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Abstract: 1. Sexual size dimorphism (SSD) can vary drastically across environments, demonstrating pronounced sex-specific plasticity. In insects, females are usually the larger and more plastic sex. However, the shortage of taxa with male-biased SSD hampers the assessment of whether the greater plasticity in females is driven by selection on size or represents an effect of the female reproductive role. Here, we specifically address the role of sex-specific plasticity of body size in the evolution of SSD reversals to disentangle sex and size effects. 2. We first investigate sex-specific body size plasticity in Sepsis punctum and Sepsis neocynipsea as two independent cases of intraspecific SSD reversals in sepsid flies. In both species, directional variation in SSD between populations is driven by stronger sexual selection on male size. Using controlled laboratory breeding, we find evidence for sex-specific plasticity and increased condition dependence of male size in populations with male-biased SSD, but not of female size in populations with female-biased SSD. 3. To extend the comparative scope, we next estimate sex-specific body size plasticity in eight additional fly species that differ in the direction of SSD under laboratory conditions. In all species with male-biased SSD we find males to be the more plastic sex, while this was only rarely the case in species with female-biased SSD, thus suggesting a more general trend in Diptera. 4. To examine the generality of this pattern in holometabolous insects, we combine our data with data from the literature in a meta-analysis. Again, male body size tends to be more plastic than female size when males are the larger sex, though female size is now also generally more plastic when females are larger. 5. Our findings indicate that primarily selection on size, rather than the reproductive role per se, drives the evolution of sex-specific body size plasticity. However, sepsid flies, and possibly Diptera in general, show a clear sexual asymmetry with greater male than female plasticity related to SSD, likely driven by strong sexual selection on males. Although further research controlling for phylogenetic and ecological confounding effects is needed, our findings are congruent with theory in suggesting that condition dependence plays a pivotal role in the evolution of sexual size dimorphism.

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The evolution of male-biased sexual size dimorphism is associated with increased body size plasticity in males

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Running head: the larger sex shows increased plasticity

Key words: adaptive canalization, condition dependence, Diptera, genic capture, Holometabola, sexual size dimorphism, sex-specific phenotypic plasticity

Abstract

1. Sexual size dimorphism (SSD) can vary drastically across environments, demonstrating pronounced sex-specific plasticity. In insects, females are usually the larger and more plastic sex. However, the shortage of taxa with male-biased SSD hampers the assessment of whether the greater plasticity in females is driven by selection on size or represents an effect of the female reproductive role. Here we specifically address the role of sex-specific plasticity of body size in the evolution of SSD reversals to disentangle sex and size effects.
2. We first investigate sex-specific body size plasticity in *Sepsis punctum* and *S. neocynipsea* as two independent cases of intraspecific SSD reversals in sepsid flies. In both species, directional variation in SSD between populations is driven by stronger sexual selection on male size. Using controlled laboratory breeding, we find evidence for sex-specific plasticity and increased condition dependence of male size in populations with male-biased SSD, but not of female size in populations with female-biased SSD, indicating no adaptive canalization of female size.
3. To extend the comparative scope, we next estimate sex-specific body size plasticity in eight additional fly species that differ in the direction of SSD under laboratory conditions. In all species with male-biased SSD we find males to be the more plastic sex, while this was only rarely the case in species with female-biased SSD, thus suggesting a more general trend in Diptera.
4. To examine the generality of this pattern in holometabolous insects, we combine our data with data from the literature in a meta-analysis. Again, male body size tends to be more plastic than female size when males are the larger sex, though female size is now also generally more plastic when females are larger.

5. Our findings indicate that primarily selection on size, rather than the reproductive role *per se*, drives the evolution of sex-specific body size plasticity. However, sepsid flies, and possibly Diptera in general, show a clear sexual asymmetry with greater male than female plasticity related to SSD, likely driven by strong sexual selection on males. Although further research controlling for phylogenetic and ecological confounding effects is needed, our findings are congruent with theory in suggesting that condition dependence plays a pivotal role in the evolution of sexual size dimorphism.

Introduction

The strength and type of selection on body size often differ between males and females, owing to their distinct reproductive roles favouring divergent fitness optima (Shine 1989; Honek 1993; Blanckenhorn 2000; Blanckenhorn 2005; Fairbairn, Blanckenhorn & Székely 2007; Fairbairn 2013). Consequently, sexual size dimorphism (SSD) is widespread across animals and varies greatly among species and sometimes populations (Fairbairn, Blanckenhorn & Székely 2007; Fairbairn 2013).

In insects, females are generally larger than males due to a strong size–fecundity relationship (Honek 1993). However, despite being rare, male-biased SSD has evolved numerous times independently across the insect phylogeny, often in association with intensified sexual selection on male size and corresponding shifts in the mating system (e.g. Rohner, Blanckenhorn & Puniamoorthy 2016). SSD can differ considerably in its extent, but rarely in its direction (i.e. males or females being the larger sex) among insect species and populations (Stillwell, Morse & Fox 2007; Rohner, Blanckenhorn & Puniamoorthy 2016), and often varies strongly across environments due to pronounced sex-specific plasticity in growth and development (Fischer & Fiedler 2001; Fairbairn 2005; Stillwell & Fox 2007). In species with female-biased SSD, females are generally more sensitive to environmental

variation (in ~70% of all species studied) and tend to grow disproportionately larger than males along a gradient from poor to good environmental quality, leading to an increase in SSD with body size (Teder & Tammaru 2005; Stillwell *et al.* 2010). The underlying evolutionary causes of this pattern are poorly understood. Whether the greater plasticity in females is the result of their reproductive role (being female) or of selection on body size (being the larger sex) remains unclear.

For instance, the sexes often differ in their nutritional requirements such that growth can be more strongly affected by nutrient limitation or quality in females than in males (Stockhoff 1993; Moreau *et al.* 2003; Lee 2010; Chapman, Simpson & Douglas 2013), which could cause body size to respond more strongly to environmental variation in females (Teder & Tammaru 2005). Alternatively, the sex that has its fitness optimum at larger body size may show a stronger response to environmental variation because of greater potential fitness gains with increasing size. In insects, disentangling these alternative mechanisms and assessing whether plasticity is indirectly driven by the reproductive roles or selection on size is inherently challenging because females are the larger sex in the overwhelming majority of species. Studying sex-specific phenotypic plasticity in closely related taxa differing in the direction of SSD can therefore prove very useful to differentiate whether sex or size effects drive variation in sex-specific size plasticity. If female size responds more strongly to environmental quality even when females are the smaller sex, the reproductive role is likely to account for sex-specific variation in plasticity independently of size. By contrast, if the level of sex-specific plasticity consistently co-varies with the magnitude and direction of SSD, variation in size plasticity is more likely to result from selection on size.

Sex-specific phenotypic plasticity is ultimately explained by two major alternative hypotheses. First, the adaptive canalization hypothesis (Fairbairn 2005) predicts decreased

plasticity in traits most strongly related to fitness in either sex due to increased developmental canalization by stabilizing selection (or directional selection counteracted by a constraint (Stearns & Kawecki 1994; Stillwell *et al.* 2010)). Alternatively, the condition dependence hypothesis posits that plasticity increases by strong directional selection for resource-use efficiency and so captures interactive genetic and environmental effects (Rowe & Houle 1996; Bonduriansky 2007a; Amend *et al.* 2013; Oudin, Bonduriansky & Rundle 2015). Although these two hypotheses predict opposing patterns of plasticity, differentiating between them is not straightforward. For example, female body size may be more plastic than male size due to directional selection on female size, but strong stabilizing selection on male size (or any other trait associated with body size such as growth rate or development time: Wiklund and Fagerstrom (1977)) could lead to an identical pattern. A rigorous test of these hypotheses thus requires knowledge of the selective forces driving the system, data on multiple traits, and/or comparative data that may reveal which sex evolved a heightened degree of body size plasticity.

Here, we address the role of sex-specific body size plasticity in the evolution of male-biased SSD in insects by integrating approaches at three different taxonomic levels: (i) within two species of black scavenger flies (Diptera: Sepsidae) that convergently evolved intraspecific reversals of SSD; (ii) among fly species dispersed across the higher Diptera clade; and (iii) in a meta-analysis across Holometabola. Sepsid flies are particularly well suited to study such patterns due to considerable SSD variation in both magnitude and direction even among closely related species and populations. Male-biased SSD evolved independently several times across the family, and the direction of SSD further varies within species. *Sepsis neocynipsea* and *S. punctum* show directional variation in SSD between North American and European populations. In *S. neocynipsea*, males are larger than females in

North America, while females are the larger sex in Europe (Rohner, Blanckenhorn & Puniamoorthy 2016). In *S. punctum*, this pattern is reversed across the same continents (Dmitriew & Blanckenhorn 2012; Puniamoorthy, Schafer & Blanckenhorn 2012; Dmitriew & Blanckenhorn 2014). In both species, male-biased SSD is derived and driven by enhanced sexual selection on male size, whereas the intensity of fecundity selection on female size does not differ between male- and female-biased populations (Puniamoorthy, Schafer & Blanckenhorn 2012; Rohner, Blanckenhorn & Puniamoorthy 2016).

Taking advantage of these two independent microevolutionary systems with known underlying selective drivers, we conducted controlled laboratory experiments to identify which sex shows greater body-size plasticity, and to test competing hypotheses based on our understanding of the underlying selective forces. If the reproductive role of females is the main driver of increased plasticity, we expected females to show greater plasticity in general, even in species with male-biased SSD. In contrast, if the larger sex is also the more plastic sex irrespective of whether males or females are larger, selection on size is likely to be a more important force. Decreased plasticity of the larger sex, in contrast, would suggest a role of adaptive canalization driven by stabilizing selection and/or directional selection, with body size otherwise being constrained at its upper limit (Fairbairn 2005). Finally, lack of any sex-specific plasticity (i.e. constant SSD across environments) would suggest that either its evolution is constrained, or that selection pressures counterbalance and thus canalize variation in SSD across environments.

Previous research has demonstrated that different environmental variables can have disparate effects on sex-specific plasticity. Whereas sex-specific plasticity is common when food quality or quantity is manipulated (Teder & Tammaru 2005; Stillwell *et al.* 2010), SSD does not seem to vary consistently with temperature across arthropods (Hirst, Horne &

Atkinson 2015). However, in Diptera, females tend to decrease more strongly in size than males with increasing temperature (leading to a reduction in female-biased SSD with increasing temperature: Hirst, Horne & Atkinson 2015). We therefore here not only manipulated food quantity, but also rearing temperature to test whether results can be generalized across multiple environmental variables.

Our second goal was to understand the evolution of sex-specific body size plasticity more broadly. To this end, we conducted a comparative study by gathering detailed data for three additional dipterans with male-biased SSD (*Sepsis lateralis, Drosophila prolongata, Scathophaga stercoraria*) and five closely related fly species with female-biased SSD (*Sepsis cynipsea, S. fulgens, Drosophila melanogaster, D. rhopaloa, Musca domestica*). We thus tested whether the association between sex-specific body size plasticity and SSD in *S. neocynipsea* and *S. punctum* extends to these additional flies in a more general pattern across the Diptera. Finally, we analyzed published data on species with contrasting SSD in a meta-analysis to test for an even broader pattern among holometabolous insects. Integrating our results from the intraspecific case studies with the comparative Dipteran and holometabolous insect data, we discuss the general role of condition dependence, sex and body size in the evolution of sexual size dimorphism and reversals thereof.

Materials and Methods

Intraspecific variation in sex-specific plasticity of *Sepsis neocynipsea* and *S. punctum*

Outbred laboratory populations of *S. neocynipsea* and *S. punctum* were established using offspring of at least ten wild-caught, gravid females of European (both species: Zurich, Switzerland) and North American (*S. neocynipsea*: Montana, USA; *S. punctum*: Georgia, USA)

origin following standard laboratory protocols (Puniamoorthy, Schafer & Blanckenhorn 2012). These populations were cultured for several generations at densities of approximately 200–300 individuals.

For egg collection, each laboratory population was provided with a petri dish filled with cow dung for oviposition. After 3 to 4 hours, depending on the number of eggs laid, this dish was removed and incubated at 18°C for 24 hours. Thereafter, the freshly hatched first-instar larvae were retrieved from the dung by rinsing it with tap water and removing larvae using a fine brush. These larvae were then randomly assigned to different environmental treatments. To maximize environmental variation, we used a factorial design (three food treatments × two temperatures) for each population. In the unlimited food treatment, we provided 10 larvae with 6 g of standardized dung in a rectangular plastic dish. We mimicked natural food limitation by filling the lids of 1.5-ml Eppendorf tubes with dung and placing either a single larva (intermediate food limitation: 0.3 g per individual) or 10 larvae (strong food limitation: 0.03 g per individual) into it. To prevent desiccation, we placed all dishes into glass vials fitted with wet cotton. For the intermediate food treatment, we combined several Eppendorf tube lids in one glass vial, whereas in the two remaining treatments only one dish/Eppendorf lid was placed per vial. These glass vials were treated as independent experimental replicates (random effect). For each population and each food x temperature treatment we generated at least 3 such replicates. When no adults emerged, we repeated the experiment to increase our sample size. The experimental procedure in these temporal blocks was identical, but we statistically accounted for this random block effect nevertheless (see below). Vials were maintained in climate chambers at either 15°C or 28°C. Upon emergence, adults were sexed and frozen. To estimate body size, we removed the hind legs of each fly and mounted them on glass slides in Euparal, which were subsequently

photographed and measured to determine the mean length of both hind tibiae. Note that hind tibia length correlates strongly with other measures of body size, and the sexes do not differ in the allometric relationship of tibia in relation to thorax length (table S1). Hind tibia length thus well represents overall size. Furthermore, studies of primarily sexual selection in the close relative *S. cynipsea* (Blanckenhorn *et al.* 2004) show no specific morphological trait targeted by selection, but rather "overall body size". Hind tibia length is thus unlikely to be a direct target of selection, except indirectly via body size effects.

To assess sex-specific plasticity within populations, we used linear mixed models with (mean) hind tibia length as a function of sex, temperature and food quantity, including all interactions. All non-significant interactions were discarded, except for the sex × food quantity and the sex × temperature interactions, which were our focus. We used replicates (the identity of the glass vial used for incubation) and experimental block (date on which replicates were set up) as random effects. In addition, we also formally tested whether the sexes differ in their body size response to food quantity between continents. To this end, we tested for a food quantity × population interaction for males and females of each species separately. A significant interaction term would suggest population differentiation in the sex-specific slope of the reaction norm (body size as response to food), whereas a significant population main effect would suggest a shift in the intercept. Replicates, temporal blocks as well as temperatures were added as random effects in these models. All analyses were conducted in R (RCoreTeam 2016) using the package *lme4* (Bates *et al.* 2015).

Interspecific variation in sex-specific plasticity in Diptera

To examine sex-specific plasticity beyond our two focal species *S. punctum* and *S. neocynipsea*, we also lab-reared several closely related dipteran species that differ in the

direction of SSD. These additional species included three other *Sepsis* spp., two with female-biased SSD (*S. cynipsea* and *S. fulgens*) and one with male-biased SSD (*S. lateralis*). We further studied two other clades of Diptera showing both directions of SSD. In the *Drosophila* clade, *D. prolongata* exhibits male-biased SSD (Rohner et al. submitted), and *D. rhopaloa* and *D. melanogaster* female-biased SSD (data for the last species derived from the literature: Miller (1964). The second clade included two calyptrate Diptera, with *Musca domestica* exhibiting female-biased and *Scathophaga stercoraria* male-biased SSD (data on the latter from (Blanckenhorn *et al.* 2010). Given that these species dwell on various substrates and are adapted to different ecological niches, we cannot directly compare environmental treatments across species. We therefore did not use identical treatments across species but crossed different larval densities (1 to 60 individuals per container) with various amounts of food (0.3 to 100g) and temperatures (15–30°C; see supplementary table S2) separately for each species. Each species thus experienced different food and temperature treatments, mimicking a strong environmental gradient within species. Although the conditions differed between species, this did not hamper our main goal, the comparison of body size variation between the sexes within species, which were of course always reared under identical environmental conditions. *Musca domestica* and *Sepsis* spp. were reared on cow dung, *D. prolongata* and *D. rhopaloa* on standard *Drosophila* medium. As traditionally different proxies of size are used for different dipteran species, we used thorax length or log adult weight for all drosophilids and *Musca domestica* but hind tibia length for all sepsids and *Scathophaga stercoraria*. We are aware that using different body size surrogates may to some extent confound the interspecific comparison. However, our research mainly focussed on between-sex comparisons within species such that the trait used to estimate body size was secondary and

unlikely to greatly confound variation in SSD (because species with both male- and female-biased SSD were scored for tibia as well as thorax length).

Data analysis

To assess sex-specific plasticity, we calculated the sex-specific mean body size for each environmental replicate (temperature × larval density) per species and regressed log(male size) against log(female size) across these replicates in reduced major-axis regressions (RMA), as is standard (Fairbairn 2007). RMA slopes equal the ratio of the standard deviations of the y- and x-axes. Hence, slopes deviating from unity in these regressions indicate sex-specific plasticity, with slopes > 1 suggesting greater variation in male size (y-axis) across environmental conditions and slopes < 1 greater female variation (x-axis). Because such ratios produce asymmetric effect-size distributions, we used the natural logarithm of the RMA slopes as index for the strength and direction of sex-specific plasticity (producing a symmetrical effect-size distribution).

We further quantified the strength and direction of SSD, either using independent datasets of our own or data retrieved from the literature (flies were raised at overabundant food in the latter cases), by calculating the sexual dimorphism index (SDI) as proposed by Lovich and Gibbons (1992). To this end, we divided the size of the larger sex by that of the smaller and subtracted 1 from this ratio, and arbitrarily assigned positive signs when females are the larger sex and negative ones when males are larger. To control for phylogenetic non-independence we used phylogenetic generalized linear models (PGLS) as implemented in the R-package *caper* (Orme *et al.* 2013), using log(RMA) as the response and mean SSD as the explanatory variable. Since detailed phylogenetic information was lacking, we constructed a

cladogram derived from published literature (Wiegmann *et al.* 2011; Zhao *et al.* 2013; Setoguchi *et al.* 2014) and set all branch lengths to one. Note that we included our above data for North American and European *S. neocynipsea* and *S. punctum* populations in these analyses as well.

Meta-analysis across Holometabola

To test for a general pattern in holometabolous insects, we gathered data from the literature, focussing, where possible, on closely related species pairs or triplets that differ in their direction of SSD (even if they do not represent sister species). In general, we followed the procedure of Teder & Tammaru (2005) and accepted studies in which diet, food amount, larval crowding, or ant attendance (for some lycaenid butterflies) were manipulated. Further, we only considered studies presenting data for at least four environmental treatment levels for females and males separately. Adult weights at eclosion as well as pupal weights were accepted as body size estimates, although the former were preferred if both were available. The nature of environmental manipulations was very diverse, including different host species for parasitoids and herbivores, or various manipulations of food quantity or quality for other species (supplementary table S3). Such treatments thus cannot be compared directly across species. To assess sex-specific plasticity quantitatively, we therefore again regressed species-specific log(RMA) slopes across environmental treatments (as above) against SDI. As independent body size data were lacking for most species, the mean SDI across environments was calculated for each species and used to estimate species-specific SSD. To account for the precision of RMA estimates per species, which increases with the number of independent treatment levels, our linear regression was weighted by the number of

treatments within species. This approach further corrects, at least to some extent, for the different magnitudes of the environmental gradient used in different studies.

Results

Intraspecific variation in sex-specific plasticity of *Sepsis neocynipsea* and *S. punctum*.

Food quantity had a strong positive effect on hind tibia length in all populations studied (table 1). Crucially, the effect of food quantity differed between the sexes in North American (NA) *S. neocynipsea* as well as in European (EU) *S. punctum* (sex × food quantity interaction in table 1). In these populations, the sexes were essentially monomorphic at low food quantity but males increased more strongly in size with increasing food quantity, leading to considerable male-biased SSD under ample food conditions (figure 1). Both independent intraspecific SSD reversals thus feature increased plasticity in males, while this pattern was absent in the sister populations with female-biased SSD (sex × food quantity interaction not significant in table 1; figure 1). In addition, we found no differences in the response of female body size to food quantity between continents in either *S. neocynipsea* (continent × food quantity interaction: $F_{1,39.07} = 0.68$, $p = 0.413$; table S4) or *S. punctum* ($F_{1,89.80} = 2.14$ $p = 0.148$; table S4). In contrast, males differed in their plastic response to food quantity between continents (continent × food quantity interaction: *S. neocynipsea*: $F_{1,17.44} = 9.49$, $p = 0.006$; *S. punctum*: $F_{1,46.30} = 37.13$, $p < 0.001$; table S4), suggesting that the differences in sex-specific plasticity observed among populations are driven by variation in male body size plasticity alone.

The effect of food quantity on tibia length further differed between temperatures in both populations of *S. neocynipsea* (food quantity × temperature interaction; table 1), although

this did not affect SSD (because the sex × food quality × temperature three-way-interactions were non-significant throughout and hence removed; *S. neocynipsea* NA: $F_{2,83.75} = 0.45$, p = 0.640; EU: $F_{2,110.67} = 0.88$, p = 0.420; *S. punctum* NA: $F_{2,156.51} = 0.79$, p = 0.460; EU: $F_{2,14.03} = 0.09$, p = 0.910). The sexes differed in their reaction to temperature only in North American *S. punctum* (sex × temperature interaction in table 1). In this population, female tibia length increased more with decreasing temperature than in males, suggesting that female body size is more plastic in response to temperature.

Interspecific variation in sex-specific plasticity in Diptera

Log(RMA) slopes were always steeper in taxa with male-biased SSD than in those with female-biased SSD (i.e. males are more plastic than females when they are the larger sex; table 2). Log(RMA) slopes decreased significantly with the degree of female bias in SSD (PGLS: $F_{1,10} = 8.03$, P = 0.018, r = -0.67, $\lambda = 0.00$ [95% CI: 0.00–0.89], slope = -0.91; figure 2a), demonstrating that taxa with relatively larger males have steeper RMA slopes. Since the reversed pattern was also observed when females were larger than males (lower right quadrant in figure 2a), the larger sex generally seems to show heightened plasticity.

Meta-analysis across holometabolous insects

Combining our own data with data from the literature, we obtained information on sex-specific plasticity for a total of 43 species (Coleoptera: 8 species; Diptera: 16 species; Hymenoptera: 4 species; Lepidoptera: 15 species; see supplementary tables S3 & S5). All these data are restricted to Holometabola, as studies of other insect groups did not fit our requirements. The number of environmental treatments per species varied from 4 to 23 (median: 7, mean ± SD: 7.3 ± 3.7). When averaging SDI across environments, 21 species

showed female-biased SSD whereas males were the larger sex in 22 species (SDI ranging from -0.41 in *D. prolongata* to 0.32 in the cowpea seed beetle *Callosobruchus maculatus*).

Across all 43 species, log(RMA) showed a negative relationship with SDI (weighted least-squares regression: $t_{1,41} = -2.48$, $p = 0.017$, slope = -0.52; figure 2b). Since the intercept is close to zero (estimate = -0.003, $t_{1,41} = -0.12$ $p = 0.907$), males tend to be more plastic than females in species with male-biased SSD, and females tend to be more plastic than males in species with female-biased SSD. When restricting the analysis to previously available data from the literature, this relationship was qualitatively similar but no longer statistically significant (weighed least-squares regression: $t_{1,29} = -1.05$, $p = 0.302$, slope = -0.29).

Discussion

Our study demonstrates an association between sex-specific body size plasticity and the strength and direction of SSD across holometabolous insects (figure 2). In general, the larger sex tends to be more plastic in response to environmental factors, thus being more condition-dependent. This result is consistent with the hypothesis that sex-specific plasticity is driven mainly by selection on size rather than selection associated with the reproductive role (i.e. being male or female). However, at least in the sepsid flies, size plasticity is not entirely symmetrical with regard to sex. Stronger condition-dependence in males is likely mediated by strong sexual selection (Bonduriansky 2007a,b; figure 1, 2a, table 2). We discuss potential evolutionary and ecological drivers of these patterns and their implications for the study of body size and SSD evolution.

Intraspecific variation in sex-specific plasticity in *Sepsis*

In North American *S. neocynipsea* and European *S. punctum*, the derived male-biased SSD is associated with increased plasticity in males (significant sex-by-food quantity interaction, RMA slope > 1), while conspecific female-biased populations show no sex-specific plasticity (sex-by-food quantity interaction not significant in table 1; RMA slope not different from 1 in table 2), a clear sexual asymmetry in condition dependence. In populations with larger males, SSD was absent in stressful environments but increased gradually with environmental quality (figure 1). This pattern can be caused either by increased condition dependence in males or developmental canalization in females. As male body size plasticity in response to food availability differs between continents, while female plasticity does not (see table S4), population differentiation must be caused by variation in male plasticity only, suggesting evolution of increased condition dependence in males in populations with male-biased SSD. This fits well with previous studies demonstrating that the intensity of sexual selection on male size is stronger in the male-biased populations while there is no indication for differences in fecundity selection on female size among populations (Puniamoorthy, Schafer & Blanckenhorn 2012; Rohner, Blanckenhorn & Puniamoorthy 2016). Hence, there is no evidence for stronger (stabilizing) selection potentially canalizing female size in male-biased populations only. We thus attribute the greater male plasticity in male-biased populations to increased directional selection on male size, consequently arguing in favour of the condition dependence hypothesis and rejecting the canalization hypothesis (Fairbairn 2005; Bonduriansky 2007a).

In contrast to food availability, temperature did not strongly affect sex-specific plasticity. This finding is common in insects (Hirst, Horne & Atkinson 2015). We found a significant temperature effect on SSD only in North American *S. punctum*, in which female

size declined more strongly than male size from low to high temperature, whereas European populations responded more plastically to food (table 1). Hirst, Horne and Atkinson (2015) also found that in Diptera SSD unusually declines with temperature. So it is possible that the extent of sex-specific plasticity and condition dependence varies with the taxon and the environmental variable in question. According to the temperature–size rule (Atkinson 1994; Atkinson & Sibly 1997), low temperatures generally produce larger individuals through physiological responses. However, this size increase does not necessarily co-vary with environmental quality (Atkinson & Sibly 1997), and it is thus unclear whether this response means increased condition (dependence), which currently hampers a functional interpretation.

Originally proposed to explain variation in ornament size via genic capture, condition dependence is predicted to link genome-wide genetic quality of an individual to the expression of its secondary sexual traits in a given environment (Rowe & Houle 1996). This opportunistic mechanism should allow individuals to invest optimally in costly traits under resource limitation, flexibly trading fitness gains in sexual selection against viability (or any other) costs. Theory thus predicts a tight association between sexual dimorphism and condition dependence (Bonduriansky 2007a, 2007b). In *Sepsis* and insects more generally, large size entails viability costs due to prolonged development time and/or increased growth rate (Blanckenhorn 2000, 2009; Teder 2014). In both sepsids and drosophilids, for example, males take longer to develop than females, possibly related to male gonad or gamete development (Blanckenhorn *et al.* 2007; Rohner, Blanckenhorn & Puniamoorthy 2016), so the costs of growing large at limited food are expected to be greater for males. This sex difference should be amplified if habitats are ephemeral and/or when sexual selection favoring large male size is particularly strong. When facing serious food limitation, males

may therefore not be able to grow larger by prolonging growth due to severe mortality risks. Instead, they may shorten their larval development and emerge as adults earlier but at smaller size. These small males are not favored by sexual selection, but by reaching the adult stage they at least maintain some potential for direct fitness. Such a "bail-out" strategy in response to food limitation has been found in several dung-dwelling beetles (Shafiei, Moczek & Nijhout 2001) and flies (Blanckenhorn 1999), and likely explains the evolution of greater male body size plasticity in *S. neocynipsea* and *S. punctum* populations with male-biased SSD. Selection on adult male size could thus indirectly lead to the evolution of condition dependence in larval growth rate and developmental time.

In contrast, the absence of sex-specific plasticity in populations or species with female-biased SSD is not congruent with the condition dependence hypothesis (sex × food quantity interaction not significant). When females are the larger sex, as is most common in ectotherms, it is equally reasonable to assume that females would benefit to a greater extent than males from investing in body size at limited resources, but they do not show increased plasticity in our data set. In females, condition dependence should be driven primarily by fecundity selection. Fecundity selection tends to be generally weaker than sexual selection on males in sepsids and other species, specifically also in the two species studied here (Puniamoorthy, Schäfer & Blanckenhorn 2012; Rohner, Blanckenhorn & Puniamoorthy 2016), and further tends to asymptote at the largest body sizes in *S. cynipsea* (Blanckehorn 2007). Perhaps as a consequence, female-biased SSD is relatively weak in *S. neocynipsea* and *S. punctum*, such that the absence of sex-specific plasticity here may be explained by rather weak divergent selection on body size, in which case other selective pressures may obscure any patterns (see also below). Alternatively, this lack of sex-specific plasticity might be confined to tibia length and not necessarily apply to other estimates of body size, which we,

however, consider unlikely because tibia length well reflects body size in many fly species (Supplementary table S1).

Interspecific variation in sex-specific plasticity in Diptera

In extension of the above argument, the magnitude and direction of SSD co-varied with sex-specific size plasticity among several species of flies, again suggesting that females are not inherently more responsive to environmental quality (e.g. due to their particular nutritional needs), but that the larger sex is generally more plastic. As predicted by theory (Bonduriansky 2007a; Bonduriansky 2007b), this suggests a pivotal role of condition dependence in the evolution of male-biased SSD, and of SSD in general.

It is important to note, however, that while RMA slopes of species with male-biased SSD are always significantly steeper than unity and often strongly so, species with female-biased SSD frequently do not show significant sex-specific size plasticity (RMA slopes not significantly shallower than unity in table 2). As argued above, this may well be caused by the relatively weak SSD of female-biased species and the concomitant low levels of divergent selection that could be counteracted by other forms of selection. This finding corroborates the results of Teder and Tammaru (2005) showing that female size plasticity is more likely to exceed that of males as the magnitude of female-biased SSD increases. Nevertheless, irrespective of whether intra-specific RMA slopes significantly deviate from unity, the overall interspecific pattern for the Diptera covered here suggests a rather strong and sexually not entirely symmetric (inverse) relationship between condition dependence and SSD (figure 2a).

A general pattern in Holometabola?

Our quantitative meta-analysis adds further evidence to the notion that the larger sex tends to be more plastic (Teder & Tammaru 2005), thus suggesting a general trend at least across the Holometabola (figure 2). This result should be treated with some caution, however, as it was not quite statistically significant when excluding the dipterans, although the pattern itself persisted, again suggesting that Diptera are somehow different. Since our experimental rearing specifically aimed at covering extreme environments including severely limited and overabundant resource availabilities that should well cover the range experienced in nature (Blanckenhorn 2009), our RMA slopes should adequately estimate the pattern with low biological error. The differences between Diptera and the rest of Holometabola might therefore merely be quantitative (as opposed to qualitative), with the larger sex generally being more plastic. Alternatively, however, as demonstrated by Hirst, Horne and Atkinson (2015), patterns of sex-specific plasticity can differ among insect orders (see also Teder & Tammaru 2005), likely caused by shared phylogenetic relatedness, life histories or habitats.

So why might sepsids, and possibly other Diptera, differ from other insects in the impact of sex-specific condition dependence on SSD expression? We can only speculate at this point. All flies studied here depend on ephemeral resources for reproduction and development. Since condition dependence is expected to be more common when resources are strongly limited, which regularly applies to the short-lived and unpredictable resources of dung flies and perhaps also *Drosophila* (Blanckenhorn 1999, 2009), this might explain the discrepancies between Diptera and other Holometabola found here. Further data on species dwelling in other substrates are therefore required to test how common the phenomenon is, although it may not explain the sexual asymmetry in sex-specific plasticity. Alternatively,

Blanckenhorn *et al.* (2007) also uncovered an asymmetric sex-specific pattern in that females of several insect groups, including sepsids and drosophilids but also water bugs, apparently can afford to grow faster than males. This suggests lower viability costs for females counteracting the generally weaker fecundity selection on female size (relative to the typically stronger sexual selection on male size: Blanckenhorn 2007; Rohner, Blanckenhorn and Puniamoorthy 2016). At least in sepsids and drosophilids, male costs are presumably exerted by the time and energy consuming production of male gonads and gametes (Blanckenhorn *et al.* 2007; Lüpold *et al.* 2016), provoking stronger viability counter-selection in males. Increased investment into body and organ size thus appears generally costlier in males, but also more rewarding as sexual selection on male size tends to be stronger than fecundity selection on female size. The evolution of stronger condition dependence in males than females, allowing to flexibly counterbalance costs depending on environmental circumstances, thus seems to have some adaptive value (Rowe & Houle 1996; Bonduriansky 2007a,b).

In conclusion, our study of species varying in the direction of SSD revealed that male-biased SSD is associated with increased phenotypic plasticity in (higher) Diptera if not all Holometabola. We corroborate theoretical predictions by demonstrating that condition dependence, particularly in males through sexual selection, plays a pivotal role in generating both quantitative and qualitative variation in sexual size dimorphism, within as well as across species. It remains yet unclear, however, whether this pattern extends to other phylogenetic clades and ecological guilds, or even beyond insects (c.f. Blanckenhorn *et al.* 2007). Further data for other taxonomic groups covering a wide range of different life histories and ecological adaptations will be needed to evaluate the generality of this phenomenon. Given the complex developmental patterns generating sex-specific plasticity

(Stillwell & Davidowitz 2010), particular attention should be paid to the underlying physiological and genetic mechanisms for a more comprehensive understanding of the evolution of SSD, plasticity and condition dependence (Davidowitz 2016; Rohner, Blanckenhorn & Schäfer 2017).

Authors' Contributions

All authors conceived the ideas and designed methodology; PTR, SL and TT collected the data; PTR analysed the data; PTR and WUB led the writing of the manuscript. All authors contributed critically to the drafts and gave final approval for publication.

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Data Accessibility

Data available from the Dryad Digital Repository: http://dx.doi.org/10.5061/dryad.f6r60 (Rohner et al. 2017)

Supporting Information

Table S1: Correlations and allometric slopes between hind tibia length and thorax length for males and females

Table S2: Sex-specific body size estimates (means) for several dipterans raised under various environmental conditions

Table S3: Sex-specific body size estimates for numerous species derived from the literature (raw data, including references)

Table S4: Linear mixed models with hind tibia length as a function of food quantity and continent separately for each sex and species.

Table S5: RMA and SSD data derived from published raw data presented in table S3 (including taxonomic authority)

Tables & Figures

Table 1: Using hind tibia length as a proxy for overall body size, we found that food quantity had a pronounced effect on size in all species/populations. However, the sexes only differed in their response to food quantity in North American populations of *S. neocynipsea* and European populations of *S. punctum*, both of which show male-biased SSD. This suggests an association between SSD reversals (i.e. the evolution of male-biased SSD) and increased condition dependence in male size. Statistics are derived from general mixed models including replicate and experimental block as random effects.

	Sepsis neocynipsea						***Sepsis punctum***					
	NORTH AMERICA male-biased SSD			EUROPE female-biased SSD			NORTH AMERICA female-biased SSD			EUROPE male-biased SSD		
Effect	df	F	P	df	F	P	df	F	P	df	F	P
sex	1,103.07	4.68	**0.03**	1,115.84	8.97	**<.001**	1,268.53	4.26	**0.04**	1,154.17	17.8	**<.001**
food quantity	2,41.05	86.6	**<.001**	2,65.14	171	**<.001**	2,86.68	102	**<.001**	2,43.64	79.9	**<.001**
temperature	1,37.8	0	0.99	1,74.03	11.2	**<.001**	1,112.55	0.32	0.57	1,50.06	3.09	0.08
sex * food quantity	2,102.74	5.14	**0.01**	2,119.22	0.11	0.9	2,245.93	0.18	0.83	2,150.98	6.31	**<.001**
sex * temperature	1,114.64	1.48	0.23	1,127.1	1.58	0.21	1,248.09	5.32	**0.02**	1,156.88	0.28	0.6
temperature * food quantity	2,38.72	5.8	**0.01**	2,74.26	21.8	**<.001**						

Table 2: Reduced Major Axis (RMA) slopes of log male size against log female size with various numbers of replicates (n) reflecting a large environmental gradient. All slopes are significantly greater than unity in taxa with male-biased SSD, whereas for female-biased taxa slopes vary around 1. We used the sexual dimorphism index (SDI), a standardized ratio, as an estimate of the direction and strength of SSD (Lovich & Gibbons 1992), derived from independent datasets in which flies were raised with ad libitum food at benign temperature. Means (95% CI) are given for the two SSD groups.

Taxon	Authority	n	R^2	RMA Slope	P	SDI Estimate
Male-biased SSD						
Drosophila prolongata	Singh & Gupta, 1977	17	0.88	**1.37**	**0.003**	-0.3
*Scathophaga stercoraria**	Linnaeus, 1758	5	0.99	**1.2**	**0.045**	-0.25
Sepsis punctum EU	Fabricius, 1794	21	0.85	**1.24**	**0.021**	-0.07
Sepsis neocynipsea NA	Melander & Spuler, 1917	17	0.96	**1.14**	**0.015**	-0.04
Sepsis lateralis	Wiedemann, 1830	15	0.91	**1.23**	**0.026**	-0.03
				1.236 (0.074)		***-0.138 (0.111)***
Female-biased SSD						
Sepsis punctum NA	Fabricius, 1794	31	0.94	0.96	0.406	0.03
Sepsis fulgens	Meigen, 1826	30	0.71	1.01	0.925	0.04
Sepsis neocynipsea EU	Melander & Spuler, 1917	16	0.83	1.09	0.459	0.05
Musca domestica	Linnaeus, 1758	21	0.81	1.06	0.557	0.07
Sepsis cynipsea	Linnaeus, 1758	26	0.97	**0.87**	**0.001**	0.07
Drosophila rhopaloa	Bock & Wheeler, 1972	15	0.79	1.14	0.324	0.11
Drosophila melanogaster+	Meigen, 1830	11	0.94	**0.69**	**0.001**	0.13
				0.974 (0.114)		***0.071 (0.027)***

** Blanckenhorn et al. 2010*

+ Miller 1964

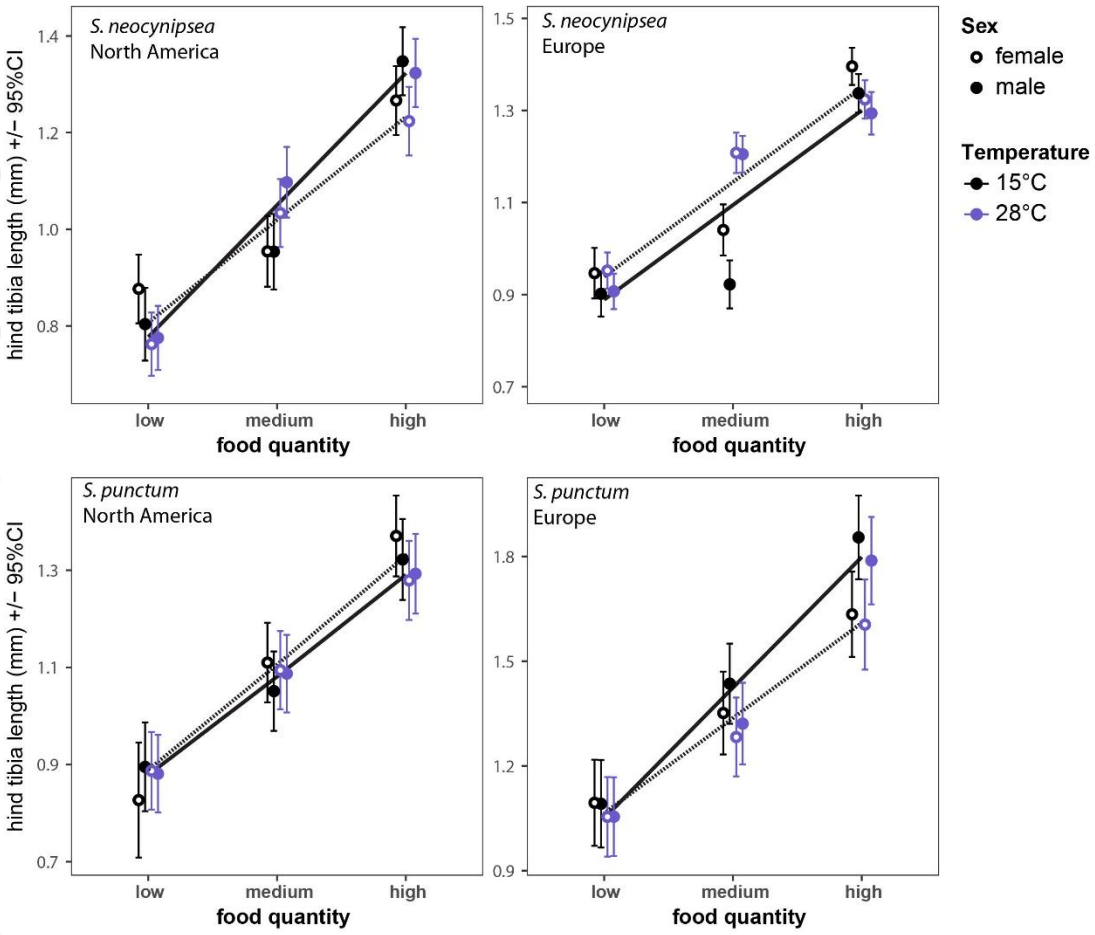

Figure 1: Mean hind tibia length of sepsid fly populations reared at three food (dung) quantities and two temperatures. Males increase more strongly in size with environmental quality in North American *S. neocynipsea* and European *S. punctum* (solid lines), the populations in which males are larger than females. In contrast, the sexes do not differ in their plastic response in populations with female-biased SSD (dotted lines). This pattern qualitatively holds in *S. punctum* and *S. neocynipsea*, although the latter shows a weaker sex-by-environment interaction. Mean ± SE estimates represent model parameters and their associated errors; random variation among blocks and replicates is thus accounted for. For simplicity, we only show average sizes across temperatures, but raw data are shown in supplementary figure S1.

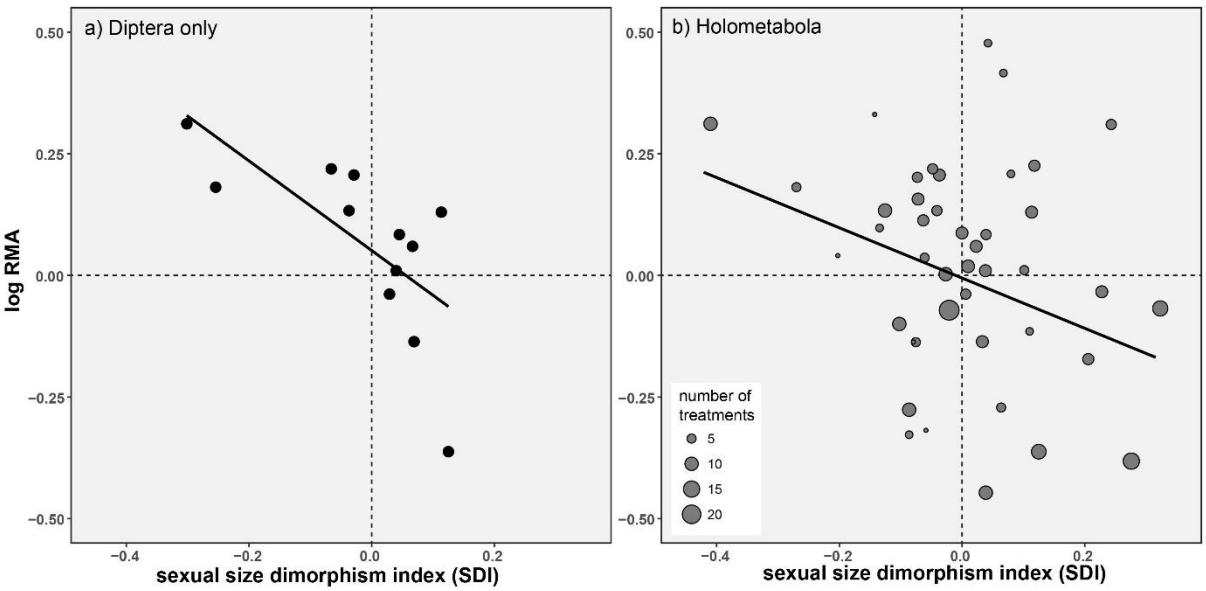

Figure 2: The relative plasticity of males (estimated by log(RMA) slopes) increases with the relative size of males (decreasing SDI) in Diptera (a) and Holometabola in general (b). This suggests that the evolution of male-biased SSD is associated with an increase in male plasticity. Log(RMA) slopes larger than zero indicate that males are more responsive to environmental variation (RMA slope = SD(males)/SD(females)), while females are more plastic if this slope is less than zero. To quantify SSD, we divided the size of the larger sex by that of the smaller and subtracted 1 from this ratio, and arbitrarily assigned positive signs when females are the larger sex and negative ones when males are larger (= SDI). While SDI of independent datasets were used in the analysis for Diptera only, we used the mean SDI across environments for the Holometabola in b). The trend line in b) gives the weighted linear regression using the number of treatments as weights (as indicated by the size of points).

References

Amend, A.S., Oliver, T.A., Amaral-Zettler, L.A., Boetius, A., Fuhrman, J.A., Horner-Devine, M.C., Huse, S.M., Welch, D.B.M., Martiny, A.C., Ramette, A., Zinger, L., Sogin, M.L. & Martiny, J.B.H. (2013) Macroecological patterns of marine bacteria on a global scale. *Journal of Biogeography,* **40,** 800-811.

Atkinson, D. (1994) Temperature and Organism Size - a Biological Law for Ectotherms. *Advances in Ecological Research, Vol 25,* **25,** 1-58.

Atkinson, D. & Sibly, R.M. (1997) Why are organisms usually bigger in colder environments? Making sense of a life history puzzle. *Trends in Ecology & Evolution,* **12,** 235-239.

Bates, D., Machler, M., Bolker, B.M. & Walker, S.C. (2015) Fitting Linear Mixed-Effects Models Using lme4. *Journal of Statistical Software,* **67,** 1-48.

Blanckenhorn, W.U. (1999) Different growth responses to temperature and resource limitation in three fly species with similar life histories. *Evolutionary Ecology,* **13,** 395-409.

Blanckenhorn, W.U. (2000) The Evolution of Body Size: What Keeps Organisms Small? *The Quarterly Review of Biology,* **75,** 385-407.

Blanckenhorn, W.U. (2005) Behavioral causes and consequences of sexual size dimorphism. *Ethology,* **111,** 977-1016.

Blanckenhorn, W.U. (2009) Causes and consequences of phenotypic plasticity in body size: the case of the yellow dung fly *Scathophaga stercoraria* (Diptera: Scathophagidae). *Phenotypic plasticity of insects: mechanisms and consequences* (eds Whitman & Anathakrishnan), pp. 369-422.

Blanckenhorn, W.U., Dixon, A.F., Fairbairn, D.J., Foellmer, M.W., Gibert, P., van der Linde, K., Meier, R., Nylin, S., Pitnick, S., Schoff, C., Signorelli, M., Teder, T. & Wiklund, C. (2007) Proximate causes of Rensch's rule: does sexual size dimorphism in arthropods result from sex differences in development time? *American Naturalist,* **169,** 245-257.

Blanckenhorn, W.U., Kraushaar, U.R., Teuschl, Y. & Reim, C. (2004) Sexual selection on morphological and physiological traits and fluctuating asymmetry in the black scavenger fly *Sepsis cynipsea*. *J Evol Biol,* **17,** 629-641.

Blanckenhorn, W.U., Pemberton, A.J., Bussiere, L.F., Roembke, J. & Floate, K.D. (2010) A review of the natural history and laboratory culture methods for the yellow dung fly, *Scathophaga stercoraria*. *J Insect Sci,* **10,** 11.

Bonduriansky, R. (2007a) The evolution of condition-dependent sexual dimorphism. *American Naturalist,* **169,** 9-19.

Bonduriansky, R. (2007b) The genetic architecture of sexual dimorphism: the potential roles of genomic imprinting and condition-dependence. *Sex, Size and Gender Roles* (eds D.J. Fairbairn, W.U. Blanckenhorn & T. Székely), pp. 176-184. Oxford University Press.

Chapman, R.F., Simpson, S.J. & Douglas, A.E. (2013) The Insects: Structure and Function. 1-929.

Davidowitz, G. (2016) Endocrine Proxies Can Simplify Endocrine Complexity to Enable Evolutionary Prediction. *Integr Comp Biol,* **56,** 198-206.

Dmitriew, C. & Blanckenhorn, W.U. (2012) The role of sexual selection and conflict in mediating among-population variation in mating strategies and sexually dimorphic traits in *Sepsis punctum*. *Plos One,* **7,** e49511.

Dmitriew, C. & 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.

Fairbairn, D.J. (2005) Allometry for Sexual Size Dimorphism: Testing Allometry Hypotheses for Rensch ' s Rule in the Water Strider *Aquarius remigis*. *American Naturalist,* **166,** 69-84.

Fairbairn, D.J. (2007) Introduction: the enigma of sexual size dimorphism. *Sex, Size and Gender Roles* (eds D.J. Fairbairnt, W.U. Blanckenhorn & T. Székely), pp. 1-12.

Fairbairn, D.J. (2013) Odd Couples: Extraordinary Differences between the Sexes in the Animal Kingdom.

Fairbairn, D.J., Blanckenhorn, W.U. & Székely, T. (2007) Sex, Size and Gender Roles: Evolutionary Studies of Sexual Size Dimorphism.

Fischer, K. & Fiedler, K. (2001) Dimorphic growth patterns and sex-specific reaction norms in the butterfly *Lycaena hippothoe sumadiensis*. *Journal of Evolutionary Biology,* **14,** 210-218.

Hirst, A.G., Horne, C.R. & Atkinson, D. (2015) Equal temperature-size responses of the sexes are widespread within arthropod species. *Proc Biol Sci,* **282,** 20152475.

Honek, A. (1993) Intraspecific Variation in Body Size and Fecundity in Insects - a General Relationship. *Oikos,* **66,** 483-492.

Lee, K.P. (2010) Sex-specific differences in nutrient regulation in a capital breeding caterpillar, *Spodoptera litura* (Fabricius). *Journal of Insect Physiology,* **56,** 1685-1695.

Lovich, J.E. & Gibbons, J.W. (1992) A review of techniques for quantifying sexual size dimorphism. *Growth Dev Aging,* **56,** 269-281.

Lupold, S., Manier, M.K., Puniamoorthy, N., Schoff, C., Starmer, W.T., Luepold, S.H., Belote, J.M. & Pitnick, S. (2016) How sexual selection can drive the evolution of costly sperm ornamentation. *Nature,* **533,** 535-538.

Miller, R.S. (1964) Larval Competition in *Drosophila-Melanogaster + D Simulans*. *Ecology,* **45,** 132.

Moreau, G., Quiring, D.T., Eveleigh, E.S. & Bauce, E. (2003) Advantages of a mixed diet: feeding on several foliar age classes increases the performance of a specialist insect herbivore. *Oecologia,* **135,** 391-399.

Orme, D., Freckleton, R., Thomas, G., Petzoldt, T., Fritz, S., Isaac, N. & Pearse, W. (2013) caper: Comparative Analyses of Phylogenetics and Evolution in R.

Oudin, M.J., Bonduriansky, R. & Rundle, H.D. (2015) Experimental evidence of condition-dependent sexual dimorphism in the weakly dimorphic antler fly *Protopiophila litigata* (Diptera: Piophilidae). *Biological Journal of the Linnean Society,* **116,** 211-220.

Puniamoorthy, N., Schafer, M.A. & Blanckenhorn, W.U. (2012) Sexual selection accounts for the geographic reversal of sexual size dimorphism in the dung fly, *Sepsis punctum* (Diptera: Sepsidae). *Evolution,* **66,** 2117-2126.

RCoreTeam (2016) R: A Language and Environment for Statistical Computing.

Rohner, P.T., Blanckenhorn, W.U. & Puniamoorthy, N. (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., Blanckenhorn, W.U. & Schäfer, M.A. (2017) Critical weight mediates sex-specific body size plasticity and sexual dimorphism in the yellow dung fly *Scathophaga stercoraria* (Diptera: Scatophagidae). *Evolution & Development,* **19,** 147-156.

Rohner P. T., Teder T., Esperk T., Lüpold S., Blanckenhorn W. U. (2017). Data from: The evolution of male-biased sexual size dimorphism is associated with increased body size plasticity in males. *Dryad Digital Repository*. doi:10.5061/dryad.f6r60

Rowe, L. & Houle, D. (1996) The lek paradox and the capture of genetic variance by condition dependent traits. *Proceedings of the Royal Society B-Biological Sciences,* **263,** 1415-1421.

Setoguchi, S., Takamori, H., Aotsuka, T., Sese, J., Ishikawa, Y. & Matsuo, T. (2014) Sexual dimorphism and courtship behavior in *Drosophila prolongata*. *Journal of Ethology,* **32,** 91-102.

Shafiei, M., Moczek, A.P. & Nijhout, H.F. (2001) Food availability controls the onset of metamorphosis in the dung beetle *Onthophagus taurus* (Coleoptera : Scarabaeidae). *Physiological Entomology,* **26,** 173-180.

Shine, R. (1989) Ecological causes for the evolution of sexual dimorphism: a review of the evidence. *Q Rev Biol,* **64,** 419-461.

Stearns, S.C. & Kawecki, T.J. (1994) Fitness Sensitivity and the Canalization of Life-History Traits. *Evolution,* **48,** 1438-1450.

Stillwell, R.C., Blanckenhorn, W.U., Teder, T., Davidowitz, G. & Fox, C.W. (2010) Sex differences in phenotypic plasticity affect variation in sexual size dimorphism in insects: from physiology to evolution. *Annu Rev Entomol,* **55,** 227-245.

Stillwell, R.C. & Davidowitz, G. (2010) A developmental perspective on the evolution of sexual size dimorphism of a moth. *Proc Biol Sci,* **277,** 2069-2074.

Stillwell, R.C. & Fox, C.W. (2007) Environmental effects on sexual size dimorphism of a seed-feeding beetle. *Oecologia,* **153,** 273-280.

Stillwell, R.C., Morse, G.E. & Fox, C.W. (2007) Geographic variation in body size and sexual size dimorphism of a seed-feeding beetle. *American Naturalist,* **170,** 358-369.

Stockhoff, B.A. (1993) Ontogenetic change in dietary selection for protein and lipid by gypsy moth larvae. *Journal of Insect Physiology,* **39,** 677-686.

Teder, T. (2014) Sexual size dimorphism requires a corresponding sex difference in development time: a meta-analysis in insects. *Functional Ecology,* **28,** 479-486.

Teder, T. & Tammaru, T. (2005) Sexual size dimorphism within species increases with body size in insects. *Oikos,* **108,** 321-334.

Wiegmann, B.M., Trautwein, M.D., Winkler, I.S., Barr, N.B., Kim, J.W., Lambkin, C., Bertone, M.A., Cassel, B.K., Bayless, K.M., Heimberg, A.M., Wheeler, B.M., Peterson, K.J., Pape, T., Sinclair, B.J., Skevington, J.H., Blagoderov, V., Caravas, J., Kutty, S.N., Schmidt-Ott, U., Kampmeier, G.E., Thompson, F.C., Grimaldi, D.A., Beckenbach, A.T., Courtney, G.W., Friedrich, M., Meier, R. & Yeates, D.K. (2011) Episodic radiations in the fly tree of life. *Proc Natl Acad Sci U S A,* **108,** 5690-5695.

Wiklund, C. & Fagerstrom, T. (1977) Why do males emerge before females? : A hypothesis to explain the incidence of protandry in butterflies. *Oecologia,* **31,** 153-158.

Zhao, L., Annie, A.S., Amrita, S., Yi, S.K. & Rudolf, M. (2013) Does better taxon sampling help? A new phylogenetic hypothesis for Sepsidae (Diptera: Cyclorrhapha) based on 50 new taxa and the same old mitochondrial and nuclear markers. *Molecular Phylogenetics and Evolution,* **69,** 153-164.