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ORIGINAL ARTICLE

Sexual selection on male size drives the evolution of male-biased sexual size dimorphism via the prolongation of male development

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Sexual size dimorphism (SSD) arises when the net effects of natural and sexual selection on body size differ between the sexes. Quantitative SSD variation between taxa is common, but directional intraspecific SSD reversals are rare. We combined micro- and macroevolutionary approaches to study geographic SSD variation in closely related black scavenger flies. Common garden experiments revealed stark intra- and interspecific variation: *Sepsis biflexuosa* is monomorphic across the Holarctic, while *S. cynipsea* (only in Europe) consistently exhibits female-biased SSD. Interestingly, *S. neocynipsea* displays contrasting SSD in Europe (females larger) and North America (males larger), a pattern opposite to the geographic reversal in SSD of *S. punctum* documented in a previous study. In accordance with the differential equilibrium model for the evolution of SSD, the intensity of sexual selection on male size varied between continents (weaker in Europe), whereas fecundity selection on female body size did not. Subsequent comparative analyses of 49 taxa documented at least six independent origins of male-biased SSD in Sepsidae, which is likely caused by sexual selection on male size and mediated by bimaturism. Therefore, reversals in SSD and the associated changes in larval development might be much more common and rapid and less constrained than currently assumed.

KEY WORDS: Body size, sepsidae, sexual selection, sexual size dimorphism.

Sexual dimorphism, the divergence of morphological, behavioral, and physiological traits between males and females, evolves when the optimal character states differ between the sexes (Bateman 1948; Hedrick and Temeles 1989). Sexually dimorphic characters are ubiquitous in nature, in large part founded by differential gametic investment (i.e., anisogamy) whereby the costs and benefits of mating usually differ between males and females. These traits can be completely sex-specific or may differ quantitatively between the sexes (Bateman 1948; Abouheif and Fairbairn 1997; Fairbairn 2013).

Body size is especially subject to sexual dimorphism due to its strong correlation with physiology and fitness (Peters 1986; Reiss 1991; Blanckenhorn 2000). Given that large females can

generally allocate more energy to reproduction and provide extra resources to more and/or higher quality offspring, fecundity selection usually favors larger body size in females (Darwin 1872; Andersson 1994). Male body size, in contrast, depends on the mating system, the degree of investment into mate acquisition, male–male competition, and/or female choice, and is typically strongly sexually selected (Kokko et al. 2014). Viability selection (via predation or parasitism, ecological selection pressures, etc.) also impacts body size of both males and females (Blanckenhorn 2000). According to the differential equilibrium model of sexual size dimorphism (SSD), the direction and strength of SSD in a species is expected to covary with the sex-specific net effects of natural and sexual selection on body size (see Price

1984; Andersson 1994; Preziosi and Fairbairn 2000; Blanckenhorn 2007). Moreover, whether females or males are the larger sex has crucial implications for the mating system (e.g., Ding and Blanckenhorn 2002); thus mating system evolution (e.g., evolution of lekking behavior, changes in female remating behavior, etc.) can be cause or consequence of shifts in the direction and strength of SSD, provided that its evolvability is not hampered by the genetic architecture, strong genetic correlations between the sexes, or developmental constraints (Badyaev 2002).

In most mammals and birds sexual selection on male body size is relatively stronger than fecundity selection on female body size, resulting in male-biased SSD (Abouheif and Fairbairn 1997). In most invertebrates, in contrast, fecundity selection on female size generally exceeds sexual selection on male size, leading to female-biased SSD (Abouheif and Fairbairn 1997; Blanckenhorn et al. 2007; Stillwell et al. 2010; Hirst and Kiorboe 2014). Nonetheless, variation in the strength and direction of SSD is common even among closely related species, and also occurs among populations within species (Teder and Tammaru 2005; Stillwell et al. 2010). Intraspecific variation in SSD is usually minor, and directional variation within species is rarely reported. Badyaev et al. (2000) described albeit slight population variation in SSD in the American house finch, whereas Stillwell et al. (2007) demonstrate clinal variation in SSD of a seed-feeding beetle ranging from male-biased SSD at low latitudes to monomorphic (and sometimes female-biased) populations at higher latitudes. However, to our knowledge, the only one striking case of an intraspecific SSD *reversal* with an underlying genetic basis has been reported by Puniamoorthy et al. (2012b; also see Dmitriew and Blanckenhorn 2014): in the black scavenger fly *S. punctum* (Fabricius 1794), males are the larger sex in Europe (EU), whereas North American (NA) populations exclusively express female-biased SSD. Intraspecific, that is, population variation in SSD is ideal, in fact the only direct way to test the differential equilibrium model at the microevolutionary, mechanistic scale because conspecific populations in various habitats across the northern hemisphere share recent evolutionary history (Puniamoorthy et al. 2012a). Such studies are very rare (Fairbairn et al. 2007). Puniamoorthy et al. (2012a,b) demonstrated a positive association between the direction of SSD and variation in the strength of sexual selection on male size. However, *S. punctum* remains a single datapoint, so the putative underlying evolutionary mechanism still lacks generality. Therefore, to test the robustness of the model, it is useful if not imperative to replicate this assessment and embed it in the framework of a phylogenetic comparative study, taking into account inter- and intraspecific variation among other closely related species.

In holometabolous insects, given their complex development, variation in adult body size and SSD can be caused by three different mechanisms: variation in egg size, the duration of the juvenile period, or the speed of growth during the latter (Teder 2014). In Diptera, there is little evidence for sex-specific differences in egg size. Therefore, development time and growth rate seem to be the major drivers of SSD variation, which has been demonstrated previously. However, it remains unclear which of the two parameters is more important due to contradicting findings in different studies. Blanckenhorn et al. (2007) found that growth rate differences between the sexes explain the Lion's share of variation in SSD, while Teder (2014) demonstrated that SSD is usually accompanied by differences in developmental durations. In this context, the proximate causes of SSD reversals (which are rare) remain elusive given the lack of comparative empirical data and are therefore of particular interest.

We here take a combined micro- and macroevolutionary approach to study the evolution of SSD in black scavenger flies (Diptera: Sepsidae). Widespread species are ideal to investigate intraspecific variation and ultimately speciation because differentiation may occur due to genetic drift alone but more likely will be facilitated by ecological specialization. We first document population variation in direction and magnitude of SSD and body size in three *Sepsis* species over a wide geographical scale: *S. biflexuosa* (Strobl 1893) and *S. neocynipsea* (Melander and Spuler 1917), which are both found in Europe (EU) and North America (NA), as well as the sister species of the latter, *S. cynipsea* (Linnaeus 1758), which is widespread only in the old world. Next, we provide a further independent test of the differential equilibrium model by estimating sexual selection on male size and fecundity selection on female size in *S. neocynipsea* populations from both continents, again expecting sexual selection on male size to co-vary with and presumably mediate the pattern of SSD, and implying that male-biased SSD derives from female-biased SSD in connection with a shift in the mating behavior and system (as documented for *S. punctum* by Puniamoorthy et al. 2012b). Finally, using the latest phylogeny for Sepsidae (Zhao et al. 2013), we document and reconstruct the evolution of SSD across 49 taxa to test whether differences in growth rate or development time between the sexes proximately account for more variation in SSD and reversals thereof.

Materials and Methods

POPULATION SAMPLING AND FLY MAINTENANCE

Wild-caught females were used to establish laboratory isofemale lines of all three species with several replicates per population per continent (Fig. 1A). All fly cultures were provided with sugar, standardized cow dung, and water ad libitum and were cultured in climate chambers at 18–24°C and 60% humidity. To ensure comparability across the studies, we followed the experimental procedure of (Puniamoorthy et al. 2012b) with minor adaptations.
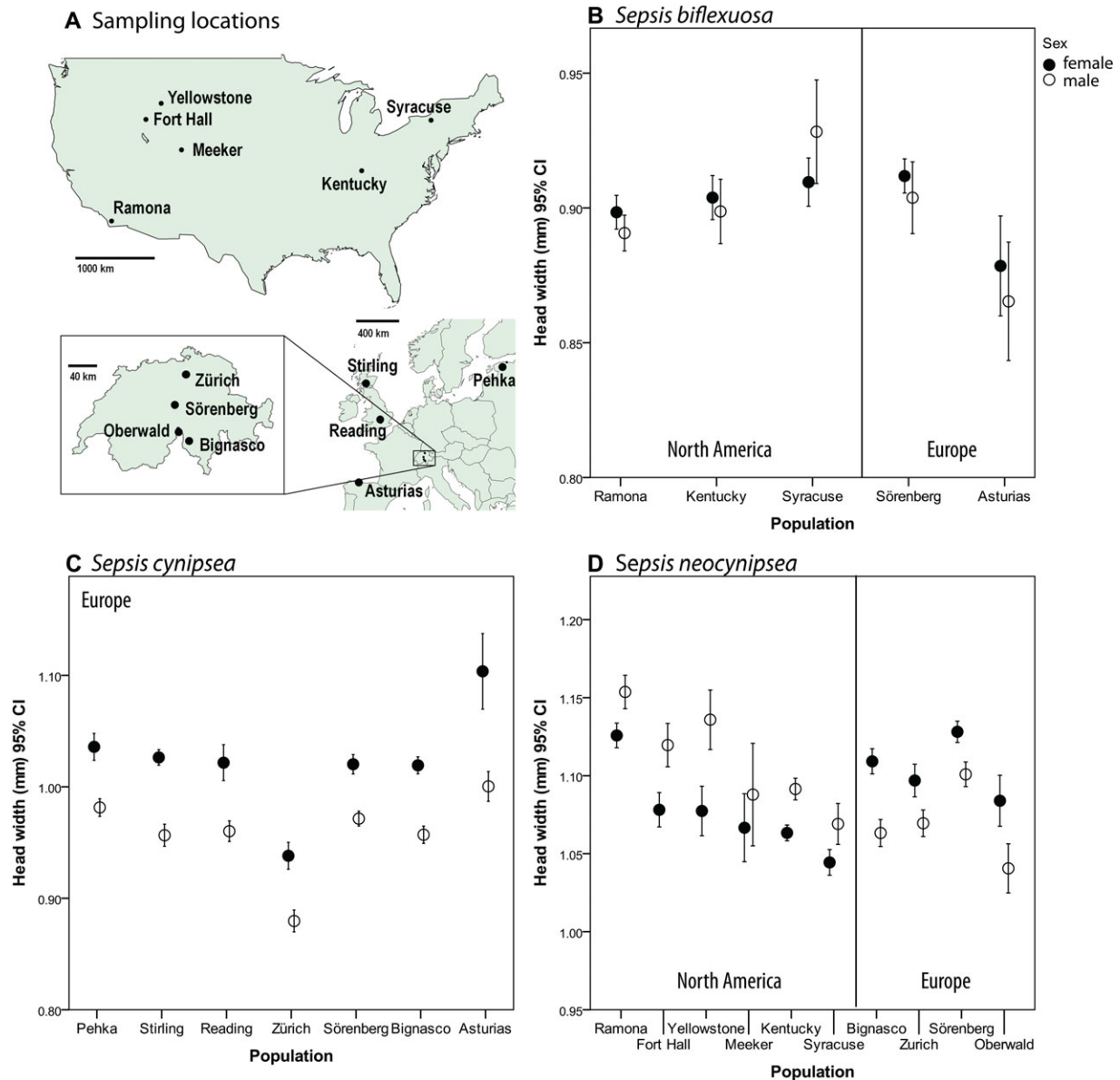
Figure 1. Common garden data gathered across a broad geographic range (A) document population variation in sexual size dimorphism (SSD) in three closely related sepsid flies. Body size in *Sepsis biflexuosa* differs between populations but not between the sexes (B). Populations of the European *Sepsis cynipsea* have exclusively female-biased SSD (C). North American populations of its widespread sister species *S. neocynipsea* show male-biased SSD, whereas females are larger than males in Europe (D).

VARIATION IN SSD ACROSS SPECIES AND POPULATIONS

To control for environmental variation in body size, F3 or later offspring of the established isofemale lines were reared in a common environment. Females were allowed to oviposit in previously frozen, standardized fresh cow dung for several hours, and the offspring were reared at low larval densities at constant 24°C, 60% humidity, and 12-h light cycle. In addition to head width, correlating strongly with overall body size (Blanckenhorn et al. 2004), development time (day of oviposition to day of adult emergence) of all individuals was recorded. These common garden data were analyzed using nested analyses of variance, with (isofemale) lines nested within populations nested within continents, and sex as a crossed factor, for each species separately.

TESTING THE DIFFERENTIAL EQUILIBRIUM MODEL IN *S. neocynipsea*

Eggs of several isofemale lines per population were collected in the same manner as for the common garden experiment, but larvae were reared under low and high densities, leading to variation in larval food competition and hence adult body size. We combined the eggs of all isofemale lines per population and then distributed

the eggs to low- and high-density treatments. Upon emergence, all eclosed flies were separated according to sex to avoid unobserved copulations and provided with water, sugar, and cow dung. Experiments were only started after four to five days, ensuring sexual maturity (Puniamoorthy et al. 2012b).

To estimate sexual selection on male size, mating trials were conducted in groups, emulating the natural situation at a dung pat, at three operational sex ratios (OSR; following a power scale): five males and five females (OSR1), 10 males and five females (OSR2), and 20 males and five females (OSR4). We lack field estimates of OSRs for *S. neocynipsea*, but the used ratios are comparable to observations for *S. cynipsea* in the wild ranging from 1:1 to 1:11 (Blanckenhorn et al. 1999, 2000). Mating trials were conducted in 1 L plastic containers equipped with sugar, water, and dung, with four to five container replicates per population and OSR. Copulating pairs were removed from the arena, thus OSRs were not held constant within a replicate container; however, this variation remains minor compared to the variation between ORS treatments. After 3–4 h, the trial was terminated and all individuals sacrificed by freezing. Subsequently, the head width of all males and females was measured using a binocular microscope.

Univariate sexual selection differentials on male body size were estimated using standard regression approaches (Arnold and Wade 1984). To calculate selection coefficients for each replicate mating container, the sizes of mated and unmated males were standardized (i.e., z-scored) by subtracting the replicate mean from each individual trait value and dividing by the standard deviation: $z_i = (x_i - \bar{x})/SD_x$. Absolute mating success of each male (0 or 1) was divided by the replicate mean mating success to yield relative mating success w_m. When regressing relative mating success on standardized male body size, $w_m = c + \beta_m z$, the slope of the regression line β_m represents the standardized linear sexual selection gradient (equal to the selection differential; note that even though the response variable is discrete, linear regression slopes are well suited for coefficient estimation: Brodie and Janzen 1996). These coefficients (with their corresponding SE) estimating the intensity of sexual selection on male body size for each mating group (i.e., replicate) were later averaged to estimate overall selection.

To estimate fecundity selection, virgin females of varying sizes were paired with a male and housed in a glass vial equipped with sugar and dung for oviposition and incubated at 24°C. The presence of eggs was checked daily until the female laid her first clutch. The number of eggs of this first clutch was then counted as an estimate of early female fecundity, and the females' head width was measured. Female fecundity selection gradients β_f were calculated analogously as relative fecundity w_f (i.e., a female's first clutch size divided by the population mean clutch size) against z-scored female size (as above): $w_f = c + \beta_f z$. We thus obtained one female fecundity coefficient for each population, with a corresponding SE.

To assess the significance of these selection coefficients, mixed generalized linear models were applied to the full dataset (one datapoint per individual) with relative fitness (binomial mating success or number of eggs) as the outcome variable and standardized size as covariate plus any other appropriate fixed factors (continent, population within continent, OSR). Therein, interactions between standardized size and fixed factors indicate variation in selection.

PHYLOGENETIC/COMPARATIVE ANALYSES

Sex-specific body size and development time data for 13 sepsid taxa were gathered under common garden conditions (21°C, low larval competition), which were complemented with data published earlier in Blanckenhorn et al. (2013) using the same experimental protocol, adding 23 taxa. Additionally, body size data from field caught individuals (nine species, one of which we obtained from Eberhard 2002: *Palaeosepsis dentatiformis*; Duda 1926) and four species that were bred in the laboratory without standardized environmental conditions were also included to achieve as broad a coverage of the phylogeny as possible, obtaining 49 taxa in total. The most recent phylogenetic hypothesis for the Sepsidae proposed by Zhao et al. (2013) and extended by Rohner et al. (2014) was used to reconstruct sex-specific body size and SSD of ancestral nodes applying Wagner's (linear) parsimony in Mesquite version 3.03 (Maddison and Maddison 2008) while setting branch lengths to one due to lacking branch length information. To test for phylogenetic clustering, terminal nodes were randomized 1000 times to estimate the frequency distribution of the parsimony score under the corresponding null model, against which we tested the parsimony score of the original tree. This analysis was conducted twice, once including body size data for field caught individuals and once with only common garden data.

Whether variation in SSD is caused more strongly by development time or growth rate differences between the sexes has major implications for the sex-specific costs and benefits (Blanckenhorn et al. 2007). Insect larvae neither grow continuously, nor in a linear fashion; thus, the estimation of growth rates is not trivial. For most species, we lack detailed sex-specific growth trajectories, which are difficult to gather in small larvae dwelling in dung, and therefore have no independent estimates of (infinitesimal) growth rates. However, growth rates, development time, and body size are biologically interrelated such that variation in SSD that is not due to sexual bimaturism must be caused by sex-specific differences in growth rates. This issue is analogous to the question of whether and how much organ size increases are produced by increases in cell size or cell number. In this literature, allometric relationships have been used to quantify their relative contribution

to body size, and we here borrow the approach of Stevenson et al. (1995) to quantify the relative contribution sexual growth rate versus development time differences have on dimorphism.

First, following Lovich and Gibbons (1992), we calculated indices for SSD (SDI) and, analogously, for differences in development time between the sexes (sexual bimaturism index: SBI) for all taxa with available data (36 species), as:

$$SDI = \frac{size \ of \ larger \ sex}{size \ of \ smaller \ sex} - 1.$$

We then used the allometric equation to express SBI as a function of SDI: $SBI = a_1 \times SDI^{b_1}$. The same applies to growth rate differences between the sexes (sexual rate index: SRI): $SRI = a_2 \times SDI^{b_2}$. Because body size differences between the sexes must conceptually equal the product of SRI and SBI, $SDI = SRI \times SBI = a_1 \times a_2 \times SDI^{b_1} \times SDI^{b_2} = a_1 \times a_2 \times SDI^{(b_1+b_2)}$. Given this equation, the sum of the two allometric slopes b_1 and b_2 should equal 1. Therefore, by calculating the allometric slope of SBI against SDI (b_1, equal to the contribution of SBI to SDI in percent), we can in turn estimate the relative contribution of growth rate differences between the sexes as $b_2 = 1 - b_1$. This is because variation in SDI not due to development time differences must be due to growth rate variation. For instance, if SBI would be constant (allometric slope, $b_1 = 0$), all variation in SDI would be due to SRI ($1 - b_1 = 1$). (Other approaches based on r^2 or ANCOVA yield qualitatively similar results.)

To account for the phylogenetic nonindependence of taxa, we used ordinary least squares (OLS) regressions, Felsenstein's (1985) independent contrasts (ICs), and phylogenetic generalized linear models (PGLSs; Freckleton et al. 2002) to calculate b_1 and subsequently b_2. All analyses were conducted in either SPSS version 22 (IBM 2013) or R version 3.1.3 (R Development Core Team 2008).

Results
COMMON GARDEN EXPERIMENTS
We found no SSD in *S. biflexuosa* (sex main effect: $F_{1,294} = 2.22$, $P = 0.137$; sex by continent interaction: $F_{1,294} = 1.78$, $P = 0.183$; Fig. 1B), and overall body size differed in magnitude among populations ($F_{3,26} = 56.39$, $P < 0.001$) and lines ($F_{26,294} = 3.02$, $P < 0.001$), but not between continents ($F_{1,3} = 0.72$, $P = 0.458$).

Although SSD in *S. cynipsea* was exclusively female-biased (sex main effect: $F_{1,24.8} = 256$, $P < 0.001$; Fig. 1C, Table S1) with variation between lines (sex by line within population interaction: $F_{28,571} = 1.67$, $P = 0.018$), the populations did not differ in SSD (sex by population interaction: $F_{6,28} = 5.46$, $P = 0.20$). Overall body size varied between populations ($F_{6,29} = 10.94$, $P < 0.001$) and lines ($F_{29,29} = 1.67$, $P < 0.001$).

SSD was clearly reversed between continents in *S. neocynipsea*, with EU populations showing female-biased and NA populations male-biased SSD (continent by sex interaction: $F_{1,8} = 92.34$, $P < 0.001$; Fig. 1D, Table S1). SSD additionally varied between lines (sex by line interaction: $F_{49,964} = 1.64$, $P = 0.004$) but not among populations within continents (sex by population interaction: $F_{8,49} = 1.10$, $P = 0.379$). Overall body size varied between lines ($F_{50,50} = 3.81$, $P < 0.001$) and populations ($F_{8,50} = 10.34$, $P < 0.001$) but not between continents ($F_{1,8} = 0.08$, $P = 0.783$).

Development time did not differ between the sexes ($F_{1,3} = 7.24$; $P = 0.072$) and continents ($F_{1,3} = 0.56$, $P = 0.507$) in *S. biflexuosa*, but varied between populations ($F_{3,26} = 20.74$, $P < 0.001$) and lines ($F_{26,291} = 8.40$, $P < 0.001$). In *S. cynipsea*, we found no main effect of sex ($F_{1,605} = 0.10$, $P = 0.748$), but development time varied between populations ($F_{6,27} = 2.65$, $P = 0.037$) and lines ($F_{29,605} = 11.71$, $P < 0.001$). Along with the SSD reversal in *S. neocynipsea* (continent by sex interaction: $F_{1,8} = 17.21$, $P = 0.003$), NA males (12.68 ± 0.05 [SE] days) took longer to develop than females (11.94 ± 0.06 days) at 24°C, whereas the sexes did not differ significantly in development time in EU (females: 12.86 ± 0.06 days; males: 12.76 ± 0.07 days). There was also variation in bimaturism between populations (population by sex interaction: $F_{8,1013} = 3.832$, $P < 0.001$). Overall, development time differed between lines ($F_{50,1013} = 9.31$, $P < 0.001$) but not between populations ($F_{8,50} = 1.13$, $P = 0.359$) or continents ($F_{1,8} = 3.38$, $P = 0.103$).

SEXUAL AND FECUNDITY SELECTION
Sexual selection differentials were overall positive (NA: 35/41; EU: 20/30), thus favoring large male size in general, and tended to increase with OSR in NA as predicted by mating systems theory (Emlen and Oring 1977) while remaining constant in EU (Fig. 2A; Table 1). On the whole, body size had a significant effect on male mating success ($\chi^2(1) = 14.4$, $P < 0.001$; Table S2a), and the intensity of sexual selection varied between continents (continent by size interaction: $\chi^2(1) = 10.3$, $P = 0.001$), among populations (populations nested within continent by size interaction: $\chi^2(6) = 14.1$, $P = 0.029$), and with OSR (OSR by size interaction: $\chi^2(2) = 7.6$, $P = 0.022$). The proportion of females that copulated in each replicate group (i.e., container) was added as a covariate to control for variation in female receptivity ($\chi^2(1) = 41.9$, $P < 0.001$; Table 1). The main effects on mating success of continent ($\chi^2(1) = 1.06$, $P = 0.303$), population ($\chi^2(6) = 1.4$, $P = 0.967$), and OSR ($\chi^2(2) = 1.0$, $P = 0.608$) were not significant.

Fecundity selection differentials were all positive (Table 1), thus larger females were more fecund ($F_{1,222} = 33.462$, $P < 0.001$; Table S2b) in the overall model. However, the effect of body size on fecundity did not differ between continents (size by continent interaction: $F_{1,222} = 0.025$, $P = 0.875$; NA: 0.18 ± 0.05 SE; EU:
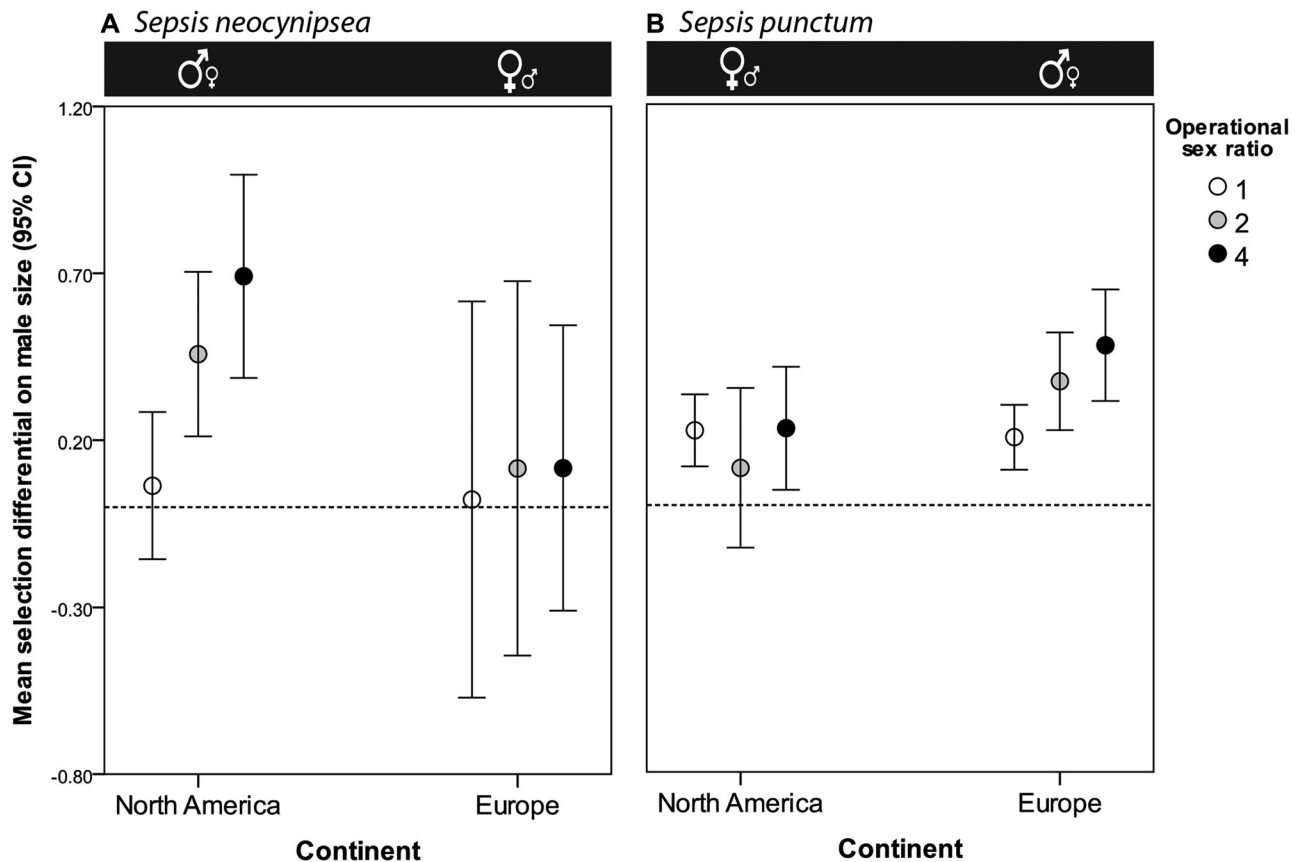
Figure 2. Sexual selection on male head width (body size) is similar in North American and European populations of *Sepsis neocynipsea* at a 1:1 operational sex ratio. However, sexual selection increases in North America with OSR (male-biased SSD), while it stays constant in Europe (female-biased). (A). This is congruent with earlier work on *Sepsis punctum* (B, data redrawn from Puniamoorthy et al. 2012b), for which European populations with male-biased SSD also feature stronger sexual selection on male size that increases with the operational sex ratio.

0.17 ± 0.04 SE). Also, the number of eggs laid did not differ between populations ($F_{5,222} = 1.86$, $P = 0.102$) or continents ($F_{1,5} = 0.00$, $P = 0.984$).

PHYLOGENETIC COMPARATIVE ANALYSES

As in most invertebrates, most sepsid species exhibit female-biased SSD, and this is most likely the ancestral state in Sepsidae (Fig. 3; Table S3). However, Wagner's parsimony suggests that male-biased SSD (SDI < 0) evolved at least six times independently within Sepsidae: in *S. neocynipsea* (NA), *S. punctum* (EU), *S. thoracica*, *S. lateralis*, *P. dentatiformis* and *Saltella nigripes* (Fig. 3). When using the full dataset, as well as when only analyzing common garden data, randomization tests revealed a phylogenetic signal for male ($P_{All} \leq 0.001$; $P_{Common\ garden} \leq 0.001$) and female body size ($P_{All} \leq 0.001$; $P_{Common\ garden} \leq 0.001$), but not for SDI ($P_{All} = 0.241$; $P_{Common\ garden} = 0.205$). We found the same pattern for development time, with sex-specific traits showing phylogenetic clustering (all $P \leq 0.001$), whereas the indices of dimorphism showed no phylogenetic signal (SBI: $P = 0.656$).

Across all taxa, the allometric slope of ln(SBI + 1) on ln(SDI + 1) amounts to nearly one half (OLS: 0.45 ± 0.07 SE; ICs: 0.48 ± 0.07; PGLS: 0.48 ± 0.07), thus growth rate differences and bimaturism seem to contribute equally to SSD. However, when only considering taxa with female-biased SSD, the contribution of sexual bimaturism drops to 10–14% (OLS: 0.10 ± 0.11 SE; ICs: 0.13 ± 0.11; PGLS: 0.14 ± 0.11; Fig. 3C), and consequently growth rate differences between the sexes account for most (86–90%) of the variation in SDI when females are the larger sex.

Discussion

Our study documents significant quantitative variation in body size, SSD, and associated juvenile life-history traits (development time and growth rate) within and among closely related species of sepsid flies. Crucially, a second case of dramatic cross-continental reversal of SSD within a species has been demonstrated for *S. neocynipsea* (cf. Puniamoorthy et al. 2012b). This SSD reversal is again associated with, and therefore likely mediated by

Q4

Table 1. XXX.

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| Continent	Population	Female clutch size		Male mating success		
		β_f	SE	OSR	β_m	SE
Europe	Sörenberg	0.285	±0.088	1	0.417	±0.322
				2	0.764	±0.372
				4	0.234	±0.348
	Bignasco	0.085	±0.081	1	0.214	±0.31
				2	0.908	±0.538
				4	0.249	±0.356
	Oberwald	0.028	±0.07	1	0.124	±0.369
				2	0.267	±0.343
				4	0.131	±0.349
	Zürich	0.312	±0.08	1	0.284	±0.35
				2	0.545	±0.432
				4	0.321	±0.421
North America	Kentucky	0.212	±0.08	1	0.038	±0.268
				2	0.731	±0.228
				4	0.952	±0.227
	Ramona	NA		1	0.059	±0.293
				2	0.634	±0.274
				4	0.013	±0.357
	Syracuse	0.173	±0.068	1	0.298	±0.232
				2	0.315	±0.226
				4	0.938	±0.227
	Yellowstone	0.127	±0.103	1	0.053	±0.331
				2	0.115	±0.221
				4	0.723	±0.314

corresponding variation in the strength of sexual selection acting on male size (Fig. 2), in agreement with the differential equilibrium model for the evolution of SSD (Fairbairn et al. 2007), thus generalizing the evolutionary mechanism. Comparative analyses further suggest that the extent and direction of SSD evolves independently of the phylogenetic history within Sepsidae, whereas female and male body sizes (as well as development times and growth rates) show phylogenetic clustering. Variation in SSD within female-biased taxa is primarily mediated by sexual divergence in growth rates, but reversals in SSD are more strongly driven by the prolongation of male development time.

By analyzing a handful of closely related, widespread sepsid fly species, we have documented variability and lability in patterns of SSD variation among populations and species. In some taxa there is no intraspecific continental variation in SSD, as exemplified by the widespread *S. biflexuosa* (Fig. 1B). *Sepsis cynipsea* is the most common and abundant species in Europe, north of the Alps (the species also occurs in Asia as far as Japan), and is historically the only well investigated species of that group from a behavioral perspective (e.g., Parker 1972; Blanckenhorn et al. 2000; Ding and Blanckenhorn 2002; Teuschl and Blanckenhorn 2007). SSD in *S. cynipsea* was always female-biased and invari-

ant among the seven distant populations investigated. However, its sister species *S. neocynipsea* presents the second known case of a strong continental reversal in SSD, paralleling that of *S. punctum* documented by Puniamoorthy et al. (2012b), but in the opposite direction. So far we do not know whether this relates in any way to the species' differing ecological niches on the two continents, presupposing sex-specific variation in ecological selection, for which we currently lack evidence.

Even though we cannot address possible ecological factors influencing the observed shift in SSD in *S. neocynipsea*, we can associate it phenomenologically with sexual selection. Theory predicts that the net effects of sex-specific selection on body size should qualitatively and quantitatively predict the degree of SSD, provided no genetic or ontogenetic constraints hamper sexual divergence (Andersson 1994; Blanckenhorn 2007). As in *S. punctum* (Puniamoorthy et al. 2012b), the continental reversal in SSD in *S. neocynipsea* is associated with corresponding patterns of variation in the strength of precopulatory sexual selection on male size, with no significant differences in fecundity selection on female size, thus supporting the generality of the selective mechanism behind the differential equilibrium model for the evolution of SSD and the paramount role of sexual selection in mediating
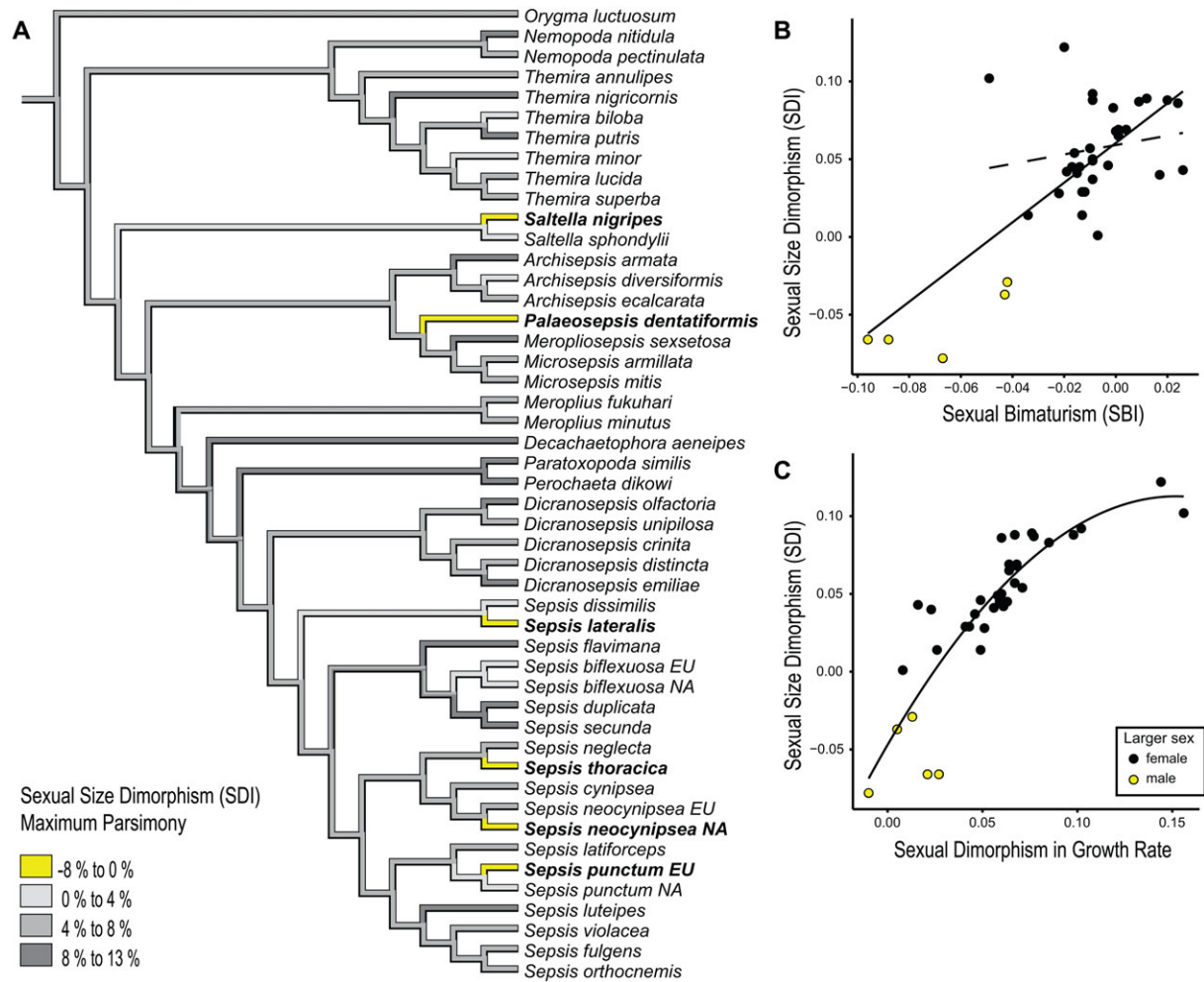
Figure 3. Phylogenetic reconstruction of sexual size dimorphism (SDI) using Wagner's linear parsimony (A) reveals that female-biased dimorphism is predominant among sepsids; however, male-biased dimorphism has evolved at least six times independently. Negative values indicate male-biased SDI (yellow), whereas positive values denote a graded extent of female-biased SDI (gray scale). Sexual bimaturism (B) explains much less variance in SDI than sexual differences in growth rates (C) in species with female-biased SSD (filled black circles), but the prolongation of male development time appears to be the major mechanism to generate male-biased dimorphism (open, yellow circles).

SSD (Fairbairn et al. 2007). Populations with male-biased SSD in both *S. punctum* and *S. neocynipsea* show stronger sexual selection on male size, the intensity of which increases with OSR in agreement with central concepts of mating systems theory (Emlen and Oring 1977; Shuker and Simmons 2014; Fig. 2).

We acknowledge that our estimation of selection differentials slightly differed between the sexes, as we calculated differentials per replicate and OSR and averaged per population in males, whereas for females we directly calculated differentials per population given the lack of experimental manipulation. Consequently the calculations of SEs differed between the sexes, but this difference is primarily statistical and mediated by the contrasting nature of the outcome variable (dichotomous mating success vs. continuous fecundity). Regardless, our main focus here was to test variation in selection between continents and populations within

the sexes, the significance of which was assessed using the full dataset with the appropriate fixed and random effects in both cases. We therefore do not believe our comparison of sexual and fecundity selection to be biased in principle as it follows standard procedures (Brodie and Janzen 1996; Janzen and Stern 1998), although ultimately some caution may be in order.

The fitness components measured here are doubtlessly important for a small and short-lived species that is presumably under strong selection in the wild. We did not however estimate other selection pressures, notably viability selection and postcopulatory selection pressures, which could act in concert with but also can counteract sexual selection on male size (Clutton-Brock 1988; Hunt et al. 2004). As discussed by Puniamoorthy et al. (2012b), who provided such an estimate (showing no continental differences), meaningful estimates of juvenile and adult viability

selection are difficult to obtain for small insects with larvae dwelling in opaque substrates. In contrast to female and male reproductive success, mortality under laboratory conditions and especially intrinsic (residual) life expectancy likely do not well reflect viability selection in the field (cf. Hamilton 1966 "wall of death"). Nevertheless, even though we lack estimates of viability selection we have little evidence that size-dependent mortality varies between the sexes to the extent that it can offset the strong continental differences in sexual selection found for both *S. punctum* and *S. neocynipsea*.

Although we have strong evidence for sexual selection differences between North American and European *S. neocynipsea* populations with differing SSD, we only have anecdotal observations as to the underlying mechanisms producing this phenomenological effect. In *S. punctum*, continental variation in sexual selection is accompanied by visible changes in male courtship (Puniamoorthy et al. 2012a,b): the males of North American populations with larger females display a precopulatory courtship dance, whereas the larger European males show no obvious courtship and simply jump on the females, which may show a rejection response (shaking similar to *S. cynipsea*: Ding and Blanckenhorn 2002). Our phylogenetic analyses suggest that the SSD of EU *S. punctum* is likely derived but their mating behavior (lack of precopulatory courtship) is likely ancestral (cf. Puniamoorthy et al. 2009). In *S. neocynipsea* however, we cannot detect obvious derived features of male courtship. Infrequently, we observed strong male–male aggression among NA males during mating trials, which essentially does not occur in EU populations. These aggressive interactions include jumping on a copulating pair, trying to displace the first male by grabbing its wing bases, which may involve several males. Eberhard (1999) documented territorial behavior of NA *S. neocynipsea* males at feeding sites of females in the wild. Thus, large male advantages during combat over females or territories could lead to stronger sexual selection on male size. This fits well with other anecdotal evidence, suggesting that in many of the sepsids with male-biased SSD (Fig. 3), male behaviors involving aggression toward other males are apparent (EU *S. punctum:* Parker 1972; Zerbe 1993; *S. thoracica*: unpublished data; *Palaeosepsis dentatiformis*: Eberhard 2002). The extent and role of male–male aggression in species with female-biased SSD remain unclear.

Although there are strong parallels between the SSD reversals of *S. punctum* and *S. neocnipsea*, there are also differences. Contrary to the pattern in *S. punctum* (Puniamoorthy et al. 2012b), the body size of *S. neocynipsea* females does not follow the expected evolutionary increase in overall body size (Fig. 1D), as European females are overall larger than North American females. This disagrees with models predicting that due to a high intersexual genetic correlation, female size should show a (weaker) correlated response to directional selection on male size, leading to greater body size of both sexes in populations with male-biased SSD (Lande 1980; Fairbairn and Preziosi 1994).

Taking a comparative angle, we again found striking quantitative variation in adult SSD across the phylogeny of sepsids, featuring at least six independent qualitative reversals (Fig. 3), even though both male and female size show phylogenetic clustering. As adult body size is necessarily mediated by variation and plasticity in juvenile growth and development, selection likely indirectly targets these latter two traits (Blanckenhorn et al. 2007; Stillwell and Davidowitz 2010), body size variation, and SSD potentially resulting as a mere consequence or epiphenomenon. In agreement with Blanckenhorn et al. (2007), we found that growth rate more than development time differences between the sexes (sexual bimaturism) mediate SSD across the majority of female-biased taxa studied here (Figs. 3b and c). However, in the few species with male-biased SSD reversals, sexual bimaturism is clearly more important. This suggests that the only way to increase relative male size drastically is by prolonging development, potentially increasing viability costs during larval development (agreeing with Teder 2014; see also Tammaru et al. 2010). This implies that growth rate cannot be increased beyond an unknown maximum. Facing evidence that dimorphisms in SSD, development and growth in sepsids are evolutionarily very dynamic (Fig. 3), the evolution of these traits seems to be rather unconstrained to vary in this group of flies. However, the juvenile growth of flies (and other insects) is complex and controlled by various endocrine cascades and genetic networks (e.g., Colombani et al. 2005; Mirth and Riddiford 2007; Testa et al. 2013; Nijhout et al. 2014). We therefore suspect that the genetic basis for sexual dimorphisms in development time and growth rates might evolve much more dynamically than currently assumed.

In conclusion, we here show that SSD can vary strongly between and also drastically within taxa. This variation is primarily meadiated by sexual selection on male body size, which is stronger than fecundity selection on female size, supporting the differential equilibrium model. In taxa with reversed SSD, selection on male size primarily targets male development time, while growth rate variation contributes more to SSD in female-biased taxa. Recent studies document that SSD is mediated by critical parameters regulating larval growth and development (Ghosh et al. 2013; Testa et al. 2013), so further studies should aim to uncover these mechanistic developmental changes in sex-specific growth leading to variation in SSD and plasticity therein, which perhaps may be the true targets of body size selection (Stillwell et al. 2010).

Q6

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Table S1. Body size (head width) and egg-to-adult development time (days) for three closely related sepsid flies.

Table S2. (a) Sexual selection on male (binomial GLM) and (b) fecundity selection on female (regular GLM) body size (head width), with nonsignificant interactions removed from the model (except for the continent by body size interaction, due to its a priori relevance).

Table S3. Body size, development time, and growth rates as well as all dimorphism indices for 49 taxa of sepsid flies.

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