

# Macroevolutionary Origin and Adaptive Function of a Polymorphic Female Signal Involved in Sexual Conflict

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**ABSTRACT:** Intersexual signals that reveal developmental or mating status in females have evolved repeatedly in many animal lineages. Such signals have functions in sexual conflict over mating and can therefore influence sexually antagonistic coevolution. However, we know little about how female signal development modifies male mating harassment and thereby sexual conflict. Here, we combine phylogenetic comparative analyses of a color polymorphic damselfly genus (*Ischnura*) with behavioral experiments in one target species to investigate the evolutionary origin and current adaptive function of a developmental female color signal. Many *Ischnura* species have multiple female color morphs, which include a male-colored morph (male mimics) and one or two female morphs that differ markedly from males (heterochrome females). In *Ischnura elegans*, males and male-mimicking females express a blue abdominal patch throughout postemergence life. Using phenotypic manipulations, we show that the developmental expression of this signaling trait in heterochrome females reduces premating harassment prior to sexual maturity. Across species this signal evolved repeatedly, but in heterochrome females its origin is contingent on the signal expressed by co-occurring male-mimicking females. Our results suggest that the co-option of a male-like trait to a novel female anti-harassment function plays a key role in sexual conflict driven by premating interactions.

**Keywords:** cross-sexual transfer, male mating harassment, male mimicry, sex-limited polymorphism, sexual dimorphism, sexually antagonistic coevolution.

## Introduction

Intersexual communication in relation to reproduction is a time-sensitive affair. The timely expression of sexual signals is crucial when the fitness costs and benefits of mating vary

during development (Kleemann and Basolo 2007; Maklakov et al. 2007; Long et al. 2010). Temporal variation in the consequences of mating may be particularly important for female fitness. When females are sexually unreceptive, male courtship and coercive mating attempts can increase the risk of predation (Rowe 1994; Zuk and Kolluru 1998), can increase disease transmission (Thrall et al. 1997), and can cause physical damage (Crudgington and Siva-Jothy 2000; Blankenhorn et al. 2002) while providing little if any benefits to females. Therefore, when females have relatively long remating latencies (Trivers 1972; Schärer et al. 2012) or sexual maturation times (Teder 2014), age- and status-dependent female signals are expected to evolve to avoid costly and premature male mating attempts (e.g., Stoltz et al. 2007). However, previous research on sex-limited signals has traditionally focused on male intrasexual competition, male ornaments, and other male secondary sexual characters (Edward and Chapman 2011). We know much less about how intersexual interactions during the course of female development have influenced the evolution of female signaling traits.

While the empirical interest in female sexual signals has recently surged (e.g., Amundsen 2000; Cornwallis and Birkhead 2007; Clutton-Brock 2009; Baldauf et al. 2011; Belliure et al. 2018), the evolution of female signaling traits is typically viewed only through the Darwinian lens of sexual selection, that is, by investigating the role of such signals in intrasexual competition for mating opportunities (Tobias et al. 2012). Thus, a considerable body of theoretical work has been devoted to exploring whether and how female sexual signals might evolve, which constitutes something of a paradox in light of traditional sex roles in animals (Kokko and Johnstone 2002; Servedio and Lande 2006; Barry and Kokko 2010; Fitzpatrick and Servedio 2017). In contrast, the potential role of female sexual signals as developmental and mating status signals to avoid male harm has only recently received theoretical attention (e.g., Rooker and Gavrillets 2018). A small

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but growing number of examples of female color traits that are expressed in coordination with reproductive development have now been documented in a wide range of taxa (Rowland et al. 1991; Weiss 2002; Baldauf et al. 2011; Takahashi and Watanabe 2011; LaPlante 2015; Belliure et al. 2018). These studies suggest that signals indicating female reproductive status may be widespread across animals.

Female visual signals that evolve to prevent males from seeking mating attempts with sexually immature partners might be advantageous for both sexes and are therefore predicted to be honest and relatively inexpensive (Maynard Smith 1994). During prereproductive development of females, the interests of both sexes are often aligned, as they share some of the costs associated with superfluous matings (Rolf and Siva-Jothy 2002; Fedorka et al. 2004; Harshman and Zera 2007). However, sexual conflict can be both age and status dependent (Kleemann and Basolo 2007; Maklakov et al. 2007). Once females become sexually mature, conflict between the sexes over mating may emerge, as optimal mating rates are often higher for males than for females (Chapman et al. 2003). Mating might incur direct fecundity costs for females (Arnqvist and Nilsson 2000) due to physical damage or impaired immunity (Fedorka and Zuk 2005; Rönn et al. 2007) or because the benefits of additional matings are outweighed by lost opportunities for female resource acquisition (Rowe 1992; Reinhardt et al. 2009). For these reasons, deceptive mating-suitability signals are expected to evolve under pervasive sexual conflict as a means to avoid premating harassment and unsought mating attempts from males (Rooker and Gavrillets 2018).

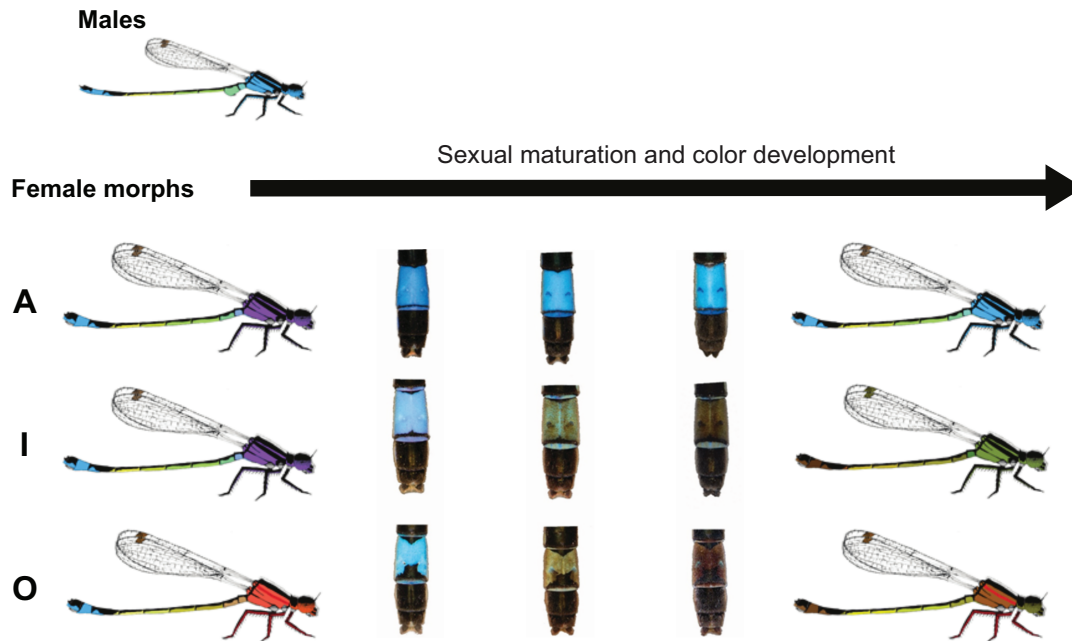
Females may avoid excessive male mating attempts through intersexual mimicry, whereby some individual females express a phenotype similar to that of males (Gosden and Svensson 2009; Neff and Svensson 2013). Such male mimicry has been associated with reduced male harassment and male-induced harm in different insect orders (Cook et al. 1994; Reinhardt et al. 2007; Gosden and Svensson 2009). Alternatively, females can reduce male mating harassment if they maintain immature characters as signals of reproductive unsuitability even after sexual maturation. Such retained juvenile traits in adults have evolved in male signaling systems (Slagsvold and Sætre 1991; Hawkins et al. 2012). For example, in some sexually dimorphic bird species, males may delay their plumage maturation and maintain female-like juvenile colors in spite of being sexually mature, thereby avoiding aggression from dominant males (Hawkins et al. 2012). However, empirical evidence of such delayed expression of mature characters in females to avoid male mating harassment is lacking.

The insect order Odonata (dragonflies and damselflies) is uniquely suited to study both honest signals that reduce male mating harassment of sexually immature females and deceptive signals that reduce male mating harassment of sexually mature females. In several odonate species males and females

go through pronounced ontogenetic color changes between sexually immature and mature phases (Corbet 1999; Futahashi et al. 2012). In the globally distributed damselfly genus *Ischnura*, such ontogenetic color changes are especially pronounced in females (Svensson et al. 2009; Sanmartin-Villar and Cordero-Rivera 2016; fig. 1). Many species in this and several other damselfly genera are also polymorphic, with multiple heritable color morphs in females that include one male-colored morph (androchrome females), typically thought to be a male mimic (Robertson 1985; Gosden and Svensson 2009; Gering 2017), and one or two female morphs that are markedly different from males (heterochrome females; Fincke et al. 2005; Svensson et al. 2009). These color polymorphisms, in combination with extensive male mating harassment and developmental color changes, make *Ischnura* damselflies ideal study systems of how signals of female reproductive development affect intersexual communication and sexual conflict.

Here, we studied the current function and traced the evolutionary origin of a potential color signal of reproductive unsuitability in females of *Ischnura* damselflies. We studied a blue male-like abdomen patch, which may be expressed in immature females of all morphs and in sexually mature females of the androchrome female morph (fig. 1). We first hypothesized that selection against premature mating in females has led to the evolutionary emergence of this immature color signal in the form of a male-like trait in all females. Thus, to test whether this potential immature signaling trait in females reduces premating male harassment, we conducted an experimental manipulation of the blue color patch in immature and mature females of a focal species, *Ischnura elegans*. Based on the observation that this male-like trait is retained throughout adult life in androchrome females of some species (e.g., fig. 1), we proceeded to investigate whether the sexually mature expression of this blue patch in androchrome females has evolved through the retention of an immature signaling trait, thereby deceiving males about the developmental stage of such females. Finally, we investigated whether the developmental expression of the blue patch has evolved independently or in a correlated fashion between androchrome and heterochrome female morphs. We addressed the latter two questions using a phylogenetic comparative approach across 41 species of *Ischnura*.

A strong genetic correlation between the female morphs due to a shared genetic architecture could be expected to result in a strong phylogenetic correlation. The existence of such a correlation between female color morphs could indicate the presence of an evolutionary constraint that could result in intralocus intermorph conflict (Abbott and Svensson 2010), similar to the intralocus sexual conflict that constrains the evolution of sexual dimorphism (Lande 1980; Bonduriansky and Chenoweth 2009). However, because the female morphs in *Ischnura* species differ in multiple phenotypic traits, including reproductive traits, physiology, behaviors,



**Figure 1:** Ontogenetic color changes in heritable female color morphs of the common bluetail (*Ischnura elegans*). The monomorphic males are shown in the upper row for comparison. Males exhibit a blue abdomen patch, which is expressed throughout their entire life on land. Sexually immature and sexually mature color phases are illustrated below for the three female morphs (A = androchrome; I = *infuscans*; O = *infuscans-obsolata*). Between them, close-up photographs of the distal portion of the females' abdomens show whether and how coloration changes over female development. Shortly after emergence from their last larval instar, males and all three female color morphs express a blue patch on the eighth abdominal segment. I- and O-females differ from males and male-like A-females in that they cover the blue abdomen patch with dark pigment as they reach sexual maturity.

and parasite resistance (Robertson 1985; Cordero et al. 1998; Gosden and Svensson 2009; Willink and Svensson 2017), it is certainly possible that the morphs have evolved some phenotypic differentiation in their signaling traits in spite of any such evolutionary constraints caused by a shared genetic architecture. We motivate our integrative research approach by a recent call to combine experimental studies and phylogenetic comparative methods in evolutionary biology (Weber and Agrawal 2012). Such integrative studies provide stronger inferences than when either approach is used in isolation (Weber and Agrawal 2012). Linking the origin and current adaptive function of female signaling traits to intersexual antagonistic interactions is an important task, and integrative studies of the kind we have carried out here are needed to understand how intra- and interlocus sexual conflict can interact during sexually antagonistic coevolution (Pennell et al. 2016).

## Methods

### *Study System: Polymorphism and Development of Female Color*

Females of our focal experimental species, *Ischnura elegans*, occur in three discrete and heritable color morphs (Cordero

et al. 1998; Svensson et al. 2009; fig. 1). In *I. elegans* as well as in the four other *Ischnura* species for which the mode of inheritance of the female polymorphism has been established, a single locus or a set of a few tightly linked loci govern color morph development (Johnson 1964, 1966; Cordero 1990; Sánchez-Guillén et al. 2005; Sanmartín-Villar and Cordero-Rivera 2016). There are three well-investigated species in the clade containing *I. elegans* (*I. elegans*, *I. genei*, and *I. graellsii*). In each one of these species females occur in three color morphs, one that is male-like in sexually mature coloration (androchrome; fig. 1) and two that are markedly different from males when mature (heterochrome; fig. 1). Morph development is governed by three segregating alleles in a dominance hierarchy at an autosomal locus with sex-limited expression to females (Cordero 1990; Sánchez-Guillén et al. 2005).

The immature color phase of androchrome females (hereafter, A-females) is characterized by violet markings on the thorax and a blue patch on the eighth abdominal segment, similar to that of males. In sexually mature A-females, these violet markings become greenish blue, but the blue abdominal patch is retained. Thus, sexually mature A-females and males (which are always monomorphic) express nearly identical color patterns, with blue thoracic stripes and a blue patch in the distal portion of the abdomen (Svensson et al.

2009; fig. 1). One of the heterochrome female morphs is denoted as *infuscans* (hereafter, I-females) and also goes through an early violet color phase, but sexual maturity is accompanied by the darkening of the areas that are blue in males and A-females (fig. 1). Finally, females of the other heterochrome morph, denoted as *infuscans-obsolata* and here referred to as O-females, express a strikingly different immature thoracic color (fig. 1). However, like A- and I-females, O-females also have a blue abdominal patch during the sexually immature stage (fig. 1). At sexual maturity, O-females develop a darker thoracic coloration, and like in I-females the blue patch is covered with dark pigment and hence no longer visible (fig. 1). In summary, the male mimics (A-females) retain the abdomen patch throughout their entire life, just like males, whereas the two sexually dimorphic female morphs (I- and O-females) subsequently conceal this blue patch (fig. 1).

#### *Mating Probabilities and Fecundities in the Field*

During three reproductive seasons (June–July, 2015–2017), we collected data on field mating frequencies of immature and mature females of the three heritable morphs in *I. elegans*. These data were collected as part of a longitudinal long-term study of populations of *I. elegans* in southern Sweden (Svensson et al. 2005; Le Rouzic et al. 2015; Willink and Svensson 2017). Sixteen populations were visited regularly during these three breeding seasons (range, 4–8 visits per season; mean  $\pm$  SD,  $6.08 \pm 1.17$ ). During these regular field visits to our study populations, we recorded the morph identity, developmental color phase, and mating status (single or mating) of all females sampled over a measured time period (sampling times per population and visit: range, 15–180 min; mean  $\pm$  SD,  $67.90 \pm 28.75$  min). Sampling was conducted by one to three researchers on each occasion, who collected all encountered individuals with a hand net as they walked along a transect next to a breeding pond. Recently emerged adults typically complete their sexual maturation in the vegetation surrounding these breeding sites (Parr 1973), so our sampling procedures were effective in detecting individuals of both sexually immature and mature color phases. Mating females were brought into our indoor laboratory at the Biology Department in Lund and were housed individually in plastic containers with moist filter paper, providing a suitable substrate for oviposition. After 72 h the egg clutches were collected and photographed at high resolution, and the fertilized eggs were counted.

We compared mating probabilities and fecundities of immature and mature females of each morph using generalized mixed effects models fitted by Markov chain Monte Carlo (MCMC) in R (R Core Team 2018) with the package MCMCglmm (Hadfield 2010). Mating success was modeled as a categorical response variable with variation be-

tween populations and seasons treated as a random interaction effect. Fecundity, measured as the count of fertilized eggs, was modeled using a Poisson error distribution and controlling for the same random effect as in the previous analysis. Models were run for 2,000,000 iterations after an initial 250,000 iterations of burn-in, saving every 1,000th to avoid autocorrelation between draws. This resulted in an effective sample size of 2,000 iterations. MCMC performance in these and all subsequent analyses was evaluated by visually inspecting independent chains, calculating the autocorrelations between draws, and using the Gelman-Rubin convergence diagnostic in the R package coda (Plummer et al. 2006). We report the mean of the posterior distribution of model parameters and 95% highest posterior density intervals. As *P* values, we used the proportion of posterior values in which immature females had higher estimates than mature females.

#### *Experimental Manipulations of Abdomen Color*

We investigated experimentally whether developmental darkening of the blue abdominal patch in I- and O-females leads to an increase in premating male harassment and higher mating probabilities. We used field-caught individuals from populations within our study area, which covers approximately  $40 \times 40$  km<sup>2</sup>. All females used in this experiment were caught early in their immature color phase and were kept in large ( $3 \times 3 \times 3$  m<sup>3</sup>) outdoor enclosures to reduce the likelihood that they had mated before the experimental trials. We conducted observations of male mating harassment and female behavior in a temperature-controlled room at 25°C under a full spectrum lamp to simulate field conditions on a warm sunny day. These behavioral experiments were conducted by placing females in cylindrical cages 24 cm in diameter and 28 cm in height. The curved surface of the cylinders was made of fine mesh on which damselflies could perch, the bottom circle was made of green polyester, and the top circle, facing the observers, was made of clear plastic. We added three grass stems to these cages to provide perches for the damselflies and a plastic dish with water to provide moisture during the experimental trial.

In each trial, two females of the same morph and color phase were introduced to a single male. The eighth abdominal segment of one of the two females was experimentally manipulated to mismatch its current developmental stage. Thus, in the trials with immature females, the color signal of experimental “forward” females was artificially concealed, thereby mimicking the sexually mature female state. In contrast, in trials with two sexually mature I- or O-females, the experimental “reverse” female obtained an artificial blue patch painted on the eighth abdominal segment, thereby mimicking its juvenile developmental stage. Because A-females never undergo darkening of the blue-colored

patch, the experimental A-females in trials with both sexually immature and mature individuals were painted as forward, to determine whether such color change would increase male mating harassment in this morph. The second female in all trials was always used as pairwise control. This control female was painted with its actual color in order to account for the potential confounding effect of the paint manipulation per se. The use of such a sham control was justified in a pilot experiment that indicated that the paint manipulation did not affect male pre-mating harassment but may have reduced female activity during the trials (tables A2, A3; tables A1–A6, S1–S5 are available online). Experimental females and sham controls were painted with Essie nail polish “fierce no fear” for forward and “strut your stuff” for reverse (fig. A1; figs. A1–A3, S1–S12 are available online).

We performed twenty 1-h trials for each morph and developmental color phase. In the trials with sexually mature I- and O-females, we used individuals that had completely lost the blue abdominal patch. In the trials with A-females, we used individuals that had not visibly changed their thoracic coloration in the last 24 h. This classification of developmental stages was evaluated in a study that quantified the time to maturation of females of the different morphs (E. I. Svensson, B. Willink, M. C. Duryea, and L. Lancaster, unpublished data), and it helped us to minimize potentially harmful effects of handling of individuals prior to the experiment. All trials were conducted by one of three possible observers, systematically distributed among trial categories. The observers recorded the number of male approaches toward females, the number of female rejections, the number of male clasping attempts, and the number of successful copulations. These different male and female behaviors are defined in table A1.

If no successful mating attempts had occurred by the end of the trial, we immediately removed the male and covered his primary and secondary genitalia with UV-reflective non-toxic powder (for methodological details, see Joron and Brakefield 2003; Gosden and Svensson 2007, 2009). We put the males back into the cages, which were placed next to a pond with normal field temperature and light conditions. After 24 h we collected the females and examined them under UV light to record whether matings had occurred. In these tests, powder heavily concentrated around the female prothorax indicated a male attempt to form a tandem, whereas additional powder transferred to the female ovipositor provided evidence that a mating had occurred (Gosden and Svensson 2007, 2009; fig. A2).

These behavioral data were also analyzed using generalized mixed effects models fitted by MCMC. We used a Poisson distribution to test for an effect of the color treatment on the number of male approaches to females within each morph and age category. Because damselfly activity differed among trials and thereby potentially influenced

the number of intersexual interactions, we included trial session as a random effect to account for nonindependence of control and experimental females in the same trial. Only about 5% of all male approaches were not rejected by females and resulted in physical clasping attempts. Thus, we analyzed whether the probability of observing at least one mating attempt depended on female abdomen color, for each morph and age category and using a categorical response model. We also used this model family to analyze mating probability over 24 h. *P* values in this case indicate the proportion of posterior values in which the female with a blue patch (either experimental or control) had a larger estimate than the seemingly older female.

#### *Phylogenetic Comparative Analysis of the Evolution of Female Color Signals*

To reconstruct the evolutionary history of the blue abdomen patch in the genus *Ischnura*, we constructed a multilocus molecular phylogeny that included 41 of the approximately 75 species in this clade (see the appendix, available online). We obtained specimens from museum and field collections and sequenced five molecular markers including nuclear and mitochondrial regions (table A4). These novel sequence data were complemented with already-published sequences, including sequences for one more mitochondrial marker, that we downloaded from the National Center for Biotechnology Information (<https://www.ncbi.nlm.nih.gov/genbank/>). Alignment of protein-coding regions was unproblematic, as there were no indels in our data set. Ribosomal and intronic regions were aligned using the phylogenetically informed algorithm in PRANK in order to avoid gap overmatching (Löytynoja and Goldman 2005, 2008). Bayesian phylogenetic inference was conducted on the concatenated gene matrix using RevBayes version 1.0.7 (Höhna et al. 2016). We used a birth-death process as the species tree prior and a general time-reversible model of molecular evolution, assuming four gamma-distributed rate categories for each data partition. The tree branches were scaled using a fixed root age and a global molecular clock.

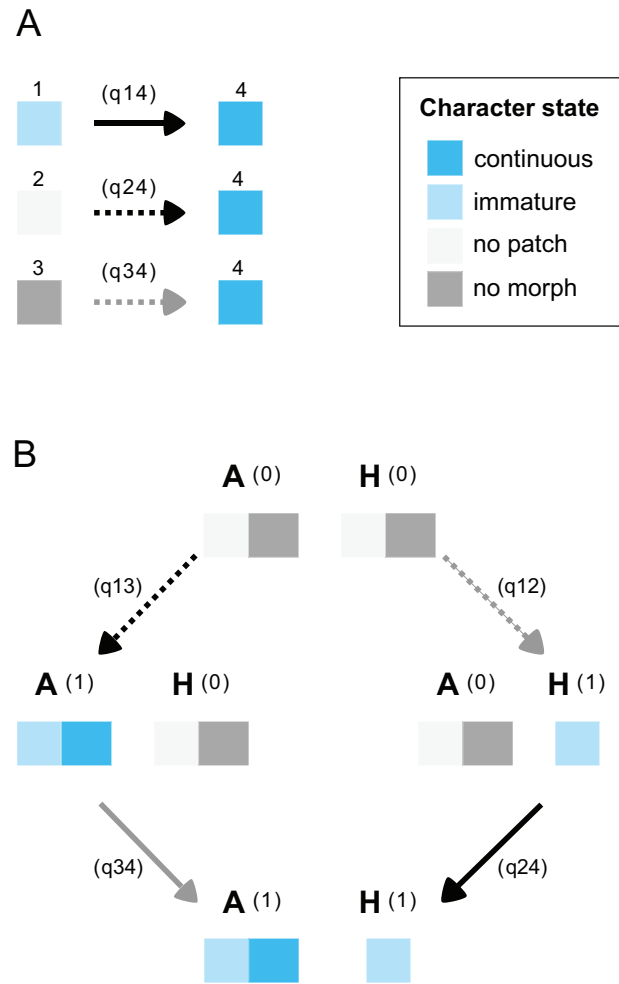
We collected phenotypic color data from published literature, examination of museum specimens, and field observations of some species. We recorded the presence of sex-limited color variation and developmental color variation in all of the species of *Ischnura* damselflies that were included in our phylogeny and in this study (table A6). We also searched the literature for information about sex- and morph-related color variation in the species of *Ischnura* for which molecular data were not available (table A6). Of the 75 species in this clade, 29 have two or three female morphs reported in the literature, and 30 either were described as monomorphic or at least no female-limited color variation has been reported. We were unable to obtain

a meaningful description of female color for 14 species, while the remaining two species are likely misidentifications (table A6). We classified the female morphs as androchrome (A-females) if their coloration and pattern was similar to males in both thorax and abdomen during at least part of the female adult life or as heterochrome (H-females) otherwise, following standard classifications of color morphs in *Ischnura* (Cordero et al. 1998; Svensson et al. 2009). Hence, in the three trimorphic species that were included in this study, I- and O-females were both classified as H-females.

For each species and female color morph, we recorded the presence or absence of a contrasting blue patch in the distal portion of the abdomen (eighth or ninth segments; fig. 1). We also recorded whether this color patch becomes concealed with age, either by pigmentation or by other mechanisms (e.g., pruinescence). Our phenotypic classification scheme resulted in a four-state categorization of female color development for each morph and species: (1) expression of the color patch only during the sexually immature color phase (“immature patch”); (2) no expression of a contrasting color patch at any point in adult life (“no patch”); (3) absence of the focal female morph, that is, the species is monomorphic for the alternative morph (“no morph”); and (4) continuous expression of the color patch throughout adult life (“continuous”).

We first used the phylogenetic inference on *Ischnura* mentioned above and this discrete character-state scheme to test the hypothesis that the continuous expression of the blue abdomen patch in sexual mature A-females has evolved via neoteny, that is, through the retention of an immature trait after sexual maturation. This hypothesis predicts that the continuous expression of the blue patch in A-females should be derived from an ancestral state of immature-only expression, as opposed to alternative ancestral states (i.e., no patch or no morph; fig. 2A). To assess support for this hypothesis, we modeled the evolution of the developmental expression of the blue patch in A-females using the multi-state method for discrete traits in BayesTraits version 3.0 (Pagel 1994; Pagel et al. 2004; Pagel and Meade 2017). Specifically, we asked whether there is evidence of a higher transition rate from immature expression to continuous expression of the blue patch compared with the two other transitions requiring de novo evolution of the continuous expression of the blue patch (i.e., transitions from no-patch and no-morph ancestors to continuous expression).

For this purpose, we used marginal likelihood approximation of evolutionary models with different parameter restrictions (Pagel and Meade 2017). The stepping-stone sampler approximates the marginal likelihood of a model over a series of points (“stones”) connecting the prior with the posterior (Xie et al. 2010). This approach allowed us to test hypotheses of whether two or more parameter estimates differ from each other by comparing the marginal



**Figure 2:** Phylogenetic hypotheses tested in this study. *A*, The continuous expression of a blue male-like patch in A-females of some species of *Ischnura* may have evolved via neoteny, that is, the retention of an immature trait after sexual maturation. If so, we predict that transitions toward a derived state of continuous expression occur at a higher rate when the ancestral state is immature expression of the color signal ( $q_{14}$ ) than when the ancestral state is either no patch ( $q_{24}$ ) or no morph ( $q_{34}$ ), as these transitions ( $q_{24}$  and  $q_{34}$ ) would involve de novo evolution of continuous expression of the blue patch. *B*, There are four possible scenarios of evolutionary covariance between female morphs in the origin of a blue patch. First, this color signal may arise independently in the two morphs. If so, the rate transition ( $q_{12}$ ) is equal to the rate of transition ( $q_{34}$ ), and likewise  $q_{13}$  equals  $q_{24}$ . Second, the expression of the blue patch in A-females may be contingent on the character state of H-females; hence,  $q_{13}$  and  $q_{24}$  differ from each other, but  $q_{12}$  and  $q_{34}$  remain equal. Similarly, if the origin of the blue patch in H-females is contingent on the character state of A-females but not vice versa,  $q_{12}$  and  $q_{34}$  would differ from each other but  $q_{13}$  and  $q_{24}$  would be equal. Finally, if the evolutionary origin of the blue patch is correlated between female morphs, both comparisons— $q_{13}$  versus  $q_{24}$  and  $q_{12}$  versus  $q_{34}$ —would be significant. We used marginal likelihood approximations of alternative evolutionary models to test which of these transition rate parameters differ from each other.

likelihood of a model where these parameters are set to be equal versus a model where they can vary. We used a stepping-stone sampler with 100 stones, each run for 100,000 iterations, and compared the marginal likelihood estimates, obtained in natural log scale, using Bayes factors. For each model, five independent runs were used to assess robustness of the stepping-stone sampler, and the median marginal likelihood was used to calculate Bayes factors (tables S4, S5). If a more parametrized model was supported, the posterior distribution of parameter estimates could then be used to determine which transition was inferred to occur at a higher rate. Here, we compared a restricted model where all transitions to continuous expression occur at equal rates, regardless of the ancestral state, to a series of models in which transitions from the immature-patch ancestral state occurred at a different rate than transitions from either other alternative or both other alternatives.

Finally, we tested whether the blue abdomen patch has originated independently or in a correlated fashion in A- and H-females across the *Ischnura* phylogeny. We used marginal likelihood approximation of alternative models in BayesTraits, as explained above, to address this question. To test for correlated evolution, character states were coded as binary for each morph. There were no extant taxa with continuous expression of the blue patch in H-females (see “Results”). Thus, immature-patch expression was counted as presence of the trait, and no morph and no patch were counted as absence of the blue patch in this morph. For A-females, we considered both immature-patch and continuous expression as presence of the trait, but in the supplemental material we present the results of analyses in which only continuous or immature-patch expression are counted as presence of the trait (table S5). Here, we compared an independent origin model in which there is a single rate of origin of the blue patch for each morph against three alternatives: (1) the origin of the blue patch in A-females is contingent on the character state of H-females, (2) the origin of the blue patch in H-females is contingent on the character state of A-females, and (3) the origin of the blue patch in each morph simultaneously depends on the character state of the other morph (fig. 2B). If any of the three alternative models was supported, we could then use the posterior distributions of parameter estimates in the supported model to determine whether the presence of the blue patch in one morph promotes or constrains the evolutionary origin of the trait in the other morph. Here, our focus was to test whether the origin of the blue patch depended on the character state of alternative and sympatric morphs. In our main analyses, we estimated four rates of backward transitions, one for each combination of character states between morphs. However, our results are statistically equivalent if a single rate of loss of the blue patch is enforced across categories (fig. S11).

Posterior distributions of rate estimates in all phylogenetic analysis were derived using MCMC algorithms run over 10,000,000 iterations sampling every 1,000th and with a burn-in of 2,500,000 iterations. Prior rate values were drawn from an exponential distribution with mean in turn drawn from a uniform hyperprior bounded between 0 and 50. We used this broad hyperprior to reduce arbitrariness in our prior specifications (Pagel et al. 2004; Pagel and Meade 2017). In each iteration, the proposed rate value was tried on a tree randomly drawn from a sample of 1,000 posterior trees. The trees were rescaled using a value of 0.001 to avoid rates from becoming very small (Pagel and Meade 2017). All data used in this study have been deposited in the Dryad Digital Repository (<https://doi.org/10.5061/dryad.d0bh91n>; Willink et al. 2019).

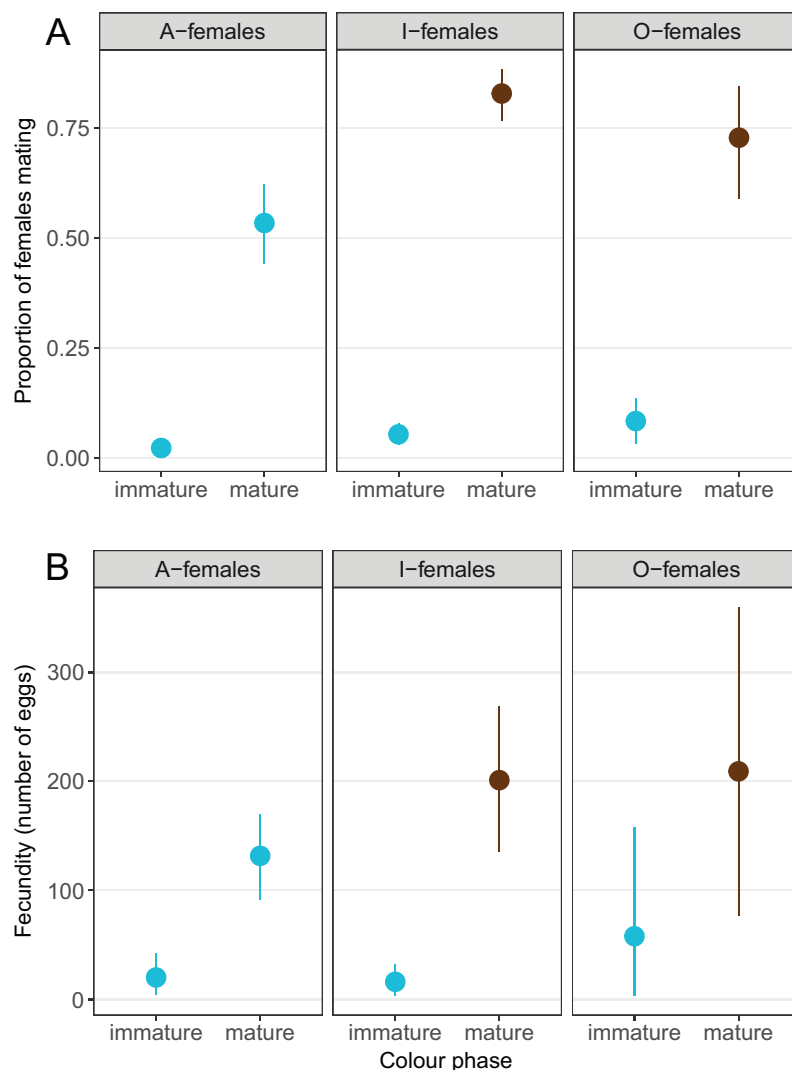
## Results

### *Mating Rates and Female Fecundities in the Field*

Over 3 years and across 16 populations in southern Sweden, we recorded the mating activity of 4,612 *Ischnura elegans* females ( $N_A = 3,071$ ,  $N_I = 1,306$ ,  $N_O = 235$ ), of which 2,275 were immature ( $N_A = 1,377$ ,  $N_I = 741$ ,  $N_O = 157$ ). Immature females of all three color morphs had lower mating probability than sexually mature females (all PMCMC < .001; fig. 3A). This age difference was even more pronounced for I-females than for either A-females (PMCMC = .041) or O-females (PMCMC = .035). We collected fecundity data for 1,197 females ( $N_A = 725$ ,  $N_I = 411$ ,  $N_O = 61$ ), of which 73 were immature ( $N_A = 28$ ,  $N_I = 34$ ,  $N_O = 11$ ). These immature females also had overall lower fecundity than mature females (PMCMC<sub>A</sub> < .001, PMCMC<sub>I</sub> < .001, PMCMC<sub>O</sub> = .032), although their fecundity was not necessarily always zero (fig. 3B). There was no evidence that the age effect on fecundity differed between female morphs (all PMCMC > .05).

### *Effects of Abdomen Color on Intersexual Interactions*

In our experimental manipulations of the female blue abdomen patch in *I. elegans*, we found that male approaches to perched females was the most common behavior across all of our trials ( $N = 20$  trials for each morph and age category). In all but one trial type, males approached the mature female phenotype significantly more often than the immature female phenotype (fig. 4). Our forward manipulation—in which a blue patch was artificially concealed, thereby advancing the apparent developmental state of females—increased the rate of male approaches in the two female morphs that naturally conceal the blue patch during their development (cf. fig. 1; PMCMC<sub>I</sub> = .016, PMCMC<sub>O</sub> = .006) as well as in the sexually mature A-females (PMCMC = .006) but not in the sexually immature A-females, which experienced



**Figure 3:** Mating rate (A) and fecundity (B) of *Ischnura elegans* females belonging to each one of the three heritable color morphs during their two developmental stages: immature and sexually mature. Field data come from 16 populations in southern Sweden. The three different female morphs have different color phases during ontogeny, including differences in the expression of a blue abdominal patch. Circles and whiskers represent the means and 95% highest posterior density intervals of posterior estimates. Female categories that exhibit a contrasting abdominal color patch are shown in blue, and female categories that have concealed this patch over development are shown in brown. Mature-colored females of all three morphs have higher mating rates in the field and higher fecundity than females expressing the immature color phase and the blue abdomen patch (all PMCMC  $\leq .050$ ).

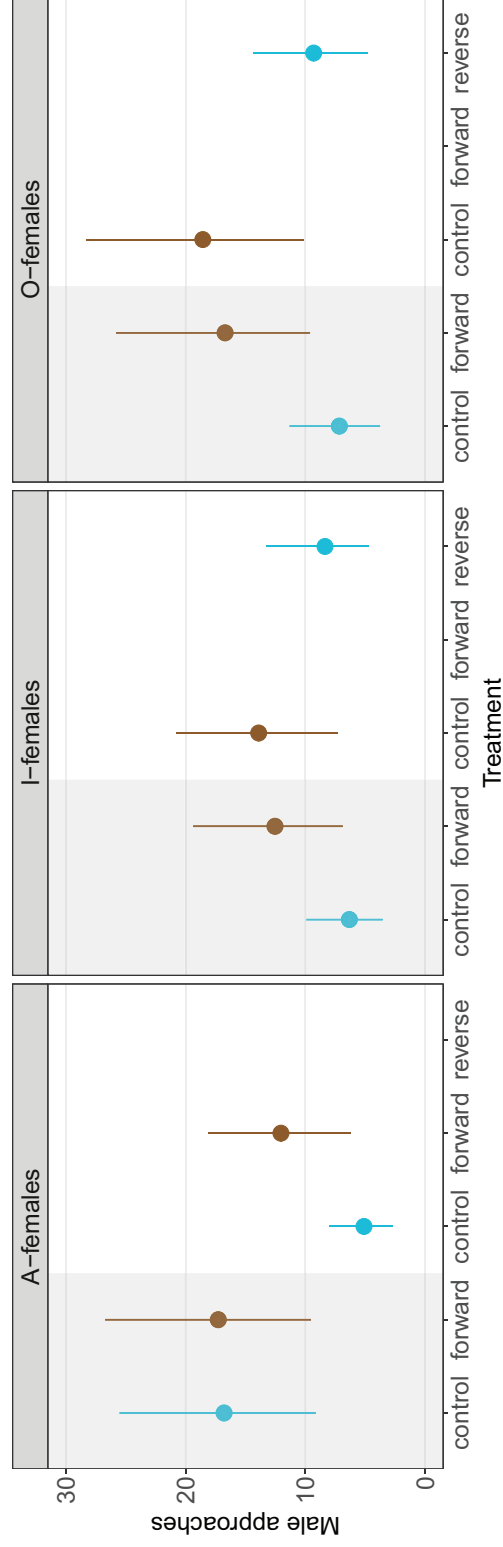
overall high approach rates (PMCMC = .463; fig. 4). In contrast, the reverse treatment, in which mature females were given an apparently younger developmental state, caused in both cases a reduction in male approaches compared with the control treatment (PMCMC<sub>I</sub> = .048, PMCMC<sub>O</sub> = .016). The experimental manipulation of abdominal color did not significantly affect the probabilities of male clasp attempts (fig. S6; table S1). Over 24 h, only the forward experimental manipulations had an effect on mating probability. Specifically, a sexually mature appearance increased mating probability in immature females and sexually mature A-females,

but experimentally reversing the signaling phenotype of sexually mature I- and O-females did not decrease their mating rate (fig. S7; table S2).

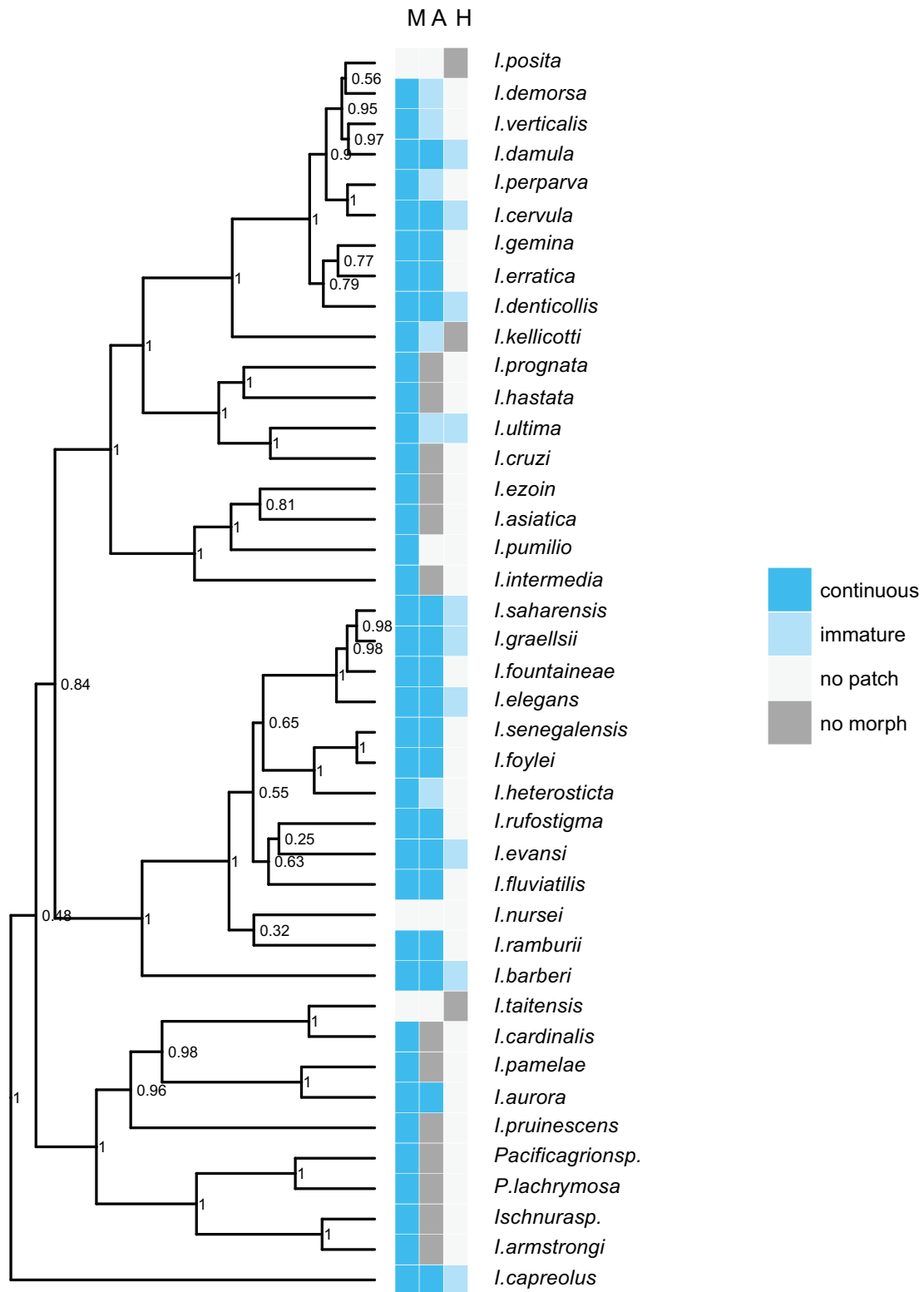
#### *Macroevolution of the Blue Patch as a Reproductive-Unsuitability Signal*

Our data revealed that the presence of the blue abdominal patch in females is phylogenetically widespread across *Ischnura* damselflies (fig. 5). Moreover, we found that the different female morphs often differ in whether developmental





**Figure 4:** Male approaches to females displaying immature and mature abdominal colorations. Experimental trials with immature females are shaded in gray. Control females display their actual coloration, forward females have been manipulated to resemble older females, and reverse females have been manipulated to show the coloration of younger individuals. Because A-females do not change their abdominal color throughout development (fig. 1), sexually mature A-females were manipulated in the same way as immature females. Circles and whiskers represent the means and 95% highest posterior density intervals of posterior estimates. Except for the comparison between control and forward immature A-females (PMCMC = .463), all manipulations resulted in increased premating harassment of seemingly mature females (all PMCMC  $\leq$  .050).



**Figure 5:** Phylogenetic distribution of developmental color changes in 41 species of *Ischnura* damselflies. For each sex and morph four character states are possible: no patch (the abdominal coloration is homogeneous throughout development), immature (the contrasting blue patch is expressed in sexually immature individuals only), continuous (the contrasting blue patch is expressed throughout development), and no morph (the focal color morph is absent and females are monomorphic for the alternative type). From left to right, the columns show character states for males (M), A-females (A), and H-females (H). Numbers indicate the posterior probability of each node in the maximum a posteriori tree. All comparative analyses were performed using a randomly drawn sample of 1,000 posterior trees.

**Table 1:** Marginal likelihood comparison between alternative models for the evolution of continuous blue patch expression in the distal abdominal segments of the male-colored female morph in the genus *Ischnura* (A-females)

Model description	Model restriction(s)	Marginal likelihood	Bayes factor versus model A	Support for less restricted model
A. All transition rates equal	$q_{14} = q_{24} = q_{34}$	-48.373	...	...
B. Immature patch different from alternative states, alternative states equal to each other	$q_{24} = q_{34}$	-48.056	.634	Weak
C. Immature patch different from alternative states, alternative states different from each other	No restrictions	-47.961	.824	Weak
D. Immature patch different only from no morph	$q_{14} = q_{24}$	-47.737	1.272	Weak
E. Immature patch different only from no patch	$q_{14} = q_{34}$	-48.528	-.310	Negative

Note: A restricted model (A) in which all possible ancestral states occur with equal probability was sequentially compared via Bayes factors against alternative models (B–E), in which the transition rates toward a derived state of continuous expression of the blue patch depended on the ancestral state. In model B the transition rate from an immature patch to continuous expression ( $q_{14}$ ) differed from both transition rates involving de novo evolution of continuous expression—no patch to continuous expression ( $q_{24}$ ) and no morph to continuous expression ( $q_{34}$ )—and the rates of  $q_{24}$  and  $q_{34}$  were equal. In model C the transition rate  $q_{14}$  was also different from the other two possible transitions toward continuous expression ( $q_{24}$  and  $q_{34}$ ), but these transition rates differed from each other. In model D  $q_{14}$  differed only from  $q_{34}$ , and in model E it differed only from  $q_{24}$ . Interpretation of log Bayes factors is based on Raftery (1996), as follows:  $<0$  = negative evidence;  $0-2$  = weak evidence (barely worth mentioning);  $2-5$  = positive evidence;  $5-10$  = strong evidence; and  $>10$  = very strong evidence.

color changes affect the expression of this female color signal (fig. 5). If the blue patch is present in H-females at all, it is always restricted to the immature color phase (fig. 5). In contrast, A-females of several species express this color trait throughout their entire postemergence life (fig. 5).

We did not find support for the hypothesis that immature expression of the abdominal blue patch precedes the evolution of its continuous expression in A-females (table 1). Comparison of alternative multistate models did not provide evidence that the transition to continuous expression of the blue patch occurred at a different rate between ancestors with immature expression of the trait and ancestors completely lacking the trait (no morph and no patch; table 1). We obtained qualitatively similar results if we treated monomorphic species with no A-females as missing data rather than as an alternative state (table S4). In contrast, we found that the evolutionary origin of the blue abdomen patch in H-females was contingent on the character state of A-females

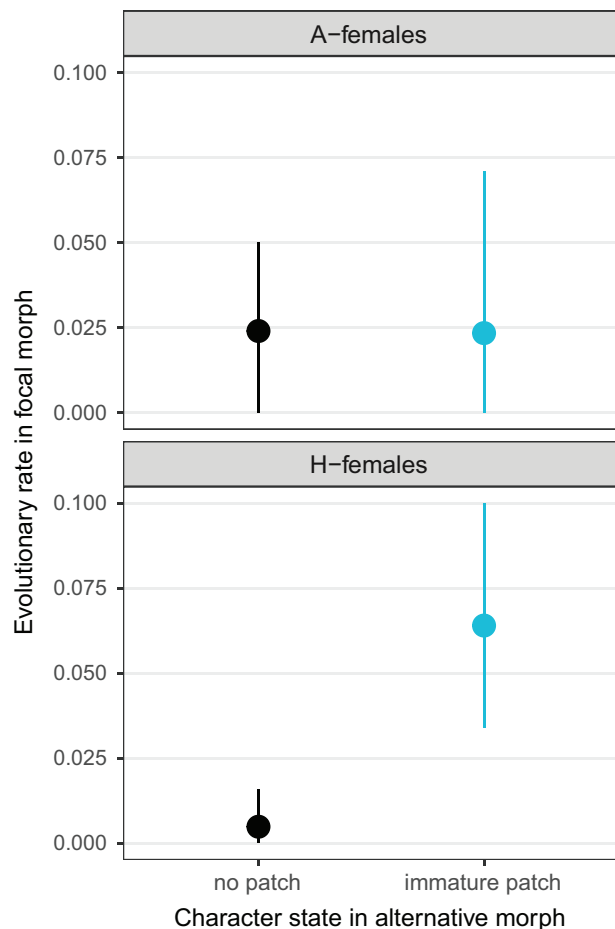
but not vice versa (table 2). H-females are more likely to gain a blue color patch in their immature state when this trait is already present in A-females (fig. 6). Lack of support for the correlated origin model over the contingent origin in H-females model (BF =  $-0.152$ ; table 2) instead suggests that A-females evolve the blue color patch independently of the character state in H-females (fig. 6; table 2).

We followed up this result with a simulation approach to investigate whether support for the contingency of blue patch evolution in H-females on A-female character state emerged spuriously due to the relatively small size of the *Ischnura* phylogeny. We used the posterior distribution of transition rate estimates from the independent origin model and the same sample of posterior trees to simulate 500 evolutionary histories. Then we asked in how many of these data sets would we erroneously infer a similar pattern as in the empirical data, where the origin of the trait in H-females depended on the character state of A-females but not vice versa

**Table 2:** Marginal likelihood comparison between alternative models for the evolution of a blue patch on the distal abdominal segments in A- and H-females of *Ischnura* damselflies

Model description	Model restrictions	Marginal likelihood	Bayes factor versus model A	Support for less restricted model
A. Both morphs independent	$q_{12} = q_{34}; q_{13} = q_{24}$	-49.109	...	...
B. Origin in A-females contingent on H-females	$q_{12} = q_{34}$	-48.989	.240	Weak
C. Origin in H-females contingent on A-females	$q_{13} = q_{24}$	-44.628	8.722	Strong
D. Morphs correlated	No restrictions	-44.704	8.570	Strong

Note: A model in which the evolutionary origin of the blue patch is independent between female morphs was sequentially compared, via Bayes factors, to alternative models in which the origin of the trait in at least one female morph depended on the character state of the other. In model B the origin of the blue patch in A-females is contingent on the character state of H-females, in model C vice versa, and in model D the presence of the trait is correlated between morphs. The evolutionary transitions in these alternative models are illustrated in figure 2B. Interpretation of log Bayes factors is based on Raftery (1996), as follows:  $<0$  = negative evidence;  $0-2$  = weak evidence (barely worth mentioning);  $2-5$  = positive evidence;  $5-10$  = strong evidence; and  $>10$  = very strong evidence.



**Figure 6:** Posterior mean and 95% highest posterior density intervals for the rates of evolutionary origin of the blue abdominal patch in A- and H-female morphs across the *Ischnura* phylogeny. The rates are estimated using an unrestricted model of correlated evolution between discrete traits (model D in table 2). *A*, We found no evidence for a higher rate of origin of the blue patch in A-females when the trait is present in H-females (PMCMC = .560). *B*, In contrast, there is strong support for a higher rate of origin of the immature blue patch in H-females when A-females already express the signaling trait at any point in their development (PMCMC < .001).

(see the supplemental material). This analysis indicated that our type I error was low, as only 1.4% of the simulated data sets would have lend support to the contingent origin model (fig. S12).

### Discussion

The results in this study suggest that conspicuous female color signals of reproductive unsuitability have important functions in reducing male mating harassment (figs. 3, 4) and that these color signals might have evolved in H-females through the co-option of a trait with signaling functions in

males and male-colored A-females (figs. 5, 6). Coupling phylogenetic comparative analyses of macroevolutionary patterns with experimental manipulations of focal traits is a powerful approach to investigate the mechanisms underlying phenotypic evolution (Sinervo and Basolo 1996; Weber and Agrawal 2012). Here, we have combined such phylogenetic comparative analyses across the damselfly genus *Ischnura* (tables 1, 2; figs. 5, 6) with experimental phenotypic manipulations of a female color signal (fig. 4) to clarify the adaptive significance of the blue abdomen patch and understand its macroevolutionary origin.

We experimentally documented a novel function of a female signal in the common bluetail (*Ischnura elegans*). In contrast to the traditional view of female color signals as selected by male choice in species with sex role reversal or biparental care (e.g., Amundsen 2000; Clutton-Brock 2009), the blue abdomen patch in *I. elegans* females seems to advertise mating unavailability, either prior to sexual maturation (I- and O-females) or continuously throughout development (A-females). Our experimental phenotypic manipulations (fig. 4) suggest that this female color signal is partly responsible for active male discrimination against sexually immature I- and O-females (figs. 4, S7). Yet while the appearance of a sexually mature status increased the rate of approaches by males (fig. 4), these approaches did not result in a higher probability of physical mating attempts (table S1; fig. S6) and had a only a modest effect on the probability of mating (table S2; fig. S7). This may seem surprising given that immature-colored females in the field have markedly lower mating rates (fig. 3). However, mating rates in the field reflect more than just male mating preferences and are certainly also affected by female avoidance behaviors and microhabitat selection (Van Gossum et al. 2001; Bots et al. 2015).

Male pond damselflies (family Coenagrionidae, in which the genus *Ischnura* is nested) approach females from behind and above when females are either flying above the water or perching from vertical stems (Bick and Bick 1963). Therefore, a bright and contrasting blue color signal displayed dorsally on the abdomen of the female (fig. 1) is the first color signal that a passing male will detect, and it will likely influence his decision to approach a female. Other elements of female coloration, such as thorax color, are likely to be detected only on closer examination, and female behavior may then influence the probability that a male approach results in mating (Bots et al. 2015). Nonetheless, premating male harassment, which is initiated by male approaches, can have negative consequences for female fitness, and because it depends more directly on male preferences it could be the primary driver of negative frequency-dependent selection on female morphs (Gosden and Svensson 2009; Bots et al. 2015).

In the damselfly genus *Ischnura*, H-females of several species exhibit another developmental color change, namely,

the darkening of bright yellow-orange thoracic coloration (table A6). This change may occur in H-females that do not display the blue abdominal patch at any point in development. Thorax color might be used by males as an alternative or additional cue of female sexual maturity (Fincke 1987; Langenbach 1993), and it may indicate unsuitability for mating (Takahashi and Watanabe 2011). Therefore, the joint presence of a blue abdomen patch and juvenile thorax color in females could contribute to reduced premating harassment, although it remains unclear why some species and female morphs exhibit only one of these two immature signals and why some express both traits simultaneously.

Unlike H-females, A-females can express the blue abdomen patch either only when immature or throughout their entire postemergence life. This signaling trait could play a similar role in immature females of all morphs, but sexually mature A-females of some species may benefit from the continuous expression of this signal throughout development by deceiving males about their sexually mature status. However, experimental and phylogenetic results do not support this hypothesis and instead point to distinct functions of the blue abdomen patch in A- and H-females. We suggest that the blue abdomen patch in sexually mature A-females has been maintained because, together with the thorax coloration, it makes male mimicry more effective and reduces male mate recognition. In support of this interpretation, we found that a phenotypic manipulation concealing the blue patch in sexually mature A-females disrupted male mimicry and increased male harassment. However, advancing the apparent developmental stage of immature A-females did not result in increased male approaches, unlike the other two female morphs (fig. 4). Furthermore, the phylogenetic comparative analyses provided no evidence that the continuous expression of the blue patch in sexually mature A-females has primarily evolved through the retention of an immature trait after sexual maturation (table 1). The continuous expression of the blue abdomen patch in A-females is therefore unlikely to exploit a preexistent immature signal.

The blue abdomen patch presumably has an ancestral adaptive function in male-male agonistic signaling, as suggested by experimental results in a recent study of male coenagrionid damselflies (Beatty et al. 2015). Consistent with such an ancestral adaptive male function, our comparative data indicate that the blue abdominal patch is indeed phylogenetically widespread in males (fig. 5). Interestingly, this blue abdominal patch is absent in males of only three extant taxa out of 41 included in our phylogeny (fig. 5) and only five taxa out of 66 for which male color descriptions were available (table A6). This suggests that selection due to male-male competition maintains this signaling trait in males. It is also suggestive that in those three species in which males lack the blue abdomen patch, the blue patch is also completely absent in females. Finally, 28 of the 38 females with

H-morphs do not express any blue patch on their abdomen at all during their entire life, and the remaining 10 express it only when immature and notably only when the A-females also express this signal at some point in their development (fig. 5).

Our phylogenetic comparative analyses further suggest that expressing the blue patch during the immature stage in H-females is a general mechanism to reduce premating harassment (fig. 6B; table 2). Strikingly, the evolutionary origin of a blue abdomen patch in H-females was restricted to lineages in which A-females already exhibit this trait but not vice versa (figs. 5, 6). This might reflect phylogenetic variation in the intensity of conflict over mating rates if both male-mimicking morphs and immature signals in H-females are more likely to arise and to be maintained in lineages with pervasive sexual conflict. Alternatively, it is also possible that the presence of A-females in a population increases male premating harassment toward H-females of all ages. If male mimicry is effective, the rate at which males attempt to mate with A-females on an encounter should be lower than the rate at which they attempt to mate with an H-female under the same circumstances (Sherratt 2001). In fact, experimental studies in another female-polymorphic damselfly, *Enallagma hageni*, suggest that males are more likely to initiate a mating attempt toward heteromorphic females precisely because they are markedly different from males and even if their color pattern is novel for the species (Xu et al. 2014). By reducing the proportion of females that are perceived as such by males, the presence of A-females may thus bias male harassment toward H-females, with most adverse effects prior to reproductive development. In this scenario, the social effects of A-females would favor the expression of honest signals of reproductive unsuitability in sexually immature H-females.

An outstanding question is how have H-females repeatedly evolved an immature patch in different clades within the *Ischnura* phylogeny (fig. 5). In her seminal book on developmental plasticity and evolution, West-Eberhard (2003) speculated that the male-like traits in female *Ischnura* damselflies might have evolved through a process of reorganization of developmental sex expression with limited genetic change, termed cross-sexual transfer. Through such process, a trait that originally evolved in one sex could become co-opted for a novel function in the other sex (Bonduriansky 2011). In insects, the transcription factor *doublesex* (*dsx*) controls sex specificity in the expression of multiple genes (Kopp 2012). Recent studies in female-polymorphic butterflies (*Papilio polytes*) and damselflies (*Ischnura senegalensis*) have shown that color development in A-females is characterized by a male-like expression pattern of splice variants of *dsx* (Kunte et al. 2014; Takahashi et al. 2018). These studies suggest that cross-sexual transfer, through the masculinization of regulatory gene expression, could occur in several

different color polymorphic insect taxa with male-like females, including the genus *Ischnura*. It is therefore possible that instead of evolving a temporary blue abdomen patch de novo, H-females in different *Ischnura* lineages have acquired this trait by modulating the expression of regulatory genes underlying sex specificity, but in a more restricted location and developmental period than in A-females.

Rapid evolutionary turnover of the blue patch precludes ancestral state reconstruction with high confidence in H-females (fig. 6B; table S3). However, our data strongly suggest that the blue patch has an adaptive function in H-females and that the benefit of this trait depends on a social environment that requires the existence of A-females. In A-females, the blue abdomen patch might have originally evolved as a correlated response to selection on males when this male-mimicking morph arose. Subsequently, this blue patch presumably became co-opted and maintained in A-females as a mating-harassment reduction mechanism. Immature H-females might have gained this blue abdomen patch in some of the lineages in which A-females occurred. Selection driven by sexual conflict on these H-females would have then lead to further co-option of this signal as a mechanism to reduce pre-mating harassment prior to sexual maturity. These results suggest a highly dynamic scenario of color evolution in the genus *Ischnura*, with a potential role for cross-sexual and even cross-morph transfer. One way to test this hypothesis in the future is to determine whether immature color signals in H-females are produced by temporal and local expression of the molecular processes that produce the blue abdomen patch in males and A-females.

### Conclusions

Our field data and experimental and comparative analyses strongly suggest that color changes in heterochromatic females (H-females) are associated with the onset of reproductive capacity and signal sexual suitability and have evolved by exploiting a male bias against a blue abdomen patch in the genus *Ischnura*. Our study adds to the accumulating empirical evidence for female color signals used in intersexual interactions (Rowland et al. 1991; Weiss 2002; Setchell et al. 2006; Barelli et al. 2007; Cornwallis and Birkhead 2007; Baldauf et al. 2011; Higham et al. 2012; LaPlante 2015). The narrow-sense theoretical framework for the evolution of male signals (intrasexual competition and mate choice) is clearly insufficient to explain the design and variation of these female signals, particularly in systems characterized by pervasive sexual conflict. The questions of how and why females evolve conspicuous signals under traditional sex roles will require a deeper understanding of how development and mating status influence sexually antagonistic interactions. Moreover, linking experiments on focal species with phylogenetic macroevolutionary analyses (Weber and Agra-

wal 2012) and incorporating information about the developmental basis of color signals will increase our understanding of both the adaptive significance and the evolutionary history of such sexually antagonistic traits and their influence on coevolutionary dynamics between males and females.

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### Literature Cited

- Abbott, J. K., and E. I. Svensson. 2010. Morph-specific variation in intersexual genetic correlations in an intra-specific mimicry system. *Evolutionary Ecology Research* 12:105–118.
- Amundsen, T. 2000. Why are female birds ornamented? *Trends in Ecology and Evolution* 15:149–155.
- Arnqvist, G., and T. Nilsson. 2000. The evolution of polyandry: multiple mating and female fitness in insects. *Animal Behavior* 60:145–164.
- Baldauf, S. A., T. C. M. Bakker, H. Kullmann, and T. Thünken. 2011. Female nuptial coloration and its adaptive significance in a mutual mate choice system. *Behavioral Ecology* 22:478–485.
- Barelli, C., M. Heistermann, C. Boesch, and U. H. Reichard. 2007. Sexual swellings in wild white-handed gibbon females (*Hylobates lar*) indicate the probability of ovulation. *Hormones and Behavior* 51:221–230.
- Barry, K. L., and H. Kokko. 2010. Male mate choice: why sequential choice can make its evolution difficult. *Animal Behaviour* 80:163–169.

- Beatty, C. D., J. A. Andrés, and T. N. Sherratt. 2015. Conspicuous coloration in males of the damselfly *Nehalennia irene* (Zygoptera: Coenagrionidae): do males signal their unprofitability to other males? *PLoS ONE* 10:e0142684.
- Belliure, J., B. Fresnillo, and J. J. Cuervo. 2018. Male mate choice based on female coloration in a lizard: the role of a juvenile trait. *Behavioural Ecology* 29:543–552.
- Bick, G. H., and J. C. Bick. 1963. Behavior and population structure of the damselfly, *Enallagma civile* (Hagen) (Odonata: Coenagrionidae). *Southwestern Naturalist* 8:57–84.
- Blanckenhorn, W. U., D. J. Hosken, O. Y. Martin, C. Reim, Y. Teuschl, and P. I. Ward. 2002. The costs of copulating in the dung fly *Sepsis cynipsea*. *Behavioral Ecology* 13:353–358.
- Bonduriansky, R. 2011. Sexual selection and conflict as engines of ecological diversification. *American Naturalist* 178:729–745.
- Bonduriansky, R., and S. F. Chenoweth. 2009. Intralocus sexual conflict. *Trends in Ecology and Evolution* 24:280–288.
- Bots, J., A. Iserbyt, H. Van Gossum, M. Hammers, and T. N. Sherratt. 2015. Frequency-dependent selection on female morphs driven by premating interactions with males. *American Naturalist* 186:141–150.
- Calvert, P. P. 1898. Odonata (dragonflies) from the Indian Ocean, and from Kashmir, collected by Dr. W. L. Abbott. *Proceedings of the Academy of Natural Sciences of Philadelphia* 50:141–154.
- Chapman, T., G. Arnqvist, J. Bangham, and L. Rowe. 2003. Sexual conflict. *Trends in Ecology and Evolution* 18:41–47.
- Clausnitzer, V., and K. D. B. Dijkstra. 2005. The dragonflies (Odonata) of Ethiopia, with notes on the status of endemic taxa and the description of a new species. *Entomologische Zeitschrift* 115:117–130.
- Clutton-Brock, T. 2009. Sexual selection in females. *Animal Behavior* 77:3–11.
- Colgan, D., A. McLauchlan, G. Wilson, S. Livingston, G. Edgecombe, J. Macaranas, G. Cassis, and M. Gray. 1998. Histone H3 and U2 snRNA DNA sequences and arthropod molecular evolution. *Australian Journal of Zoology* 46:419–437.
- Cook, S. E., J. G. Vernon, M. Bateson, and T. Guilford. 1994. Mate choice in the polymorphic African swallowtail butterfly, *Papilio dardanus*: male-like females may avoid sexual harassment. *Animal Behaviour* 47:389–397.
- Corbet, P. S. 1999. Dragonflies: behavior and ecology of Odonata. Harley, Colchester.
- Cordero, A. 1990. The inheritance of female polymorphism in the damselfly *Ischnura graellsii* (Rambur) (Odonata: Coenagrionidae). *Heredity* 64:341–346.
- Cordero, A., S. S. Carbone, and C. Utzeri. 1998. Mating opportunities and mating costs are reduced in androchrome female damselflies, *Ischnura elegans* (Odonata). *Animal Behavior* 55:185–197.
- Cornwallis, C. K., and T. R. Birkhead. 2007. Experimental evidence that female ornamentation increases the acquisition of sperm and signals fecundity. *Proceedings of the Royal Society B* 274:583–590.
- Crudgington, H. S., and M. T. Siva-Jothy. 2000. Genital damage, kicking and early death. *Nature* 407:855–856.
- Dijkstra, K. D. B., V. J. Kalkman, R. A. Dow, F. R. Stokvis, and J. Van Tol. 2014. Redefining the damselfly families: a comprehensive molecular phylogeny of Zygoptera (Odonata). *Systematic Entomology* 39:68–96.
- Dijkstra, K. D. B., and R. Lewington. 2006. Field guide to the dragonflies of Britain and Europe: including western Turkey and north-western Africa. British Wildlife Publishing, Dorset.
- Dumont, H. J. 1974. *Ischnura intermedia* spec. nov. from Turkey, and its relations to *I. forcipata* Morton, 1907 and *I. pumilio* (Charpentier, 1825) (Anisoptera: Coenagrionidae). *Odonatologica* 3:153–165.
- Edward, D. A., and T. Chapman. 2011. The evolution and significance of male mate choice. *Trends in Ecology and Evolution* 26:647–654.
- Englund, R. A., and D. A. Polhemus. 2010. A review of the damselfly fauna of the Austral Islands, French Polynesia, with descriptions of two new species (Odonata: Zygoptera: Coenagrionidae). *Tijdschrift voor Entomologie* 153:25–40.
- Fedorka, K. M., and M. Zuk. 2005. Sexual conflict and female immune suppression in the cricket, *Allonemobius socius*. *Journal of Evolutionary Biology* 18:1515–1522.
- Fedorka, K. M., M. Zuk, and T. A. Mousseau. 2004. Immune suppression and the cost of reproduction in the ground cricket, *Allonemobius socius*. *Evolution* 58:2478–2485.
- Ferreira, S., M. O. Lorenzo-Carballa, Y. Torres-Cambas, A. Cordero-Rivera, D. J. Thompson, and P. C. Watts. 2014. New EPIC nuclear DNA sequence markers to improve the resolution of phylogeographic studies of coenagrionids and other odonates. *International Journal of Odonatology* 17:135–147.
- Fincke, O. M. 1987. Female monogamy in the damselfly *Ischnura verticalis* Say (Zygoptera: Coenagrionidae). *Odonatologica* 16:129–143.
- Fincke, O. M., R. Jödicke, D. R. Paulson, and T. D. Schultz. 2005. 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.
- Fitzpatrick, C. L., and M. R. Servodio. 2017. Male mate choice, male quality, and the potential for sexual selection on female traits under polygyny. *Evolution* 71:174–183.
- Fraser, F. 1949. The Zygoptera of Mauritius (order Odonata). *Transactions of the Royal Entomological Society of London* 100:135–146.
- . 1953. Further notes on Samoan Odonata belonging to the Ischnurine complex of species, with descriptions of two new species and some unknown females. *Proceedings of the Royal Entomological Society of London B: Taxonomy* 22:119–126.
- Futahashi, R., R. Kurita, H. Mano, and T. Fukatsu. 2012. Redox alters yellow dragonflies into red. *Proceedings of the National Academy of Sciences of the USA* 109:12626–12631.
- Gering, E. J. 2017. Male-mimicking females increase male-male interactions, and decrease male survival and condition in a female-polymorphic damselfly. *Evolution* 71:1390–1396.
- Gosden, T. P., and E. I. Svensson. 2007. Female sexual polymorphism and fecundity consequences of male mating harassment in the wild. *PLoS ONE* 2:e580.
- . 2009. Density-dependent male mating harassment, female resistance, and male mimicry. *American Naturalist* 173:709–721.
- Hadfield, J. D. 2010. MCMC methods for multi-response generalized linear mixed models: the MCMCglmm R package. *Journal of Statistical Software* 33:1–22.
- Haritonov, A. Y. 1988. Strekozy roda *Ischnura* Charp. (Insecta: Odonata) fauny SSSR. Pages 32–46 in G. S. Zolotarenko, ed. *Taksonomiya zhivotnyh Sibiri*. Nauka, Novosibirsk.
- Harshman, L. G., and A. J. Zera. 2007. The cost of reproduction: the devil in the details. *Trends in Ecology and Evolution* 22:80–86.
- Hawkins, G. L., G. E. Hill, and A. Mercadante. 2012. Delayed plumage maturation and delayed reproductive investment in birds. *Biological Reviews* 87:257–274.

- Heckman, C. 2008. *Encyclopedia of South American aquatic insects: Odonata-Zygoptera*. Springer, Washington, DC.
- Higham, J. P., M. Heistermann, C. Saggau, M. Agil, D. Perwitasari-Farajallah, and A. Engelhardt. 2012. Sexual signalling in female crested macaques and the evolution of primate fertility signals. *BMC Evolutionary Biology* 12:1.
- Höhna, S., M. J. Landis, T. A. Heath, B. Boussau, N. Lartillot, B. R. Moore, J. P. Huelsenbeck, and F. Ronquist. 2016. RevBayes: Bayesian phylogenetic inference using graphical models and an interactive model-specification language. *Systematic Biology* 65:726–736.
- Huang, S. C., and J. Reinhard. 2012. Color change from male-mimic to gynomorphic: a new aspect of signaling sexual status in damselflies (Odonata, Zygoptera). *Behavioral Ecology* 23:1269–1275.
- Johnson, C. 1964. The inheritance of female dimorphism in the damselfly, *Ischnura damula*. *Genetics* 49:513.
- . 1966. Genetics of female dimorphism in *Ischnura demorsa*. *Heredity* 21:453–459.
- Joron, M., and P. M. Brakefield. 2003. Captivity masks inbreeding effects on male mating success in butterflies. *Nature* 424:191–194.
- Joshi, S., P. Koparde, K. Subramanian, and P. Roy, eds. 2016. *Odonata of India version 1.10*. Accessed November 30, 2016. <http://www.indianodonata.org>.
- Kalkman, V. J., and A. G. Orr. 2013. *Field guide to the damselflies of New Guinea*. Nederlandse Vereniging voor Libellenstudie, Netherlands.
- Kambhampati, S. 1995. A phylogeny of cockroaches and related insects based on DNA sequence of mitochondrial ribosomal RNA genes. *Proceedings of the National Academy of Sciences of the USA* 92:2017–2020.
- Kjer, K. M., R. J. Blahnik, and R. W. Holzenthal. 2001. Phylogeny of Trichoptera (caddisflies): characterization of signal and noise within multiple datasets. *Systematic Biology* 50:781–816.
- Kleemann, G. A., and A. L. Basolo. 2007. Facultative decrease in mating resistance in hermaphroditic *Caenorhabditis elegans* with self-sperm depletion. *Animal Behavior* 74:1339–1347.
- Kokko, H., and R. A. Johnstone. 2002. Why is mutual mate choice not the norm? operational sex ratios, sex roles and the evolution of sexually dimorphic and monomorphic signalling. *Philosophical Transactions of the Royal Society B* 357:319–330.
- Kompier, T. 2015. *A guide to the dragonflies and damselflies of the Serra dos Orgaos, south-eastern Brazil: Guia dos Anisoptera e Zygoptera Da Serra dos Órgãos, Sudeste do Brasil*. REGUA Publications, Belgium.
- Kopp, A. 2012. *Dmrt* genes in the development and evolution of sexual dimorphism. *Trends in Genetics* 28:175–184.
- Kosterin, O. E. 2015. *Ischnura foylei* sp. nov. (Odonata, Coenagrionidae) from the highlands of Sumatra. *Zootaxa* 4032:179–189.
- Kunte, K., W. Zhang, A. Tenger-Trolander, D. Palmer, A. Martin, R. Reed, S. Mullen, and M. Kronforst. 2014. *Doublesex* is a mimicry supergene. *Nature* 507:229–232.
- Lande, R. 1980. Sexual dimorphism, sexual selection, and adaptation in polygenic characters. *Evolution* 34:292–305.
- Langenbach, A. 1993. Time of color change in female *Ischnura pumilio* (Charpentier) (Zygoptera: Coenagrionidae). *Odonatologica* 22:469–477.
- LaPlante, L. H. 2015. Female belly color and bobbing behavior advertise sexual receptivity in a pelagic-spawning coral reef fish. *Behavior* 152:705–725.
- Le Rouzic, A., T. F. Hansen, T. P. Gosden, and E. I. Svensson. 2015. Evolutionary time-series analysis reveals the signature of frequency-dependent selection on a female mating polymorphism. *American Naturalist* 185:E182–E196.
- Long, T. A. F., A. Pischedda, R. V. Nichols, and W. R. Rice. 2010. The timing of mating influences reproductive success in *Drosophila melanogaster*: implications for sexual conflict. *Journal of Evolutionary Biology* 23:1024–1032.
- Lohmann, H. 1979. A new species of *Ischnura* from New Guinea (Zygoptera: Coenagrionidae). *Odonatologica* 8:221–225.
- Löytynoja, A., and N. Goldman. 2005. An algorithm for progressive multiple alignment of sequences with insertions. *Proceedings of the National Academy of Sciences of the USA* 102:10557–10562.
- . 2008. Phylogeny-aware gap placement prevents errors in sequence alignment and evolutionary analysis. *Science* 320:1632–1635.
- Machado, A. 2012. A new species of *Ischnura* (Odonata: Coenagrionidae) from high altitude eastern Andes, of Colombia. *Zoologia (Curitiba)* 29:598–600.
- Maklakov, A. A., N. Kremer, and G. Arnqvist. 2007. The effects of age at mating on female life-history traits in a seed beetle. *Behavioral Ecology* 18:551–555.
- Marinov, M., O. FossatiGaschignard, and M. Schorr. 2016. Faunistic studies in Southeast Asian and Pacific island Odonata. *Journal of the International Dragonfly Fund* 18:1–12.
- Marinov, M., M. Schmaedick, D. Polhemus, and R. L. Stirne. 2015. Faunistic and taxonomic investigations on the Odonata fauna of the Samoan archipelago with particular focus on taxonomic ambiguities in the “Ischnurine complex.” *Journal of the International Dragonfly Fund* 91:1–56.
- Maynard Smith, J. 1994. Must reliable signals always be costly? *Animal Behavior* 47:1115–1120.
- Museum of Natural History, ed. 2003–2018. *National Inventory of Natural Heritage*. Accessed June 2, 2017. <https://inpn.mnhn.fr>.
- Neff, B. D., and E. I. Svensson. 2013. Polyandry and alternative mating tactics. *Philosophical Transactions of the Royal Society B* 368:20120045.
- Ozono, A., I. Kawashima, and R. Futahiashi. 2012. *Dragonflies of Japan*. Bunichi-Sogo Syuppan, Tokyo.
- Pagel, M. 1994. Detecting correlated evolution on phylogenies: a general method for the comparative analysis of discrete characters. *Proceedings of the Royal Society B* 255:37–45.
- Pagel, M., and A. Meade, eds. 2017. *BayesTraits version 3*. <http://www.evolution.rdg.ac.uk>.
- Pagel, M., A. Meade, and D. Barker. 2004. Bayesian estimation of ancestral character states on phylogenies. *Systematic Biology* 53:673–684.
- Parr, M. J. 1973. Ecological studies of *Ischnura elegans* (Vander Linden) (Zygoptera: Coenagrionidae). I. Age groups, emergence patterns and numbers. *Odonatologica* 2:139–157.
- Paulson, D. 2009. *Dragonflies and damselflies of the west*. Princeton University Press, Princeton, NJ.
- . 2011. *Dragonflies and damselflies of the east*. Princeton University Press, Princeton, NJ.
- Pennell, M. W., J. M. Eastman, G. J. Slater, J. W. Brown, J. C. Uyeda, R. G. FitzJohn, M. E. Alfaro, and L. J. Harmon. 2014. geiger v2.0: an expanded suite of methods for fitting macroevolutionary models to phylogenetic trees. *Bioinformatics* 30:2216–2218.
- Pennell, T. M., F. J. H. de Haas, E. H. Morrow, and G. S. van Doorn. 2016. Contrasting effects of intralocus sexual conflict on sexually antagonistic coevolution. *Proceedings of the National Academy of Sciences of the USA* 113:E978–E986.



- Polhemus, D., A. Asquith, and S. Miller. 2000. A new species of *Ischnura* from Rota (Odonata: Coenagrionidae), and a discussion of zygopteran zoogeography in the insular tropical Pacific. Occasional Papers of Bernice P. Bishop Museum 62:5–12.
- Plummer, M., N. Best, K. Cowles, and K. Vines. 2006. CODA: convergence diagnosis and output analysis for MCMC. R News 6:7–11.
- R Core Team. 2018. R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna.
- Raftery, A. E. 1996. Hypothesis testing and model selection. Pages 163–188 in W. R. Gilks, S. Richardson, and D. J. Spiegelhalter, eds. Markov chain Monte Carlo in practice. Chapman & Hall, London.
- Ramage, T., P. Martins-Simoes, G. Mialdea, R. Allemand, A. Duploux, P. Rousse, N. Davies, G. K. Roderick, and S. Charlat. 2017. A DNA barcode-based survey of terrestrial arthropods in the Society Islands of French Polynesia: host diversity within the SymbioCode Project. European Journal of Taxonomy 272:1–13.
- Realpe, E. 2010. Two new Andean species of the genus *Ischnura*. Odonatologica 39:121–131.
- Reinhardt, K., E. Harney, R. Naylor, S. Gorb, and M. T. Siva-Jothy. 2007. Female-limited polymorphism in the copulatory organ of a traumatically inseminating insect. American Naturalist 170:931–935.
- Reinhardt, K., R. A. Naylor, and M. T. Siva-Jothy. 2009. Situation exploitation: higher male mating success when female resistance is reduced by feeding. Evolution 63:29–39.
- Revell, L. J., L. J. Harmon, and D. C. Collar. 2008. Phylogenetic signal, evolutionary process, and rate. Systematic Biology 57:591–601.
- Robertson, H. M. 1985. Female dimorphism and mating behaviour in a damselfly, *Ischnura ramburi*: females mimicking males. Animal Behavior 33:805–809.
- Rolff, J., and M. T. Siva-Jothy. 2002. Copulation corrupts immunity: a mechanism for a cost of mating in insects. Proceedings of the National Academy of Sciences of the USA 99:9916–9918.
- Rönn, J., M. Katvala, and G. Arnqvist. 2007. Coevolution between harmful male genitalia and female resistance in seed beetles. Proceedings of the National Academy of Sciences of the USA 104:10921–10925.
- Rooker, K., and S. Gavrilets. 2018. On the evolution of visual female sexual signalling. Proceedings of the Royal Society B 285:20172875.
- Rowe, L. 1992. Convenience polyandry in a water strider: foraging conflicts and female control of copulation frequency and guarding duration. Animal Behavior 44:189–202.
- . 1994. The costs of mating and mate choice in water striders. Animal Behavior 48:1049–1056.
- Rowe, R. J. 1987. The dragonflies of New Zealand. Auckland University Press, Auckland.
- Rowland, W. J., C. L. Baube, and T. T. Horan. 1991. Signalling of sexual receptivity by pigmentation pattern in female sticklebacks. Animal Behavior 42:243–249.
- Sánchez-Guillén, R. A., H. Van Gossom, and A. Cordero Rivera. 2005. Hybridization and the inheritance of female color polymorphism in two ischnurid damselflies (Odonata: Coenagrionidae). Biological Journal of the Linnean Society 85:471–481.
- Sanmartin-Villar, I., and A. Cordero-Rivera. 2016. The inheritance of female color polymorphism in *Ischnura genei* (Zygoptera: Coenagrionidae), with observations on melanism under laboratory conditions. PeerJ 4:e2380.
- Sanmartin-Villar, I., H. Zhang, and A. Cordero-Rivera. 2016. Colour polymorphism and ontogenetic colour changes in *Ischnura rufostigma* (Odonata: Coenagrionidae). Odonatologica 45:77–86.
- Schärer, L., L. Rowe, and G. Arnqvist. 2012. Anisogamy, chance and the evolution of sex roles. Trends in Ecology and Evolution 27:260–264.
- Schneider, T., D. Ikemeyer, and H. J. Dumont. 2015. New records of dragonflies (Odonata) from Belutschistan-e-Sistan province in Iran. Zoology in the Middle East 61:288–290.
- Servedio, M. R., and R. Lande. 2006. Population genetic models of male and mutual mate choice. Evolution 60:674–685.
- Setchell, J. M., E. J. Wickings, and L. A. Knapp. 2006. Signal content of red facial coloration in female mandrills (*Mandrillus sphinx*). Proceedings of the Royal Society B 273:2395–2400.
- Sherratt, T. 2001. The evolution of female-limited polymorphisms in damselflies: a signal detection model. Ecology Letters 4:22–29.
- Sinervo, B., and A. L. Basolo. 1996. Testing adaptation using phenotypic manipulations. Pages 149–185 in M. R. Rose and G. V. Lauder, eds. Adaptation. Academic Press, New York.
- Slagsvold, T., and G.-P. Sætre. 1991. Evolution of plumage color in male pied flycatchers (*Ficedula hypoleuca*): evidence for female mimicry. Evolution 45:910–917.
- Stoltz, J. A., J. N. McNeil, and M. C. B. Andrade. 2007. Males assess chemical signals to discriminate just-mated females from virgins in redback spiders. Animal Behavior 74:1669–1674.
- Svensson, E. I., J. Abbott, and R. Härdling. 2005. Female polymorphism, frequency dependence, and rapid evolutionary dynamics in natural populations. American Naturalist 165:567–576.
- Svensson, E. I., J. K. Abbott, T. P. Gosden, and A. Coreau. 2009. Female polymorphisms, sexual conflict and limits to speciation processes in animals. Evolutionary Ecology 23:93.
- Takahashi, M., Y. Takahashi, and M. Kawata. 2018. Candidate genes associated with color morphs of female-limited polymorphisms of the damselfly *Ischnura senegalensis*. Heredity 122:81–92.
- Takahashi, Y., and M. Watanabe. 2011. Male mate choice based on ontogenetic color changes of females in the damselfly *Ischnura senegalensis*. Journal of Ethology 29:293–299.
- Teder, T. 2014. Sexual size dimorphism requires a corresponding sex difference in development time: a meta-analysis in insects. Functional Ecology 28:479–486.
- Theischinger, G., and J. Hawking. 2006. The complete field guide to dragonflies of Australia. CSIRO, Australia.
- Thrall, P. H., J. Antonovics, and J. D. Bever. 1997. Sexual transmission of disease and host mating systems: within-season reproductive success. American Naturalist 149:485–506.
- Tobias, J. A., R. Montgomerie, and B. E. Lyon. 2012. The evolution of female ornaments and weaponry: social selection, sexual selection and ecological competition. Philosophical Transactions of the Royal Society B 367:2274–2293.
- Trivers, R. 1972. Parental investment and sexual selection. Pages 136–179 in B. Campbell, ed. Sexual selection and the descent of man. Aldine, Chicago.
- Van Gossom, H., R. Stoks, and L. De Bruyn. 2001. Frequency-dependent male mate harassment and intra-specific variation in its avoidance by females of the damselfly *Ischnura elegans*. Behavioral Ecology and Sociobiology 51:69–75.
- Vick, G. S., and D. A. L. Davies. 1988. A new species of *Ischnura* from New Caledonia (Zygoptera: Coenagrionidae). Odonatologica 17:281–287.
- Von Ellenrieder, N., and R. Garrison. 2007. Dragonflies of the Yungas (Odonata): a field guide to the species from Argentina. Pensoft, Sofia.

- Ware, J., M. May, and K. Kjer. 2007. Phylogeny of the higher Libelluloidea (Anisoptera: Odonata): an exploration of the most speciose superfamily of dragonflies. *Molecular Phylogenetics and Evolution* 45:289–310.
- Weber, M. G., and A. A. Agrawal. 2012. Phylogeny, ecology, and the coupling of comparative and experimental approaches. *Trends in Ecology and Evolution* 27:394–403.
- Weiss, S. L. 2002. Reproductive signals of female lizards: pattern of trait expression and male response. *Ethology* 108:793–813.
- West-Eberhard, M. J. 2003. *Developmental plasticity and evolution*. Oxford University Press, New York.
- Willink, B., M. C. Duryea, and E. I. Svensson. 2019. Data from: Macroevolutionary origin and adaptive function of a polymorphic female signal involved in sexual conflict. *American Naturalist*, Dryad Digital Repository, <http://doi:10.5061/dryad.d0bh91n>.
- Willink, B., and E. I. Svensson. 2017. Intra- and intersexual differences in parasite resistance and female fitness tolerance in a polymorphic insect. *Proceedings of the Royal Society B* 284:20162407.
- Xie, W., P. O. Lewis, Y. Fan, L. Kuo, and M.-H. Chen. 2010. Improving marginal likelihood estimation for Bayesian phylogenetic model selection. *Systematic Biology* 60:150–160.
- Xiong, B., and T. D. Kocher. 1991. Comparison of mitochondrial DNA sequences of seven morphospecies of black flies (Diptera: Simuliidae). *Genome* 34:306–311.
- Xu, M., A. L. Cerreta, T. D. Schultz, and O. M. Fincke. 2014. Selective use of multiple cues by males reflects a decision rule for sex discrimination in a sexually mimetic damselfly. *Animal Behaviour* 92:9–18.
- Yanybaeva, V., H. Dumont, A. Y. Haritonov, and O. Popova. 2006. The Odonata of South Ural, Russia, with special reference to *Ischnura aralensis* Haritonov, 1979. *Odonatologica* 35:167–185.
- Zuk, M., and G. R. Kolluru. 1998. Exploitation of sexual signals by predators and parasitoids. *Quarterly Review of Biology* 73:415–438.

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Mating wheel of the common bluetail damselfly (*Ischnura elegans*). The male and male-mimicking female are nearly identical in coloration. Photo credit: E. I. Svensson.