Sexual dichromatism in wing pigmentation of New World dragonflies follows Rensch's rule

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Abstract

Many animal taxa that display sexual size dimorphism (SSD) exhibit a positive allometric relationship in which the degree of dimorphism increases with body size. This macroevolutionary pattern is known as Rensch's rule. Although sexual selection is hypothesized to be the main mechanism causing this pattern, body size is influenced by several selective forces, including natural and sexual selection. Therefore, by focusing exclusively on SSD one cannot ascertain which of these selective forces drives Rensch's rule. If sexual selection is indeed the main mechanism underlying Rensch's rule, we predict that other sexually selected traits, including coloration-based ornaments, will also exhibit interspecific allometric scaling consistent with Rensch's rule. We tested this prediction using wing pigmentation of 89 species of dragonflies. Studies show that male wing pigmentation is generally under strong intra- and intersexual selection, so that sexual dichromatism in this trait should follow Rensch's rule. Conversely, the available evidence suggests that male body size is usually not sexually selected in dragonflies, so we do not expect SSD to follow Rensch's rule. First, we found that sexual dichromatism in wing pigmentation was consistent with Rensch's rule. The phylogenetic major axis regression slope was significantly greater than one. We also showed that the allometric slope for SSD was not different from unity, providing no support for Rensch's rule. Our results provide the first evidence that a trait which appears to be under strong sexual selection exhibits a pattern consistent with Rensch's rule.

Introduction

Sexual dimorphism, the difference in a trait such as size, coloration and behaviour, between males and females of a species, is widespread in animals (Andersson, 1994; Fairbairn *et al.*, 2007). This pattern has attracted considerable attention ever since Darwin (1871), particularly because of extreme cases of sexual dimorphism in body and ornament size reported for some species (Fairbairn *et al.*, 2007). Although there are several postulated hypotheses to explain the evolution of sexual dimorphism (Fairbairn, 1997; Dale *et al.*, 2007; Fairbairn *et al.*, 2007), sexual and fecundity selections are the primary mechanisms used to explain both

the direction and magnitude of sexual dimorphism. The magnitude of sexual dimorphism, in particular, is currently understood as the sex-specific equilibrium of sexual selection acting mainly on males, fecundity selection acting on females and natural selection acting on both sexes (Blanckenhorn, 2005). Irrespective of the proposed mechanism influencing the evolution of sexual dimorphism in a trait, a macroevolutionary pattern known as the Rensch's rule often emerges: there is an interspecific allometric scaling between the degree of sexual dimorphism and the magnitude of the phenotypic trait in question (Rensch, 1960). For instance, the interspecific relationship between male and female body size is usually positively allometric, so that the slope of this relationship exceeds one (Fairbairn *et al.*, 2007). The consequence of this allometric scaling is that the magnitude of sexual size dimorphism increases with overall body size across species when males are the larger sex.

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Rensch's rule is a macroevolutionary pattern that has been extensively shown for a range of taxa, including both vertebrates and invertebrates (Abouheif & Fairbairn, 1997; Fairbairn, 1997; Cullum, 1998; Colwell, 2010; Ceballos et al., 2013). Sexual selection is the main mechanism invoked to explain such a pattern (Fairbairn, 1997). Briefly, sexual selection through male–male competition and/or female mate choice is known to be a source of strong and directional selection favouring larger body sizes (Hoekstra et al., 2001; Kingsolver et al., 2001). In the presence of this selective force, sexual size dimorphism is expected, because male body size is under strong directional sexual selection, which could be neutral or even deleterious for females (Lande, 1980). However, despite many studies, it is still uncertain whether sexual selection is the main mechanism responsible for the emergence of Rensch's rule (Fairbairn et al., 2007). Body size is influenced by several selective forces so there is uncertainty regarding which processes drive Rensch's rule (Hedrick & Temeles, 1989; Blanckenhorn, 2005). For instance, body size could be influenced concomitantly by sexual and fecundity selection (Honek, 1993). By focusing exclusively on sexual size dimorphism, it is difficult to assess whether sexual selection is the main mechanism responsible for Rensch's rule.

Nevertheless, if sexual selection is a key processes underlying Rensch's rule, then a wide range of sexually selected traits should also exhibit interspecific allometric scaling consistent with Rensch's rule. Take, for example, ornamental traits that are under intra- and/or intersexual selection, such as coloration-based ornamental traits (Andersson, 1994). The prediction is that with an increment in the degree of ornamentation in one sex, the magnitude of sexual dimorphism in ornamentation should increase, so that the expected allometric slope is greater than one. Consequently, using an ornamental trait that is generally assumed to be under strong sexual selection in one sex (but not the other), it should be possible to test whether sexual selection is generally responsible for Rensch's rule. In fact, Rensch in his original proposition argued that selection on secondary sexual characters was responsible for sexual dimorphism (Rensch, 1960). To the best of our knowledge, most tests of Rensch's rule to date have focused on size dimorphism (but see Outomuro et al., 2014).

Here, we asked whether a presumed sexually selected trait in males, wing pigmentation, presents a Rensch's rule-like pattern across species of libellulid dragonflies (Odonata: Anisoptera). More specifically, we tested whether wing pigmentation exhibits a greater degree of sexual dichromatism in species that have more wing pigmentation. Odonates are a suitable group to test this prediction because they are highly visually oriented organisms, which use body coloration patterns in the context of intra- and interspecific signalling (Corbet, 1999; Svensson & Waller, 2013). Moreover, empirical evidence demonstrates that male wing coloration is under intra- and intersexual selection in several species of dragonflies and damselflies (Moore, 1990; Contreras-Garduno et al., 2008; Svensson & Waller, 2013). Finally, melanic wing ornamentation in many species of odonates is a sexually selected trait with an underlying mechanism that suggests that it is an honest signal of male immunocompetence (Moore, 1990; Rantala et al., 2000; Siva-Jothy, 2000). We should note, however, that the production and expression of melanin pigments is related to disease resistance and thermoregulation, so that it might be under natural selection in some odonates (e.g. Wilson et al., 2001; Outomuro & Ocharan, 2011; Dubovskiy et al., 2013). Although it is not obviously clear why this would lead to sex-specific natural selection. Given that the evidence for an influence of odonate body size on male reproductive success is inconclusive (Koenig, 2008; Serrano-Meneses et al., 2008a), and that female reproductive success is positively influenced by body size due to fecundity selection (Sokolovska et al., 2000), we also predict that sexual dimorphism in libellulid body size should not increase with an increase in male body size across species. By contrasting the macroevolutionary patterns of two traits putatively under different selective regimes, we provide a test of the generality of sexual selection as a key factor underlying Rensch's rule.

Materials and methods

We collected data on wing size and wing pigmentation of male and female libellulid dragonflies from specimens deposited in two of the main repositories of New World Odonata collections: Florida State Collection of Arthropods (FSCA) and Rosser W. Garrison's personal collection at the California Department of Food and Agriculture (Garrison et al., 2006). We obtained records of wing size and pigmentation for 89 species of New World libellulids, comprising 3072 individuals, including 1615 males and 1457 females. We measured wing size (maximum length of the right anterior wing) and wing ornamentation from at least three males and three females per species (individuals measured per species: median males = 15, range males = 3–138; median females = 15, range females = 3–106). Only specimens whose wings were intact were included in the data set. We estimated wing pigmentation as the proportion of the right posterior wing that was covered by dark pigments. Wing pigmentation was quantified from the posterior wing as this wing could be fully observed on preserved specimens, whereas we measured wing length from the anterior wing because morphological anchoring points for measurement were easier to observe. We calculated the proportion of the wing area that was pigmented to account for a weak positive correlation between the area of wing pigmentation and wing length ($r = 0.173$, 95% CI: 0.018–0.409). The extent of dark

pigmentation was measured using an iterative threshold adjustment process to quantify pigmented areas against the white standardized background of the image in the program IMAGEJ (US National Institutes of Health, Bethesda, MD, USA http://imagej.nih.gov/ij). We found no effect of the date the specimen was collected on the amount of wing pigmentation (β_{time} = −0.034, 95% CI: −0.077–0.004), which rules out the possibility that pigments fade with time.

One premise of our main analysis is that dark pigmentation is a good proxy of the total amount (different hues) of wing pigmentation. Thus, we gathered information on the number of different colours on the wings of live dragonflies (we found information for 81 of the 89 species in our data set, see Table S1) to assess whether the proportion of the wing that was covered with dark pigments was influenced by the number of other colours. A negative effect would suggest that our variable of dark pigmentation is a poor proxy of overall pigmentation, and could be interpreted as a trade-off between other hues and dark pigmentation. However, the lack of a relationship or a positive relationship would be indicative that there is no trade-off or that species that express more colour also express more dark pigmentation, respectively. Both of the latter situations suggest that our pigmentation variable is generally a good proxy. Most species in our data set only had one colour of wing pigmentation (n = 43 species with dark pigmentation), eight species had two different colours, and the remaining 30 species were classified as having clear wings, because it was not possible to determine from the images whether these species had any wing pigmentation. We found that the number of colours expressed on the wings had a significant positive effect on the proportion of the wing that was covered by dark pigments (β = 0.919, 95% CI: 0.638–1.182), indicating that our variable is a good proxy of overall wing pigmentation.

To score sexual dichromatism for each species, we calculated a sexual wing dichromatism index (SWD) as the ratio of the proportion of pigmentation in the posterior wing of males to that of females. SWD values greater than one indicate that males are more ornamented in a given species, and values smaller than one indicate that females are more ornamented (which, as with body size, occurs in some species). Three of the 79 species included in this subset (*Erythemis collocata*, *Orthemis ferruginea discolor* and *Sympetrum corruptum*) had male and female proportion of wing pigmentation values of zero. Therefore, we manually set their SWD estimates to one, which means that these species are monomorphic with respect to their wing pigmentation. Using the SWD index, we aimed to make the sexual dichromatism scores intuitively interpretable as a surrogate for the strength of sexual selection on males (see also Friedman & Remeš, 2016).

Because of shared ancestry, species data are not statistically independent (Harvey & Pagel, 1991).

Therefore, in addition to the traditional reduced major axis (RMA) regression to calculate allometric slopes (McArdle, 1988), we carried out phylogenetically controlled analysis using the phylogenetic RMA (pRMA) regression (Revell, 2010). For this analysis, we used a recently published molecular phylogeny of Libellulidae (Carle *et al.*, 2015). After pruning, 75 species from the data set were available in the supertree for the pRMA regression using the PHYTOOLS package (Revell, 2011) in R version 3.1.2 (R Core Team, 2014). In order to test for an analogous pattern to Rensch's rule in wing ornamentation, we used the *phyl.RMA* function in PHYTOOLS to estimate the slope of logit-transformed proportion of pigmentation of the posterior wing of males against that of females. Because of the variation in sample size among species used to estimate wing pigmentation, we also built a model that accounted for measurement error. The results of this model were qualitatively similar to those presented below (see Appendix S1 for model description and results), indicating that our results are robust to the variation in sample size across species. We tested for Rensch's rule in body size by fitting a pRMA regression to log-transformed anterior wing length of males against that of females. Deviation from isometry was considered statistically significant if the test statistic between the estimated and an isometric slope resulted in a P value ≤ 0.05.

As a proxy for different mating systems, hence potential differences in sexual selection regimes, we noted whether species are territorial (i.e. males remain faithful to and defend a resource or site for at least 1 day) or nonterritorial (i.e. males not faithful to a specific resource or site) (Serrano-Meneses *et al.*, 2008b). We then split the data set between the two types of mating systems and conducted RMA analyses on each subset for both wing pigmentation and wing length.

Results

For those species that have wing pigmentation (n = 86), the average proportion of posterior wing pigmentation ranged from 3.26% in *Leucorrhinia intacta* males to 100% in males from *Diastatops obscura*, *Perithemis intensa*, *P. mooma* and *P. tenera*. The proportion of the wing that is pigmented varied considerably among the sexes, from species with little pigmentation that were on average female-more pigmented to species in which males were far more highly pigmented (Figs 1 and 2).

We found that sexual dichromatism in wing pigmentation had a pattern analogous to Rensch's rule, as the phylogenetic major axis regression slope was significantly greater than one (β = 1.485, n = 75, $t_{59.4}$ = 6.15, P < 0.001, R^2 = 68.1%, Fig. 2). Similarly, RMA revealed a significant positively allometric relationship between male and female wing pigmentation (β = 1.755, 95% CI: 1.531–2.033, n = 89, R^2 = 69.3%). Sexual dimorphism in wing length, however, did not
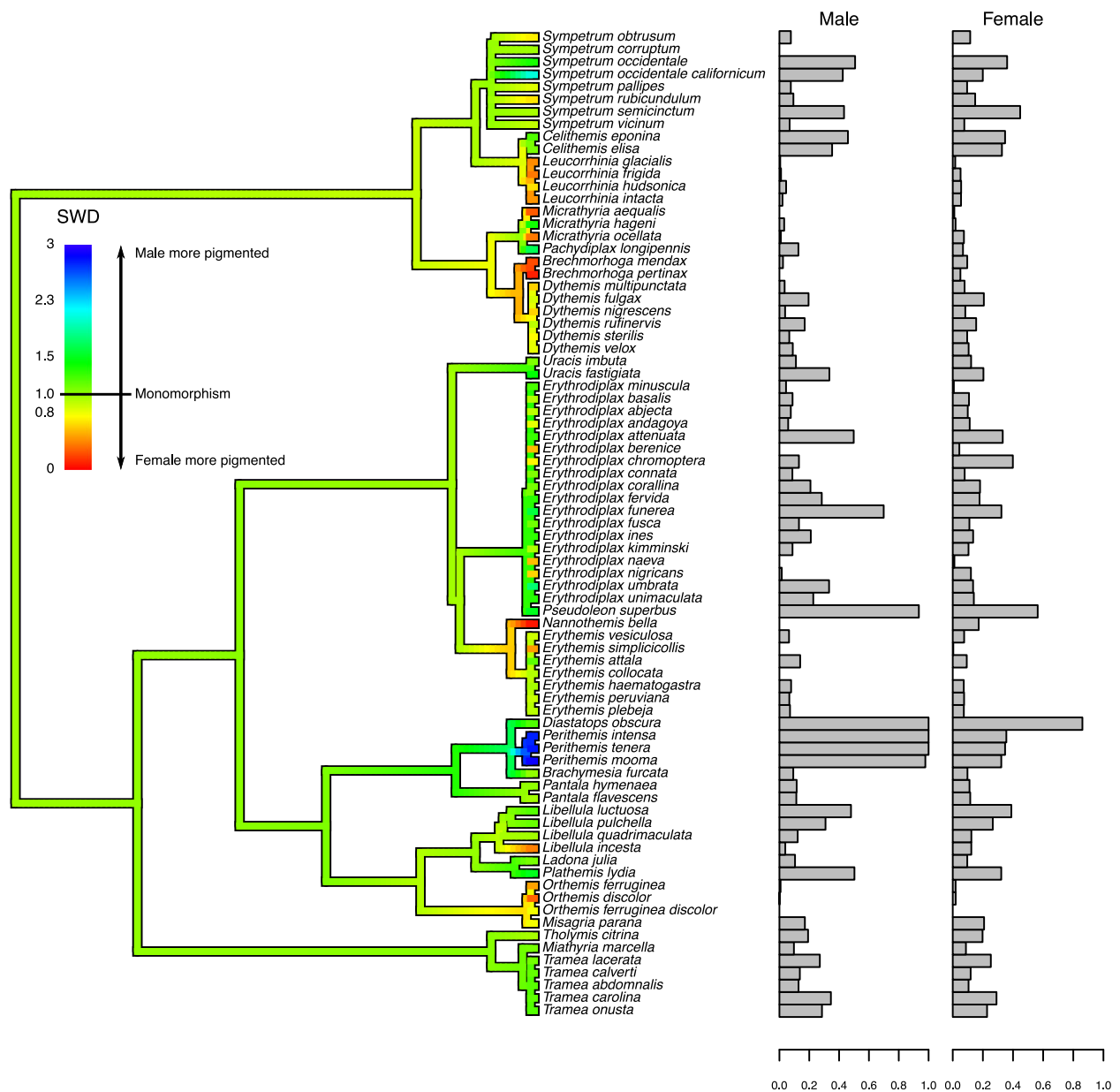
Fig. 1 Sexual wing dichromatism (SWD) of New World libellulid dragonflies (n = 79 species) and reconstructed ancestral dichromatism for the group. Bar plots on the right side of the supertree represent the proportion of the posterior wing that is pigmented in males and females of each species.

differ from unity (pRMA: β = 0.976, n = 75, $t_{51.1}$ = 1.22, P = 0.225, R^2 = 97.1%, Fig. 3), which suggests little evidence for Rensch's rule for sexual size dimorphism.

After splitting the data set between territorial and nonterritorial species, we found the same results as presented above in both groups. There were positively allometric relationships between male and female wing pigmentation (territorial: β = 1.813, 95% CI: 1.546–2.158, n = 59, R^2 = 72.0%; nonterritorial: β = 1.956,

95% CI: 1.471–2.757, n = 12, R^2 = 84.2%), but isometric relationships between male and female wing length (territorial: β = 0.965, 95% CI: 0.928–1.005, n = 59, R^2 = 97.7%; nonterritorial: β = 0.954, 95% CI: 0.893–1.019, n = 12, R^2 = 99.1%).

Discussion

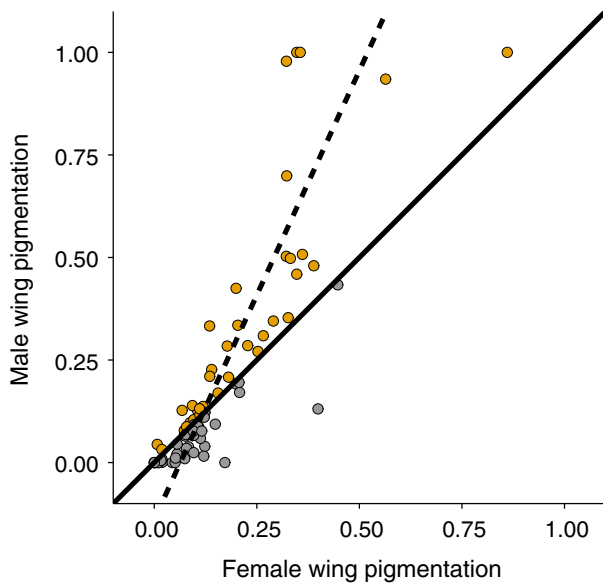In this study, we investigated whether a trait, namely wing pigmentation, that seems to be under strong

Fig. 2 Allometric relationship between male and female proportion of pigmentation of the posterior wing in New World libellulid dragonflies and phylogenetic reduced major axis regression (dashed line) and isometric slope (solid line). Species in which males are more pigmented are depicted in orange symbols (above solid line), and those in which females are more pigmented are in grey (below solid line).
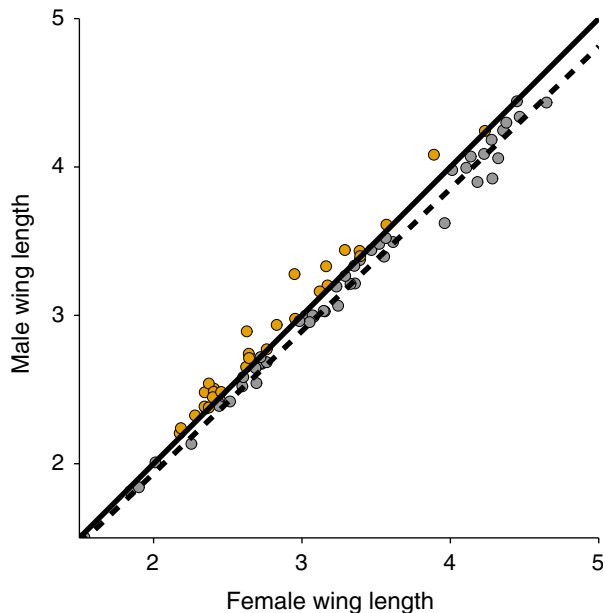

Fig. 3 Allometric relationship between male and female wing length (cm in New World libellulid dragonflies and phylogenetic reduced major axis regression (dashed line) and isometric slope (solid line). Species in which males are larger are depicted in orange symbols (above solid line), and those in which females are more pigmented are in grey (below solid line).

sexual selection in males presented an allometric pattern consistent with Rensch's rule across species of New World libellulid dragonflies. This is an insect group with enormous variation in the degree of wing sexual dichromatism, from female-more ornamented species to exceptionally male-more ornamented ones (Fig. 1). Our findings show that a considerable part of this variation is explained by the positive allometry of male wing ornamentation when scaled against the wing pigmentation of conspecific females. Specifically, the macroevolutionary pattern of sexual dichromatism reported here is consistent with Rensch's rule, predicting an increase in relative male pigmentation with increasing wing pigmentation of the species. Furthermore, male size, which is generally considered not to be under sexual selection in most odonates species studied so far (Koenig, 2008; Serrano-Meneses et al., 2008a), does not show positive allometric scaling against female wing size. The patterns we encounter do not covary with the type of mating system of the dragonflies, which implies that the underlying process is applicable to a wide range of contexts, and is not confined to species under more intense sexual selection. To our knowledge, this is the first time that Rensch's rule is tested in the same set of species for two different traits, one that appears to be sexually selected in only one sex, and another that does not.

The observation that allometric relationships consistent with Rensch's rule are a widespread pattern for secondary sexual traits or body size dimorphism has led to an exploration for a unifying mechanistic explanation (Fairbairn, 1997, 2005; Lindenfors, 2002). Because nearly all studies focus on size-related traits, the main proposed explanation for the faster divergence of male size relies on sexual selection acting either on size per se, or on the size of a sexually selected trait that is correlated with body size (see Fairbairn, 2005 and references therein). Rensch originally proposed that sexual selection caused the macroevolutionary pattern of faster male divergence through selection on the size of sexually selected traits (Rensch, 1960). Evidence for this hypothesis is, however, almost exclusively based on correlations between the ratio of male to female body size, or the ratio of the structural size of a sexually selected trait. This evidence could be confounded by other selective forces on body size that lead to the same pattern. The lack of support for a pattern consistent with Rensch's rule for sexual size dimorphism in anisopteran dragonflies, for instance, is a case in point (Serrano-Meneses et al., 2008a; this study). Among the proposed explanations for the isometric relationship between male and female body size in anisopterans is fecundity selection for large body size in females, which may mask the effect of sexual selection on male body size and result in a macroevolutionary pattern not consistent with Rensch's rule (Honek, 1993; Fairbairn et al., 2007; Serrano-Meneses et al., 2008a).

Rensch (1960) was specific about the mechanism that lead to faster male divergence in traits, namely sexual selection on males. Here, we took his original proposition and extended it from the usual focus on body size to consider a presumably sexually selected trait, wing pigmentation. The allometry of male and female wing pigmentation is consistent with Rensch's rule, which is consistent with sexual selection driving this macroevolutionary pattern. The inferential power of our approach relies on using an ornamental trait that (i) is only slightly correlated with body size and (ii) seems to be under strong sexual selection. There is empirical evidence that wing coloration is an ornament used by males both in intra- and intersexual selection in several dragonfly species (Moore, 1990; Contreras-Garduno et al., 2008; Svensson & Waller, 2013; Outomuro et al., 2014). More generally, sexually dimorphic coloration is implicated in sexual selection in a diverse range of situations and across a wide range of taxa (Andersson, 1994; Searcy & Nowicki, 2010). Our findings that a sexually selected ornamental trait exhibits a macroevolutionary pattern consistent with Rensch's rule could be put to further test using more detailed and extensive available data on other organisms, most notably birds (e.g. Dale et al., 2015). Additionally, one could investigate meta-analytically the strength of the evidence from male–female regressions of traits that are assumed not to be under sexual selection to determine how often such traits exhibit positive allometry consistent with Rensch's rule.

In some species included in our data set females have more wing pigmentation, on average, than conspecific males (Figs 1 and 2). When this pattern occurs, the proportion of the hind wing that is pigmented never crosses 50%, which contrasts with male-more pigmented species in which some species have 100% of the wing pigmented. Although we do not have an available explanation for such pattern, it is possible that natural selection for disease resistance (e.g. Wilson et al., 2001; Dubovskiy et al., 2013) or thermoregulation (Outomuro & Ocharan, 2011) might influence the degree of sexual dichromatism. These two fitness components are directly related to melanin in odonates, so that selection for higher female investment in melanin combined with resource allocation trade-offs in males might have promoted female-biased wing dichromatism. Alternatively, female ornamentation could be due to selection for signalling needs. The possibility of intense sexual selection on females has received increased attention in recent years (see Clutton-Brock, 2009 and references therein). Libellulid species with highly pigmented females and poorly pigmented males, including *Cannaphila insularis* and *Erythrodiplax chromoptera* (Fig. 1), offer good opportunities to study the evolution of female-biased wing dichromatism.

For over 50 years, evidence consistent with Rensch's rule has come almost exclusively from studies of body size or size-linked traits. Most of these studies argued that sexual selection is the main mechanism responsible for this macroevolutionary pattern. However, with the exception of a few studies showing that sexual selection predicts the extent of Rensch's rule in birds, particularly through mating competition and male agility (Székely et al., 2004, 2007), the role of sexual selection in promoting Rensch's rule is still poorly explored. Here, we show that a trait other than body size – wing pigmentation, which appears to be predominantly under sexual selection in odonates – exhibits a pattern consistent with Rensch's rule. This finding provides independent evidence that sexual selection might be a general process underlying Rensch's rule, but more such studies are needed to test the generality of this claim.

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Supporting information

Additional Supporting Information may be found online in the supporting information tab for this article:

Table S1 Dragonfly species for which we found information on wing coloration from live specimens.

Appendix S1 Description of model used to estimate the slope of male vs. female pigmentation while accounting for phylogenetic relatedness and differences in sample size across dragonfly species.