

## Research

### Sexual size dimorphism is associated with reproductive life history trait differentiation in coexisting sepsid flies

Wolf U. Blanckenhorn, Julian Baur, Juan Pablo Busso, Athene Giesen, Natalia Gourgoulianni, Nicola van Koppenhagen, Jeannine Roy, Martin A. Schäfer, Alexandra Wegmann and Patrick T. Rohner

W. U. Blanckenhorn (<https://orcid.org/0000-0002-0713-3944>) ✉ ([wolf.blanckenhorn@ieu.uzh.ch](mailto:wolf.blanckenhorn@ieu.uzh.ch)), J. Baur, J. P. Busso, A. Giesen, N. Gourgoulianni, N. van Koppenhagen, J. Roy, M. A. Schäfer (<https://orcid.org/0000-0002-0982-1468>), A. Wegmann and P. T. Rohner (<https://orcid.org/0000-0002-9840-1050>), Dept of Evolutionary Biology and Environmental Studies, Univ. of Zurich, Winterthurerstrasse 190, CH-8057, Zurich, Switzerland.

Oikos

129: 1152–1162, 2020

doi: 10.1111/oik.07036

Subject Editor: Morgan Kelly

Editor-in-Chief: Dries Bonte

Accepted 23 March 2020

Organismal life histories evolve as syndromes, resulting in correlated evolutionary differentiation of key traits that ultimately aid in discerning species. Reproductive success depends both on the absolute body size of an individual and its size relative to the opposite sex: sexual size dimorphism. In an attempt to further elucidate their coexistence and ecological diversification, we compared standard life history (first reproduction, clutch size, egg size) and associated reproductive trait differentiation of 15 widespread European sepsid fly species (Diptera: Sepsidae) under laboratory common garden conditions. Despite relatively uniform body sizes, sexual dimorphism ranged from female-to male-biased, and development time varied twofold across species. We expected, and found, the abundant and relatively large species (*Sepsis cynipsea*, *punctum*, *thoracica*) with often male-biased SSD to lay larger but fewer eggs and show fast-developing, fast-reproducing life histories with aggressive (coercive) mating behavior characterized by short mating latencies and male conflict. In contrast, the smaller and more dispersed species with female-biased SSD (*S. flavimana*, *orthocnemis*, *violacea*) laid smaller but more eggs, showing a generally slower life history with long and delayed copulation and oviposition, high mating reluctance fostering extensive inter-sexual conflict, and more elaborate male (pre-)copulatory courtship. Two *Saltella* species were exceptional, being large, developing slowly, nevertheless copulating soon after adult emergence, profusely and briefly. The documented life history differentiation seems partly driven by sexual selection leading to male-biased dimorphism, rather than undetermined ecological selection, but regardless appears insufficient to explain the coexistence and diversification of these sepsid species in European pastoral landscapes.

Keywords: coexistence, Diptera, dung flies, egg size–number tradeoff, life history, mating behavior, reproductive traits, sexual size dimorphism

### Introduction

According to the competitive exclusion principle of community ecology, species are expected to inhabit different ecological niches. Niche differentiation typically involves spatial and/or temporal partitioning of resources, leading to the evolutionary



differentiation of key traits that ultimately characterize a species' natural history (Abrams 1987, Holt 2009, Levine and HilleRisLambers 2009). This first and foremost concerns life history traits, i.e. the traits most closely linked to fitness, such as growth rate, age and size at first reproduction, offspring number, longevity, propagule size, etc. These traits rarely evolve singly but rather in a concerted manner, leading to correlated evolution (Lande 1980, 1982, Roff 1992, Stearns 1992). Traits covary because energy and time investment into one trait often comes at the expense of another (Schluter et al. 1991, Roff 1992, Stearns 1992, Rowe and Houle 1996). For instance, larger size often requires longer or faster development and/or growth (Roff 1980, 1992); or increased investment into specific offspring typically results in a reduced number of other offspring (Smith and Fretwell 1974, Blanckenhorn and Heyland 2004). Due to such tradeoffs, life histories often evolve as syndromes with several traits changing in a correlated fashion, similarly across species (e.g. *r/K* or fast/slow strategists: Begon et al. 1986, Tauber et al. 1986, Roff 1992, Stearns 1992, Nylin and Gotthard 1998). Life history traits further co-vary with various other characteristics of a species, as organismal life histories have been found to be associated with the expression of secondary sexual traits (e.g. male forehead coloration in flycatchers: Gustafsson et al. 1995), behavior (Blanckenhorn 2005, Shuker and Simmons 2014; e.g. mobility and aggression in water striders: Blanckenhorn et al. 1995), physiology (e.g. lipid accumulation towards the winter: Tauber et al. 1986), or even genome size (body size and growth in crustaceans: Persson and Hessen 2009).

Body size is a trait closely associated with many life history traits and individual fitness. This is because the evolutionary benefits of being large are manifold (and the benefits of small size few and enigmatic: Blanckenhorn 2000), such that natural selection for large size is common in males but also females (Honek 1993, Andersson 1994, Kingsolver and Pfennig 2004, Fairbairn et al. 2007). In gonochoristic species with separate sexes, reproductive success often strongly depends on the number and quality of mating partners, i.e. sexual selection (Bateman 1948, Andersson 1994). Because sexual, fecundity and viability selection pressures are sex-specific and contingent upon the mating system (Emlen and Oring 1977, Fairbairn et al. 2007, Shuker and Simmons 2014), reproductive life history traits are expected to differ between the sexes and across different mating systems. Moreover, reproductive success not only depends on the absolute size of an individual but also on its size relative to the opposite sex (i.e. sexual size dimorphism, SSD), because the larger sex may in general be better able to control mating (Andersson 1994, Eberhard 1996, Ding and Blanckenhorn 2002, Blanckenhorn 2005). Thus, when females are larger, they tend to be better at rejecting males by various behavioral mechanisms and therefore at choosing whether to accept a mate or not. Consequently, species with female-biased SSD are expected to show strong female choice and/or scramble competition (Eberhard 1996). In contrast, males may coerce females if they are larger and/or

stronger, such that species with male-biased SSD tend to show strong male–male competition, as in the classic cases of resource defense polygyny of large, group-living mammals (Wilson 1975, Emlen and Oring 1977, Andersson 1994, Blanckenhorn 2005). All this implies that the mating system, the direction of dimorphism, and whether males show copulatory courtship and/or coercive behavior are, at least conceptually, closely intertwined. For example, the frequently observed interspecific increase of male-bias in SSD with average species size, termed Rensch's rule (after Rensch 1950), is often associated with sexual selection on male size, even when males are smaller than females (Fairbairn 1990, 2005, Abouheif and Fairbairn 1997, Blanckenhorn et al. 2007a, b).

Comparative studies often investigate distantly related groups of species that strongly differ in their ecology (Cheverud et al. 1985, Abouheif and Fairbairn 1997, Blanckenhorn et al. 2007a, b). However, it is also necessary to compare closely related species with similar ecologies, thereby reducing confounding environmental effects and selective pressures (Fairbairn 1990, Andersen 1997). We here provide such a comparative study of European black scavenger flies (Diptera: Sepsidae; Pont and Meier 2002, Ang et al. 2013; <<http://sepsidnet-rmbr.nus.edu.sg/>>). These flies are a common part of many coprophagous (i.e. dung-eating) invertebrate communities in natural and (human-)managed grasslands worldwide (Hammer 1941, Rohner et al. 2015, Jochmann and Blanckenhorn 2016) and generally depend on decaying organic matter for reproduction and juvenile development. Multiple widespread species with apparently very similar ecological niches coexist in Europe, and all 12 *Sepsis* species occurring in Switzerland may be found on the same pasture (Rohner et al. 2014, 2015, 2019). Sepsids have received considerable attention in behavioral ecology and evolutionary research because of their diverse mating systems (Eberhard 2001a, 2003, Kraushaar and Blanckenhorn 2002, Mühlhäuser and Blanckenhorn 2002, Martin and Hosken 2004, Tan et al. 2011, Puniamoorthy 2014, Rohner and Blanckenhorn 2018) and extraordinary secondary sexual morphology of forelegs and convergently evolved abdominal appendages (Eberhard 2001b, Bowsher and Nijhout 2009, Bowsher et al. 2013, Herath et al. 2015, Baur et al. 2020a, b). Mating systems range from species with classic female choice and scramble competition to systems with pronounced male competition (cf. Shuker and Simmons 2014), with associated changes in morphology (e.g. male forelegs), life history (e.g. growth and development rates) and mating behavior involving various traits such as mating duration, mate choice, intra-specific aggression, pre-copulatory and copulatory courtship, which together co-define the mating system of any particular species (Eberhard 2001a, b, 2003, Pont and Meier 2002, Puniamoorthy et al. 2009, Rohner et al. 2016, 2018). We have described at least two independent intraspecific shifts from the ancestral female-biased SSD to male-biased SSD (Rohner et al. 2016) with a mating system characterized by male–male competition associated with increased investment in male ornaments or armaments (Dmitriew and

Blanckenhorn 2012, 2014, Puniamoorthy et al. 2012a, b, Rohner et al. 2016, 2018), in addition to some similar inter-specific shifts (Rohner et al. 2016, Rohner and Blanckenhorn 2018). We thus suspect that mating systems evolve fast in this insect group, without much phylogenetic inertia (cf. Puniamoorthy et al. 2009, Puniamoorthy 2014).

Despite considerable research on the behavior and life history of sepsids it has not been systematically assessed whether and how mating system variation relates to general life history syndromes, reproduction and dimorphism at the comparative level in this fly guild. We here combine two long-term laboratory data sets on the most common European sepsid species, which were collected over several years using standardized methods, to investigate interspecific associations between variation in SSD, mating behavior and life history. To ultimately better understand the coexistence and ecological diversification of multiple similar sympatric sepsid species (Pont and Meier 2002, Holt 2009, Levine and HilleRisLambers 2009), we test for interrelations among several life history and behavioral characters, thereby also providing essential natural history information for this insect group that is currently lacking. As behavioral variation associated with a given mating system may evolve to be genetically fixed (Ding and Blanckenhorn 2002), we expected species with male-biased SSD to show rapid and aggressive (coercive) mating behavior characterized by short mating latencies and male conflict. Species with large males should also lay smaller clutches of large eggs, if large eggs indeed mediate fast offspring development and growth (Fox and Czesak 2000). In contrast, species with female-biased SSD should show high levels of mating reluctance fostering extensive inter-sexual conflict and more elaborate male (pre-)copulatory courtship, in combination with high female egg output (Eberhard 1996, Blanckenhorn et al. 2000). These groupings largely reflect the classic slow-fast reproductive life history axis, but also e.g. the common egg size – egg number tradeoff (Roff 1992, Stearns 1992).

## Methods

### General laboratory methods and fly husbandry

Standard life history traits are often listed in taxonomic compendia for many animal groups, as they aid in characterizing and differentiating closely related species (Pont and Meier 2002 for sepsids). Such compendia typically derive taxonomic, natural history and other biological information from field observations or extraneous reports in the literature, at whatever environmental conditions or locations. At least for small species such as insects, life history and behavioural traits can alternatively be systematically assessed comparatively in the laboratory using so-called ‘common garden’ approaches. Such controlled experiments have the advantage of making traits more directly comparable by minimizing confounding extraneous environmental effects (Roff 1992), although laboratory data may not well reflect the situation in the wild for any particular parameter or species.

Over several years, we have assembled two separate though overlapping (in terms of species) data sets for 15 (female reproductive traits) and 9 (behavioral traits) sepsid taxa common in Europe, which we here summarize and use for the purposes outlined above. Species composition was haphazard because we had different sets of species/populations in culture at the different times when the data were collected. Because the widespread *Sepsis punctum* (Dmitriew and Blanckenhorn 2012, Puniamoorthy et al. 2012a, b) and *S. neocynipsea* (Rohner et al. 2016, Giesen et al. 2017, 2019, Rohner and Blanckenhorn 2018) also occur in North America, and because continental populations display strong intraspecific variation in mating system and size dimorphism (Baur et al. 2020a, b; op. cit.), we included a North American population of both species in one of our data sets for direct comparison (from Ottawa, Ontario (45°40'N, 75°65'W; ca 100 m altitude) and Lamar Valley, Montana (45°01'N, 110°02'W, ca 2300 m), respectively), treating these as independent evolutionary lineages (i.e. subspecies; Fig. 1). European test individuals of all species came from around Zurich, Switzerland (47°34'N, 8°54'E; ca 450 m), except *S. neocynipsea*, which stemmed from nearby Sörenberg, Switzerland (46°87'N, 8°27'E; ca 1100 m), as it is rare in the lowlands.

All flies used were derived from cultures held in the laboratory in standard ways for variable amounts of time that were originally derived from multiple (at least 10, often many more) field-caught females (see Blanckenhorn et al. 2000, 2002, Puniamoorthy et al. 2012a, b, Rohner et al. 2016 for more details on our general rearing methods). At least two replicate 3-liter (2.2 × 1.2 × 1.2 dm<sup>3</sup>) plastic containers equipped with ad libitum sugar, water and cow dung were available per species/population, which typically held ca 200 adult flies and were kept at 20–21 °C (i.e. room temperature), 60% RH and 14 h light.

To obtain test individuals, laboratory cultures were provided with 2–5 oviposition plates filled with homogenized, previously frozen cow dung. After 24 h, these oviposition plates were moved into another container kept at 20–21 °C. Emergence of adult flies was checked daily (after ca 2–3 weeks). Newly emerged test flies were collected within 24 h upon eclosion, separated by sex (to avoid untimely mating), and subsequently kept singly in glass vials equipped with sugar and a small plastic dish (22 × 22 × 11 mm) filled with cow dung. In case of the female reproductive data set, the oviposition plates were large petri dishes to generate females of maximal body size at low larval densities; in the case of the mating behavior data set, flies of a wide body size range were generated by varying dung quantity and/or larval density (as in Rohner et al. 2018).

### Female reproductive trait differentiation

To quantify key reproductive traits of 15 (sub)species early in adult life, we allowed focal females to mate with at least one male starting 24 h after eclosion. Because adult sepsid individuals live for variable amounts of time in the laboratory (typically for 1–2 months) and may mate repeatedly, we

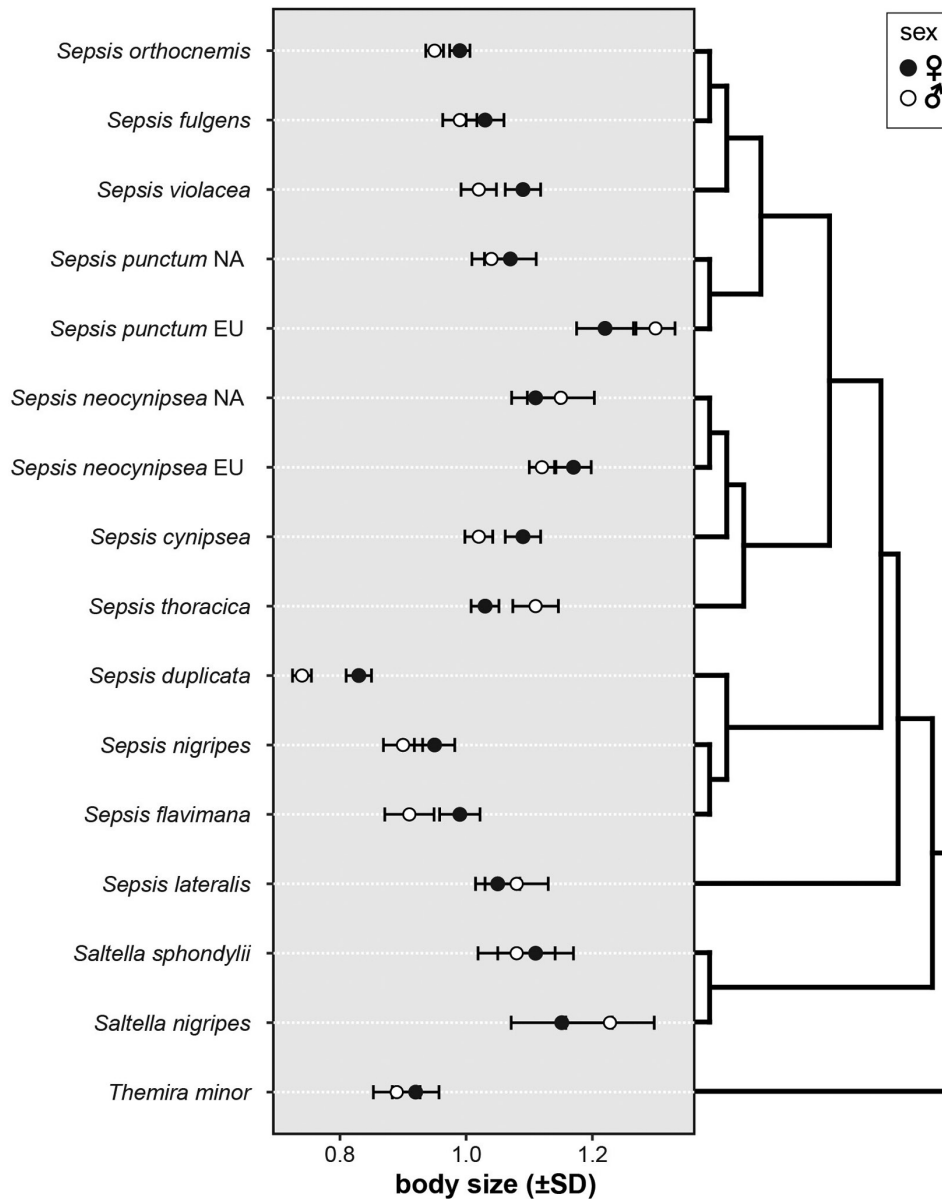


Figure 1. Sexual body size dimorphism (SSD; i.e. female and male head width  $\pm$  SD) of all species covered in this study, all estimated under ideal nutritional (i.e. competition-free) laboratory common garden conditions, mapped onto the underlying phylogenetic relationships (data from Rohner et al., 2016). The original female-biased SSD (females larger) has evolutionarily reversed multiple times towards male-based SSD (males larger), especially in large species.

focused on early reproduction, which is more directly comparable across species and more relevant under field conditions. About 20–30 virgin test females per species were kept singly in glass vials and provided with mature males haphazardly chosen from individuals emerging from the petri dishes on a daily basis. To assure fertility and mating experience, males were 5–10 days older than the focal females (cf. Teuschl and Blanckenhorn 2007); beyond copulation males were irrelevant for this (female) data set. We observed all pairs for a total of 1 h per day, 3–5 at a time. Copulations were only scored as such if genital contact was established. Whenever copulation took place, we scored mating latency (the time since the start

of the mating trial on that day) as well as copulation duration (to the minute). Thereafter, males were removed and females were provided with fresh dung. In case females did not copulate, the mating trial was repeated on the next day with another random male. Females that did not copulate by the age of 14 days were discarded.

After copulation, we scored the female's age (i.e. day) at first reproduction, egg number (clutch size) and egg size to quantify early fecundity and female reproductive investment. To do so, we checked for oviposition every 24 h. Once females laid their first clutch, we counted all eggs and photographed 3–5 eggs (depending on the total number available)

Table 1. Summary of assessed female reproductive traits for 15 (sub)species of sepsid flies. (\* Data from Rohner et al. 2016.)

species	n	Hind tibia length (mm ± SE)	Age 1st copulation (days ± SE)	Age 1st reproduction (days ± SE)	Clutch size (± SE)	Egg volume (mm <sup>3</sup> × 10 <sup>-3</sup> ± SE)	Copulation duration (mins ± SE)	Mating latency (mins ± SE)	Development time female 21°C (days)	Sexual size dimorphism index (SDI) <sup>†</sup>
<i>Saltella nigripes</i>	6	1.57 (±0.11)	1.50 (±0.24)	4.00 (±0.91)	58.37 (±3.71)	12.94 (±0.35)	4.33 (±0.54)	14.00 (±4.40)	18.3	-0.07
<i>Saltella sphondylii</i>	29	1.5 (±0.01)	1.76 (±0.17)	7.5 (±0.82)	48.43 (±1.99)	7.07 (±0.15)	4.24 (±0.28)	24.52 (±2.54)	19.67	0.03
<i>Sepsis cynipsea</i>	37	1.47 (±0.01)	3.83 (±0.20)	7.34 (±0.30)	47.05 (±2.01)	17.49 (±0.37)	23.74 (±0.57)	23.17 (±4.89)	12.31	0.07
<i>Sepsis duplicata</i>	23	1.07 (±0.01)	3.75 (±0.28)	6.91 (±0.21)	57.09 (±1.86)	6.74 (±0.18)	25.17 (±1.06)	-	20.61	0.12
<i>Sepsis flavimana</i>	23	1.32 (±0.01)	5.35 (±0.56)	8.86 (±0.59)	86.27 (±5.03)	7.15 (±0.16)	36.87 (±2.78)	33.43 (±5.82)	22.03	0.09
<i>Sepsis fulgens</i>	62	1.38 (±0.01)	3.90 (±0.19)	6.40 (±0.21)	55.04 (±1.79)	8.37 (±0.18)	19.22 (±0.37)	-	15.77	0.04
<i>Sepsis lateralis</i>	31	1.41 (±0.01)	2.16 (±0.07)	7.67 (±0.49)	71.74 (±8.73)	8.11 (±0.28)	16.35 (±0.72)	11.10 (±1.02)	16.19	-0.03
<i>Sepsis neocynipsea</i> NA	36	1.46 (±0.01)	3.03 (±0.16)	6.22 (±0.22)	49.14 (±3.69)	12.15 (±0.14)	26.03 (±1.19)	12.11 (±1.81)	14.04	-0.04
<i>Sepsis neocynipsea</i> EU	29	1.59 (±0.01)	4.28 (±0.42)	9.79 (±0.57)	62.29 (±7.28)	12.16 (±0.53)	26.79 (±1.32)	10.62 (±1.95)	16.91	0.04
<i>Sepsis nigripes</i>	15	1.26 (±0.01)	2.27 (±0.12)	9.14 (±1.06)	58.57 (±7.21)	5.58 (±0.12)	21.47 (±1.51)	24.47 (±7.71)	19.76	0.06
<i>Sepsis orthocnemis</i>	17	1.32 (±0.01)	10.18 (±1.01)	14.13 (±1.01)	87.88 (±7.67)	6.96 (±0.13)	38.12 (±1.84)	42.76 (±5.60)	18.04	0.06
<i>Sepsis punctum</i> NA	32	1.44 (±0.02)	5.34 (±0.29)	8.81 (±0.44)	42.71 (±4.64)	10.81 (±0.18)	19.94 (±0.98)	24.63 (±3.32)	13.65	0.03
<i>Sepsis punctum</i> EU	34	1.67 (±0.01)	3.05 (±0.12)	8.44 (±0.61)	49.74 (±1.96)	15.04 (±0.36)	23.62 (±0.87)	11.28 (±2.13)	15.22	-0.07
<i>Sepsis thoracica</i>	22	1.38 (±0.02)	3.05 (±0.17)	6.64 (±0.50)	56.98 (±3.60)	14.79 (±0.37)	18.43 (±0.56)	8.38 (±0.76)	11.28	-0.08
<i>Sepsis violacea</i>	21	1.47 (±0.01)	5.71 (±0.30)	11.94 (±0.63)	74.50 (±7.58)	8.26 (±0.13)	63.33 (±2.48)	45.86 (±3.59)	21.32	0.07

per clutch using a camera mounted on a microscope to later estimate egg volume using the formula for an ellipsoid (egg volume =  $1/6 \times \pi \times \text{egg length} \times \text{egg width}^2$ ). Even though sepsids tend to lay more or less discrete clutches, females sometimes take more than 24 h to lay all eggs. Hence, we also counted the number of eggs a female laid on the following day (only ~17% of all (i.e. 71 of 417) females laid additional eggs on the second day). Thereafter, females were stored in 70% ethanol to later measure their hind tibia length as an estimate of body size. Females that did not lay eggs after 14 days upon copulation were discarded.

### Mating behavior differentiation

To quantify the (male and female) mating behavior of 9 sepsid species early in adult life, we conducted mating trials similar to those described above. Virgin females eclosed 2–4 days before were kept singly in glass vials and provided haphazardly with a virgin male. Males mature earlier than females and already come with sperm from the juvenile phase but were typically of similar age (Teuschl and Blanckenhorn 2007). We observed all pairs for a total of 30 minutes or until copulation occurred. We scored 1) the time of the first male mating attempt (= male mating latency), 2) the total number of male mating attempts that occurred (in 30 min, or until copulation occurred), 3) the cumulative duration of female shaking to dislodge the male (before copulation occurred), and 4) whether copulation occurred or not, to estimate the overall mating probability of virgin flies. The first two traits largely reflect male aggressiveness in obtaining matings (Ding and Blanckenhorn 2002), the third trait reflects female reluctance to mate and/or female choice (Blanckenhorn et al. 2000, 2002), while copulation ultimately results from the actions of both mating partners. As above, we measured both individuals' body size (head width) after the mating trial. We did not correct our data for the shorter observation period in case copulation occurred, on which some of the behavioral data are contingent, as we primarily wanted to characterize the behavior and mating system of the species.

### Statistical analysis

For both data sets separately, we subjected the correlation matrix of species means for all traits measured to a phylogenetically corrected principal component analysis (PCA) to find the major axes of species differentiation, using the R-package phytools (Revell 2012). To account for phylogenetic non-independence, we constructed a composite cladogram based on published phylogenies (Su et al. 2008, Rohner et al. 2014, Lei et al. 2013; Fig. 1). As mating latency data were not available for all species, this trait was eventually excluded from the overall analysis but is nevertheless documented for the sake of completeness. Because PCA finds the axis that best differentiates between species but not necessarily the axis most strongly associated with sexual size dimorphism, we also used partial least squares regression (which in this case is similar to multivariate regression: Geladi and

Kowalski 1986), as implemented in the R-package *plsdepot* (Sanchez 2012) to find the linear combination of all life history or mating behavior variables that best explains variation in SSD.

Though quantitatively (but only rarely qualitatively) variable within species, the species' dimorphism was deliberately estimated by the sexual dimorphism index (SDI) of Lovich and Gibbons (1992) using extraneous data from Rohner et al. (2016) and Rohner and Blanckenhorn (2018) of flies reared at overabundant food (i.e. dung), to obtain a representative, reliable value (as recommended by Fairbairn et al. 2007). To calculate SDI, we divided the mean size of the larger sex by that of the smaller sex and subtracted 1 from this ratio, and arbitrarily assigned positive signs when females are the larger sex and negative signs when males are larger. Thus, for both data sets separately, the actual body size of the flies was used for the analyses (because this value affects reproductive traits), while SSD (SDI) was estimated from the data in Fig. 1.

## Results

### Female reproductive trait differentiation

The phylogenetically corrected PCA including all female reproductive traits revealed that life histories of closely related European sepsid fly species are diversified predominantly along the body size – development time and the female reproductive trait (particularly egg size and egg number) axes (Table 1, Fig. 2). The presumably maximal mean body size of females in situations of low to no larval competition (here estimated by hind tibia length, a common proxy for body size in these flies) varied from 1.07 mm in the by far smallest *Sepsis duplicata* to 1.67 mm in European *S. punctum*, the largest species in our dataset (Table 1). Mean development time varied more across species, from roughly 11 days (at 21°C) in *S. thoracica*, a relatively large species, to 22 days in *S. flavimana*, a rather small species (Table 1). First clutch sizes varied between 45 and 60 for most species, with *S. flavimana*, *S. orthocnemis* and *S. violacea* being exceptional in laying 75–90 eggs. The latter species at the same time laid particularly small eggs (around 8 mm<sup>3</sup>), whereas the eggs of similarly-sized *S. cynipsea*, *S. punctum* and *S. thoracica* are almost double that size (Table 1). *Sepsis flavimana*, *S. orthocnemis* and *S. violacea* further feature the slowest initiation of reproduction (adult age at first reproduction > 9 days) and the longest copulation durations (> 30 min), while for the other *Sepsis* these two traits average around 20–25 min and 7 days, respectively. In these three species, the late and long copulations were accompanied by prolonged pre-copulatory stimulation of the female abdomen by the male with his paired genital claspers ('surstyli'; cf. Eberhard 1996, 2001a, Puniamoorthy et al. 2009; data not shown). *Saltella* are exceptional in that they copulate already 1–2 days after adult emergence, profusely and briefly (ca 5 min; cf. Martin and Hosken 2004, Tan et al. 2011). Differences between the continental *S. neocynipsea* and *S. punctum* populations/subspecies were minor in

comparison, especially when accounting for their size difference (Fig. 2, Table 1).

### Mating behavior differentiation

Analogous PCA of the behavioral data set showed diversification of sepsid species with regard to mating latencies and whether female shaking occurs or not in response to recurring male mating attempts (Fig. 2). *Sepsis orthocnemis* and *S. violacea*, but also *S. fulgens* and *S. duplicata* feature long male mating latencies (= time to first mating attempt), while *Saltella sphondylii* and also European *S. punctum* initiated mating quickly and aggressively (Table 2). The frequency of male mating attempts did not vary greatly among species, but only *S. cynipsea*, *S. punctum* EU and *Themira minor* displayed significant female reluctance in terms of shaking off mounted males (cf. Blanckenhorn et al. 2000, 2002, Mühlhäuser and Blanckenhorn 2002; Table 2). Mating probabilities were high (> 50%) in those (large) species that mate aggressively and whose mating system is characterized by sexual conflict (*S. cynipsea*, *thoracica*, *punctum* EU and *Saltella sphondylii*; Blanckenhorn et al. 2002, Martin and Hosken 2004), but low in all other species.

### Interrelations between SSD, life history and behavior

Variation in sexual size dimorphism, which is substantial and likely not strongly constrained by phylogeny (Fig. 1), and which in our analyses provided the common link between the two data sets, aligns well with behavioral and reproductive trait variation among species. Based on the loadings on the first principal component (PC1) (as well as the loadings on PLS1; Supplementary material Appendix 1 Table A1), the evolution of more female-biased SSD (i.e. increasing SDI) is associated with a longer male mating latency, less female shaking (i.e. less sexual conflict), small average body size, and a low mating probability (Fig. 2, right panel for the behavioral data set; Table 3). A more strongly female-biased SSD across species further associates with longer and later copulation and reproduction, increased clutch size with smaller eggs, and prolonged development (Fig. 2, left panel for the female reproductive trait data set; Table 3).

## Discussion

Our comparative life history study of 15 coexisting European sepsid taxa revealed that these flies are diversified predominantly along the body size–development time (which was found before: Blanckenhorn et al. 2007a, 2013, Rohner and Blanckenhorn 2018) and the female reproductive trait axes (which is our new contribution: first reproduction, clutch size and especially egg size; Fig. 2, Table 1). Traits characterizing male and female mating behavior furthermore align well along the slow–fast reproductive continuum (Table 2). The most abundant and relatively large species *Sepsis cynipsea*, *S.*

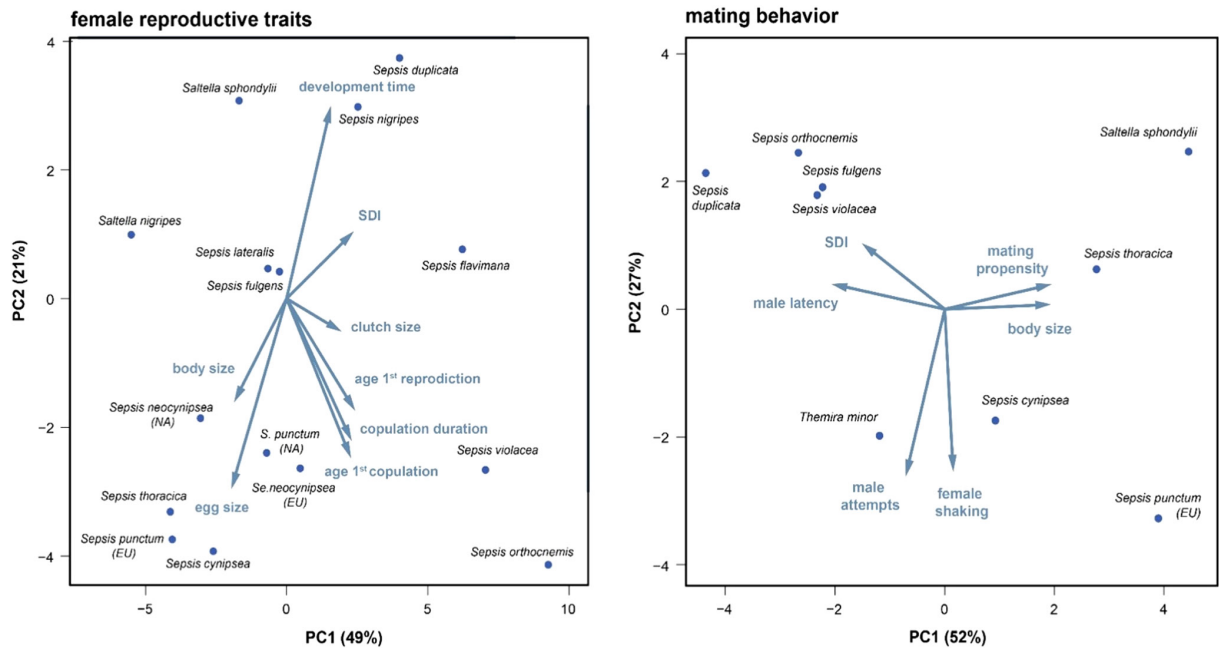


Figure 2. Female reproductive trait (left) and mating behavior trait diversification (right) for 15 and 9 (respectively) European sepsid (sub) species based on their phylogenetic principal components (Supplementary material Appendix 1 Table A1).

*punctum* and *S. thoracica* (Rohner et al. 2015, 2019, Busso and Blanckenhorn 2018a; Fig. 1) feature clutch sizes of around 45–60 large eggs (ca 16 mm<sup>3</sup>), whereas the rarer and more dispersed *S. flavimana*, *S. orthocnemis* and *S. violacea* lay much smaller (ca 8 mm<sup>3</sup>) but more eggs (75–90), suggesting an egg size – egg number tradeoff across species (Smith and Fretwell 1974, Roff 1992). The latter species are further characterized by late reproduction and long copulations (> 30 min), indicating a generally slow life history, in contrast to the former species and especially *Saltella*. The relatively large (Fig. 1) *Saltella* are exceptional in that they copulate very soon (1–2 days) after emergence, featuring brief and repeated genital insertions (ca 5 min; see also Martin and Hosken 2004, Tan et al. 2011). The faster life history of the first group of species is further indicated by frequent copulations, fast and recurrent male mating attempts, and female reluctance to mate (expressed by shaking) in the behavioral assays (Table 2). Most of the slow-reproducing species show less aggressive

mating behaviour, except perhaps the very small (Fig. 1) *Sepsis duplicata*, the only species here that displays elaborate male precopulatory courtship (cf. Puniamoorthy et al. 2009). Importantly, low SDI indices (indicating male-biased sexual dimorphism) are associated with high mating probabilities in those large species that mate aggressively or whose mating system is characterized by sexual conflict (Blanckenhorn et al. 2002, Martin and Hosken 2004; *S. cynipsea*, *S. thoracica*, *S. punctum* and *Saltella sphondylii*), but low in all other species. We could thus roughly identify three life history groupings, which are detectable in Fig. 2, based on their reproductive strategies, in this closely related guild of European sepsid dung flies, and documented classic life history differentiation outlined by standard theory (Stearns 1992, Roff 1992, Fox and Czesak 2000) that aligns well with mating system variation.

By focusing on sexual size dimorphism (SSD), which provided the main common link between our two disjunct,

Table 2. Summary of assessed male and female mating traits for 9 species of sepsid flies (behavioral data set).

Species	Head width (mm ± SE)		n	Male latency (min)	Male attempts (no.)	Female shaking (min)	Proportion mated	Total pairs
	Males	Females						
<i>Saltella sphondylii</i>	1.00 (± 0.02)	1.01 (± 0.02)	42	3.31 (± 0.78)	1.86 (± 0.19)	0.32 (± 0.10)	0.65 (± 0.07)	55
<i>Sepsis cynipsea</i>	0.83 (± 0.01)	0.92 (± 0.01)	189	7.60 (± 0.42)	2.19 (± 0.13)	5.93 (± 0.64)	0.49 (± 0.03)	267
<i>S. duplicata</i>	0.67 (± 0.01)	0.73 (± 0.01)	33	13.55 (± 0.72)	2.29 (± 0.16)	0.40 (± 0.19)	0.20 (± 0.04)	128
<i>S. fulgens</i>	0.77 (± 0.01)	0.85 (± 0.01)	62	11.94 (± 0.73)	2.13 (± 0.17)	0.12 (± 0.03)	0.06 (± 0.02)	176
<i>S. orthocnemis</i>	0.78 (± 0.01)	0.83 (± 0.01)	27	11.98 (± 1.67)	2.00 (± 0.32)	0.11 (± 0.02)	0.01 (± 0.01)	120
<i>S. punctum EU</i>	0.92 (± 0.01)	0.97 (± 0.01)	61	4.14 (± 0.64)	2.84 (± 0.41)	3.57 (± 0.83)	0.56 (± 0.05)	111
<i>S. thoracica</i>	0.86 (± 0.01)	0.85 (± 0.01)	83	8.02 (± 0.56)	2.20 (± 0.18)	0.55 (± 0.12)	0.63 (± 0.04)	173
<i>S. violacea</i>	0.85 (± 0.01)	0.95 (± 0.01)	11	13.17 (± 2.11)	2.27 (± 0.45)	0.18 (± 0.02)	0.01 (± 0.01)	79
<i>Themira minor</i>	0.81 (± 0.01)	0.88 (± 0.01)	35	9.02 (± 1.17)	2.89 (± 0.41)	2.65 (± 1.26)	0.16 (± 0.05)	52

Table 3. Phylogenetically corrected inter-specific correlations (with 95% confidence limits) among the female reproductive life history traits of the reproductive data set (A; n=15 species), and the female and male behavioral traits of the behavioral data set (B; n=9 species). Significant relationships are in bold.

	Hind tibia length	Age at first copulation	Age at first reproduction	Clutch size	Egg volume	Copulation duration	Development time
(A)							
SDI	<b>-0.64 [-0.82, -0.18]</b>	<b>0.52 [0.00, 0.76]</b>	<b>0.54 [0.03, 0.77]</b>	0.17 [-0.35, 0.58]	<b>-0.57 [-0.79, -0.07]</b>	0.50 [-0.02, 0.76]	<b>0.57 [0.08, 0.79]</b>
Hind tibia length		-0.24 [-0.62, 0.3]	0.00 [-0.48, 0.48]	0.06 [-0.44, 0.52]	<b>0.51 [0.00, 0.76]</b>	0.01 [-0.47, 0.49]	-0.06 [-0.51, 0.44]
Age at first copulation			<b>0.78 [0.46, 0.89]</b>	<b>0.60 [0.12, 0.80]</b>	-0.24 [-0.62, 0.3]	<b>0.75 [0.39, 0.87]</b>	0.26 [-0.27, 0.63]
Age at first reproduction				0.48 [-0.04, 0.75]	-0.49 [-0.75, 0.04]	<b>0.73 [0.36, 0.87]</b>	0.39 [-0.15, 0.70]
Clutch size					-0.38 [-0.69, 0.16]	<b>0.53 [0.02, 0.77]</b>	0.37 [-0.17, 0.69]
Egg volume						-0.18 [-0.59, 0.34]	<b>0.66 [0.23, 0.83]</b>
Copulation duration							
(B)							
SDI	<b>0.79 [0.09, 0.93]</b>	Male attempts	Female shaking	Mating probability	Body size		
Male latency		-0.28 [-0.72, 0.43]	-0.04 [-0.68, 0.64]	<b>-0.68 [-0.87, -0.03]</b>	-0.52 [-0.81, 0.21]		
Male attempts		0.00 [-0.60, 0.59]	-0.19 [-0.68, 0.49]	<b>-0.89 [-0.95, -0.55]</b>	<b>-0.88 [-0.95, -0.54]</b>		
Female shaking			0.56 [-0.16, 0.83]	-0.46 [-0.79, 0.28]	-0.29 [-0.72, 0.42]		
Mating probability				0.09 [-0.55, 0.64]	0.03 [-0.58, 0.61]		
					<b>0.67 [0.00, 0.87]</b>		

descriptive laboratory data sets analyzed, we specifically addressed sexual selection as a potential factor responsible for the diversification present. As expected by theory (Emlen and Oring 1977, Fairbairn et al. 2007, Shuker and Simmons 2014), we found the mating system variation of sepsids to be associated with life history, morphological and behavioral differentiation. Species with low SDI (i.e. more male-biased SSD) showed increased male reproductive aggression leading to high mating rates, suggesting strong precopulatory male-male competition. Because the evolution of more male-biased SSD is associated with larger body sizes (Fairbairn 1990, Kraushaar and Blanckenhorn 2002, Fairbairn et al. 2007), this confirms the link between the intensity (or opportunity) of sexual selection and Rensch's rule (cf. Abouheif and Fairbairn 1997, Blanckenhorn et al. 2007b). That is, (pre-copulatory) sexual selection on males could drive the evolution of male size and also elicits a (genetically) correlated, weaker response in female size, leading to more male-biased SSD but also larger mean size of both sexes. At the other end of the SSD continuum, some species show very little male aggression and particularly long copulations (up to more than an hour in *Sepsis violacea*, and more than 30 min in *S. orthocnemis* and *S. flavimana*). As these long copulations were preceded by copulatory courtship (a repeated, apparently ritualized stimulation of the female abdomen with the male genital claspers), this suggests a dominant role of pre- (and possibly post-) copulatory female choice (Eberhard 1996).

Even though the ecological niche differentiation in terms of life history and reproductive traits found here may appear minor, we could identify some common life history syndromes despite the close relatedness of all species (Tauber et al. 1986, Roff 1992, Stearns 1992, Fox and Czesak 2000). There first and foremost appears to be an egg size – egg number (i.e. clutch size – egg volume) tradeoff across sepsid species, however no body size – development time tradeoff. Both tradeoffs are expected by theory though not necessarily present within or between some species groups (Smith and Fretwell 1974, Roff 1992, 2000, Blanckenhorn and Heyland 2004, Fox and Czesak 2000). The two *Saltella* species stand out in exhibiting a live-fast-die-young reproductive strategy with quick and frequent copulations leading to early death due to high costs of reproduction and potentially strong sexual conflict (Arnqvist and Nilsson 2000, Martin and Hosken 2004). This interpretation aligns with strong reluctance to mate expressed by female shaking in several *Sepsis* species (*S. cynipsea*: Blanckenhorn et al. 2000, 2002, Mühlhäuser and Blanckenhorn 2002, Teuschl and Blanckenhorn 2007, Giesen et al. 2017; European *S. punctum*: Puniamorthy et al. 2009, 2012b; *S. thoracica*: Busso and Blanckenhorn 2018b). The latter three abundant European *Sepsis* species, plus *Saltella* (Martin and Hosken 2004), are all characterized by relatively large body size and, in the extreme, male-biased sexual size dimorphism or at least low SDI (Table 1; Rohner and Blanckenhorn 2018); these flies mate selectively and aggressively (i.e. exhibit potentially intense sexual selection), and subsequently invest strongly in



reproduction by laying (for their size) many large eggs fast. The tiny and slow-growing *S. duplicata* is rather exceptional, as it shows elaborate precopulatory male courtship dances and oviposits in beetle tunnels to reach the moist interior of old and crusty dung pats (Hammer 1941).

Fox and Czesak (2000) identified body size, latitude and predation as the main selective forces driving inter-specific variation in propagule or offspring size and number. With the exception of *S. duplicata*, the body size of the sepsid flies covered here does not vary tremendously. We know little about predation or parasite pressure on sepsids in the field (but see Busso and Blanckenhorn 2018c), but doubt that it differs strongly for the species treated here given they largely share the same (micro)habitat on pastures (Rohner et al. 2015, 2019). As we primarily studied species from north-central Switzerland, latitudinal variation was not an issue here. However, we have previously examined intraspecific latitudinal (and altitudinal) variation across Europe (and North America) of some of these species, and generally found merely minor differentiation (Blanckenhorn 1997, Puniamoorthy et al. 2012a, b, Berger et al. 2013, Rohner et al. 2016, Giesen et al. 2017, 2019, Busso and Blanckenhorn 2018a, Roy et al. 2018). Clinal variation at least in egg size was somewhat more pronounced and in accordance with expectations (larger at higher latitude) in the much larger yellow dung fly (Bauerfeind et al. 2018; but see Roy et al. 2018 for contrary results for *S. fulgens*), however also not marked. We therefore conclude that sexual size dimorphism, but probably not so much seasonality (for which altitude and latitude are common proxies; cf. Blanckenhorn and Demont 2004) or predation (and parasitoids), contribute to the life history differentiation documented here to some extent.

The differences in female reproductive traits found here between the continental *S. neocynipsea* and *S. punctum* populations were minor in comparison, as could be expected given that they are (still) considered the same species (Pont and Meier 2002) and hybridization is possible in the laboratory (Schulz 1999, Giesen et al. 2017, 2019). The extent of continental differentiation remained within the lower left quadrant in Fig. 2, and can probably largely be explained by the known differences in body size, dimorphism and reproductive behavior between the populations/subspecies (Puniamoorthy et al. 2012a, b, Rohner et al. 2016, Giesen et al. 2017, 2019, Baur et al. 2020a).

Although all species investigated here (except *T. minor*) commonly co-occur on livestock excrements (at least in central Europe), the mechanisms that allow more than 10 closely related species of the genus *Sepsis* to co-exist in the same habitat remain contentious even after this study. Previous studies suggested little larval substrate specialization (Laux et al. 2019) and only minor geographic, altitudinal or seasonal (including diurnal) variation in occurrence (Rohner et al. 2014, 2015, 2019). Therefore these species have largely overlapping ecological niches. We here hypothesized that sepsid diversity could be associated with life history and behavioral differentiation that may be largely mediated by sexual rather

than ecological selection. Although our study revealed such differentiation, we conclude that it appears to be too subtle to explain the coexistence of so many similar sympatric species by ecological diversification (Pont and Meier 2002, Holt 2009, Levine and HilleRisLambers 2009). A relationship with sexual size dimorphism could be established in that large *Sepsis* species with (evolutionarily derived) male-biased dimorphism tend to show fast-reproducing life histories and aggressive mating behavior (Table 1, 2, Fig. 1; Rohner et al. 2016, Rohner and Blanckenhorn 2018). Moreover, flies could further be clustered into two (three) crude reproductive groupings recognized by standard life history theory (Roff 1992, Stearns 1992, Fox and Czesak 2000; Fig. 2). Nevertheless, further studies will be necessary to elucidate the phylogenetic and ecological differentiation of this fly guild.

### Data availability statement

Data are available from the Dryad Digital Repository: <<http://dx.doi.org/10.5061/dryad.sbcc2fr3c>> (Blanckenhorn et al. 2020).

*Acknowledgements – Funding* – This work was supported over the years by the University (Museum) of Zurich and by several grants from the Swiss National Science Foundation, most recently grant no. 31003A\_143787.

*Author contributions* – WUB, MAS and PTR designed the project; all authors contributed to the data collection; PTR and WUB analyzed the data and led the writing of the manuscript.

### References

- Abouheif, E. and Fairbairn, D. J. 1997. A comparative analysis of allometry for sexual size dimorphism: assessing Rensch's rule. – *Am. Nat.* 149: 540–562.
- Abrams, P. A. 1987. Alternative models of character displacement and niche shift. I. Adaptive shifts in resource use when there is competition for nutritionally nonsubstitutable resources. – *Evolution* 41: 651–661.
- Andersen, N. M. 1997. A phylogenetic analysis of the evolution of sexual dimorphism and mating systems in water striders (Hemiptera: Gerridae). – *Biol. J. Linn. Soc.* 61: 345–368.
- Andersson, M. 1994. Sexual selection. – Princeton Univ. Press.
- Ang, Y. C. et al. 2013. Sepsidnet: a plea for digital reference collections and other science-based digitization initiatives in taxonomy. – *Syst. Entomol.* 38: 637–644.
- Arnqvist, G. and Nilsson, T. 2000. The evolution of polyandry: multiple mating and female fitness in insects. – *Anim. Behav.* 60: 145–164.
- Bauerfeind, S. S. et al. 2018. Replicated latitudinal clines in reproductive traits of European and North American yellow dung flies. – *Oikos* 127: 1619–1632.
- Baur, J. et al. 2020a. Exaggerated male forelegs are not more differentiated than wing morphology in two widespread sister species of black scavenger flies. – *J. Zool. Syst. Evol. Res.* 58: 159–173.
- Baur, J. et al. 2020b. Intraspecific mating system evolution and its effect on complex male secondary sexual traits: does male–male

- competition increase selection on size or shape? – *J. Evol. Biol.* 33: 297–308.
- Begon, M. et al. 1986. *Ecology. Individuals, populations and communities.* – Blackwell Scientific.
- Berger, D. et al. 2013. Quantitative genetic divergence and standing genetic (co)variance in thermal reaction norms along latitude. – *Evolution* 67: 2385–2399.
- Bateman, A. J. 1948. Intra-sexual selection in *Drosophila*. – *Heredity* 2: 349–368.
- Blanckenhorn, W. U. 1997. Altitudinal life history variation in the dung flies *Scatophaga stercoraria* and *Sepsis cynipsea*. – *Oecologia* 109: 342–352
- Blanckenhorn, W. U. 2000. The evolution of body size: what keeps organisms small? – *Q. Rev. Biol.* 75: 385–407.
- Blanckenhorn, W. U. 2005. Behavioral causes and consequences of sexual size dimorphism. – *Ethology* 111: 977–1016.
- Blanckenhorn, W. U. and Demont, M. 2004. Bergmann and converse Bergmann latitudinal clines in arthropods: two ends of a continuum? – *Integr. Comp. Biol.* 44: 413–424.
- Blanckenhorn, W. U. and Heyland, A. 2004. The quantitative genetics of two life history tradeoffs in the yellow dung fly in abundant and limited food environments. – *Evol. Ecol.* 18: 385–402.
- Blanckenhorn, W. U. et al. 1995. Time and energy constraints and the evolution of sexual size dimorphism – to eat or to mate? – *Evol. Ecol.* 9: 369–381.
- Blanckenhorn, W. U. et al. 2000. Female choice, female reluctance to mate and sexual selection on body size in the dung fly *Sepsis cynipsea*. – *Ethology* 106: 577–593.
- Blanckenhorn, W. U. et al. 2002. The costs of copulating in the dung fly *Sepsis cynipsea*. – *Behav. Ecol.* 13: 353–358.
- Blanckenhorn, W. U. et al. 2007a. Proximate causes of Rensch's rule: does sexual size dimorphism in arthropods result from sex differences in development time? – *Am. Nat.* 169: 245–257.
- Blanckenhorn, W. U. et al. 2007b. Rensch's rule in insects: patterns among and within species. Sex, size and gender roles: evolutionary studies of sexual size dimorphism. Fairbairn, D. J. et al. (eds). – Oxford Univ. Press, pp. 60–70.
- Blanckenhorn, W. U. et al. 2013. Standardized laboratory tests with 21 species of temperate and tropical sepsid flies confirm their suitability as bioassays of pharmaceutical residues in cattle dung. – *Ecotox. Environ. Safety* 89: 21–28.
- Blanckenhorn, W. U. et al. 2020. Data from: Sexual size dimorphism is associated with reproductive life history trait differentiation in coexisting sepsid flies. – Dryad Digital Repository, <<http://dx.doi.org/10.5061/dryad.sbcc2fr3c>>.
- Bowsher, J. H. et al. 2013. Deciphering the evolutionary history and developmental mechanisms of a complex sexual ornament: the abdominal appendages of Sepsidae (Diptera). – *Evolution* 67: 1069–1080.
- Bowsher, J. H. and Nijhout, H. F. 2009. Partial co-option of the appendage patterning pathway in the development of abdominal appendages in the sepsid fly *Themira biloba*. – *Dev. Genes Evol.* 219: 577–587.
- Busso, J. P. and Blanckenhorn, W. U. 2018a. Climatic factors shape plastic tradeoffs in the polyphenic black scavenger fly *Sepsis thoracica* (Diptera: Sepsidae). – *J. Biogeogr.* 45: 593–603.
- Busso, J. P. and Blanckenhorn, W. U. 2018b. Disruptive sexual selection on male body size in the polyphenic black scavenger fly *Sepsis thoracica* (Diptera: Sepsidae). – *Behav. Ecol.* 29: 769–777.
- Busso, J. P. and Blanckenhorn, W. U. 2018c. Viability selection by invertebrate predators in the polyphenic black scavenger fly *Sepsis thoracica* (Diptera: Sepsidae). – *Behav. Ecol.* 29: 992–1000.
- Cheverud, J. M. et al. 1985. The quantitative assessment of phylogenetic constraints in comparative analyses: sexual dimorphism in body weight among primates. – *Evolution* 39: 1335–1351.
- Ding, A. and Blanckenhorn, W. U. 2002. The effect of sexual size dimorphism on mating behavior in two dung flies with contrasting dimorphism. – *Evol. Ecol. Res.* 4: 1–15.
- Dmitriew, C. and Blanckenhorn, W. U. 2012. The role of sexual selection and conflict in mediating among-population variation in mating strategies and sexually dimorphic traits in the black scavenger fly *Sepsis punctum*. – *PLoS One* 7: e49511.
- Dmitriew, C. and Blanckenhorn, W. U. 2014. Condition dependence and the maintenance of genetic variance in a sexually dimorphic black scavenger fly. – *J. Evol. Biol.* 27: 2408–2419.
- Eberhard, W. G. 1996. *Female control: sexual selection by cryptic female choice.* – Princeton Univ. Press.
- Eberhard, W. G. 2001a. Species-specific genitalic copulatory courtship in sepsid flies (Diptera, Sepsidae, *Microsepsis*) and theories of genitalic evolution. – *Evolution* 55: 93–102.
- Eberhard, W. G. 2001b. Multiple origins of a major novelty: moveable abdominal lobes in male sepsid flies (Diptera: [S]epsidae), and the question of developmental constraints. – *Evol. Dev.* 3: 206–222.
- Eberhard, W. G. 2003. Sexual behavior and morphology of *Themira minor* (Diptera: Sepsidae) males and the evolution of male sternal lobes and genitalic surstyli. – *Can. Entomol.* 135: 569–581.
- Emlen, S. T. and Oring, L. W. 1977. Ecology, sexual selection and the evolution of mating systems. – *Science* 197: 215–223.
- Fairbairn, D. J. 1990. Factors influencing sexual size dimorphism in temperate waterstriders. – *Am. Nat.* 136: 61–86.
- Fairbairn, D. J. 2005. Allometry for sexual size dimorphism: testing allometry hypotheses for Rensch's rule in the water strider *Aquarius remigis*. – *Am. Nat.* 166: 69–84.
- Fairbairn, D. J. et al. 2007. Sex, size and gender roles: evolutionary studies of sexual size dimorphism. – Oxford Univ. Press.
- Fox, C. W. and Czesak, M. E. 2000. Evolutionary ecology of progeny size in arthropods. – *Annu. Rev. Entomol.* 45: 341–369.
- Geladi, P. and Kowalski, B. R. 1986. Partial least-squares regression – a tutorial. – *Anal. Chim. Acta* 185: 1–17.
- Giesen, A. et al. 2017. Behavioral mechanisms of reproductive isolation between two hybridizing dung fly species (*Sepsis cynipsea* and *S. neo-cynipsea*: Diptera: Sepsidae). – *Anim. Behav.* 132: 155–166.
- Giesen, A. et al. 2019. Patterns of postzygotic isolation between two closely related sepsid fly species (*Sepsis cynipsea* and *S. neo-cynipsea*; Diptera: Sepsidae). – *J. Zool. Syst. Evol. Res.* 57: 80–90.
- Gustafsson, L. et al. 1995. Tradeoffs between life-history traits and a secondary sexual character in male collared flycatchers. – *Nature* 375: 311–313.
- Hammer, O. 1941. Biological and ecological studies on flies associated with pasturing cattle and their excrement. – *Vid. Med. Dansk. Naturhistorisk Forening* 105: 140–393.
- Herath, B. et al. 2015. Selection on bristle length has the ability to drive the evolution of male abdominal appendages in the sepsid fly *Themira biloba*. – *J. Evol. Biol.* 28: 2308–2317.
- Holt, R. D. 2009. Bringing the Hutchinsonian niche into the 21st century: ecological and evolutionary perspectives. – *Proc. Natl Acad. Sci. USA* 106: 19659–19665.
- Honek, A. 1993. Intraspecific variation in body size and fecundity in insects – a general relationship. – *Oikos* 66: 483–492.

- Jochmann, R. and Blanckenhorn, W. U. 2016. Non-target effects of ivermectin on trophic groups of the cow dung insect community replicated across an agricultural landscape. – *Basic Appl. Ecol.* 17: 291–299.
- Kingsolver, J. G. and Pfennig, D. W. 2004. Individual-level selection as a cause of Cope's rule of phyletic size increase. – *Evolution* 58: 1608–1612.
- Kraushaar, U. and Blanckenhorn, W. U. 2002. Population variation in sexual selection and its effect on size allometry in two dung fly species with contrasting sexual size dimorphism. – *Evolution* 56: 307–321.
- Lande, R. 1980. Sexual dimorphism, sexual selection and adaptation in polygenic characters. – *Evolution* 34: 292–305.
- Lande, R. 1982. A quantitative genetic theory of life-history evolution. – *Ecology* 63: 607–615.
- Laux, A. et al. 2019. The role of larval substrate specialization and female oviposition in mediating species diversity of closely-related sepsid flies. – *Eur. J. Entomol.* 116: 75–84.
- Lei, Z. et al. 2013. Does better taxon sampling help? A new phylogenetic hypothesis for Sepsidae (Diptera: Cyclorhapha) based on 50 new taxa and the same old mitochondrial and nuclear markers. – *Mol. Phylog. Evol.* 69: 153–164.
- Levine, J. M. and Hillerislambers, J. 2009. The importance of niches for the maintenance of species diversity. – *Nature* 461: 254.
- Lovich, J. E. and Gibbons, J. W. 1992. A review of techniques for quantifying sexual size dimorphism. – *Growth Dev. Aging* 56: 269–281.
- Martin, O. Y. and Hosken, D. J. 2004. Copulation reduces male but not female longevity in *Saltella sphondylii* (Diptera: Sepsidae). – *J. Evol. Biol.* 17: 357–362.
- Mühlhäuser, C. and Blanckenhorn, W. U. 2002. The costs of avoiding matings in the dung fly *Sepsis cynipsea*. – *Behav. Ecol.* 13: 359–365.
- Nylin, S. and Gotthard, K. 1998. Plasticity in life history traits. – *Annu. Rev. Entomol.* 43: 63–83.
- Persson, J. and Hessen, D. O. 2009. Genome size as a determinant of growth and life-history traits in crustaceans. – *Biol. J. Linn. Soc.* 98: 393–399.
- Pont, A. C. and Meier, R. 2002. The Sepsidae (Diptera) of Europe. – *Fauna Entomol. Scand.* 37: 1221.
- Puniamoorthy, N. 2014. Behavioral barriers to reproduction may evolve faster than sexual morphology among populations of a dung fly (Sepsidae). – *Anim. Behav.* 98: 139–148.
- Puniamoorthy, N. et al. 2009. From kissing to belly stridulation: comparative analysis reveals surprising diversity, rapid evolution and much homoplasy in the mating behavior of 27 species of sepsid flies (Diptera: Sepsidae). – *J. Evol. Biol.* 22: 2146–2156.
- Puniamoorthy, N. et al. 2012a. Sexual selection accounts for geographic reversal of sexual size dimorphism in the dung fly *Sepsis punctum* (Diptera: Sepsidae). – *Evolution* 66: 2117–2126.
- Puniamoorthy, N. et al. 2012b. Differential investment in pre- versus post-copulatory sexual selection reinforces a cross-continental reversal of sexual size dimorphism in *Sepsis punctum* (Diptera: Sepsidae). – *J. Evol. Biol.* 25: 2253–2263.
- Rensch, B. 1950. Die Abhängigkeit der relativen Sexualdifferenz von der Körpergröße. – *Bonner Zool. Beiträge* 1: 58–69.
- Roff, D. A. 1980. Optimising development time in a seasonal environment: the 'ups and downs' of clinal variation. – *Oecologia* 45: 202–208.
- Roff, D. A. 1992. The evolution of life histories. – Chapman and Hall.
- Roff, D. A. 2000. Tradeoffs between growth and reproduction: an analysis of the quantitative genetic evidence. – *J. Evol. Biol.* 13: 434–445.
- Revell, L. J. 2012. phytools: an R package for phylogenetic comparative biology (and other things). – *Methods Ecol. Evol.* 3: 217–223.
- Rohner, P. T. and Blanckenhorn, W. U. 2018. A comparative study of the role of sex-specific condition dependence in the evolution of sexually dimorphic traits. – *Am. Nat.* 192: E202–E215.
- Rohner, P. T. et al. 2014. Genetic data confirm the species status of *Sepsis nigripes*, Meigen 1826 (Diptera: Sepsidae) and adds one species to the Alpine fauna while questioning the synonymy of *Sepsis helvetica*, Munari 1985. – *Invert. Syst.* 28: 555–563.
- Rohner, P. T. et al. 2015. Distribution, diversity gradients and Rapoport's elevational rule in the black scavenger flies of the Swiss Alps (Diptera: Sepsidae). – *Insect Conserv. Divers.* 8: 367–376.
- Rohner, P. T. et al. 2016. Sexual selection on male size drives the evolution of male-biased sexual size dimorphism via the prolongation of male development. – *Evolution* 70: 1189–1199.
- Rohner, P. T. et al. 2018. The evolution of male-biased sexual size dimorphism is associated with increased body size plasticity in males. – *Funct. Ecol.* 32: 581–591.
- Rohner, P. T. et al. 2019. Temporal niche partitioning of Swiss black scavenger flies in relation to season and substrate age (Diptera: Sepsidae). – *Alpine Entomol.* 3: 1–10.
- Rowe, L. and Houle, D. 1996. The lek paradox and the capture of genetic variance by condition dependent traits. – *Proc. R. Soc. B* 263: 1415–1421.
- Roy, J. et al. 2018. Life history traits along a latitudinal cline in European *Sepsis fulgens*. – *Oecologia* 187: 851–862.
- Sanchez, G. 2012. plsdepot: partial least squares (PLS) data analysis methods. – R package, ver. 0.1.17. <<https://CRAN.R-project.org/package=plsdepot>>
- Schluter, D. et al. 1991. Conflicting selection pressures and life history tradeoffs. – *Proc. R. Soc. B* 246: 11–17.
- Schulz, K. S. 1999. The evolution of mating systems in black scavenger flies (Diptera: Sepsidae). – PhD thesis, Dept of Entomology, Univ. of Arizona.
- Shuker, D. M. and Simmons, L. W. 2014. The evolution of insect mating systems. – Oxford Univ. Press.
- Smith, C. C. and Fretwell, S. D. 1974. The optimal balance between size and number of offspring. – *Am. Nat.* 108: 499–506.
- Stearns, S. C. 1992. The evolution of life histories. – Oxford Univ. Press.
- Su, K. F. Y. et al. 2008. Morphology versus molecules: the phylogenetic relationships of Sepsidae (Diptera: Cyclorhapha) based on morphology and DNA sequence data from ten genes. – *Cladistics* 24: 902–916.
- Tan, D. S. H. et al. 2011. New information on the evolution of mating behavior in Sepsidae (Diptera) and the cost of male copulations in *Saltella sphondylii*. – *Org. Divers. Evol.* 11: 253–261.
- Tauber, M. J. et al. 1986. Seasonal adaptations of insects. – Oxford Univ. Press.
- Teuschl, Y. and Blanckenhorn, W. U. 2007. The reluctant fly: what makes *Sepsis cynipsea* females willing to copulate? – *Anim. Behav.* 73: 85–97.
- Wilson, E. O. 1975. Sociobiology – the new synthesis. – Harvard Univ. Press.

Supplementary material (available online as Appendix oik-07036 at <[www.oikosjournal.org/appendix/oik-07036](http://www.oikosjournal.org/appendix/oik-07036)>). Appendix 1.