Ecology and sexual conflict drive the macroevolutionary dynamics of female-limited colour polymorphisms

Beatriz Willink[1*] Tammy Ai Tian Ho[2] Erik I Svensson[3]

[1] Department of Entomology, Cornell University, Ithaca, NY 14853, USA

[2] Department of Biology, Aarhus University, Aarhus C 8000, Denmark

[3] Department of Biology, Division for Biodiversity and Evolution, Ecology Building, Lund University, Lund 223-62, Sweden

[*] Corresponding author email: beatriz.willink@cornell.edu

Abstract

Sexual conflict over mating has been documented in many species, both in the field and in experimental studies. In pond damselflies (family Coenagrionidae), sexual conflict maintains heritable female colour polymorphisms, with one female morph typically being a male mimic. However, it is not known whether sexual conflict can also explain the evolutionary origin of female-limited colour morphs, and if so, what ecological factors play a role in this macroevolutionary transition, by modulating the strength of the conflict. Furthermore, the effects of sexual conflict on phylogenetic diversification remain controversial, in particular, whether sexual conflict elevates speciation rates, extinction rates, or both. Here, we use phylogenetic comparative methods to show that female colour polymorphisms are more likely to evolve when population densities at breeding sites are high, and that these demographic conditions are more common at high latitudes and in open landscapes. We show that female-limited polymorphisms typically evolve from sexually dimorphic ancestors through the addition of a male-like female morph, consistent with the hypothesis of selection for male mimicry. Female colour polymorphisms increase both speciation and extinction rates, leading to higher evolutionary turnover of polymorphic lineages. We conclude that female colour polymorphisms evolve in a predictable fashion, and are likely driven by ecological conditions that increase the rate of pre-mating interactions and thus the intensity of sexual conflict. The effects of female colour polymorphisms on extinction differ from previous intraspecific studies and indicate contrasting long-term effects of sexual conflict at micro- and macroevolutionary scales.

Introduction

A fundamental goal in evolutionary biology is to understand the link between the processes that fuel microevolutionary change and the patterns of trait variation that occur across groups of related organisms (1–3). One way to bridge this gap is to start from a well-understood microevolutionary force, and ask what phenotypic differences between species should we expect, had this process been causal throughout the evolutionary history of a particular clade (e.g. 4, 5, 6). Through this approach, phylogenetic comparative methods, which have traditionally been viewed as exclusively correlational, can be incorporated into an explicit causal framework (7, 8). Here, we use this causal approach to investigate the macroevolutionary dynamics of female-limited colour polymorphisms in damselflies. Specifically, we asked if the macroevolutionary origins of the polymorphisms could be explained by the same process of sexual conflict over mating rates that maintains sympatric morphs within populations.

Inter-locus sexual conflict arises whenever the fitness consequences of mating and reproduction ultimately differ between the sexes (9). If so, the evolution of traits involved in pre-mating interactions, such as mating harassment, will reflect genetic conflicts of interest between alleles segregating at different loci (10). For example, sexual conflict can drive evolutionary change in male-specific behaviours that bias the outcome of pre-mating interactions in favour of male fitness, and at the expense of female fitness. This will in turn impose selection on other loci, governing female behavioural, morphological, or physiological defence traits against such antagonistic pre-mating interactions (11). One possible outcome of inter-locus sexual conflict is the continuous reiteration of this coevolutionary process, propelling an "arms race" between the sexes (12–16). Alternatively, inter-locus sexual conflict can, under certain circumstances, result in female diversification into multiple genetic clusters, or morphs, to which all males cannot simultaneously adapt (17–19). Consistent with theory (18, 19), the sympatric co-occurrence of multiple heritable female morphs that differ in mating-related traits has been reported in several different taxa, particularly in birds and insects (20–27).

In damselflies, female-limited heritable colour morphs display alternative reproductive tactics (22), involving a suite of correlated behavioural, morphological, physiological, and life-history traits (28–31). Female-limited colour polymorphisms in damselflies typically include a male-coloured morph (i.e. "androchrome" females) and one or more female morphs markedly different from males (32, 33). In the species in which such polymorphisms have been studied in detail, empirical evidence indicates that negative frequency-dependent selection via pre-mating male harassment maintains these genetic polymorphisms (34–38). Visually-oriented mate-searching males are thought to disproportionally direct their mating attempts towards common female

morphs, and the excessive mating and pre-mating interactions encountered by targeted females can reduce their fecundity (36, 39–42). If avoidance of male mating harassment is the primary driver of the evolution of female-limited colour polymorphisms in damselflies, then these polymorphisms should generally evolve via the origin of male-like females, that can obtain a frequency-dependent fitness advantage from male mimicry. Moreover, we expect that damselfly lineages with marke male pre-mating harassment would more often evolve and maintain inter-sexual mimicry and thus female-limited colour polymorphisms, compared to lineages with lower intensity of sexual conflict.

Mating interactions are shaped by demographic conditions, particularly population densities and sex ratios (43–47), which can in turn be governed by environmental conditions (48–51). Empirical evidence from microevolutionary studies suggests that high population densities and male-biased sex ratios increase the intensity of sexual conflict in several taxa, including damselflies (22, 36, 52–56). Such demographic conditions are expected when reproduction is restricted to a short seasonal window, leading to increased adult synchrony and thus higher local density and a greater potential intense sexual selection in males for male-male scramble competition over mating opportunities (57–60). We therefore hypothesized that the concurrence of mating interactions in the warmer months of the year should ultimately drive higher evolutionary rates of female-limited polymorphisms in temperate areas, where breeding seasons are shorter compared to the tropics (Fig. 1a). Apart from temporal constraints on mating opportunities, spatial constraints can also promote sexual conflict over mating rates. For instance, in damselfly species that breed in open habitats, which tend to be relatively homogeneous environments, individuals typically stay at or near their reproductive sites throughout most of their adult life (e.g. 61, 62), resulting in high adult densities and high encounter rates between the sexes (Fig. 1a). Conversely, sexual conflict should become more relaxed in structurally complex environments, where females can more readily escape male mating harassment, such as in forests or other habitats with pronounced three-dimensional structure (63–65).

Here, we first confirm that female-limited colour polymorphisms in damselflies do typically evolve via the evolutionary origin of male-coloured females, consistent with an advantage of reduced male-mating harassment, as shown in previous population studies (22, 37, 66). We then address the two hypotheses outlined above, linking ecological factors to demographic conditions and to the evolution of female-limited polymorphisms in an explicitly causal framework (Fig. 1). An alternative scenario is that while temperate regions and open landscapes are indeed associated with the occurrence of female-limited polymorphisms, these effects may not be mediated by demographic conditions as we have hypothesized (Fig. 1b). For example, temperature may have a direct effect on male mating effort (67) or male harm upon mating (68), modulating sexual conflict independently of demographic variation. We evaluate the empirical support for these two scenarios and pro-

vide evidence in support of a role of high population density in open habitats and temperate regions driving the evolution of male mimicry and female-limited polymorphisms in damselflies. Finally, we investigate the consequences of female polymorphisms for the diversification dynamics in this ancient insect group.

The role of sexual conflict in speciation has been explored in theoretical models (69) and investigated empirically in experimental evolution studies, yet it remains contentious (52, 70–76). Female-limited polymorphisms may hamper speciation if genetic diversification gives females the upper hand on the conflict, effectively impeding male diversification (18). Even if males also diversify into genetic morphs tracking the females, sexual conflict may prevent speciation as long as assortative mating between compatible male and female morphs acts as a stabilizing force in both sexes (24, 77). Alternatively, female-limited polymorphisms might instead promote diversification (78–80). Genetic and phenotypic variance and covariance are built up and preserved in polymorphic populations (81). When populations become allopatric due to a dispersal or vicariance events and the polymorphism is lost, the release of such genetic variation from frequency-dependent selection could accelerate the evolution of reproductive isolation (78, 79). Here, we show that female-limited colour polymorphisms in damselflies are associated with relatively high rates of speciation but also accelerated extinction, leading to higher turnover rates in polymorphic lineages.

Results

Macroevolutionary origin of female-limited colour variation

We used a Hidden-State Speciation and Extinction (HiSSE) model to reconstruct the evolutionary history of sex-related colour variation in pond damselflies (Odonata:Coenagrionidae), and to investigate trait-dependent diversification dynamics (see *Diversification consequences of female colour and colour variation*, below). We obtained sex-related colour data for 418 species (out of ~ 1,400) in the most comprehensive phylogeny of pond damselflies to date (82). Each species was classified as sexually monomorphic (SM), if males and females have the same hues and pattern across the thorax and most abdomen segments, sexually dimorphic (SD), if males and females have distinctly different colour patterns, or female polymorphic (FP), if females occur in two or more distinct colour patterns, one of which is similar to males (see Methods). The most common character state was sexual dimorphism with 44% of all species, while 28% of taxa were sexually monomorphic and another 28% were female-polymorphic.

Our HiSSE analysis did not resolve the colour state of the most recent common ancestor of all pond damselflies, which was inferred as either sexually dimorphic (posterior probability (PP) = 0.565) or as female-polymorphic (PP = 0.393; Fig. 2a). Nonetheless, we found moderate support for FP lineages arising with

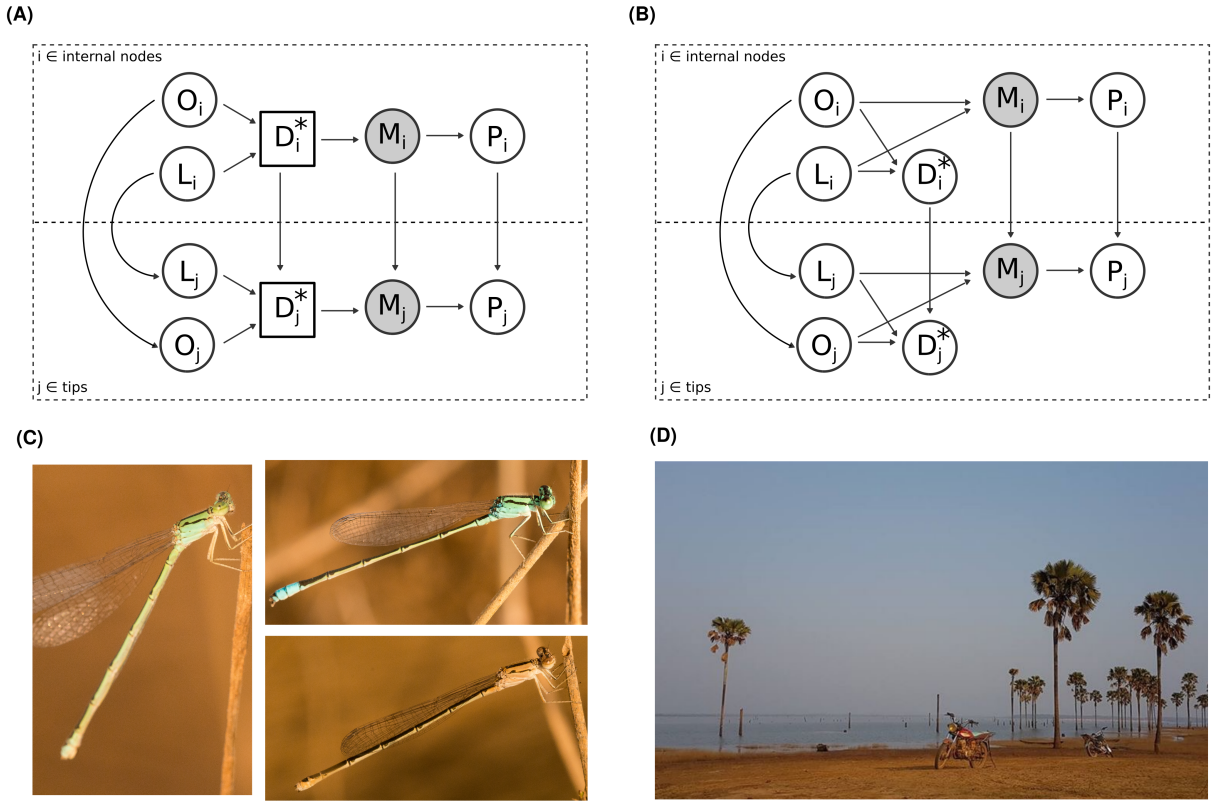

Figure 1. Ecological and demographic factors hypothesized to promote the evolution of female-limited colour polymorphisms in damselflies. **(A, B)** Directed acyclical graphs (DAGs) showing alternative scenarios for the role of ecological variation on the evolution of female-limited polymorphisms. Empty circles represent observed variables, and gray circles represent latent variables (not directly quantified). L = latitudinal range, O = habitat openness, D = density at breeding sites, M = male mating harassment, P = presence of female-limited polymorphisms. *The diagrams show density (D) as a demographic predictor of female polymorphism, but a similar causal hypothesis (not shown) was addressed using operational sex ratio (OSR) instead. In each scenario, the upper panel represents effects in ancestral lineages (i) and the lower panel shows causal relationships in extant taxa (j). Arrows connecting the two panels thus reflect the role of history (i.e. phylogenetic non-independence). **(A)** Density (D) is a mediator (represented by a square node) of the effects of latitudinal range (L) and habitat openness (O) on male-mating harassment (M). **(B)** Latitudinal range (L) and habitat openness (O) impact both density (D) and male-mating harassment (M), but the effects on M are not mediated by D. **(C)** *Pseudagrion nubicum*, an example of a tropical species treated as female-polymorphic in this study. This taxon lacked previous literature data on female-limited colour variation. We sampled 81 individuals of *P. nubicum* at their breeding habitat in Cameroon **(D)** and recorded males (top right), male-like females (left) and non-mimicking females (bottom right). Male-like females were relatively common; of 30 adult females observed, 8 were male-coloured, 12 had tan colour markings, distinctly different from males, and 10 had an intermediate muddy blue-green colouration. Photos: EI Svensson.

greater probability from SD ancestors than from SM ancestors (difference in transition rate: posterior mean (PM) = 0.004, 95% highest posterior density (HPD) interval = -0.001 - 0.010, PMCMC = 0.044; Fig. 2b-c). Stochastic character mapping resulted in a median of 42 gains of female-limited polymorphisms from SD ancestors (95% HPD interval = 4 - 66) versus only three origins of novel female morphs in ancestrally SM lineages (95% HPD interval = 1 - 12). In contrast, female-limited polymorphisms were lost with similar probability to SD and SM descendants (difference in transition rate: PM = 0.001, HPD interval = -0.007 - 0.009, PMCMC = 0.473; Fig. 2b,d).

Ecological factors associated with female-limited colour polymorphisms

Non-ambiguous latitudinal range and habitat openness data were available for 297 of 418 taxa included in our phylogeny (Table S1). Using a Bayesian Phylogenetic Mixed Model (BPMM) (*Model 1*), we found that both latitudinal range and habitat openness predicted the macroevolution of female-limited colour polymorphisms in damselflies. Specifically, female-limited polymorphisms were more common in temperate regions, across both habitat types (temperate vs tropical in open habitats: PM = 0.358, 95% HPD interval = 0.115 - 0.592, PMCMC $<$ 0.001; closed habitats PM = 0.322, 95% HPD interval = 0.060 - 0.653, PMCMC = 0.001; Fig. 3a). FP taxa were weakly associated with open habitats in tropical regions (PM = 0.056, 95% HPD interval = -0.025 - 0.166, P = 0.077), but not in temperate regions (PM = 0.092, 95% HPD interval = -0.210 - 0.416, P = 0.275; Fig 3a). Unlike female polymorphisms, the occurrence of sexual dimorphism was largely independent from these broad ecological predictors, whereas the presence of sexual monomorphism was associated with Tropical ranges and closed habitats (Table S3-S4; Fig. S1).

Female-limited colour polymorphisms occurred with highest probability in open habitats of temperate regions, in both the larger literature-data analysis (*Model 1*, N = 297) and in the analysis including only the taxa surveyed in the field (*Model 2*, N = 80). However, our field-data analysis underestimated the occurrence of female polymorphisms in temperate-closed habitats relative to the literature-data analysis (Fig. 3a-b). Female-limited polymorphisms in the field-data analysis were associated with temperate regions in open-habitat lineages (PM = 0.695, 95% HPD interval = 0.355 - 0.9126, PMCMC = 0.001), but not in closed-habitat lineages (PM = -0.011, 95% HPD interval = -0.215 - 0.305, PMCMC = 0.557). Similarly, female-limited polymorphisms were associated with open habitats in temperate regions than in the field-data analysis (open vs closed habitats in temperate regions: PM = 0.466, 95% HPD interval = 0.047 - 0.840, PMCMC = 0.005), but not literature-data analysis (Fig. 3a-b). Temperate-closed habitats are the least common habitat type in both data sets, accounting only for ~5% of the taxa. Thus, limited taxon sampling, especially in the smaller field-collected dataset, likely accounts for the disparity between the two analyses.

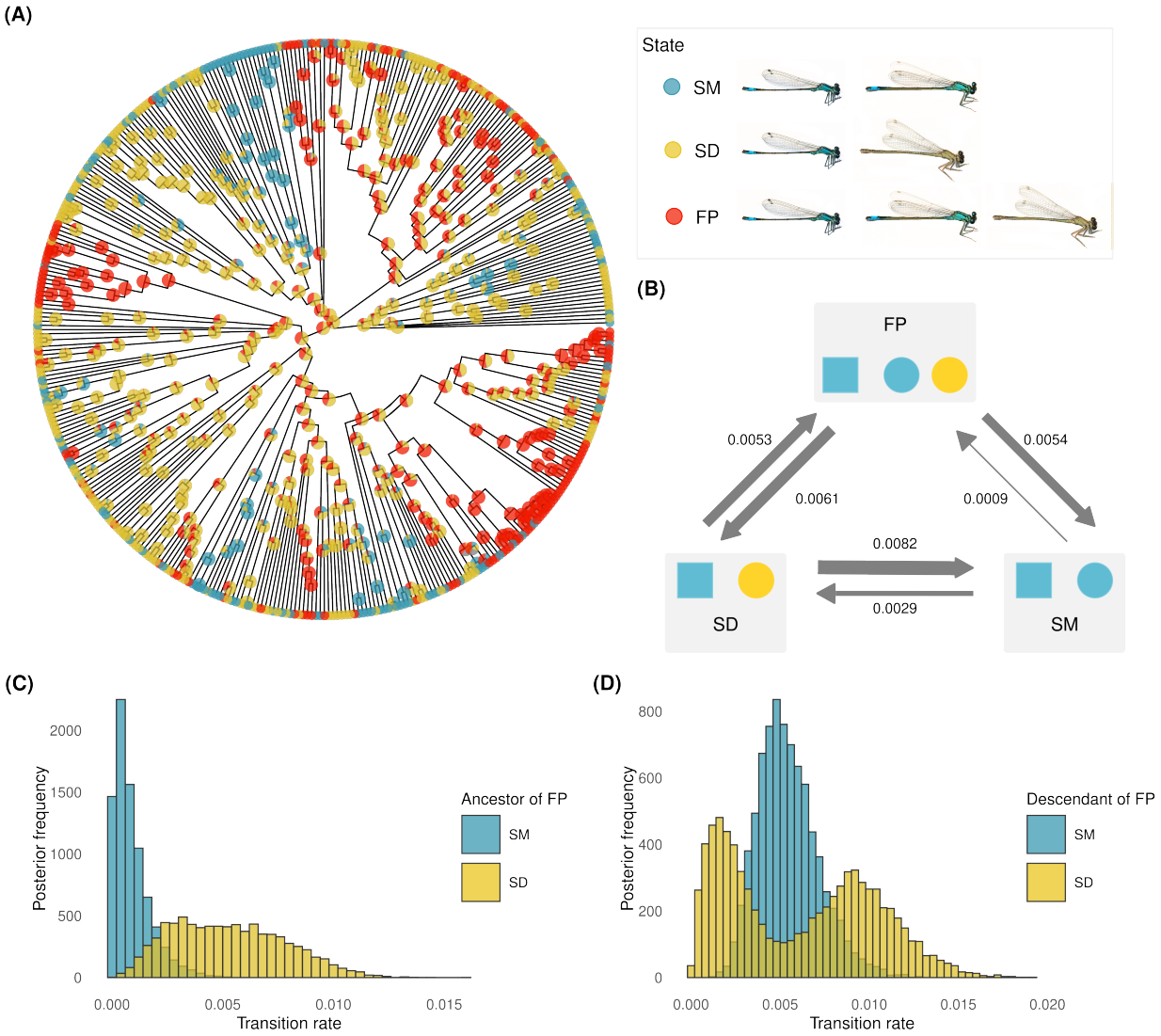

Figure 2. Macroevolutionary history of female-limited colour variation **(A)** Ancestral state reconstruction of sex-related colour states in pond damselflies (family Coenagrionidae). Colours at the tips show colour states in extant taxa. Pies indicate indicate joint conditional posterior probabilities of sex-related colour states in ancestral nodes. **(B)** Summary of transition rates between sex-related colour states in pond damselflies. Arrow width is proportional to estimates of transition rates. Numbers next to arrows represent the mean rate for each transition type. **(C)** Histogram of posterior estimates of the rate of evolution of female polymorphisms, depending on the ancestral colour state. **(D)** Histogram of posterior estimates of the rate of loss of female polymorphisms, depending on the descendant colour state. Abbreviations: FP = female-limited colour polymorphism, SM = sexual monomorphism in colour, SD = sexual dimorphism in colour.

Finally, within the tropics, we obtained a qualitatively similar result in both analyses, indicating a lower probability of FP in closed habitats. In both the literature- and the field-data analyses the difference between open and closed habitats was of about 5%. However, in the field-data analysis this mean difference was bounded by higher uncertainty (PM = 0.046, 95% HPD interval = -0.151 - 0.327, P = 0.281).

Linking ecology, demography, and sexual conflict in damselflies

We investigated two potential demographic mediators for the ecological factors associated with female-limited polymorphisms: population density and male-biased OSR at breeding sites (Fig. 3c). In *Model 3* we found that female-limited colour polymorphisms occurred with increasing probability at higher population densities (slope of log-odds ratio between FP and SM: PM = 1.564, 95% HPD interval = 0.907 - 2.257, PMCMC vs $0 < 0.001$; slope of log-odds ratio between FP and SD: PM = 1.094, 95% HPD interval = 0.325 - 1.862, PMCMC vs 0 = 0.004; Fig. 3d). In contrast, a male-biased OSR, was not correlated with the incidence of polymorphic females (*Model 4*; slope of log-odds ratio between FP and SM: PM = 0.008, 95% HPD interval = -0.877 - 0.987, PMCMC vs 0 = 0.994; slope of log-odds ratio between FP and SD: PM = -0.537, 95% HPD interval = -1.529 - 0.362, PMCMC vs 0 = 0.268; Fig. S2-S3).

To determine if population density is a mediator of these ecological effects on the evolution and occurrence of female-limited colour polymorphisms, we asked in *Model 5* whether the ecological predictors that were associated with female-limited colour polymorphisms (temperate regions and open landscapes) were also associated with high population density. Once accounting for sampling effort, population densities were indeed higher in temperate-open sites than in all other latitude-habitat categories (Table S5; Fig. 3e, S4). Population densities were lowest in tropical-closed habitats and intermediate in both tropical-open and temperate-closed sites (Table S5; Fig. 3e, S4). Finally, in *Model 6* we asked if controlling for population density would reduce the expected probability of female-limited colour polymorphism in latitude-habitat categories with higher occurrence of such polymorphisms (temperate-open and, to a lesser extent, tropical-open sites). We found that the log-odds ratio of FP vs alternative states was markedly reduced in temperate-open sites when we controlled for population density (Fig. 3f), indicating that this demographic variable likely mediates the macroecological distribution of female-limited colour polymorphisms. In tropical-open sites, accounting for density decreased the log-odds ratio of FP relative to SM, as expected, but increased the log-odds ratio of FP relative to SD (Fig. 3f). This increased log-odds ratio suggests that SD lineages are relatively more common in high-density tropical-open landscapes (Table S3; Fig. S1).

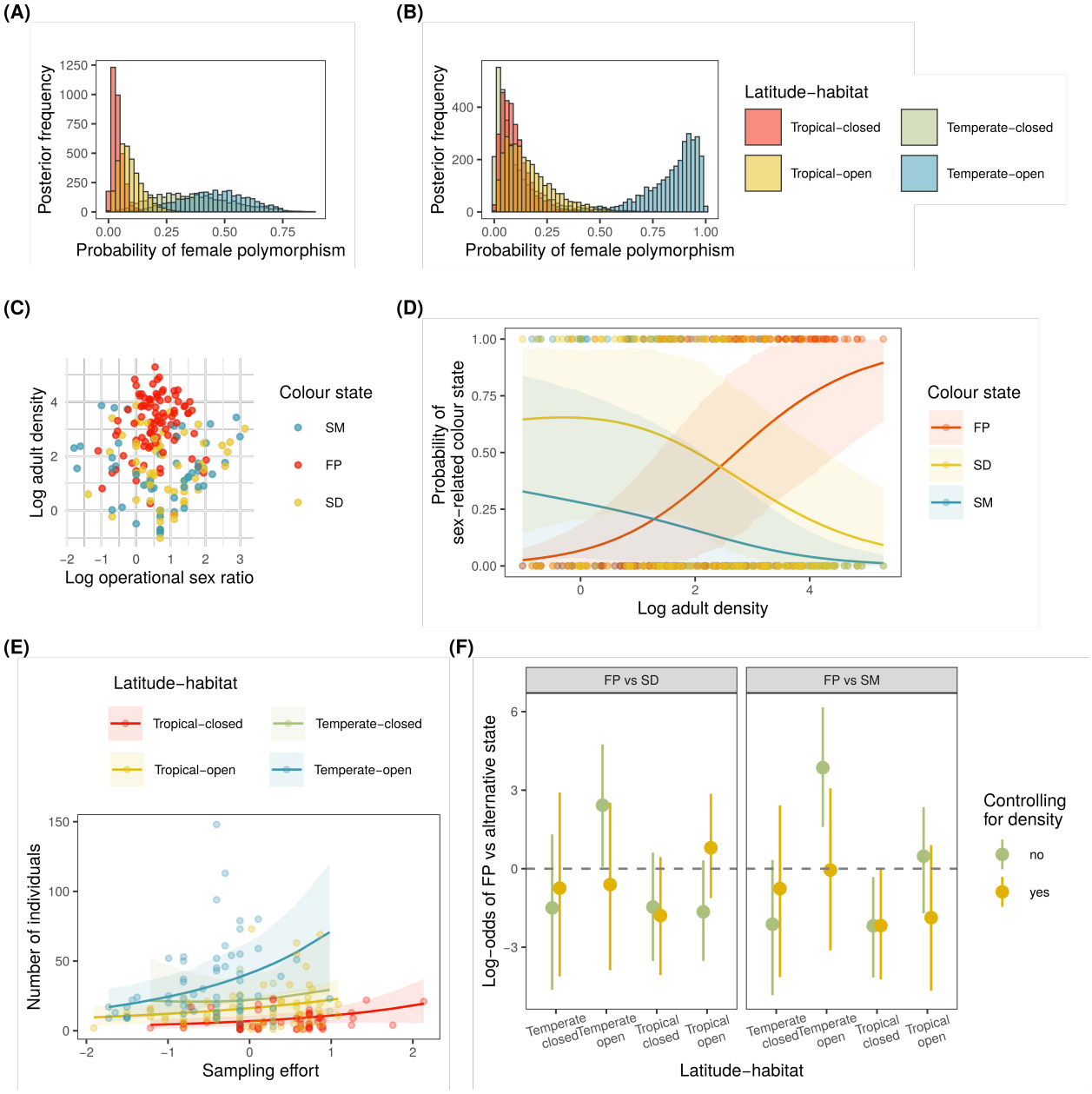

Figure 3. Demography mediates ecological effects on the occurrence of female-limited colour variation **(A)** Posterior frequency of female-limited polymorphisms depending on latitudinal range and habitat openness and based on the literature-data analysis (*Model 1*) **(B)** Posterior frequency of female-limited polymorphisms depending on latitudinal range and habitat openness and based on the field-data analysis (*Model 2*) **(C)** Operational sex ratio (OSR) and adult population density in the field data set. Each dot represents a population (n = 208) of a total of 80 species. Data are log transformed. **(D)** Predicted probability of each sex-related colour state with increasing (log) population density (*Model 3*). Shaded areas represent 95% HPD intervals. **(E)** Expected number of sampled individuals with increasing sampling effort in each latitude-habitat combination (*Model 5*). Dots show data points and shaded areas represent 95% HPD intervals. Sampling effort is mean centered, so intercept differences represent differences in density between latitude-habitat categories. **(F)** Log-odds ratio of female-limited polymorphisms vs alternative sex-related colour states in models with (*Model 6*) and without (*Model 2*) controlling for (log) adult density. Circles represent posterior means and lines represent 95% HPD intervals. Abbreviations: FP = female-limited polymorphism, SD = sexual dimorphism, SM = sexual monomorphism.

Diversification consequences of female colour and colour variation

We estimated the effects of female colour polymorphisms on diversification rates, while controlling for background diversification heterogeneity among clades (Fig. S5). The HiSSE model inferred higher speciation rates in FP lineages compared to SD lineages, which in turn speciated at a faster rate than SM lineages (Table S6; Fig. 4a). Female-limited colour polymorphisms were also associated with a trend towards greater extinction probability than both sexually monomorphic (SM) and sexually dimorphic (SD) lineages (Table S6; Fig. 4b). Net diversification (speciation - extiction) was similarly high in both FP lineages, which had high speciation and high extinction rates, and in SD lineages, which had moderate speciation rates and relatively low extinction rates (Table S6; Fig. 4c). Net diversification was instead reduced in SM lineages, which reflected slow speciation (Table S6; Fig. 4c). Finally, the combination of high speciation and extinction rates resulted in a weak but noticeable trend of higher lineage turnover (extinction/speciation) among FP clades of pond damselflies (Table S6; Fig. 4d).

Discussion

Our results revealed a demographic link between ecology and sexual conflict in the evolution of female-limited colour polymorphisms in pond damselflies (Fig. 3). Temperate and open landscapes across the globe have the highest densities of reproductive individuals, which in turn results in a higher incidence of female-polymorphic taxa. In the tropics, a similar pattern is observed in open landscapes, where damselfly species typically occur at higher densities than in the forest, and female-limited colour polymorphisms are also more common than in closed habitats. These results are in line with theoretical expectations and intraspecific studies in several different insect taxa, that have argued or showed that increased population density, elevates the intensity of sexual conflict over mating, by accelerating the rate of pre-mating interactions (22, 43, 52, 53, 83). Moreover, our findings suggest that female-limited polymorphisms have arisen predictably and repeatedly across the evolutionary history of pond damselflies (Fig. 2). The multiple independent evolutionary origins of female-limited polymorphisms are associated with a social selection regime (sexual conflict over mating) that is thought to maintain female-polymorphisms in multiple damselfly species across different genera (34–38). Our results thus indicate that in pond damselflies, density-dependent selection driven by sexual conflict and shaped by regional and local environments promotes the evolution of discrete and genetic female morphs.

In the present study, male-biased operational sex ratio (OSR) was not associated with any sex-related colour state (Fig. S2-S3). Large-scale comparative evidence indicates that male bias in the OSR increases the opportunity for sexual selection on males (45). We thus expected male-biased populations to be associated

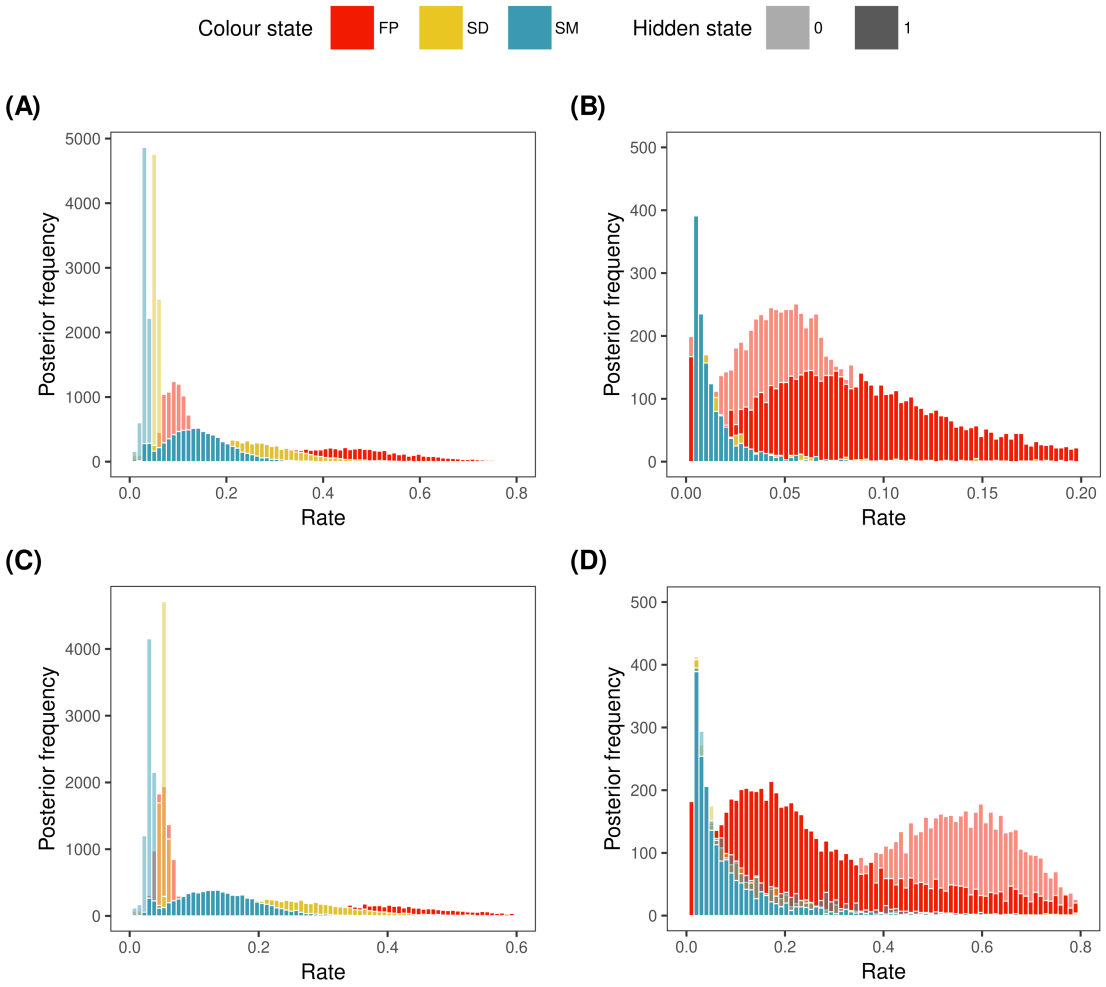

Figure 4. Sex-related colour variation influences diversification dynamics in pond damselflies. Histograms represent posterior distributions of state-dependent diversification rates, in a Hidden State-dependent Speciation and Extinction (HiSSE) model. Background heterogeneity in diversification was modeled as a hidden trait with two values (0 = low background diversification, 1 = high background diversification). **(A)** Speciation. **(B)** Extinction. **(C)** Net diversification (speciation - extinction). **(D)** Lineage turnover (extinction/speciation). Abbreviations: FP = female-limited polymorphism, SD = sexual dimorphism, SM = sexual monomorphism.

with a higher probability of sexual dimorphism. While a male-biased OSR likely increases the opportunity for sexual selection on damselfly males as in many other taxa, this selection regime may not translate into greater sexual dimorphism in colour. In territorial damselflies, OSR is often male-biase, with males defending small patches along breeding sites and females visiting these sites only when reproductively available (e.g. 84, 85). In these species, females often select mates based on the quality of oviposition sites in their territories and male-male competition for suitable sites might result in sexual dimorphism in size, instead of colour (e.g. 85, 86, 87).

The absence of a clear effect of OSR on female-limited colour polymorphisms in the present study was also at odds with expectations from previous experimental studies, in which male-biased OSR has been found to increase the intensity of sexual conflict over mating (54, 55, 88, 89). We therefore expected that female-limited colour polymorphisms would increase in frequency with more male-based OSR, as well as with higher adult density. Unlike experimental and modelling studies in which the effects of these demographic factors can be neatly teased apart, natural populations may only occupy a viable subset of all possible parameter combinations. Even if the combination of high adult density and male-biased OSR would likely exacerbate the rate of male-mating harassment, such a demographic environment may be too hostile to female fitness be sustained in the long term (90). In our field data set, only 2.4% of all populations (five populations of four species) were in the top 20% of both adult density and male-biased OSR (Fig. S3). While all of these populations were polymorphic, as expected, our data suggest that even with the evolution of female-limited polymorphisms, combinations of demographic conditions that are extremely adverse for females may be rare, as populations inhabiting such environments might suffer from elevated extinction risk (91, 92).

We hypothesized that environmental conditions in the temperate zone and open habitats promote the synchronous emergence of adults and their permanence at breeding sites, in turn leading to high adult densities and male-male scramble competition that could override female choice (Fig. 1a). When each female is likely to encounter multiple males, males benefit more from repeated pre-mating attempts and females suffer a greater harassment cost of being single (83). As a result, female choosiness is reduced and overall mating rates increase. Under these circumstances of high population density and female costs from both excessive mating and pre-mating harassment, the emergence of a novel female morph that avoids excessive male attention could be favoured by natural selection. Such a morph could then increase in frequency and subsequently be maintained at some intermediate level by negative frequency-dependent selection (34–36). In line with this idea, our HiSSE model revealed that sexual dimorphism was the most likely ancestral state to female-limited colour polymorphisms in pond damselflies (Fig. 2). These results suggest that female-limited colour polymorphisms typically evolve through the origin of a novel male-coloured female morph in a sexually

dimorphic background, consistent with this female morph being a male mimic.

We note that non-selective genetic mechanisms might operate alongside a selective advantage of male mimicry at high density. Female-limited colour morphs in several orders of insects differ from each other in the expression of regulatory elements that interact with sex-determination loci (93–95). Thus, while the origin of female morph with a novel colour pattern likely requires evolution of sex-limited expression, for instance, through the evolution of sex-specific *doublesex* isoforms (96), a novel male-coloured female morph can arise via any mutation that disrupts pre-existing sex-specific regulation. Male-coloured females could therefore arise at higher rates than othe novel morphs, even without any fitness advantage. Yet, such neutral female-limited polymorphisms are likely short-lived and eventually eroded by genetic drift unless maintained by some form selection (97). Consistent with such a scenario of mutation bias, the HiSSE model also revealed a higher rate of transition from sexual dimorphism to sexual monomorphism than *vice versa* (Fig. S6).

Female-limited colour polymorphisms enhanced speciation rates, but also extinction rates (Fig. 4). A high speciation rate in FP lineages is consistent with at least two alternative mechanisms. First, the "Buridan's ass" selection regime, in which females obtain a fitness advantage of evolving polymorphisms, is temporary, and is followed by male diversification, leading to sympatric speciation in large populations (18). Higher population densities in FP taxa, suggest suitable conditions for female colour polymorphisms to promote speciation. Alternatively, high speciation rates in FP lineages are also consistent with "morphic speciation", whereby different sympatric morphs are lost due to genetic drift in allopatric populations, releasing genetic divergence and promoting reproductive isolation (78, 79). Future studies addressing the spatial and historical dynamics of speciation in FP lineages would be fruitful to elucidate these and other speciation scenarios.

There is disagreement on whether heritable polymorphisms contribute to the preservation of existing lineages or accelerate extinction (98–101). We found moderate evidence that female-limited colour polymorphisms are associated with greater extinction risk in pond damselflies (Fig. 4b). However, the elevated extinction rate in these lineages need not be a consequence of the polymorphism *per se*, but could be driven by the same processes that promote the evolution of female-limited colour polymorphisms in the first place. Sexual conflict over mating rates could increase population extinction risk through its negative effects on female fitness, an idea supported both in theory (90), and by empirical evidence (102, 103). Female fitness costs of sexual conflict might be partially alleviated by the evolution of alternative female morphs, as previously shown experimentally in single-species studies of polymorphic damselflies (42). The comparative results in the present study suggest that sexual conflict can have contrasting effects on extinction risk at micro- and macroevolutionary scales. In pond damselflies, sexual conflict over mating therefore seems to maintain genetic polymorphisms within populations (34–38), constrain inter-population divergence (reviewed in 101),

and give rise to the evolution of FP lineages that might speciate faster but also go extinct earlier than female-monomorphic taxa (Fig. 4d).

Conclusions

Ecologists have long acknowledged the power of density-dependent natural selection, promoting genetic and phenotypic divergence (104, 105). While the role of ecological factors on the intensity and outcome of sexual conflict has recently attracted research interest (106–108), the mechanisms for such ecological effects have rarely been examined (68, e.g. 109). Here, we have shown that population density links environmental heterogeneity to the phylogenetic distribution of female colour polymorphisms in damselflies (Fig. 3). High population density and elevated sexual conflict at high latitudes and in open habitats has repeatedly favoured the evolution of female-limited colour polymorphisms, in support of the hypothesis that male mimicry is an evolved female response to male mating harassment (Fig. 1a). A similar trend has been recently reported in birds, for which the strength of sexual selection peaks at higher latitudes, as breeding seasons become shorter and more intense (60). Taken together with previous microevolutionary studies in damselflies (22, 34–38, 88), these results indicate that sexual conflict plays an important role both in the evolutionary origin and in the maintenance of female-limited colour polymorphisms. However, the effects of sexual conflict on female fitness seem to influence the macroevolutionary fate of female-polymorphic lineages, which speciate faster but are also more prone to extinction (Fig. 4). Our results reveal how phylogenetic comparative analyses can be enriched by incorporating predictions from microevolutionary studies to reveal the ecological drivers and macroevolutionary consequences of sexual conflict.

Methods

Field work and collection of comparative phenotypic data

We obtained comparative phenotypic data from field surveys, literature, and online database searches and examination of museum specimens. Field surveys are described in detail below. For literature data, we mined field guides, species descriptions, and primary scientific literature, including taxonomic revisions and regional checklists. We also obtained data from online resources managed or contributed by expert odonatologists, and from descriptions accompanying museum specimens, housed in the Florida State Collection of Arthropods (https://iodonata.updog.co/index_files/fscaodon.html) and in the New Zealand Arthropod Collection (https://www.landcareresearch.co.nz/resources/collections/nzac). All comparative data and references to the sources used for each species are available as Supporting Material (Table S1-S2) and will be uploaded to

Dryad and to the Odonate Phenotypic Database (https://doi.org/10.5061/dryad.15pm5qc).

For each species, we recorded the following variables whenever available: sex-related colour variation (see below), latitudinal range (tropical or temperate), habitat openness, population density and operational sex ratio. Taxa (n = 418) were classified as sexually monomorphic (SM), sexually dimorphic (SD) or female polymorphic (FP), depending on whether females are male-coloured (*androchrome*), markedly different from males (*heterochrome*), or if multiple discrete female colour morphs have been documented (*polymorphic*). Females were classified as androchrome if they exhibit similar hues (e.g. black, blue, green, yellow, red, brown) and similar patterning (i.e. plain or patterned) as males in both their thorax and most of the length of their abdomen (segments 1-7). Females were otherwise classified as heterochromatic (i.e. different from males in colour). Abdominal segments 8-9 are relatively short in damselflies and dragonflies, and males often display brightly-coloured patches in one or both of these segments, which may be absent, duller, or incomplete in females, even when the overall colouration is similar between the sexes (6). In cases were males and females only differed by these last abdomen segments, we coded females as male-coloured. The potential bias of implementing a relatively strict threshold for sexual dimorphism is to reduce the number of sexual dimorphic lineages, which is expected to reduce the rate of transitions from sexual dimorphism to female polymorphism (as there will be fewer sexually dimorphic ancestors). This potential bias runs counter to our hypothesis of how female-limited polymorphism more often evolve (see Introduction), and is therefore conservative.

We note that not in every species has a genetic basis of female polymorphism been confirmed by breeding experiments or molecular studies. Thus, while the data in many cases more accurately describe polychromatisms (i.e. multiple colour types of unknown basis within a population), we here tentatively refer to all female-limited colour variation as female-limited colour polymorphisms. We justify this because in every damselfly species with co-occurring androchrome and heterochrome female types in which discrete colour morphs have been experimentally raised in the laboratory or investigated in depth in the field, a genetic basis of the polymorphism has been confirmed (110–115). We recently characterized the genomic basis of the colour polymorphism that is shared between several species of *Ischnura*, and demonstrated that the alternative alleles underpinning this polymorphism arise from structural variation (i.e. insertions and inversions) at an automosal locus (116). In some species, multiple heterochromatic female types co-occur without any male-coloured females, but these colour types reflect developmental colour phases, which are often also present in males (117–119). Such purely developmental and non-genetic colour variation was not considered in the present study. For these species, we report only the colour patterns of sexually mature adults. Finally, in no case did we find evidence of heritable male-limited polymorphisms.

Latitudinal distribution data were taken from Willink et al. (82), where species distributions were recorded as presence or absence from administrative areas, namely countries and states, provinces, or territories. The resulting distribution ranges were then classified as warm-adapted (tropical) or temperate or cold-adapted (temperate) based on the biome classification of the areas they occupy. Species that covered both latitudinal ranges were treated as missing data (n = 83). Breeding habitats were categorized as open or closed based on the vegetation type and cover. In the field, we classified habitats as open if the prevailing vegetation were grasses and shrubs, and as closed for species collected within the forest understory. Similarly, when gathering habitat openness data from the literature we considered descriptions including the words "open", "marsh", "bog", and "grassland" or "grasses" as indicating open habitats, and descriptions including the words "closed", "shaded", "wooded", and "forest" as indicating closed habitats. Here too, species with mixed habitat descriptions in the literature (n = 106) were treated as ambiguous, unless the literature source explicitly referred to one habitat type being more common than the other.

Density and operational sex ratio data were collected only for species surveyed in the field, and for one additional species (*Xathocnemis zealandica*, in New Zealand) that was surveyed in a previous study using comparable field methods as ours (120). We conducted field surveys in four continents and six countries (Argentina, Costa Rica, Guyana, Cameroon, Sweden, Cyprus, and USA) in order to sample a wide ecological and phylogenetic range of taxa. In each country, we surveyed 9-29 breeding sites, for an average of 2.6 netting hours per site (sd = 3.2 h, min = 12 min, max = 16.3 h). At each site, one to three researchers captured damselflies as they were encountered along a transect, during a time-recorded capture session. All captured specimens were subsequently identified to species, sex, and colour morph whem applicable. If more than 8 individuals per species were collected, these timed surveys were used to calculate an index of population density as the number of individuals caught per researcher per hour, and an index of operational sex ratio (OSR), defined as the ratio of males per females at the breeding sites. While the population density index should reflect the overall intensity of intra-specific interactions, the OSR is often taken as a measure for the opportunity of sexual selection (45). In some species, only members of one sex were observed (typically males). In order to include these sex-biased populations in our OSR analysis, we estimated OSR in all taxa as (number of males + 1)/(number of females + 1), providing a lower boundary estimate of the extent of male bias. Restricting the analysis to only populations in which both males and females were observed did not qualitatively change our results.

Estimating transition rates and diversification dynamics

We addressed two questions about the evolutionary history of female-limited colour variation. First, we asked which ancestral state, sexual monomorphism (SM) or sexual dimorphism (SD), more often precedes the evolution of female-limited colour polymorphism (FP). A higher transition rate from SD to FP would indicate that that female-limited polymorphisms typically evolve from sexually dimorphic ancestors, and through the origin of a novel male-like female phenotype, consistent with the male-mimicry hypothesis (121). Next, we asked whether there was any bias in the female morph that persists if and when female-limited colour polymorphisms are lost. To answer these questions we inferred the transition rates between sex-related colour states (SM, SD, and FP) across the phylogeny of pond damselflies, using a Hidden-State Speciation and Extinction (HiSSE) model. We chose to model the evolution of sex-related colour variation while accounting for heterogeneity in diversification rates because ignoring such diversification asymmetries can result in an overestimation of transitions towards diversification-enhancing states (122), and our preliminary analysis suggested that diversification dynamics differed between character states (see Results).

We implemented the HiSSE analysis in RevBayes v. 1.1.1 (123). This model allows for character-dependent and background heterogeneity in diversification rates, by modelling the diversification effects of an unobserved trait. For convenience, we assumed that the hidden character influencing background heterogeneity in diversification had two alternative states. Also, for simplicity, we assumed that the evolution of female colouration occurred only along branches and not during speciation events. We based the analysis on the *maximum a posteriori* (MAP) tree from Willink et al. (82), and explicitly considered the proportion of missing taxa as randomly distributed across the tree.

We used equally distributed log-uniform priors (mean = 1e-6, sd = 100) for the three trait-dependent speciation and extinction rates. The effects of the hidden trait on lineage speciation and extinction were drawn from a lognormal with mean in turn sampled from an exponential (rate = 1.0) and standard deviation such that the 95% interval of the hidden rates spans 1 order of magnitude. These hidden rates were then used to scale the trait-dependent diversification rates, resulting in a total of six rates, two (one relatively slow and one relatively fast) for each female-colour state. Transition rates between character states (observed and unobserved) were independently drawn from identical exponential distributions, with mean equal to 10 character transitions across the tree. A birth-death processed conditioned on lineage survival was used as a the tree prior.

We ran two independent replicates of the HiSSE model on the MAP tree from Willink et al. (82). Each replicate was run for 400,000 iterations with a burn-in of 40,000 iterations, with parameter proposals updated

every 1000 iterations. We used stochastic character mapping (124) in these runs to sample character histories and estimate the number of evolutionary origins of female limited polymorphisms in pond damselflies. We also ran a separate HiSSE model without any character state data, to confirm that our results were not driven by the choice of priors. MCMC diagnostics were conducted using the package *convenience* v 1.0.0 (125) in R v 4.2.2 (126), and ensuring chain convergence and effective sample size (ESS) per run for all model parameters > 300. Ancestral state reconstructions were plotted using the R package *RevGadgets* v 1.1.0 (127).

Ecological and demographic effects on sex-related colour variation

We investigated if the evolution of female-limited colour polymorphisms could be predicted by environmental factors, and if these effects are mediated by demographic conditions (see Introduction). In *Model 1*, we explored the relationship between the presence of female-limited colour polymorphisms and two ecological factors: latitudinal range, a proxy for the duration and intensity of the mating season, and habitat openness, as a proxy for the isolation of reproductive habitats and the potential for females to escape male mating harassment through local movements. We conducted this and subsequent analyses in R v 4.2.2 (126), using Bayesian phylogenetic mixed-effect models (BPMM) implemented with the package *MCMCglmm* v. 2.33 (128). Sex-related colour state (the response variable) was coded as a multinomial variable with the same three categories as above: sexual monomorphism (SM), sexual dimorphism (SD), and female-limited colour polymorphism (FP). The fixed latitude and habitat predictors had two levels each: 'tropical' and 'temperate', and 'open' and 'closed', respectively, and with ambiguous taxa coded as missing data and thus excluded from the analyses.

For this and subsequent multinomial models, we used a Kronecker prior (mu = 0, V = σ^2units + $\pi^{2/3}$) for the fixed effects, as it is relatively flat for the marginal two-way probabilities (i.e. SD vs. SM, SD vs. FP, SM vs. FP) when a logit link function is used (129). We used a parameter-expanded χ^2 distribution (V = 1, nu = 1,000, alpha.mu = 0, alpha.V = 1) with two degrees of freedom as the prior for the phylogenetic variance components (130). The residual variance, which cannot be identified in multinomial models, was fixed to 1.

In our subsequent BPMMs (*Model 2* - *Model 6*), we used only the data from species which we sampled ourselves in the field, or for which comparable demographic data was available from published studies (Table S2). In *Model 2*, we specified the same model as outlined above and corroborated that the qualitative effects of latitudinal range and habitat openness were similar with the reduced data set. Then, we investigated if the effects of these ecological factors on the evolution of sex-related colour variation are mediated by demographic conditions, specifically, local density and sex ratio at breeding sites. To do this, we first tested for an effect

of the demographic variables on the probability of female-limited colour polymorphisms, using multinomial BPMMs and observed densities (log number of individuals per hour, *Model 3*) and operational sex ratios (log number of males per female, *Model 4*) as predictors. Because in many cases different species were surveyed in the same breeding sites and some species were surveyed in multiple breeding sites, we included the breeding site identity as an additional random effect in all BPMMs using field data. We used parameter-expanded priors for this non-phylogenetic random effect (e.g. site; V=1, nu=2, alpha.mu=1, alpha.V=1000).

After observing that only one of the demographic variables, population density, predicts the occurrence of female-limited colour polymorphisms (see Results), we conducted two additional tests to determine whether population density is a mediator of broad-scale ecological factors (latitudinal range and habitat openness). In *Model 5*, we tested for differences in population density between habitats and latitudinal ranges, modelling the number of individuals caught per sampling event with a Poisson distribution, and using the sampling effort (catching time) as a mean-centered covariate. We allowed for different slopes in each latitude-habitat combination (tropical-open, tropical-closed, temperate-open, temperate-closed) by fitting the interaction term with sampling effort. For this analysis with a continuous response variable, we assumed an inverse-Wishart prior with a low degree of believe for the residual covariance matrix (V=1, nu=0.002), and we used the default large-variance Gaussian prior (mu = 0, V = I1e+10) for the fixed effects.

Finally, we asked if ecological predictors become less informative when population density is held constant. If open and temperate habitats indeed have more female-polymorphic lineages *because* they promote higher adult densities at breeding sites, we would then expect that once we know the adult density of a population, knowing its habitat type and latitudinal range should not change our expectation of finding female-limited morphs. To test this hypothesis in *Model 6*, we included both ecological factors and population density as predictors in a single multinomial model. We report the log-odds ratio of FP vs each of the two alternative states (SM and SD) for each latitude-habitat combination, with and without accounting for population density. We predicted that the log-odds ratio in latitude-habitat categories that are associated with female-limited polymorphisms should drop to the null expectation (the log ratio of relative frequencies) when density is held at a low value (log density = 0, or 1 adult per hour).

For all BPMMs, we ran two independent replicates of each model to diagnose convergence and autocorrelation between posterior samples, using the R package *coda* (131). We report the posterior means (PM) and 95% highest posterior density intervals (HPD) for parameter estimates. Following Muff et al. (132), we communicate our results in the language of evidence. To ease interpretation, we compute differences in posterior estimates and report the percentage of posterior samples in which the sign of this difference was opposite to our prediction. For example, if we predicted a higher probability of female-limited colour polymorphisms

in temperate regions, but female-limited colour polymorphisms occurred with higher probability in tropical regions in 5% of the posterior, we report a PMCMC value of 0.05, as moderate evidence in favour of our prediction.

Acknowledgements

We are grateful to all the field assistants who helped with the damselfly population surveys, especially Robin Pranter, Hanna Bensch, Yassin Tschinda, Geovanna Rojas, and Emmanuel Venegas. These surveys were conducted with research permits from the Guyana Environmental Protection Agency (Reference No. 010715 BR001), the Administración de Parques Nacionales, Argentina (Ref No. NEA 401), from the Cameroon Ministry of Scientific Research and Innovation and Ministry of Forestry and Wildlife (Ref. No. 0000034), and from the Sistema Nacional de Áreas de Conservación, Costa Rica (M-P-SINAC-ACAT-083-2019, SINAC-ACC-PI-R-122-2019, R-SINAC-PNI-ACLAC-065-2019, SINAC-ACOSA-DT-PI-R-004-2020). Also, we wish to acknowledge the logistic support from the Iwokrama International Centre for Rain Forest Conservation and Development, Guyana, the Karanambu Trust, Guyana, the National Park Administration, Argentina, and the Congo Basin Institute, Cameroon. The Swedish National Infrastructure for Computing (SNIC) for provided computational resources for the comparative analyses and the Centre for scientific and technical computing at Lund University (LUNARC) provided technical assistance. Funding for this study was provided by research grants from The Swedish Research Council (VR: grants no. 2016-03356 and 2020–03123), the Swedish Foundation for International Cooperation in Research and Higher Education (STINT) and Erik Philip Sörensens Stiftelse to E.I.S. B.W. was supported by a “Faculty for the Future” scholarship from the Schlumberger Foundation and a international Postdoc Grant from the Swedish Research Council (VR; grant no. 2019-06444).

Author contributions

EIS and **BW** planned the study and collected the field data. **TATH** and **BW** compiled the literature data. **BW** designed and conducted the phylogenetic comparative analyses. **BW** wrote the manuscript with input from **EIS** and **TATH**.

References

1. D. N. Reznick, R. E. Ricklefs, Darwin's bridge between microevolution and macroevolution. *Nature* **457**, 837–842 (2009).

2. M. G. Weber, A. A. Agrawal, Phylogeny, ecology, and the coupling of comparative and experimental approaches. *Trends in Ecology and Evolution* **27**, 394–403 (2012).

3. S. J. Arnold, Phenotypic evolution: The ongoing synthesis: (American Society of Naturalists Address). *American Naturalist* **183**, 729–746 (2014).

4. M. G. Weber, L. Mitko, T. Eltz, S. R. Ramírez, Macroevolution of perfume signalling in orchid bees. *Ecology Letters* **19**, 1314–1323 (2016).

5. L. J. Harmon, *et al.*, Detecting the macroevolutionary signal of species interactions. *Journal of Evolutionary Biology* **32**, 769–782 (2019).

6. B. Willink, M. C. Duryea, E. I. Svensson, Macroevolutionary origin and adaptive function of a polymorphic female signal involved in sexual conflict. *American Naturalist* **194**, 707–724 (2019).

7. C. K. Cornwallis, *et al.*, Cooperation facilitates the colonization of harsh environments. *Nature Ecology & Evolution* **1**, 0057 (2017).

8. J. C. Uyeda, R. Zenil-Ferguson, M. W. Pennell, Rethinking phylogenetic comparative methods. *Systematic Biology* **67**, 1091–1109 (2018).

9. G. Arnqvist, L. Rowe, *Sexual conflict* (Princeton University Press, 2005).

10. M. A. Schenkel, I. Pen, L. W. Beukeboom, J.-C. Billeter, Making sense of intralocus and interlocus sexual conflict. *Ecology and Evolution* **8**, 13035–13050 (2018).

11. W. R. Rice, B. Holland, The enemies within: Intergenomic conflict, interlocus contest evolution (ICE), and the intraspecific red queen. *Behavioral Ecology and Sociobiology* **41**, 1–10 (1997).

496 12. W. R. Rice, Sexually antagonistic male adaptation triggered by experimental arrest of female evolution. *Nature* **381**, 232–234 (1996).

497 13. S. Gavrilets, G. Arnqvist, U. Friberg, The evolution of female mate choice by sexual conflict. *Proceedings of the Royal Society of London B: Biological Sciences* **268**, 531–539 (2001).

498 14. O. Y. Martin, D. J. Hosken, Reproductive consequences of population divergence through sexual conflict. *Current Biology* **14**, 906–910 (2004).

499 15. W. R. Rice, *et al.*, Inter-locus antagonistic coevolution as an engine of speciation: Assessment with hemiclonal analysis. *Proceedings of the National Academy of Sciences* **102**, 6527–6534 (2005).

500 16. J. C. Perry, C. J. Garroway, L. Rowe, The role of ecology, neutral processes and antagonistic coevolution in an apparent sexual arms race. *Ecology Letters* **20**, 1107–1117 (2017).

501 17. E. Franke, R. Babcock, C. Styan, Sexual conflict and polyspermy under sperm-limited conditions: In situ evidence from field simulations with the free-spawning marine echinoid *Evechinus chloroticus*. *American Naturalist* **160**, 485–496 (2002).

502 18. S. Gavrilets, D. Waxman, Sympatric speciation by sexual conflict. *Proceedings of the National Academy of Sciences* **99**, 10533–10538 (2002).

503 19. T. I. Hayashi, M. Vose, S. Gavrilets, Genetic differentiation by sexual conflict. *Evolution* **61**, 516–529 (2007).

504 20. S. E. Cook, J. G. Vernon, M. Bateson, T. Guilford, Mate choice in the polymorphic African swallowtail butterfly, *Papilio dardanus*: Male-like females may avoid sexual harassment. *Animal Behaviour* **47**, 389–397 (1994).

505 21. K. Reinhardt, E. Harney, R. Naylor, S. Gorb, M. T. Siva-Jothy, Female-limited polymorphism in the copulatory organ of a traumatically inseminating insect. *American Naturalist* **170**, 931–935 (2007).

506 22. T. P. Gosden, E. I. Svensson, Density-dependent male mating harassment, female resistance, and male mimicry. *American Naturalist* **173**, 709–721 (2009).

507 23. K. K. Green, A. Kovalev, E. I. Svensson, S. N. Gorb, Male clasping ability, female polymorphism and sexual conflict: Fine-scale elytral morphology as a sexually antagonistic adaptation in female diving beetles. *Journal of the Royal Society Interface* **10**, 20130409 (2013).

508 24. L. L. Iversen, E. I. Svensson, S. T. Christensen, J. Bergsten, K. Sand-Jensen, Sexual conflict and intrasexual polymorphism promote assortative mating and halt population differentiation. *Proceedings of the Royal Society B* **286**, 20190251 (2019).

509 25. J.-W. Lee, H.-N. Kim, S. Yoo, J.-C. Yoo, Common cuckoo females may escape male sexual harassment by color polymorphism. *Scientific Reports* **9**, 1–9 (2019).

510 26. J. J. Falk, M. S. Webster, D. R. Rubenstein, Male-like ornamentation in female hummingbirds results from social harassment rather than sexual selection. *Current Biology* **31**, 4381–4387 (2021).

511 27. A. Hausmann, *et al.*, Does sexual conflict contribute to the evolution of novel warning patterns? *Journal of Evolutionary Biology* **36**, 563–578 (2022).

512 28. J. Abbott, E. I. Svensson, Phenotypic and genetic variation in emergence and development time of a trimorphic damselfly. *Journal of Evolutionary Biology* **18**, 1464–1470 (2005).

513 29. E. I. Svensson, J. Abbott, Evolutionary dynamics and population biology of a polymorphic insect. *Journal of Evolutionary Biology* **18**, 1503–1514 (2005).

514 30. L. T. Lancaster, R. Y. Dudaniec, B. Hansson, E. I. Svensson, Do group dynamics affect colour morph clines during a range shift? *Journal of Evolutionary Biology* **30**, 728–737 (2017).

515 31. B. Willink, E. I. Svensson, Intra-and intersexual differences in parasite resistance and female fitness tolerance in a polymorphic insect. *Proceedings of the Royal Society of London B: Biological Sciences* **284**, 20162407 (2017).

516 32. P. S. Corbet, *Dragonflies: Behavior and ecology of odonata* (Harley, 1999).

517 33. O. M. Fincke, R. Jödicke, D. R. Paulson, T. D. Schultz, The evolution and frequency of female color morphs in holarctic odonata: Why are male-like females typically the minority? *International Journal of Odonatology* **8**, 183–212 (2005).

518 34. E. I. Svensson, J. Abbott, R. Härdling, Female polymorphism, frequency dependence, and rapid evolutionary dynamics in natural populations. *American Naturalist* **165**, 567–576 (2005).

519 35. Y. Takahashi, J. Yoshimura, S. Morita, M. Watanabe, Negative frequency-dependent selection in female color polymorphism of a damselfly. *Evolution* **64**, 3620–3628 (2010).

520 36. A. Iserbyt, J. Bots, H. Van Gossum, T. N. Sherratt, Negative frequency-dependent selection or alternative reproductive tactics: Maintenance of female polymorphism in natural populations. *BMC Evolutionary Biology* **13**, 1 (2013).

521 37. J. Bots, A. Iserbyt, H. Van Gossum, M. Hammers, T. N. Sherratt, Frequency-dependent selection on female morphs driven by premating interactions with males. *American Naturalist* **186**, 141–150 (2015).

522 38. A. Le Rouzic, T. F. Hansen, T. P. Gosden, E. I. Svensson, Evolutionary time-series analysis reveals the signature of frequency-dependent selection on a female mating polymorphism. *American Naturalist* **185**, E182–E196 (2015).

523 39. H. Van Gossum, R. Stoks, L. De Bruyn, Reversible frequency-dependent switches in male mate choice. *Proceedings of the Royal Society of London B: Biological Sciences* **268**, 83–85 (2001).

524 40. T. P. Gosden, E. I. Svensson, Female sexual polymorphism and fecundity consequences of male mating harassment in the wild. *PLoS ONE* **2**, e580 (2007).

525 41. Y. Takahashi, M. Watanabe, Diurnal changes and frequency dependence in male mating preference for female morphs in the damselfly *Ischnura senegalensis* (Rambur) (Odonata: Coenagrionidae). *Entomological Science* **12**, 219–226 (2009).

526 42. Y. Takahashi, K. Kagawa, E. I. Svensson, M. Kawata, Evolution of increased phenotypic diversity enhances population performance by reducing sexual harassment in damselflies. *Nature Communications* **5**, 4468 (2014).

527 43. H. Kokko, D. J. Rankin, Lonely hearts or sex in the city? Density-dependent effects in mating systems. *Philosophical Transactions of the Royal Society of London B: Biological Sciences* **361**, 319–334 (2006).

528 44. A. Liker, R. P. Freckleton, T. Székely, The evolution of sex roles in birds is related to adult sex ratio. *Nature Communications* **4**, 1–6 (2013).

529 45. T. Janicke, E. H. Morrow, Operational sex ratio predicts the opportunity and direction of sexual selection across animals. *Ecology Letters* **21**, 384–391 (2018).

530 46. J. T. Rotenberry, M. Zuk, Alternative reproductive tactics in context: How demography, ecology, and behavior affect male mating success. *American Naturalist* **188**, 582–588 (2016).

531 47. K. O. Maeno, *et al.*, Density-dependent mating behaviors reduce male mating harassment in locusts. *Proceedings of the National Academy of Sciences* **118**, e2104673118 (2021).

532 48. D. L. Field, M. Pickup, S. C. H. Barrett, Ecological context and metapopulation dynamics affect sex-ratio variation among dioecious plant populations. *Annals of Botany* **111**, 917–923 (2013).

533 49. C. A. Morrison, R. A. Robinson, J. A. Clark, J. A. Gill, Causes and consequences of spatial variation in sex ratios in a declining bird species. *Journal of Animal Ecology* **85**, 1298–1306 (2016).

534 50. S. Pironon, *et al.*, Geographic variation in genetic and demographic performance: New insights from an old biogeographical paradigm. *Biological Reviews* **92**, 1877–1909 (2017).

535 51. J. S. Lewis, *et al.*, Biotic and abiotic factors predicting the global distribution and population density of an invasive large mammal. *Scientific Reports* **7**, 1–12 (2017).

536 52. O. Y. Martin, D. J. Hosken, The evolution of reproductive isolation through sexual conflict. *Nature* **423**, 979–982 (2003).

537 53. D. R. Levitan, Density-dependent sexual selection in external fertilizers: Variances in male and female fertilization success along the continuum from sperm limitation to sexual conflict in the sea urchin *Strongylocentrotus franciscanus*. *American Naturalist* **164**, 298–309 (2004).

538 54. S. Wigby, T. Chapman, Female resistance to male harm evolves in response to manipulation of sexual conflict. *Evolution* **58**, 1028–1037 (2004).

539 55. Ł. Michalczyk, *et al.*, Experimental evolution exposes female and male responses to sexual selection and conflict in *Tribolium castaneum*. *Evolution* **65**, 713–724 (2011).

540 56. L. J. Eberhart-Phillips, *et al.*, Demographic causes of adult sex ratio variation and their consequences for parental cooperation. *Nature Communications* **9**, 1–8 (2018).

541 57. J. W. Grant, M. J. Bryant, C. E. Soos, Operational sex ratio, mediated by synchrony of female arrival, alters the variance of male mating success in japanese medaka. *Animal Behaviour* **49**, 367–375 (1995).

542 58. B. A. Buzatto, R. Macías-Ordóñez, G. Machado, "Macroecology of harvestman mating systems" in *Sexual Selection*, (Elsevier, 2013), pp. 115–162.

543 59. S. A. Heldstab, Latitude, life history and sexual size dimorphism correlate with reproductive seasonality in rodents. *Mammal Review* **51**, 256–271 (2021).

544 60. R. A. Barber, *et al.*, Climate and ecology predict latitudinal trends in sexual selection inferred from avian mating systems. *PLoS Biology* **22**, e3002856 (2024).

545 61. K. A. Allen, D. J. Thompson, Movement characteristics of the scarce blue-tailed damselfly, *Ischnura pumilio*. *Insect Conservation and Diversity* **3**, 5–14 (2010).

546 62. D. Keller, R. Holderegger, Damselflies use different movement strategies for short-and long-distance dispersal. *Insect Conservation and Diversity* **6**, 590–597 (2013).

547 63. L. Yun, P. J. Chen, A. Singh, A. F. Agrawal, H. D. Rundle, The physical environment mediates male harm and its effect on selection in females. *Proceedings of the Royal Society of London B: Biological Sciences* **284**, 20170424 (2017).

548 64. H. L. Malek, T. A. F. Long, Spatial environmental complexity mediates sexual conflict and sexual selection in *Drosophila melanogaster*. *Ecology and Evolution* **9**, 2651–2663 (2019).

549 65. D. Berger, J. Liljestrand-Rönn, Environmental complexity mitigates the demographic impact of sexual selection. *Ecology Letters* e14355 (2024).

550 66. R. A. Sánchez-Guillén, *et al.*, Alternative reproductive strategies and the maintenance of female color polymorphism in damselflies. *Ecology and Evolution* **7**, 5592–5602 (2017).

551 67. B. J. Marcellino, P. Yee, S. J. McCauley, R. L. Murray, Too hot to handle: Male dragonflies decrease time spent mating at higher temperatures. *Animal Behaviour* **207**, 109–118 (2024).

552 68. R. García-Roa, V. Chirinos, P. Carazo, The ecology of sexual conflict: Temperature variation in the social environment can drastically modulate male harm to females. *Functional Ecology* **33**, 681–692 (2019).

553 69. S. Gavrilets, Rapid evolution of reproductive barriers driven by sexual conflict. *Nature* **403**, 886–889 (2000).

554 70. G. Arnqvist, M. Edvardsson, U. Friberg, T. Nilsson, Sexual conflict promotes speciation in insects. *Proceedings of the National Academy of Sciences* **97**, 10460–10464 (2000).

555 71. S. Wigby, T. Chapman, No evidence that experimental manipulation of sexual conflict drives premating reproductive isolation in *Drosophila melanogaster*. *Journal of Evolutionary Biology* **19**, 1033–1039 (2006).

556 72. L. D. Bacigalupe, H. S. Crudgington, F. Hunter, A. J. Moore, R. R. Snook, Sexual conflict does not drive reproductive isolation in experimental populations of *Drosophila pseudoobscura*. *Journal of Evolutionary Biology* **20**, 1763–1771 (2007).

557 73. D. Hosken, O. Martin, S. Wigby, T. Chapman, D. Hodgson, Sexual conflict and reproductive isolation in flies. *Biology Letters* **5**, 697–699 (2009).

558 74. S. M. Ghosh, A. Joshi, Evolution of reproductive isolation as a by-product of divergent life-history evolution in laboratory populations of *Drosophila melanogaster*. *Ecology and Evolution* **2**, 3214–3226 (2012).

559 75. A. Plesnar-Bielak, *et al.*, No evidence for reproductive isolation through sexual conflict in the bulb mite *Rhizoglyphus robini*. *PLoS ONE* **8**, e74971 (2013).

560 76. A. P. S. Carvalho, *et al.*, Is sexual conflict a driver of speciation? A case study with a tribe of brush-footed butterflies. *Systematic Biology* **70**, 413–420 (2021).

561 77. R. Härdling, J. Bergsten, Nonrandom mating preserves intrasexual polymorphism and stops population differentiation in sexual conflict. *American Naturalist* **167**, 401–409 (2006).

562 78. M. J. West-Eberhard, Alternative adaptations, speciation, and phylogeny (a review). *Proceedings of the National Academy of Sciences* **83**, 1388–1392 (1986).

563 79. A. Corl, A. R. Davis, S. R. Kuchta, B. Sinervo, Selective loss of polymorphic mating types is associated with rapid phenotypic evolution during morphic speciation. *Proceedings of the National Academy of Sciences* **107**, 4254–4259 (2010).

564 80. A. F. Hugall, D. Stuart-Fox, Accelerated speciation in colour-polymorphic birds. *Nature* **485**, 631–634 (2012).

565 81. B. Sinervo, E. Svensson, Correlational selection and the evolution of genomic architecture. *Heredity* **89**, 329–338 (2002).

566 82. B. Willink, J. Ware, E. I. Svensson, Tropical origin, global diversification and dispersal in the pond damselflies (Coenagrionoidea) revealed by a new molecular phylogeny. *Systematic Biology* **73**, 290–307 (2024).

567 83. G. Arnqvist, Pre-copulatory fighting in a water strider: Inter-sexual conflict or mate assessment? *Animal Behaviour* **43**, 559–567 (1992).

568 84. L. D. Hamilton, R. D. Montgomerie, Population demography and sex ratio in a Neotropical damselfly (Odonata: Coenagrionidae) in Costa Rica. *Journal of Tropical Ecology* **5**, 159–171 (1989).

569 85. O. M. Fincke, Consequences of larval ecology for territoriality and reproductive success of a neotropical damselfly. *Ecology* **73**, 449–462 (1992).

570 86. K. Matsubara, M. Hironaka, Postcopulatory guarding behaviour in a territorial damselfly, *Pseudagrion p. Pilidorsum* (Brauer), for submerged ovipositing females (Zygoptera: Coenagrionidae). *Odonatologica* **34**, 387–396 (2005).

571 87. R. Guillermo-Ferreira, K. Del-Claro, Territoriality and male-biased sexual size dimorphism in *Argia reclusa* (Odonata: Zygoptera). *acta ethologica* **15**, 101–105 (2012).

572 88. A. Iserbyt, *et al.*, Frequency-dependent variation in mimetic fidelity in an intraspecific mimicry system. *Proceedings of the Royal Society of London B: Biological Sciences* **278**, 3116–3122 (2011).

573 89. M. A. Gomez-Llano, H. M. Bensch, E. I. Svensson, Sexual conflict and ecology: Species composition and male density interact to reduce male mating harassment and increase female survival. *Evolution* **72**, 906–915 (2018).

574 90. D. J. Rankin, U. Dieckmann, H. Kokko, Sexual conflict and the tragedy of the commons. *American Naturalist* **177**, 780–791 (2011).

575 91. K. Grieshop, D. Berger, G. Arnqvist, Male-benefit sexually antagonistic genotypes show elevated vulnerability to inbreeding. *BMC Evolutionary Biology* **17**, 1–10 (2017).

576 92. E. O. Flintham, V. Savolainen, C. Mullon, Male harm offsets the demographic benefits of good genes. *Proceedings of the National Academy of Sciences* **120**, e2211668120 (2023).

577 93. K. Kunte, *et al.*, Doublesex is a mimicry supergene. *Nature* **507**, 229–232 (2014).

578 94. A. Yassin, *et al.*, The pdm3 locus is a hotspot for recurrent evolution of female-limited color dimorphism in *Drosophila*. *Current Biology* **26**, 2412–2422 (2016).

579 95. M. Takahashi, G. Okude, R. Futahashi, Y. Takahashi, M. Kawata, The effect of the *doublesex* gene in body colour masculinization of the damselfly *Ischnura senegalensis*. *Biology Letters* **17**, 20200761 (2021).

580 96. B. R. Hopkins, A. Kopp, Evolution of sexual development and sexual dimorphism in insects. *Current Opinion in Genetics & Development* **69**, 129–139 (2021).

581 97. S. M. Gray, J. S. McKinnon, Linking color polymorphism maintenance and speciation. *Trends in Ecology & Evolution* **22**, 71–79 (2007).

582 98. P. E. Bolton, L. A. Rollins, S. C. Griffith, Colour polymorphism is likely to be disadvantageous to some populations and species due to genetic architecture and morph interactions. *Molecular Ecology* **25**, 2713–2718 (2016).

583 99. P. E. Bolton, L. A. Rollins, S. C. Griffith, The danger within: The role of genetic, behavioural and ecological factors in population persistence of colour polymorphic species. *Molecular Ecology* **24**, 2907–2915 (2015).

584 100. A. Forsman, Is colour polymorphism advantageous to populations and species? *Molecular Ecology* **25**, 2693–2698 (2016).

585 101. E. I. Svensson, Back to basics: Using colour polymorphisms to study evolutionary processes. *Molecular Ecology* **26**, 2204–2211 (2017).

586 102. J. F. Le Galliard, P. S. Fitze, R. Ferrière, J. Clobert, Sex ratio bias, male aggression, and population collapse in lizards. *Proceedings of the National Academy of Sciences* **102**, 18231–18236 (2005).

587 103. L. Yun, *et al.*, Competition for mates and the improvement of nonsexual fitness. *Proceedings of the National Academy of Sciences* **115**, 6762–6767 (2018).

588 104. B. Charlesworth, Selection in density-regulated populations. *Ecology* **52**, 469–474 (1971).

589 105. J. Roughgarden, Density-dependent natural selection. *Ecology* **52**, 453–468 (1971).

590 106. J. C. Perry, L. Rowe, Sexual conflict in its ecological setting. *Philosophical Transactions of the Royal Society B: Biological Sciences* **373**, 20170418 (2018).

591 107. R. Bacon, D. Washington, M.-K. Johnson, M. Burns, The geography of sexual conflict: A synthetic review. *American Naturalist* **201**, 429–441 (2023).

592 108. D. Satomi, C. Koshio, H. Tatsuta, S. Kudo, Y. Takami, Latitudinal variation and coevolutionary diversification of sexually dimorphic traits in the false blister beetle *Oedemera sexualis*. *Ecology and Evolution* **9**, 4949–4957 (2019).

593 109. N. W. Burke, R. Bonduriansky, The geography of sex: Sexual conflict, environmental gradients and local loss of sex in facultatively parthenogenetic animals. *Philosophical Transactions of the Royal Society B: Biological Sciences* **373**, 20170422 (2018).

594 110. C. Johnson, The inheritance of female dimorphism in the damselfly, *Ischnura damula*. *Genetics* **49**, 513 (1964).

595 111. C. Johnson, Genetics of female dimorphism in *Ischnura demorsa*. *Heredity* **21**, 453–459 (1966).

596 112. A. Cordero, The inheritance of female polymorphism in the damselfly *Ischnura graellsii* (Rambur)(Odonata: Coenagrionidae). *Heredity* **64**, 341–346 (1990).

597 113. J. A. Andrés, A. Cordero, The inheritance of female colour morphs in the damselfly *Ceriagrion tenellum* (Odonata, Coenagrionidae). *Heredity* **82**, 328–335 (1999).

598 114. R. A. Sánchez-Guillén, H. Van Gossum, A. Cordero Rivera, Hybridization and the inheritance of female color polymorphism in two ischnurid damselflies (Odonata: Coenagrionidae). *Biological Journal of the Linnean Society* **85**, 471–481 (2005).

599 115. I. Sanmartín-Villar, A. Cordero-Rivera, The inheritance of female color polymorphism in *Ischnura genei* (Zygoptera: Coenagrionidae), with observations on melanism under laboratory conditions. *PeerJ* **4**, e2380 (2016).

600 116. B. Willink, *et al.*, The genomics and evolution of inter-sexual mimicry and female-limited polymorphisms in damselflies. *Nature Ecology & Evolution* **8**, 83–97 (2024).

601 117. I. Sanmartín-Villar, A. Rivas-Torres, M. V. Gabela-Flores, A. C. Encalada, A. Cordero-Rivera, Female polymorphism and colour variability in *Argia oculata* (Coenagrionidae: Zygoptera). *Neotropical Biodiversity* **3**, 203–211 (2017).

602 118. M. K. Khan, Female prereproductive coloration reduces mating harassment in damselflies. *Evolution* **74**, 2293–2303 (2020).

603 119. S. Joshi, D. Agashe, Ontogenic colour change, survival, and mating in the damselfly *Agriocnemis pygmaea* Rambur (Insecta: Odonata). *Ecological Entomology* **45**, 1015–1024 (2020).

604 120. W. Crumpton, Adult behaviour of *Xanthocnemis zealandica* McLachlan and *Austrolestes colensonis* White at selected South Island (N. Zealand) habitats (Zygoptera: Coenagrionidae, Lestidae). *Odonatologica* **4**, 149–168 (1975).

121. H. M. Robertson, Female dimorphism and mating behaviour in a damselfly, *Ischnura ramburi*: Females mimicking males. *Animal Behavior* **33**, 805–809 (1985).

122. W. P. Maddison, Confounding asymmetries in evolutionary diversification and character change. *Evolution* **60**, 1743–1746 (2006).

123. S. Höhna, *et al.*, RevBayes: Bayesian phylogenetic inference using graphical models and an interactive model-specification language. *Systematic Biology* **65**, 726–736 (2016).

124. W. A. Freyman, S. Höhna, Stochastic character mapping of state-dependent diversification reveals the tempo of evolutionary decline in self-compatible Onagraceae lineages. *Systematic Biology* **68**, 505–519 (2019).

125. L. Fabreti, S. Hoehna, A. Magee, convenience*: Convergence assessment for phylogenetic inference* (2023).

126. R Core Team, *R: A language and environment for statistical computing* (R Foundation for Statistical Computing, 2022).

127. C. Tribble, *et al.*, *RevGadgets: Visualization and post-processing of 'RevBayes' analyses* (2023).

128. J. D. Hadfield, MCMC methods for multi-response generalized linear mixed models: The MCMCglmm R package. *Journal of Statistical Software* **33**, 1–22 (2010).

129. J. Hadfield, *MCMCglmm course notes* (Available at: https://cran.r-project.org/web/packages/MCMCglmm/vignettes/CourseNotes.pdf, 2015).

130. P. Villemereuil, O. Gimenez, B. Doligez, Comparing parent-offspring regression with frequentist and bayesian animal models to estimate heritability in wild populations: A simulation study for gaussian and binary traits. *Methods in Ecology and Evolution* **4**, 260–275 (2013).

131. M. Plummer, N. Best, K. Cowles, K. Vines, CODA: Convergence diagnosis and output analysis for MCMC. *R News* **6**, 7–11 (2006).

132. S. Muff, E. B. Nilsen, R. B. O'Hara, C. R. Nater, Rewriting results sections in the language of evidence. *Trends in Ecology & Evolution* (2021).