsids: Baur et al., 2019; Rohner et al., 2019; Rohner et al., 2015). Yet, after accounting for allometric scaling, we found little evidence for direct precopulatory sexual selection on fore femur (or wing) morphology beyond the documented selection on overall body size (Puniamoorthy, Schafer, et al., 2012b; Rohner

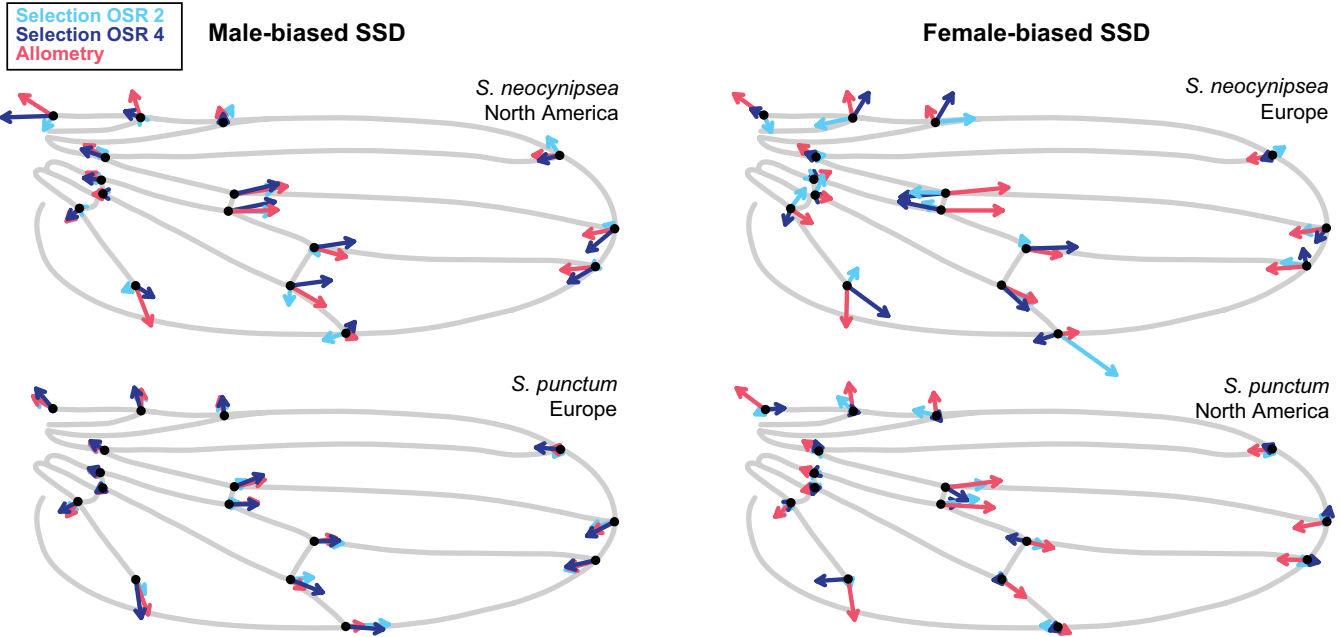


FIGURE 3 Wing shape deformations associated with mating success (= PLS coefficients) under different sex ratios (OSR2: light blue; OSR4: dark blue). Red arrows indicate the shape change associated with an increase in body size. See Table 4 for estimates of the magnitude of the selection differentials

TABLE 4 Procrustes distance between mean shape before and after selection, the norm of the selection differential ($\|S\|$), as well as the significance of the two-block PLS analyses for wing morphology by species, direction of SSD and operational sex ratio (OSR).

Wing	SSD	Continent	OSR	Procrustes distance $\times 10^3$	$\ S\ \times 10^2$	P_{PLS} full shape	P_{PLS} allometry-corrected	$r_{S,A}$
<i>S. neocynipsea</i>	Female-biased	Europe	2	4.92	0.31	0.440	0.267	0.07 [0.01, 0.17]
	Female-biased	Europe	4	5.24	0.52	0.322	0.140	0.06 [0.01, 0.16]
	Male-biased	N. America	2	4.75	0.01	0.210	0.443	0.75 [0.23, 0.97]
	Male-biased	N. America	4	8.55	0.14	<0.001	0.021	0.91 [0.86, 0.98]
<i>S. punctum</i>	Female-biased	N. America	2	5.39	0.14	0.870	0.988	0.73 [0.23, 0.97]
	Female-biased	N. America	4	3.37	0.56	0.849	0.437	0.04 [0.01, 0.06]
	Male-biased	Europe	2	4.36	1.91	<0.001	0.265	0.89 [0.73, 0.99]
	Male-biased	Europe	4	8.19	5.55	<0.001	0.154	0.93 [0.87, 0.98]

Note: Sexual selection on shape was only significant in populations with male-biased SSD, yet the shape difference between successful and unsuccessful males was strongly correlated with the allometric shape change ($r_{S,A}$). After accounting for allometry, there was no statistical support for selection on any aspects of shape in any population or species except for North American *S. neocynipsea*. Significant values in bold ($P < 0.05$). The correlations between the vectors of allometry (A) and the shape changes associated to selection (S) are given with corresponding 95% confidence intervals.

et al., 2016; this study). This suggests that selection acts mainly on size and not directly on the relative size of secondary sexual traits. However, size and shape are often evolutionarily entwined (Gould, 1966; Huxley, 1932; Thompson, 1917). We in turn discuss the function and adaptive value of body size and allometric scaling of secondary sexual traits in the evolution of mating systems.

The evolutionary benefits of being large are manifold (Blanckenhorn, 2000), and sexual or fecundity selection for large size is arguably common in both males and females (Andersson, 1994; Fairbairn et al., 2007; Honek, 1993; Kingsolver & Pfennig, 2004). However, apart from ecological selection (e.g. via predation, parasitism or foraging success), the advantage of being large

must depend on the mating system and critically so also on the (relative) size of the opposite sex. This is because the larger sex is likely able to effect greater control over copulation and reproduction. For instance, species that exhibit female choice or scramble competition tend to display female-biased SSD, whereas species with larger males demonstrate male-male competition and resource defence polygyny, and males are frequently able to coerce copulations (Andersson, 1994; Blanckenhorn, 2005). That is, the advantage of being a large male should be greater in species where males are larger than females (Ding & Blanckenhorn, 2002). Accordingly, we here found larger males to have higher mating success in populations that display male-biased SSD (the derived character state) relative

to the ancestral, female-biased populations. In these populations, the strength of selection further increases with the operational sex ratio. The variation in strength and direction of sexual selection on overall body size shown here and in the two previous studies therefore follows theoretical expectations (Andersson, 1994).

In contrast, we did not find any congruent patterns for relative trait size. Secondary sexual traits are expected to strongly covary with an individual's reproductive success (Cotton et al., 2004), and investment in relative size, complexity or elaborateness is expected to be subject to sexual selection. However, because large individuals almost always have larger secondary sexual traits, it is not always clear whether selection acts directly on trait size or indirectly via selection on overall body size, strength or stature (Eberhard et al., 2018; Gould, 1974). This is not merely a statistical problem, but might underlie biological function. If selection acts on trait size, individuals must be able to wield a large weapon or to carry larger, more elaborate ornaments (Tomkins, Kotiaho, & LeBas, 2005), so trait exaggeration requires a certain stature. Similarly, for selection to act on overall body size, conspecifics must somehow be able to sense and assess the size of a competitor or a potential mate (Charlton & Reby, 2016). Secondary sexual traits may therefore also act in communicating body size. However, due to sensory biases innate to most stimuli (Weber-Fechner law: Cohen, 1984), it has been argued that an absolute increase in trait size is more difficult to sense in large individuals compared to a smaller conspecific. For structures to be perceived as relatively larger, large males need a disproportionately large structure in order to make sure a similarly sized male recognizes him as larger, hence superior and not worth fighting with. This should particularly be the case in species where males engage in time consuming or energetically costly combat, and/or where ritualized contest replaces actual aggressive and potentially harmful behaviour (Emlen & Nijhout, 2000).

The absence of selection on relative trait size in the sepsids studied here might thus reflect selection on an indicator trait that is directly coupled to overall body size (Emlen & Nijhout, 2000; Wallace, 1987). In North American *S. neocynipsea* and European *S. punctum* (populations of the respective species with male-biased SSD), males engage in aggressive interactions, including displacements of competing males already mounted on a female by grabbing their competitor with their forelegs. As such, the male foreleg is a trait used in inter- as well as intrasexual contact behaviour that could therefore signal male size, stature or overall quality. Moreover, given the typically nonlinear, sigmoidal allometry of coloration of the male fore femur (demonstrated in *S. thoracica* by Busso & Blanckenhorn, 2018), but more subtly present in most *Sepsis* species; *unpublished*), which is also subject to sexual selection (Busso & Blanckenhorn, 2018), one could speculate that this structure broadcasts an individuals' size and strength (similar to e.g. vocal signalling in mammals: Charlton & Reby, 2016). The increased allometry of fore femur size in populations with male-biased SSD (Rohner & Blanckenhorn, 2018) also fits the expectations of a trait used in threat signalling (Eberhard et al., 2018). Therefore, although we did not find current directional selection on fore femur morphology after controlling for the effect of allometry

in our laboratory setting, we cannot exclude that this structure plays a role in selection on overall size, either visually via the coloration patterns, or via tactile stimulation when interacting with a female or a rival male. The detailed function, and in particular the role in post-copulatory selection, however, awaits further scrutiny.

Wings are important for dispersal and are thus usually thought to be subject to viability (natural) selection via flight performance, and such selection is not necessarily expected to differ between the sexes (Harrison, 1980; Hill, Griffiths, & Thomas, 2011; Hill, Thomas, & Blakeley, 1999; Hochkirch & Damerau, 2009; Roff & Fairbairn, 1991; Stevens, Turlure, & Baguette, 2010). However, in various flying organisms, wings and flight ability also play a role in finding mates and reproduction (e.g. (Hoikkala, Aspi, & Suvanto, 1998; Møller et al., 1998; Siva-Jothy, 1999). Wings could also play a role in male aggression and courtship in some sepsid species (Araujo, Tuan, Yew, & Meier, 2014; Eberhard, 2002; Puniamorthy et al., 2009), but there is no obvious wing signalling in the species studied here. Although known to play a pronounced role in mating behaviour in other Diptera (e.g. various Drosophilidae or Tephritidae), we did not find evidence for sexual selection on wings here other than via overall body size. This is despite the fact that wings show sexual shape dimorphism (Rohner et al., 2019), which is common at least in flies (Gidaszewski, Baylac, & Klingenberg, 2009) and probably insects in general. Sepsid wings might be naturally selected and there is evidence for adaptive clinal variation in wing shape (Rohner et al., 2019), but whether wing shape is also subjected to other sources of natural selection remains to be tested directly.

5 | CONCLUSIONS

In agreement with sexual selection theory, we here found the evolution of male-biased SSD and increased male-male competition to be associated with stronger selection on the size and shape of male secondary sexual traits. However, the same patterns were evident in the weakly sexually dimorphic wings that are unlikely to function in the mating context. As the covariation between male morphology and reproductive success can be explained to a large extent by allometry, most if not all selection may be explained by mere size. However, allometric scaling relationships might themselves be the result of sexual selection (O'Brien et al., 2017), and therefore, disentangling whether sexual selection acts on body size, the relative or absolute trait size, or even on the allometric relationship itself (i.e. a reaction norm) remains difficult, at least in this specific case. Despite the lack of evidence of a direct role of the sepsid fore femur morphology in mating success, secondary sexual traits rarely have only one function (Eberhard et al., 2018; McCullough, Miller, & Emlen, 2016), and the sepsid fore femur may be no exception. Future investigations of other fitness components under natural conditions will be necessary.

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ORCID

Julian Baur  <https://orcid.org/0000-0002-4739-2756>

Martin A. Schäfer  <https://orcid.org/0000-0002-0982-1468>

Nalini Puniamoorthy  <https://orcid.org/0000-0003-0651-8356>

Wolf U. Blanckenhorn  <https://orcid.org/0000-0002-0713-3944>

Patrick T. Rohner  <https://orcid.org/0000-0002-9840-1050>

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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