



Comparative sexual selection in field and laboratory in a guild of sepsid dung flies

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Phenomenological and behavioural studies have greatly advanced the study of natural selection. Field studies of selection well appraise the natural situation, but is this also true for laboratory studies, which are typically more mechanistic? We compared precopulatory sexual selection (mating differential based on pairing success) in field and laboratory of several closely related, ecologically similar black scavenger dung flies (Diptera: Sepsidae). Selection on fore femur (sexual trait) and wing size (nonsexual trait) and shape varied considerably among seven species and continental populations in agreement with variation in their mating system and sexual size dimorphism. Selection on trait size was mostly positive or nil, but never significantly negative, implying mating advantages of large males in most species. Strongest selection was found in species/populations with male-biased size dimorphism, associating evolutionary shifts from female- to male-biased dimorphism with intensified sexual selection for large male size by adding male–male competition to a mating system previously driven primarily by female choice. Although sexual selection on shape was closely aligned with allometric shape variation, selection on fore femur shape was more consistent than selection on wing shape, which was absent in most species. Sexual selection intensities, but not necessarily the underlying behavioural mechanisms, were overall similar in field and laboratory, suggesting that laboratory assessments well represent the natural situation. If this conclusion can be generalized, it would lend credence to the strategy of using controlled laboratory mating studies to better understand natural selection, behaviour and ecology, at least for smaller animals that can be held in captivity.

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Ever since the times of Darwin and Wallace, the study of natural selection has been one of the main hallmarks of evolutionary research. Starting out as little more than qualitative narrative, this framework was expanded during the first half of the 20th century by population geneticists such as R.A. Fisher (1930) and E.B. Ford (1964), and later by quantitative geneticists. These conceptual developed statistical approaches and techniques have since been applied to many species, fitness components and environments (Arnold & Wade, 1984a, b; Lande & Arnold, 1983; Arnold, 1994; Arnold & Duvall, 1994; Brodie, Moore, & Janzen, 1995; Janzen & Stern, 1998). Several prominent comparative (meta-)analyses resulted over the years that have greatly enhanced our understanding of the process and evolutionary consequences of natural

(including sexual) selection (Blanckenhorn, 2000, 2007; Cox & Calsbeek, 2009; Endler, 1986; Gotanda, Correa, Turcotte, Rolshausen, & Hendry, 2015; Kingsolver et al., 2001; Kingsolver & Pfennig, 2004; Siepielski, DiBattista, & Carlson, 2009, 2013). Nevertheless, both phenomenological and mechanistic (behavioural) studies of sexual, fecundity and viability selection in the field and the laboratory continue to be central tools in the toolbox of evolutionary and ecological researchers (e.g. Blanckenhorn, Mühlhäuser, Morf, Reusch, & Reuter, 2000; Blanckenhorn, Kraushaar, & Reim, 2003; Busso & Blanckenhorn, 2018; Jann, Blanckenhorn, & Ward, 2000; Rohner, Puniamoorthy, & Blanckenhorn, 2016).

A long-standing discussion centres around whether and how the typically mechanistic (i.e. behavioural) laboratory studies of selection adequately reflect the situation in the wild. Field studies of natural and sexual selection well appraise the natural situation (Andersson, 1994; Clutton-Brock, 1988; Endler, 1986), but have the

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disadvantage that they are largely phenomenological and therefore merely correlational, meaning that the underlying behavioural mechanisms often remain unassessed and hence undetected (Evans & Garcia-Gonzales, 2016; Anthes, Häderer, Michiels, & Janicke, 2017; e.g. ; Jann et al., 2000). By contrast, laboratory studies typically test particular (mechanistic) hypotheses in often rather specific, highly controlled environmental settings, consequently lacking generality. Nevertheless, both types of study ultimately produce situation-specific estimates of sexual selection, mate choice and/or associated quantitative genetic and life history parameters that reflect particular contexts, populations, environments etc., and therefore can fluctuate considerably (Arnold & Wade, 1984a,b; Mousseau & Roff, 1987; Falconer, 1989; Roff, 1997; Shuker & Simmons, 2014; Bailey, Marie-Orleach, & Moore, 2018; Dougherty, 2020; e.g. Grant & Grant, 2002; Blanckenhorn, Morf, Mühlhäuser, Reusch, 1999 and Blanckenhorn, Mühlhäuser, Morf, Reusch, & Reuter, 2000).

Most large animals cannot be reasonably kept and studied in the laboratory or in settings emulating their natural environments. Smaller animals, such as insects, however, often can. Nevertheless, even small species may regularly not breed well or not at all in the laboratory for a multitude of reasons, rendering choice of test species somewhat arbitrary. A fruitful approach is to study a subset of closely related species, say an entire genus, that all breed well in the laboratory because their natural histories are sufficiently similar (e.g. Pitnick et al., 1995; Arnqvist & Rowe, 2002; Bonduriansky, 2003; Björk & Pitnick, 2006). In this way one can cover and compare a diversity of natural environments or mating systems to generate representative assessments of, for instance here, sexual selection and precopulatory mate choice.

We here qualitatively and quantitatively compare sexual selection in the field and the laboratory across a guild of closely related black scavenger, ensign or dung flies (Diptera: Sepsidae; Pont & Meier, 2002; Ang et al., 2013) in a microevolutionary context. Multiple widespread species of this group with similar ecological niches coexist in Europe and beyond. For example, all 12 species of the genus *Sepsis* present in Switzerland may be found on the same pasture (Rohner et al., 2014, 2015, Rohner and Haenni et al., 2019).

Sepsid flies have received considerable research attention in evolutionary ecology because of their diverse, fast-evolving mating behaviours (Eberhard, 2001a, 2003; Kraushaar & Blanckenhorn, 2002; Martin & Hosken, 2004; Mühlhäuser & Blanckenhorn, 2002; Puniamoorthy, 2014; Tan, Ng, & Meier, 2011) and their conspicuous secondary sexual morphology (male forelegs and genitals: Eberhard, 2001b; Pont & Meier, 2002; Bowsher & Nijhout, 2009; Bowsher, Ang, Ferderer, & Meier, 2013; Herath, Dochtermann, Johnson, Leonard, & Bowsher, 2015; Rohner & Blanckenhorn, 2018). Mating systems range from species with classic female choice and male courtship to species with male scramble or contest competition, with associated changes in mating behaviour, morphology and life history (Puniamoorthy et al. 2008, 2009; Rohner & Blanckenhorn, 2018). At least four sepsid species commonly studied populate both Europe and North America (*Saltella sphondylii*, *Sepsis biflexuosa*, *Sepsis cynipsea*, *Sepsis punctum*: Pont & Meier, 2002), of which the latter two feature independent intraspecific shifts from the ancestral female-choice-dominated mating system with female-biased sexual size dimorphism (SSD) to a system characterized by male–male competition and male-biased SSD that is associated with an increase in investment in male ornaments or armaments (Dmitriew & Blanckenhorn, 2012, 2014; Puniamoorthy, Blanckenhorn, & Schäfer, 2012; Puniamoorthy, Schäfer, & Blanckenhorn, 2012; Rohner, Blanckenhorn, & Puniamoorthy, 2016; Rohner & Blanckenhorn, 2018; Table 1). Most species can be held, bred and observed in the laboratory under seminatural conditions on the same substrate (cow dung), permitting direct comparison of (among other things) mating behaviour and mate choice.

To assess our ability to approximate natural conditions by studying laboratory populations, we here compare selection estimates generated in the laboratory to those generated in the field for several species. We produce standard mating differentials based on male pairing success (a binary trait) for the size of the sexually monomorphic wing and the strongly dimorphic fore femur of seven closely related sepsid species with similar ecology (Table 1; Lande & Arnold, 1983; Arnold & Wade, 1984a,b; Arnold & Duvall, 1994; Brodie & Janzen, 1996; Janzen & Stern, 1998; Jones, 2009). In addition, we use geometric morphometric approaches to quantify

Table 1
Species and continental populations studied in the field and/or laboratory, their typical body sizes and dimorphism, with effective operational sex ratios derived from this study (OSR)

Species	Continent	Population	Head width (mm) ^a			Effective OSR	
			Male	Female	SDI*	Mean (SE; min, max)	
<i>Saltella sphondylii</i>	Field	Europe	Zürich (CH)	1.08	1.11	0.03	2.08 (0.202; 1.43, 2.83)
	Lab		No data				
	Field	North America	Syracuse (NY)	1.08			3.47 (0.154; 2.89, 4.13)
	Lab		No data				
<i>Sepsis biflexuosa</i>	Field	Europe	No data	0.91	0.93	0.01	2.01 (0.013; 1.81, 2.14)
	Lab		No data				
	Field	North America	Syracuse	0.90	0.90	0.00	2.01 (0.013; 1.81, 2.14)
	Lab		No data				
<i>Sepsis cynipsea</i>	Field	Europe	Sörenberg (CH)	1.02	1.09	0.07	1.90 (0.023; 1.56, 2.25) 3.34 (0.389; 1.67, 10.00)
	Lab		4 populations				
<i>Sepsis fulgens</i>	Field	Europe	Zürich	0.99	1.03	0.04	2.19 (0.017; 2.12, 2.30)
	Lab		No data				
<i>Sepsis neocynipsea</i>	Field	Europe	Sörenberg	1.12	1.17	0.04	0.72 (0.432; 0.53, 0.91) 5.70 (0.440; 2.00, 7.00)
	Lab		4 populations				
	Field	North America	Syracuse	1.12	1.08	−0.04	2.56 (0.181; 2.09, 3.60) 3.39 (0.188; 1.80, 7.00)
	Lab		4 populations				
<i>Sepsis punctum</i>	Field	Europe	Zürich	1.30	1.22	−0.07	2.10 (0.104; 1, 4) 2.73 (0.074; 1.60, 5.00)
	Lab		4 populations				
	Field	North America	Syracuse	1.04	1.07	0.03	2.11 (0.113; 1.40, 4.75) 3.78 (0.216; 1.75, 8.00)
	Lab		3 populations				
<i>Sepsis thoracica</i>	Field	Europe	Zürich	1.11	1.03	−0.08	2.59 (0.126; 1.33, 5.00) 2.93 (0.140; 1.50, 4.00)
	Lab		Petroia (I)				

SDI: sexual dimorphism index (size larger sex/size smaller sex)−1.

^a Typical mean sizes from Rohner et al. (2016).

how well the relationship between shape and fitness can be replicated in the laboratory. Four of the species were studied in both North America and Europe. (1) We sought to directly contrast sexual selection exerted on morphology across all species living in the same habitat, which we expected to differ depending on their particular mating system (described in more detail below), thus ultimately contributing to the diversification and coexistence of these species. We predicted more strongly positive sexual selection favouring large trait size (and shape) in those species and continental populations with male-biased SSD (Puniamoorthy, Blanckenhorn, & Schäfer, 2012; Puniamoorthy, Schäfer, & Blanckenhorn, 2012; Rohner et al., 2016, 2018; Rohner & Blanckenhorn, 2018; see above). (2) For a subset of the seven species, we further systematically compared mating differentials measured under natural field conditions with those generated in the laboratory under seminatural conditions at similar operational sex ratios (OSR). Only if the laboratory reflected the natural situation well did we expect sexual selection to be at least qualitatively (if not quantitatively) similar in field and laboratory. Else, if the typical mating system of the species was misjudged in the laboratory in terms of population density, OSR, etc., parameters that are of central quantitative importance for the calculation of selection intensities (Arnold & Wade, 1984a,b; Lande & Arnold, 1983; Brodie et al., 1995; Blanckenhorn, Reuter, et al., 1999), we would obtain significant discrepancies indicating that laboratory studies of selection are not necessarily representative of the field situation, strengthening the view that sexual selection and/or mate choice studies are highly context dependent (Dougherty, 2020).

METHODS

Study Species

We studied seven sepsid species that commonly co-occur on pastures near Zürich (47.34°N, 8.54°E; ca. 450 m altitude) or Sörenberg (46.87°N, 8.27°E; ca. 1150 m), Switzerland (CH): *Saltella sphondylii*, *Sepsis biflexuosa*, *Sepsis cynipsea*, *Sepsis fulgens*, *Sepsis neocynipsea*, *Sepsis punctum* and *Sepsis thoracica*. In parallel, and for direct comparison, we also investigated North American populations of *S. sphondylii*, *S. biflexuosa*, *S. neocynipsea* and *S. punctum* from around Syracuse, New York (42.94°N, 76.21°W; ca. 150 m). We multiply assessed mating differentials in the field for all species using standard methods (explained below) at haphazard times over the 2016 season (cross-sectional sampling). We additionally separately assessed mating differentials in the laboratory using fly cultures from several geographical populations of these species. Laboratory estimates for *S. neocynipsea* and *S. punctum* were derived from our previously published studies by Puniamoorthy, Blanckenhorn, & Schäfer (2012), Puniamoorthy, Schäfer, & Blanckenhorn (2012) and Rohner, Puniamoorthy, & Blanckenhorn (2016), whereas the laboratory estimates presented for *S. cynipsea* and *S. thoracica* are hitherto unpublished.

Our standard laboratory rearing conditions are described in detail in the last-cited publications. In brief, flies were housed in isofemale line or population groups of up to several hundred individuals in large 3.5-litre (2.2 x 1.2 x 1.2 dm³) or small 1.5 litre (1 x 1 x 1.4 dm³) plastic containers supplied with fresh cow dung (for food and breeding), sugar (for energy) and water ad libitum. These flies had been held and bred in the laboratory for varying periods of time prior to our assessments.

Sexual Selection Estimation in the Laboratory

We reanalysed two previous laboratory studies of sexual selection in European and North American *S. punctum* by Puniamoorthy,

Blanckenhorn, & Schäfer (2012) and in *S. neocynipsea* by Rohner, Puniamoorthy, & Blanckenhorn (2016). In both studies, tested flies were acquired by rearing the offspring of multiple laboratory isofemale lines belonging to several geographical populations (Table 1) under variable food quantities, resulting in extensive adult body size variation. Males and females were separated upon eclosion and kept in single-sex containers for 4–5 days, ensuring sexual maturity. To assess selection on male morphology, mating trials were conducted in groups of OSR=2 (10 males with five females) and OSR=4 (20 males with five females) in 1.5-litre (see above) plastic containers containing water, sugar and cow dung ad libitum. Copulating pairs were removed from the arena, so the effective OSR in the container changed somewhat due to varying numbers of matings, losses or deaths of some individuals (Table 1). Depending on the number of copulations per replicate, mating trials were terminated after 2–4 h. All (paired and unpaired) individuals were subsequently frozen and stored in 70% ethanol until dissected for morphometric analysis, in accordance with Swiss animal rights regulations.

Laboratory studies of the other species were modelled after the above set-ups. A total of 13 replicate groups from four *S. cynipsea* populations (Zürich, Switzerland; Buogonovo, Italy: 46.32°N, 9.41°E; Petroia, Italy: 43.25°N, 12.55°E; Ludwigshafen, Germany: 49.48°N, 8.42°E) were tested at OSR=2 (eight males with four females; Table 1). *Sepsis thoracica* from one Italian population (Petroia) were also tested at OSR=2 (two males with one female; Table 1). (Note that Busso and Blanckenhorn (2018) tested four males with two females as well as eight males with four females, but provided no wing measurements.)

Sexual Selection Estimation in the Field

Trait-dependent mating success was estimated at haphazard times for the various species by sampling and subsequently comparing paired and unpaired males from multiple replicate dung pats in the summer of 2016 (cross-sectional sampling; Table 1; see Blanckenhorn, Morf, Mühlhäuser, & Reusch, 1999, 2004 for detailed sampling methods). Exceptions were *S. fulgens*, which are infrequent on cattle pastures but often gather on large dung piles potentially hosting thousands of flies on sunny days, and European *S. punctum*, which naturally tend to defend small dung portions (such as dog droppings) at very low densities, regularly being territorial (Table 1). Typically, however, multiple males of a given species wait on and around fresh cow pats for females coming to lay eggs (e.g. *S. cynipsea*: Ward, Hemmi, & Rösli, 1992; Blanckenhorn, Morf, et al., 1999, 2004). Operational sex ratios are usually male biased, varying between 0.5 and 8 (male:female) in our experiments depending on species (Table 1), although in nature they can be even higher. In these situations of scramble competition at high densities, direct aggressive or territorial interactions among males are rare and brief, except for *S. thoracica*; in this species, large amber males regularly raid relatively empty cow dung pats (see Busso & Blanckenhorn, 2018). Again, all paired and unpaired individuals caught in the field were subsequently frozen and stored in 70% ethanol until dissected for morphometric analysis.

Geometric Morphometric Analysis

Male forelegs and wings of all individuals were removed from the fly's thorax and dried at room temperature to allow evaporation of the ethanol. They were then embedded in Euparal resin (Carl Roth GmbH, Karlsruhe, Germany) on a glass slide. Slides were placed on a 35 °C heating plate for 5 min to liquefy the 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.

Fifteen homologous landmarks were defined to describe wing morphology in all species (Fig. 1). Because fore femur morphology differs strongly between species (Fig. 1), we could not use the same

landmarks for all species but instead used a varying number of full and semi-landmarks. Individuals with 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 our estimate of the overall structural size.

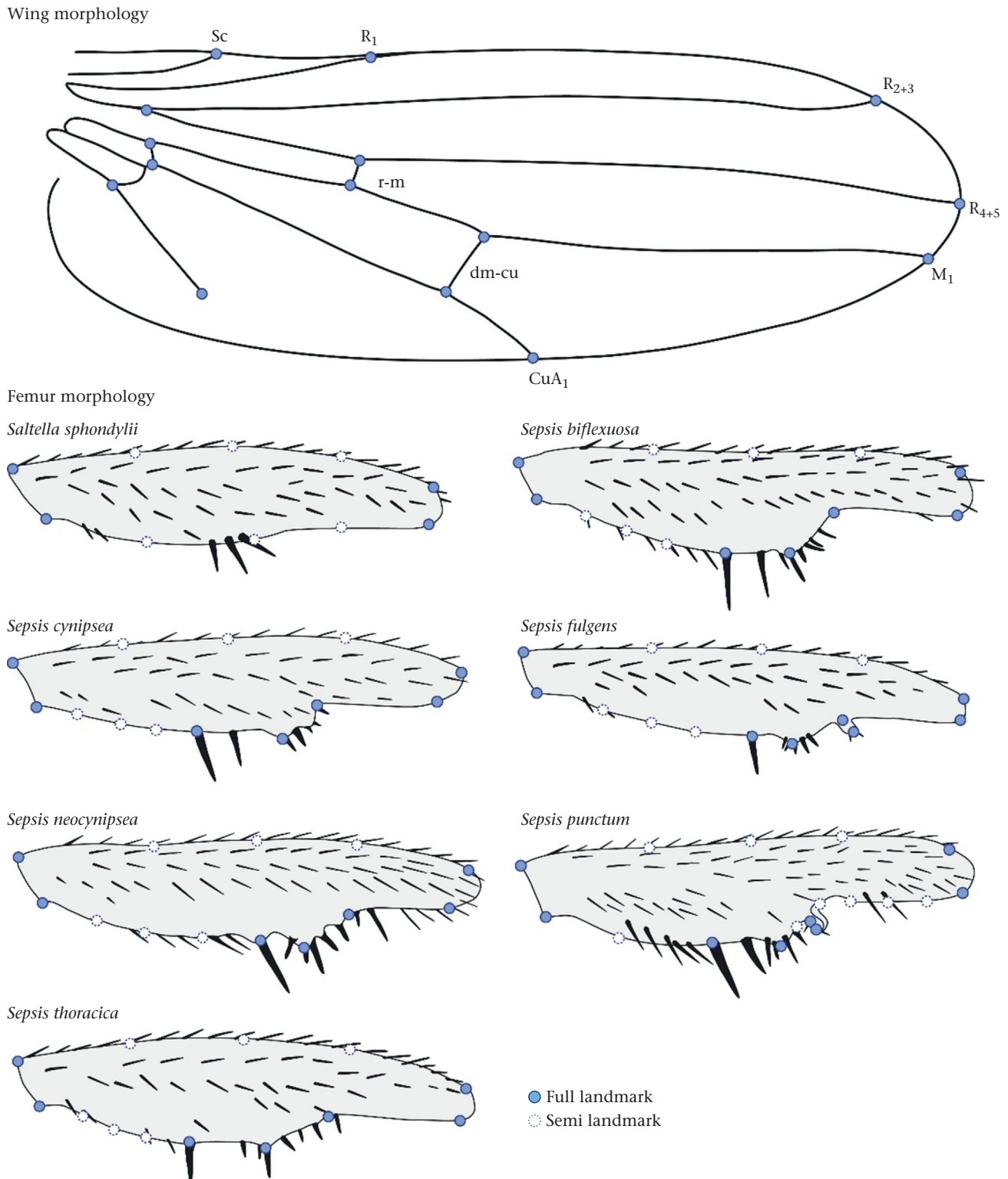


Figure 1. Landmarks used to describe wing and femur morphology in seven sepsid fly species.

Selection on Size

We calculated separate standardized univariate linear (β_{uni}) and corresponding nonlinear (γ_{uni}) selection coefficients (here mating differentials) for fore femur size and wing size (following Lande & Arnold, 1983; Arnold & Wade, 1984a,b; Brodie et al., 1995), separately for field and laboratory. Corresponding bivariate selection coefficients were also calculated, but because leg and wing (centroid) size of an individual are highly collinear these are not reliable and hence not reported (see Baur et al., 2020). For each species/continental population and trait, we calculated one overall (weighted) mean mating differential (binary variable: mated/unmated) using all data by entering population and/or temporal sample (for the field estimates), or replicate container (for the laboratory estimates), as random effects in the model. Standardized z scores for trait x were computed by subtracting the sample mean from each value and dividing by the standard deviation: $z_i = (x_i - \text{mean}(X))/\text{SD}(X)$. Relative male pairing success was computed as absolute pairing success (1 or 0) divided by the sampled proportion of mated males, which is equivalent to each sample's OSR (Brodie & Janzen, 1996; Blanckenhorn, Reuter, et al., 1999). The a priori OSR treatments of the original laboratory studies were consequently ignored (see Puniamoorthy, Blanckenhorn, & Schäfer, 2012; Puniamoorthy, Schäfer, & Blanckenhorn, 2012; Rohner, Puniamoorthy, & Blanckenhorn, 2016; Table 1). We used the univariate model of relative fitness on standardized (fore femur or wing) size $w' = c + \beta_{\text{uni}} \cdot z$ to estimate the linear selection coefficients β_{uni} , and the corresponding quadratic model $w' = c + \beta_1 \cdot z + 0.5\gamma_{\text{uni}} \cdot z^2$ to estimate corresponding univariate nonlinear selection coefficients γ_{uni} (note that $\beta_{\text{uni}} \neq \beta_1$). These coefficients (gradients) reflect the combined effects of direct and indirect selection on size (Ender, 1986). As shown by Janzen and Stern (1998; see also Blanckenhorn, Reuter, et al., 1999), these coefficients are equal to those derived from a corresponding logistic regression after back-transformation, with which their significance was tested.

Selection on Shape

To assess sexual selection on shape, we tested for a relationship between fitness and the Procrustes variables. Following Klingenberg and Monteiro (2005), we used a two-block partial least squares analysis (PLS). In essence, this technique uses a singular value decomposition of a matrix of covariances between two sets (or blocks) of variables to find the linear combinations of variables in each set that have maximum covariation with each other (Klingenberg & Marugán-Lobón, 2013; Rohlf & Corti, 2000). This method can be used to extract the linear combination of shape variables that best describes the covariation between phenotype (Procrustes coordinates) and fitness (bivariate mating success). The resulting PLS vectors (linear combinations) then represent a scaled version of the selection gradient (Klingenberg & Monteiro, 2005).

For each species, continent and field/laboratory data set, we used this approach to test for a relationship between shape (Procrustes coordinates) and mating success (paired/unpaired; coded as 1 and 0). Owing to the hierarchical (nested) experimental designs of our field and laboratory data, the unit of observation (= mating container in the laboratory or dung pat in the field) represents the appropriate level of analysis (equivalent to a random effect). To account for differences between the replicates, we used a pooled within-group PLS. In doing so, we calculated each individual's deviation from its group mean shape and used these residuals for further analysis. When using these shape residuals in one block and pairing success (0 or 1) in the other block (see above), the direction of the first PLS vector (S) represents the direction of selection

within replicates. The significance of selection was tested by means of randomization (10 000 random samples).

To compare selection qualitatively between field and laboratory data sets, we compared the significance of PLS vectors between laboratory and field data. To further quantify the similarity of the action of sexual selection under laboratory and field settings, we compared the direction of PLS vectors for laboratory (S_L) and field data (S_F) for each species by calculating vector correlations between selection vectors as:

$$r_{S_L, S_F} = \frac{|S_L \cdot S_F|}{\|S_L\| \times \|S_F\|}$$

That is, we standardized the dot product of the selection vectors S_L and S_F for species i by their norm (Claude, 2008; Klingenberg & Marugán-Lobón, 2013). If selection estimates in the laboratory are unrelated to patterns found in the field, correlations are expected to be close to 0 and nonsignificant. If, in contrast, laboratory data well emulate the natural condition, correlations are expected to be considerably larger than 0 and significant.

Because size and shape are often closely interrelated, selection on size could manifest in selection on allometric shape variation (Baur et al., 2020). To test to which extent the combined effects of sexual selection on overall size and allometric scaling of shape influence the apparent selection on shape, we assessed the similarity between the selection and allometry vectors. To quantify allometric shape change we used multivariate regressions of shape against size and extracted the vector of allometric shape change (A ; i.e. the vector of partial coefficients). To quantify the similarity between allometry and selection, we again computed the vector correlation between A and S for field and laboratory data.

To test for selection on nonallometric shape aspects (i.e. to correct for selection that is merely due to selection on overall size), we then statistically removed the effect of allometry from our shape data (by extracting the residuals of a multivariate regression of shape on size), and again performed a PLS analysis as described above.

RESULTS

Sexual Selection on Fore Femur and Wing Size

Mating differentials (based on pairing success) for fore femur and wing size varied considerably among species/continental populations ($P < 0.001$ for the species-by-trait-size interaction in separate generalized linear models for the field and laboratory data with species/continental population as fixed factor), in agreement with previous studies and our expectations relating to the flies' mating system (Tables 1, 2). Mating differentials were either nil or positive, but never strongly negative, indicating male size advantages for most species when pairing (Table 2, Fig. 2). Particularly strong and significant sexual selection was found primarily in species/continental populations with male-biased dimorphism (*S. thoracica*, European *S. punctum*, North American *S. neocynipsea*), but also in North American *S. biflexuosa*, a species with no significant sexual size dimorphism (Rohner, Puniamoorthy, & Blanckenhorn, 2016; Table 2). Importantly, a combined overall GLM for the subset of species for which field and laboratory data were available (Tables 1, 2) with field/laboratory as an additional fixed factor indicated no overall differences in sexual selection between the field and the laboratory estimates (field/laboratory*trait size interaction: $P > 0.2$), suggesting that our laboratory assessments generally well reflect the natural situation.

Table 2
Linear and nonlinear (quadratic) sexual selection coefficient (SE) for male wing and fore femur size based on pairing success in field and laboratory of seven species of sepsid flies

Species	Continent		Field No. of males/ no. of replicates	Femur	Wing	Laboratory No. of males/ no. of replicates	Femur	Wing
<i>Saltella sphondyli</i>	Europe	Linear (SE)	27/2	0.076 (0.244)	0.060 (0.243)	-/-		
		Nonlinear (SE)		0.224 (0.505)	0.222 (0.554)			
	North America	Linear (SE)	59/2	0.313 (0.213)	0.324 (0.214)	-/-		
		Nonlinear (SE)		-0.106 (0.253)	0.071 (0.272)			
<i>Sepsis biflexuosa</i>	Europe	Linear (SE)	-/-			-/-		
		Nonlinear (SE)						
	North America	Linear (SE)	175/4	0.179 (0.076)	0.153 (0.077)	-/-		
		Nonlinear (SE)		0.183 (0.121)	0.130 (0.115)			
<i>Sepsis cynipsea</i>	Europe	Linear (SE)	152/8	0.050 (0.082)	-0.048 (0.082)	117/13	0.015 (0.202)	-0.104 (0.200)
		Nonlinear (SE)		-0.054 (0.122)	-0.049 (0.111)		-0.280 (0.339)	-0.131 (0.333)
<i>Sepsis fulgens</i>	Europe	Linear (SE)	59/2	0.216 (0.145)	0.178 (0.146)	-/-		
		Nonlinear (SE)		-0.574 (0.252)	-0.644 (0.235)			
<i>Sepsis neocynipsea</i>	Europe	Linear (SE)	-/-			285/24	0.036 (0.157)	-0.003 (0.160)
		Nonlinear (SE)					0.225 (0.301)	0.264 (0.294)
	North America	Linear (SE)	41/4	0.148 (0.221)	0.078 (0.220)	173/21	0.384 (0.138)	0.404 (0.136)
		Nonlinear (SE)		-0.642 (0.480)	-0.768 (0.487)		0.063 (0.242)	0.029 (0.235)
<i>Sepsis punctum</i>	Europe	Linear (SE)	101/40	0.746 (0.139)	0.608 (0.149)	409/32	0.455 (0.072)	0.417 (0.070)
		Nonlinear (SE)		0.273 (0.284)	0.170 (0.293)		0.068 (0.153)	0.158 (0.161)
	North America	Linear (SE)	120/4	0.017 (0.114)	0.068 (0.148)	291/24	0.268 (0.118)	0.175 (0.119)
		Nonlinear (SE)		-0.179 (0.186)	-0.259 (0.208)		0.126 (0.204)	-0.031 (0.180)
<i>Sepsis thoracica</i>	Europe	Linear (SE)	150/16	0.525 (0.119)	0.475 (0.121)	126/36	0.481 (0.179)	0.477 (0.180)
		Nonlinear (SE)		0.018 (0.224)	-0.126 (0.205)		0.170 (0.627)	0.059 (0.634)

Replicates were groups within geographical populations in the laboratory or dung pats sampled in the field. Bold indicates significant coefficients ($P < 0.05$) and italics results with $P < 0.1$.

Sexual Selection on Fore Femur and Wing Shape

Mating success was significantly associated with fore femur shape in *S. biflexuosa*, North American *S. neocynipsea*, both continental populations of *S. punctum*, *S. thoracica*, and marginally in

S. cynipsea (Table 3). Sexual selection on wing shape was evident in *S. thoracica* and *S. punctum* from both continents and North American *S. neocynipsea* in the laboratory (Table 3). This suggests associations between femur and wing shape with mating success in most but not all species/continental populations studied. However,

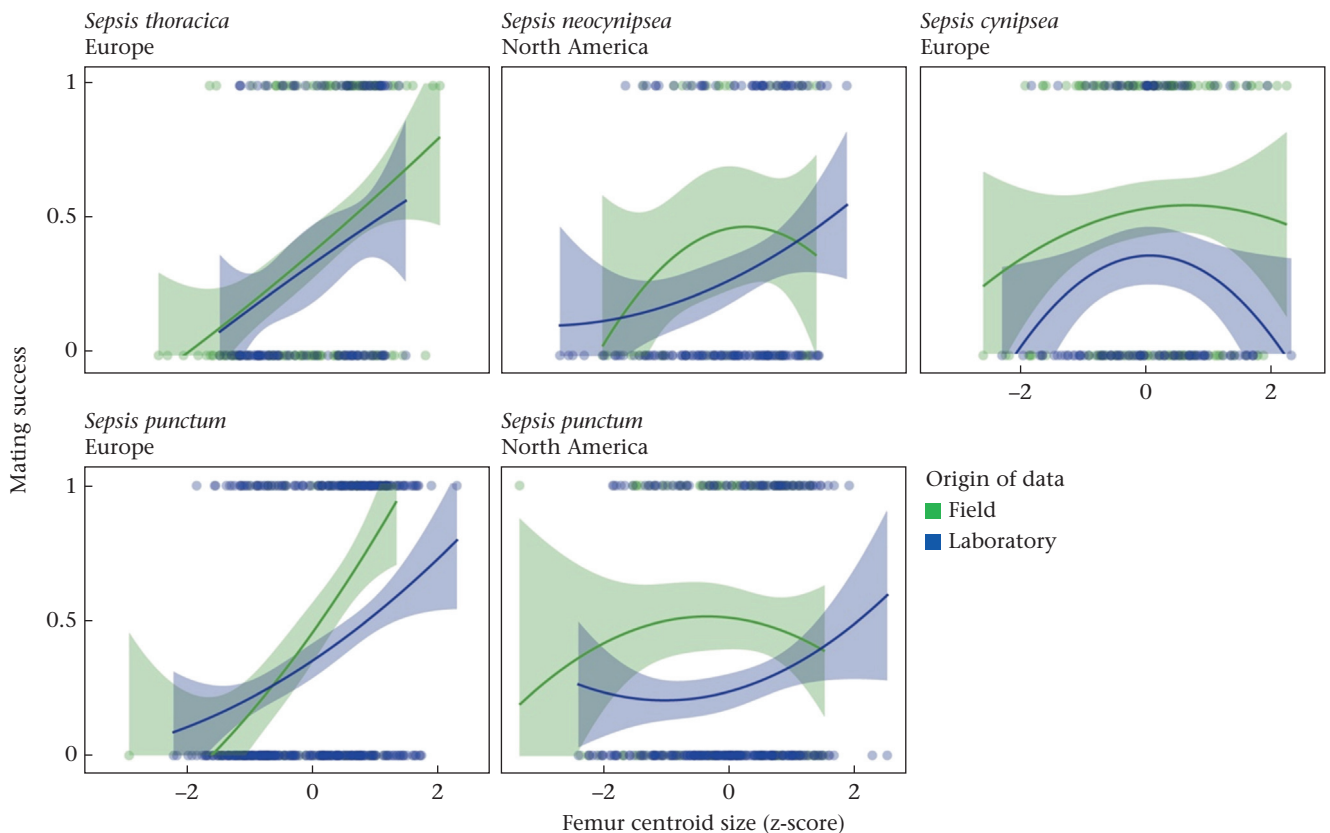


Figure 2. Relationship between mating success and standardized fore femur centroid size in the field and the laboratory for the (sub)species for which both are available.

Table 3

The partial least squares vectors between fitness and overall ($|S|$) or allometry-adjusted shape ($|S^i|$) and their significance are given for fore femur and wing shape under field and laboratory conditions

Species	Continent	Study	Fore femur				Wing							
			$ S \times 10^3$	<i>P</i>	$r_{S,A}$	<i>P</i>	$ S^i \times 10^3$	<i>P</i>	$ S \times 10^3$	<i>P</i>	$r_{S,A}$	<i>P</i>	$ S^i \times 10^3$	<i>P</i>
<i>Saltella sphondylii</i>	Europe	Field	12.6	0.258	0.51	0.009	11.4	0.238	9.4	0.851	0.13	0.248	9.4	0.790
<i>Saltella sphondylii</i>	North America	Field	7.2	0.577	0.57	0.004	5.9	0.762	9.3	0.554	0.5	0.002	8.2	0.708
<i>Sepsis biflexuosa</i>	North America	Field	10.3	0.03	0.88	<0.001	5.6	0.398	4.9	0.319	0.46	0.005	4.4	0.421
<i>Sepsis cynipsea</i>	Europe	Field	10.8	0.073	0.47	0.008	10.0	0.094	6.4	0.12	0.19	0.844	5.7	0.131
<i>Sepsis cynipsea</i>	Europe	Lab	7.9	0.099	0.28	0.078	8.5	0.042	5.2	0.279	0.55	0.001	6.2	0.062
<i>Sepsis fulgens</i>	Europe	Field	6.6	0.787	0.16	0.213	6.6	0.760	6.3	0.588	0.53	0.001	5.9	0.669
<i>Sepsis neocynipsea</i>	Europe	Lab	7.7	0.228	0.44	0.011	7.9	0.072	4.4	0.361	0.24	0.097	4.5	0.241
<i>Sepsis neocynipsea</i>	North America	Field	10.4	0.75	0.78	<0.001	6.5	0.921	10.0	0.202	0.47	0.004	9.0	0.133
<i>Sepsis neocynipsea</i>	North America	Lab	16.9	0.004	0.91	<0.001	8.5	0.053	8.8	0.001	0.9	<0.001	5.8	0.040
<i>Sepsis punctum</i>	Europe	Field	37.5	<0.001	0.95	<0.001	17.1	0.010	8.0	0.042	0.81	<0.001	4.9	0.427
<i>Sepsis punctum</i>	Europe	Lab	22.2	<0.001	0.99	<0.001	5.2	0.311	9.6	<0.001	0.93	<0.001	4.3	0.031
<i>Sepsis punctum</i>	North America	Field	14.6	0.003	0.15	0.801	16.6	<.001	9.0	0.002	0.22	0.884	10.1	<0.001
<i>Sepsis punctum</i>	North America	Lab	6.7	0.061	0.52	0.001	6.9	0.014	2.6	0.591	0.56	0.001	2.7	0.472
<i>Sepsis thoracica</i>	Europe	Field	14.1	0.005	0.7	0.001	10.2	0.045	3.5	0.812	0.72	<0.001	2.4	0.989
<i>Sepsis thoracica</i>	Europe	Lab	37.4	<0.001	0.96	<0.001	12.2	0.079	18.0	<0.001	0.98	<0.001	7.5	0.018

P values for PLS vectors were derived from 10 000 randomizations. The alignment between the direction of selection and static allometry is given by vector correlations ($r_{S,A}$), with their significance tested as proposed by Klingenberg and Marugán-Lobón (2013) using the closed-form expression for the area of a hypersphere cap by Li (2011). Bold indicates significant results ($P < 0.05$) and italics results with $P < 0.1$.

except for North American field *S. punctum*, the allometric component of shape was mostly significant and overall strong for both wings and fore femurs (Table 3). The often strong correlations between allometric vectors and the significant selection vectors further indicate that selection on size (shown above) contributes considerably to shape differences between successful and unsuccessful males (Table 3).

Irrespective of whether selection was significant or not, we can ask how well the laboratory selection and allometry data predict patterns in the field. To do so, we computed the similarity between selection and allometry vectors generated under laboratory conditions with those from the field. Except for North American *S. punctum*, the laboratory data predict sexual selection patterns found in the field for fore femur shape relatively well, with correlations ranging between 0.34 and 0.94. Correlations for wing shape tended to be weaker (Table 4). In contrast to fore femur morphology, sexual selection on size-corrected wing shape generally differed between field and laboratory (Table 4, Fig. 3), being weak to nil in most species (Table 3). These results suggest that femur shape, but not wing shape, plays some consistent role in sexual selection. In contrast to selection estimates, which had medium effect sizes, the estimates of allometry measured under laboratory conditions reflected patterns found for wild individuals very well (all $r > 0.72$).

DISCUSSION

Our comparative study of sexual selection investigating pairing success in several closely related sepsid fly species yielded two

salient results. First, although ecologically very similar, sexual selection on male fore femur and wing size and shape varied considerably among the seven species and continental populations (Table 2, Fig. 2). This is in agreement with variation in their mating system summarized in more detail in Table 5. Sexual selection on trait size was either positive or nil, although never significantly negative, generally implying mating advantages of large males in most species. Particularly strong and significant selection was found in species/continental populations with male-biased sexual size dimorphism (*S. thoracica*, European *S. punctum*, North American *S. neocynipsea*), in addition to North American *S. biflexuosa*, a species with no significant dimorphism (Rohner, Puniamoorthy, & Blanckenhorn, 2016; Table 1), but not in species with female-biased size dimorphism (see also Blanckenhorn et al., 2000). Whereas selection on trait size was similar for forelegs and wings, the size of which naturally correlates strongly among individuals, results for selection on shape were more complex. Although in general selection on the shape of both traits can be attributed to selection on size, allometry-corrected sexual selection on fore femur shape was more consistent than on wing shape, which was absent in most species (Tables 3, 4). Second, sexual selection intensities on size were overall at least qualitatively similar in field and laboratory. Similarly, the direction of selection on fore femur shape of laboratory and field data sets correlated in four of our five species. Together, this suggests that our laboratory assessments and set-ups well represent the natural situation, at least for the species investigated here. If this conclusion can be generalized, it would lend credence to the strategy of using

Table 4

Vector correlations r_{S_i, S_f} between the first PLS selection vectors *S* for laboratory and field quantify how well laboratory data predict patterns of selection in the wild for each species

Species	Continent	Fore femur						Wing					
		<i>S</i>		<i>A</i>		<i>Sⁱ</i>		<i>S</i>		<i>A</i>		<i>Sⁱ</i>	
		r_{S_i, S_f}	<i>P</i>	r_{A_i, A_f}	<i>P</i>	r_{S_i, S_f}	<i>P</i>	r_{S_i, S_f}	<i>P</i>	r_{A_i, A_f}	<i>P</i>	r_{S_i, S_f}	<i>P</i>
<i>Sepsis cynipsea</i>	Europe	0.53	0.002	0.79	<0.001	0.57	0.001	0.02	0.456	0.78	<0.001	0.01	0.527
<i>Sepsis neocynipsea</i>	North America	0.65	<0.001	0.85	<0.001	0.30	0.065	0.44	0.007	0.91	<0.001	0.10	0.304
<i>Sepsis punctum</i>	North America	0.14	0.792	0.96	<0.001	0.39	0.011	0.05	0.608	0.96	<0.001	0.19	0.158
<i>Sepsis punctum</i>	Europe	0.94	<0.001	0.96	<0.001	0.73	<0.001	0.72	<0.001	0.72	<0.001	0.17	0.173
<i>Sepsis thoracica</i>	Europe	0.34	0.043	0.88	<0.001	0.55	0.002	0.35	0.026	0.85	<0.001	0.49	0.003

Static allometry vectors *A*, representing coefficients of a multivariate regression of shape on size, were compared between field and laboratory. Because allometry can contribute to selection on shape if larger males have a fitness advantage, we additionally assessed selection on allometry-corrected shape *Sⁱ*. Bold indicates significant results ($P < 0.05$) and italics results with $P < 0.1$.

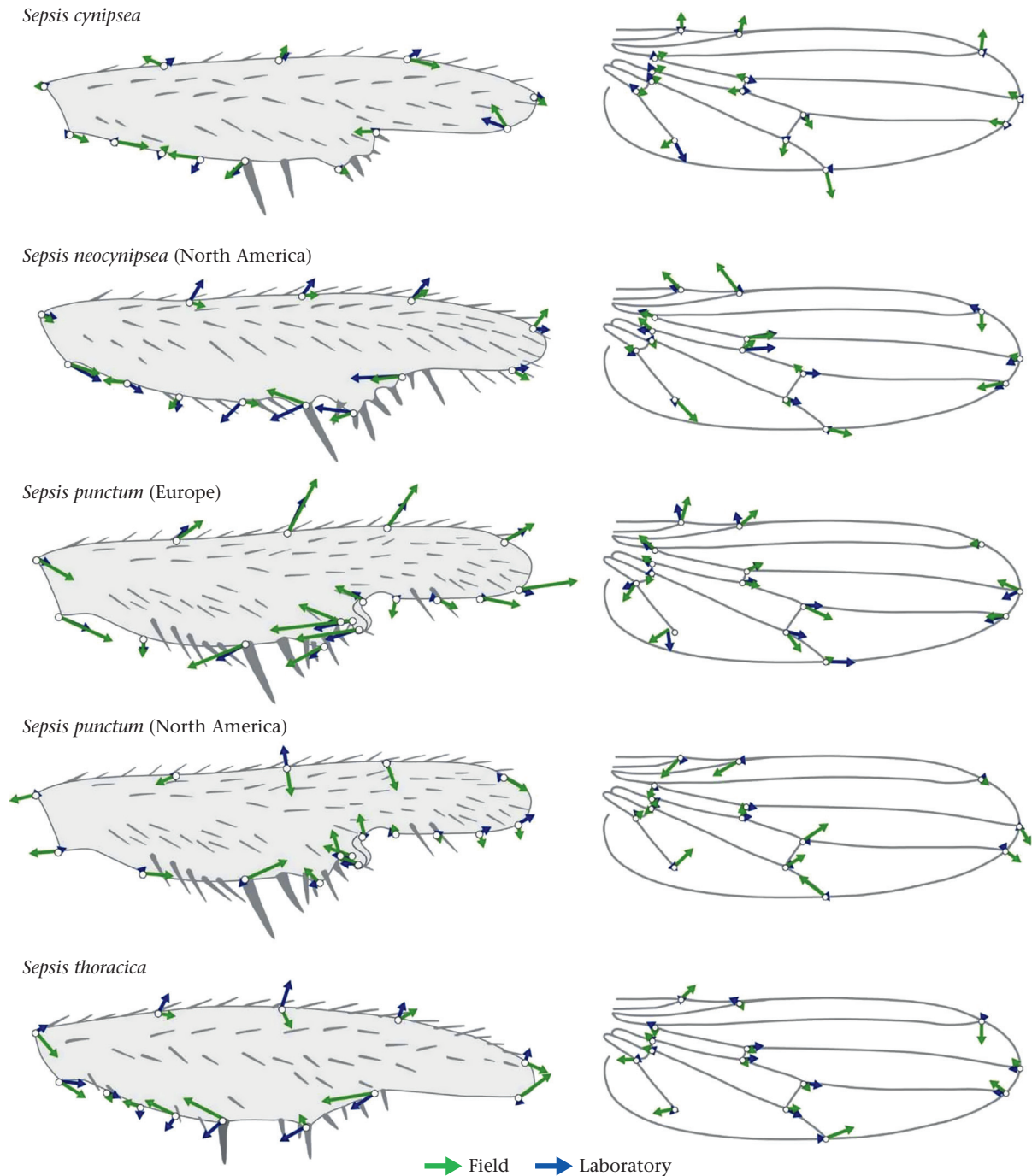


Figure 3. Direction of sexual selection on wing and femur morphology in the field and the laboratory for the (sub)species for which both were measured.

controlled laboratory mating studies to better understand natural selection, behaviour and ecology, at least for those species that can be studied in captivity.

Mating System Differentiation and Sexual Selection in Closely Related Sepsid Flies

One of our central objectives was to investigate sexual selection in closely related, ecologically similar, but nevertheless diversified dung fly species. Although their natural history is overall similar, the strength and nature of selection acting on morphological traits varied considerably (Tables 1, 2, 5). The best predictor of the intensity

of sexual selection in this guild is SSD (Blanckenhorn et al., 2020; Puniamorthy, Blanckenhorn, & Schäfer, 2012; Puniamorthy, Schäfer, & Blanckenhorn, 2012; Rohner, Puniamorthy, & Blanckenhorn, 2016). In species with male-biased SSD (European *S. thoracica*, European *S. punctum*, North American *S. neocynipsea*), large males had a strong mating advantage in the field and the laboratory (Table 2). In species with female-biased SSD (*S. sphondylii*, *S. cynipsea*, *S. fulgens*, North American *S. punctum*; no field data for European *S. neocynipsea*), by contrast, directional selection on size was not significantly positive, although *S. fulgens* and North American *S. neocynipsea* showed significant stabilizing selection in the field (negative quadratic selection coefficient in Table 2). In North

Table 5
Description of the mating system and typical behaviour of all sepsid fly species and continental populations assessed

Species	Mating site	Typical fly densities	Typical OSR	Copulation	Mating system	Source
<i>S. sphondylii</i> EU	Cow dung pat (10–40 cm)	10–100	Male bias +	Fast and frequent	Male scrambles, no apparent female choice	Pont & Meier, 2002; Martin & Hosken, 2004
<i>S. sphondylii</i> NA	Cow dung pat (10–40 cm)	10–100	Male bias +	Fast and frequent	Male scrambles, no apparent female choice	Pont & Meier, 2002; N. Puniamoorthy & P. T. Rohner, personal observation
<i>S. biflexuosa</i> EU	Cow dung pat (10–40 cm)	<5	Unknown	Unknown	Unknown	Rohner, Puniamoorthy, & Blanckenhorn (2016)
<i>S. biflexuosa</i> NA	Cow dung pat (10–40 cm)	10–100	Male bias +	Unknown	Male scrambles, female choice (?)	Pont & Meier, 2002; Rohner, Puniamoorthy, & Blanckenhorn, 2016; N. Puniamoorthy & P. T. Rohner, personal observation.
<i>S. cynipsea</i> EU	Cow dung pat (10–40 cm)	10–500	Male bias ++	Multiple	Male scrambles, female choice & reluctance	Ward et al. (1992); Blanckenhorn et al., 2000
<i>S. fulgens</i> EU	Large dung pile (2–10 m)	100–1000	Male bias +	Rare	Male scrambles, female choice (?)	J. Roy & P. T. Rohner, personal observation
<i>S. neocynipsea</i> EU	Cow dung pat (10–40 cm)	5–50	Female to male bias	Rare	Male scrambles, female choice & reluctance	Rohner, Puniamoorthy, & Blanckenhorn, 2016; Giesen, Blanckenhorn, & Schäfer, 2017
<i>S. neocynipsea</i> NA	Cow dung pat (10–40 cm)	10–100	Male bias +	Multiple	Male competition, female choice & reluctance	Eberhard, 1999; Schulz, 1999; Rohner et al., 2016; Giesen et al., 2017
<i>S. punctum</i> EU	Small dung pat (5 cm)	1–10	Female to male bias	Multiple	Male territoriality & competition, female choice & reluctance	Schulz, 1999; Puniamoorthy, Blanckenhorn, & Schäfer, 2012
<i>S. punctum</i> NA	Cow dung pat (10–40 cm)	10–100	Male bias +	Multiple	Male courtship, female choice	Schulz, 1999; Puniamoorthy, Schäfer, & Blanckenhorn, 2012
<i>S. thoracica</i> EU	Cow dung pat (10–40 cm)	5–30	Male bias +	Rare	Male territoriality & direct male-male interactions, female choice & reluctance	Busso and Blanckenhorn (2018)

EU: European; NA: North American. OSR: operational sex ratio.

American *S. biflexuosa*, a species with males and females of roughly equal size (no SSD), large males also had a mating advantage in the field.

Apart from sexual selection on (presumably overall) size, we also found significant associations between male mating success and fore femur shape, as was expected because the male forelegs function in grasping the female's wing base during pairing (Dmitriew & Blanckenhorn, 2012; Puniamoorthy, Su, & Meier, 2008). This occurred predominantly in species and continental populations with male-biased SSD, although some (marginally nonsignificant) indication of covariation between fitness and femur shape was also apparent in *S. cynipsea* and in North American *S. punctum* (Table 3), both featuring female-biased SSD. By contrast, significant allometry-corrected sexual selection on wing shape was rarely found (Table 4). Wings are generally primarily expected to be subject to natural (i.e. viability) rather than sexual selection, at least in species without precopulatory courtship by song (as exhibited e.g. by *Drosophila*: Ray, Nakata, Henningson, & Bomphrey, 2016; Schäfer et al., 2018). Nevertheless, regardless of trait and species, the covariance between shape and fitness was closely aligned with static allometry in most species, suggesting that the joint effects of selection on overall size and allometry contribute to the apparent selection on shape.

The mechanistic link between variation in mating differentials and SSD documented here is likely to be behavioural. At least in sepsids, but also in numerous vertebrates, there is an established association between male-biased SSD and competitive interactions among males during mating (Rohner, Puniamoorthy, & Blanckenhorn, 2016; Rohner, Teder et al., 2018; Andersson, 1994; Clutton-Brock, 1988; Fairbairn, Blanckenhorn, & Székely, 2007; Rohner & Blanckenhorn, 2018). Males of species with male-biased

SSD probably show territorial behaviour and lack precopulatory courtship (e.g. Busso & Blanckenhorn, 2018; Eberhard, 1999). Aggressive male behaviour is of course also evident in sepsids with female-biased SSD, for instance towards females in *S. cynipsea*, *S. biflexuosa* and *S. fulgens*, in which males scramble to mate with females that often respond with strong reluctance behaviour (Ward et al., 1992; Blanckenhorn et al., 2000; Baena & Eberhard, 2007; Table 5). However, in these species, aggression is typically only incidentally directed to other males (on occasion also when mounted).

Female-biased SSD is the rule and the plesiomorphic (original) character state in sepsid flies (Rohner, Puniamoorthy, & Blanckenhorn, 2016). We thus assume, and for some species we know, that in those species female choice of some sort is the main driver selecting for larger male size (Eberhard, 1996; e.g. in *S. cynipsea*: Ward et al., 1992; Blanckenhorn et al., 2000; also *Archiseptis diversiformis*: Baena & Eberhard, 2007; Puniamoorthy, 2014; see Crean, Dunn, Day, & Gilburn, 2000 and Hunt, Breuker, Sadowski, & Moore, 2009, for similar evidence in other insects). We favour the conclusion that some level of female preference for large males when mating, mediated by various mechanisms such as courtship performance, male persistence, song, volatile or cuticular scent etc., may be the baseline mechanism in sepsids (and probably other species as well), typically resulting in positive, albeit not necessarily significant sexual selection on body size (e.g. in *S. cynipsea*: Blanckenhorn et al., 1999, 2000, 2004). Once male-biased SSD evolved by such sexual selection or otherwise (Fairbairn et al., 2007; Puniamoorthy, Blanckenhorn, & Schäfer, 2012; Puniamoorthy, Schäfer, & Blanckenhorn, 2012; Rohner et al., 2016), males are expected to be able to effect greater control over mating and reproduction (Ding & Blanckenhorn, 2002),

leading to concomitant changes in the mating system (Table 5; Puniamoorthy, 2014). This in turn promotes and reinforces male – male competition and/or territoriality (such as in European *S. punctum* or *S. thoracica*) to further intensify sexual selection (Table 2; see Hunt et al., 2009). Male – male competition added on top of female choice may generally explain the intensification of sexual selection in species with male-biased SSD relative to those with female-biased SSD (Table 2).

Remarkably, *S. sphondylia* (as well as some other *Saltella*), which copulates soon after adult emergence, profusely and briefly (few minutes, as opposed to ca. 20 min for most *Sepsis*: e.g. Puniamoorthy, Blanckenhorn, & Schäfer, 2012), exhibit a live-fast–die-young reproductive strategy with quick and frequent copulations, leading to early death due to strong sexual conflict and/or high costs of reproduction (Martin & Hosken, 2004). Regardless, the resulting sexual selection intensity on trait size obtained here was also either positive or nil (Table 2).

Sexual Selection in the Laboratory versus the Field

Our second major objective was to test whether sexual selection as estimated in the laboratory reflects corresponding selection in the field. Despite our limited and certainly nonrandom sample size of five species/continental populations, our data suggest that field and laboratory estimates correspond very well (Tables 2, 3). This supports the study of mating behaviour in the laboratory for reasons of better control and ease of experimentation. At least for the species tested here, we apparently well emulated the competitive situation in nature.

While these findings are generally promising, it is worth pointing out that the laboratory was not well suited for investigating the mating behaviour of all sepsids, and full congruence was not achieved in every case. For instance, although European *S. neocynipsea* mating could be investigated in the laboratory, we could not observe mating of this species in nature using the same methods as for its sister species *S. cynipsea* (Ward et al., 1992; Blanckenhorn et al., 1999, 2000, 2004). Towards the end of the day at our observation site (Sörenberg, Switzerland), multiple females regularly gathered around fresh dung pats, and could be observed foraging, but neither males nor females displayed any courtship or aggressive behaviour. We can only suspect that mating takes place somewhere else or at some other time in nature. Another species for which mating is difficult to observe in the field (this study) as well as in the laboratory, to the extent that we could not present it here, is *S. fulgens* (Pont & Meier, 2002; Puniamoorthy, Kotrba, & Meier, 2010). Their mating behaviour is rather nondescript, despite breeding exceptionally easily in the laboratory in terms of population sizes. Moreover, in *S. thoracica*, but also European *S. punctum*, mating behaviour appears very flexible and may differ depending on the local environment and social context. For instance, in the field, large *S. thoracica* males (see Busso & Blanckenhorn, 2018) frequently displayed highly aggressive and territorial behaviour on dung pats, which we rarely observed in the laboratory at low fly densities. In contrast, *S. punctum* observed in the field in Switzerland occur at very low densities in most places, where they tend to defend small dung portions against male intruders. Notwithstanding, at higher densities in the field (Zerbe, 1993) and the laboratory (Puniamoorthy, Blanckenhorn, & Schäfer, 2012; Puniamoorthy, Schäfer, & Blanckenhorn, 2012) they seem to abandon territorial strategies and behave more like *S. cynipsea* (Blanckenhorn et al., 2000; Ward et al., 1992). Thus, while our sexual selection estimates in field and laboratory are similar, this is not necessarily the case for the underlying behavioural mechanisms.

Methodological Obstacles to Quantifying Sexual Selection in the Wild

The issues with quantifying selection as outlined above highlights a common problem in diversity research. That is, even slight variation in the mating system of species (e.g. in mating duration, population density, copulation duration, OSR, etc.) can lead to highly situation-specific variation that may in the extreme strongly affect the opportunity of observing mating and sexual selection in the wild, to the extent that often it is rendered impossible in many species or populations. This has purely biological implications (as discussed above), but also methodological consequences (reviewed e.g. by Hunt et al., 2009; Evans & Garcia-Gonzales, 2016; Anthes et al., 2017; Bailey et al., 2018; Dougherty, 2020).

The closely related species studied here illustrate these problems rather well. First, some species are simply too rare to be assessed at all. This applies to European *S. biflexuosa* and some other species that are rare throughout central Europe (*Sepsis luteipes*, *Sepsis nigripes*). Second, even though most species appear to mate in groups gathering around vertebrate dung (Pont & Meier, 2002), there are species that mate clandestinely, solitarily, briefly or at unknown places. For this reason, we could not gather data on European *S. neocynipsea* in the field (mentioned above). This renders any comparative ecological and behavioural data set nonrandom.

Third, particular behavioural traits of species (e.g. whether males are territorial or not) will strongly influence the mating system and population density at the mating site. This in turn affects the estimation of selection coefficients, which among other things strongly depend on properly knowing the local competitive circumstances, i.e. the OSR (Arnold & Wade, 1984a,b; Brodie & Janzen, 1996; Janzen & Stern, 1998; Blanckenhorn, Reuter, et al., 1999; Klug, Heuschele, Jennions, & Kokko, 2010). We here used the OSRs (i.e. the proportion mated of all males) defined by our sample to estimate sexual selection, and not the OSR that occurred at the particular sampling site (dung pat) in the field, which may have substantially differed at the time the flies were caught. Although the range of OSRs of our entire sample was large and therefore most likely representative of the natural situation overall (Table 1), we know from experience with *S. cynipsea* that OSRs can be much more male biased in nature on occasion (Blanckenhorn et al., 1999, 2000, 2004). If known or estimated prior to sampling, such sampling bias in terms of OSR can be corrected post hoc (Blanckenhorn, Reuter, et al., 1999), although this will often not be possible or done in practice. We therefore think that, for many if not most species, the coefficients presented here (Tables 2, 3) probably under- rather than overestimate the true mating differentials at the mating sites, as fly densities comprising multiple species on pastures around dung pats are often very high. This is particularly true for *S. fulgens*, which we caught mating on a large dung pile with thousands of competitors around. Many of the flies present may not truly be competing for mates at any one time, if only because they were foraging at the time, but this cannot easily be judged by the observer. In the end, all selection estimates have to be taken with a large grain of salt, implying that many independent samples are needed to arrive at unbiased and representative overall estimates of the action of sexual or natural selection (Lande & Arnold, 1983; Arnold & Wade, 1984a; e.g. ; Jann et al., 2000). We here offer estimates subsuming a variety of environmental situations (and populations), which therefore are likely to be representative.

Conclusions

In conclusion, mating differentials of morphological trait size and shape varied considerably among species and continental populations in accordance with variation in their mating system,

while laboratory and field estimates were surprisingly congruent, particularly for those species that show pronounced male–male interactions. Laboratory studies are therefore not necessarily as 'artificial' as sometimes argued. However, this congruence of course depends on a general understanding of all species' ecology and behaviour that ultimately enables researchers to design laboratory settings that adequately resemble those found in the wild, emphasizing the perpetual need and usefulness of basic information about the natural history of one's pet species.

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