

# Sexual conflict and ecology: Species composition and male density interact to reduce male mating harassment and increase female survival

Miguel A. Gomez-Llano,<sup>1,2,3</sup>  Hanna M. Besch,<sup>2</sup> and Erik I. Svensson<sup>2</sup>

<sup>1</sup>School of Earth and Environmental Sciences, University of Manchester, United Kingdom

<sup>2</sup>Evolutionary Ecology Unit, Department of Biology, Lund University, Sweden

<sup>3</sup>E-mail: miguel.gomez@biol.lu.se

Received June 27, 2017

Accepted February 15, 2018

Sexual conflict is a pervasive evolutionary force that can reduce female fitness. Experimental evolution studies in the laboratory might overestimate the importance of sexual conflict because the ecological conditions in such settings typically include only a single species. Here, we experimentally manipulated conspecific male density (high or low) and species composition (sympatric or allopatric) to investigate how ecological conditions affect female survival in a sexually dimorphic insect, the banded demoiselle (*Calopteryx splendens*). Female survival was strongly influenced by an interaction between male density and species composition. Specifically, at low conspecific male density, female survival increased in the presence of heterospecific males (*C. virgo*). Behavioral mating experiments showed that interspecific interference competition reduced conspecific male mating success with large females. These findings suggest that reproductive interference competition between con- and heterospecific males might indirectly facilitate female survival by reducing mating harassment from conspecific males. Hence, interspecific competitors can show contrasting effects on the two sexes thereby influencing sexual conflict dynamics. Our results call for incorporation of more ecological realism in sexual conflict research, particularly how local community context and reproductive interference competition between heterospecific males can affect female fitness.

**KEY WORDS:** Community ecology, facilitation, indirect ecological effects, interspecific competition, male mating harassment, reproductive interference, sexual conflict.

Sexual conflict is regarded as a pervasive evolutionary force that can drive male–female coevolution (Arnqvist and Rowe 2005; Chapman 2006). Sexual conflict occurs when the reproductive interests of one sex diverge from the interests of the opposite sex (Parker 1979). Many previous experimental studies have shown that sexual conflict between males and females over mating rates can have different effects, such as reducing female survival (Rice 1996; Arnqvist and Rowe 2005; Long et al. 2009), reducing female fecundity (Arnqvist and Nilsson 2000), influence sexual selection (Gavrilets et al. 2001), and promote or inhibit speciation (Arnqvist et al. 2000; Gavrilets and Waxman 2002). In many species, sexual conflict has promoted the evolutionary diversifica-

tion of male genitalia and other mating-related structures to attach males to females (e.g., spines), or to remove sperm from male rivals from previous copulations (Waage 1979; Arnqvist 1998; Rivera et al. 2004), whereas females have evolved traits to avoid such sperm removal (Córdoba-Aguilar et al. 2003). This coevolution of male and female reproductive traits has been suggested to play a crucial role in population divergence and speciation (Arnqvist et al. 2000; Gavrilets 2000; Gavrilets and Waxman 2002).

Most of our knowledge about sexual conflict is based on results from laboratory experiments that typically involve only a single species, and often model organisms like *Drosophila* (Rice 1996; Arnqvist and Rowe 2005; Long et al. 2009). One limitation

of such previous studies is that they ignore the potential role of interspecific interactions in a local natural community. Thus, almost all previous sexual conflict studies typically do not take into account how interspecific interactions influence male-female interactions and male mating harassment (*but see* Magurran and Seghers 1994b).

Heterospecific interactions such as competition or predation would be expected to influence the direction and strength of sexual conflict if they affect reproductive behavior. For instance, in a study with Trinidad guppies (*Poecilia reticulata*), the researchers found that in populations with high predation risk females suffer more harassment from male sneaky mating attempts than in low predation risk populations (Magurran and Seghers 1994a,b). This was the result of a shift in male mating behavior from courting to sneaky mating as a response to a reduced life expectancy due to predation (Magurran and Seghers 1994b). These researchers quantified a 25% decrease in female foraging time as a result of harassment due to high population density in another study of the same species, revealing high potential fitness costs of male mating harassment on females (Magurran and Seghers 1994a). However, this study is an exception, as most previous research on the role of interspecific interactions in sexual selection and speciation is focused on either reproductive character displacement (Pfennig and Pfennig 2009) or indirect fitness costs through hybridization (Abbott et al. 2013). In general, we know little about how interspecific interactions affect male and female fitness components in natural settings (Svensson 2013; Grether et al. 2017).

Damselflies (Odonata: Zygoptera) is a suborder of insects whose mating systems are characterized by intense male-male competition over females (Corbet 1999). Such intense male-male mating competition often leads to male mating harassment, which leads intense sexual conflict (Svensson et al. 2005; Gosden and Svensson 2007, 2009; Takahashi et al. 2014). Calopterygid damselflies have been used to study the effect of interspecific male-female interactions, and such interspecific interactions have been demonstrated to drive the evolution of species-specific traits used in species recognition and as targets of female mate choice (Waage 1979; Tynkkynen et al. 2004, 2005; Svensson et al. 2007; Verzijden and Svensson 2016). Agonistic interspecific interference interactions between males of the banded demoiselle (*Calopteryx splendens*) and the dominant congeneric beautiful demoiselle (*C. virgo*), have shown to select against heterospecific-similar male phenotypes (i.e., males with large wing patches) in *C. splendens* (Tynkkynen et al. 2005). Moreover, differential aggression from heterospecific *C. virgo* males directed against *C. splendens* males with large wing patches (i.e., more *C. virgo* like phenotypes) reduced the survival of *C. splendens* males with large wing patches (Tynkkynen et al. 2005). This interspecific aggression has resulted in a classic geographic pattern of character displacement that is driven by male-male interactions rather than by female

choice (Tynkkynen et al. 2004, 2005; Honkavaara et al. 2011). In sympatric populations, *C. splendens* males have been shown to avoid males with experimentally enlarged melanized wing patches (i.e., with a heterospecific male wing phenotype), suggesting that they actively avoid interactions with this behaviorally dominant aggressive competitor (Svensson et al. 2018). However, we do not know how such agonistic interactions between males of these two species could indirectly shape intersexual conflict over mating between *C. splendens* males and females.

Here, we investigated if male-male intra- and interspecific interference competition could indirectly affect female survival and reproductive success in *C. splendens*. Our point of departure, based on the previous studies of these two interacting and often sympatric species and their general natural history (see above), was that males of the dominant of these co-occurring species (*C. virgo*) should negatively influence male mating success of the subordinate species (*C. splendens*). The negative effect of the dominant competitor on the subordinate species could have a cascading effect on *C. splendens* females, because females might benefit from heterospecific male interference through reduced conspecific mating harassment that would indirectly facilitate *C. splendens* female survival. Such a positive effect of the presence of heterospecific males, if present, would then be an example of an indirect ecological effect (Walsh 2013; terHorst et al. 2015) and an example of facilitation caused by interspecific interactions (Stachowicz 2001; Day and Young 2004).

To test this hypothesis, we investigated experimentally how the presence of heterospecific *C. virgo* males influenced intraspecific male-male competition for females among *C. splendens* males. High conspecific male density is expected to increase male mating harassment on females and reduce their survival, but some empirical evidence suggests that increased male competition can sometimes decrease male condition and increase male mortality (Plaistow and Siva-Jothy 1996; Opaev and Panov 2016; Gering 2017). Reduced male condition could therefore reduce harassment on females, which would in turn have a positive effect on female survival. These different lines of evidence might suggest that the relationship between conspecific male density and the degree of mating harassment, and resulting sexual conflict should not be taken for granted and should be investigated empirically.

To evaluate these different scenarios, we performed mesocosm experiments in large outdoor cages where we simultaneously manipulated male density (high or low) and species composition (“Allopatric”: *C. splendens* alone or “Sympatric”: *C. splendens* mixed with *C. virgo*). The number of males and females in the low-density treatments was the same, while in the high-density treatments there were twice as many more males than in the low-density treatments. We used these male densities as they are in the natural range for the species in the field, based

on our previous large-scale geographic population surveys and long-term studies (57–89%; Svensson et al. 2018; E. I. Svensson, unpubl. data). In these mesocosm experiments, we recorded female survival and investigated the main and interaction effects of the presence of heterospecifics and male density. We also performed a follow-up experiment in the form of mating trials, where we manipulated male density and species composition, and recorded failed and successful male mating attempts. We synthesized the results from these two complementary experiments to clarify if con- and heterospecific reproductive interference competition is likely to reduce male mating harassment on females. If so, we would expect such interspecific male reproductive interference competition to mitigate sexual conflict by indirectly facilitating *C. splendens* female survival.

## Methods

### STUDY ORGANISMS

Our two main study species *C. splendens* and *C. virgo* emerge as adults from their larval aquatic stage from late May until late July, in a metapopulation setting of microallopatric and sympatric populations in southern Sweden (Svensson et al. 2004). Males of these two species are differentiated principally by the extent of melanic wing coloration, with small dark wing patches in *C. splendens* (covering about 50% of the wing) and entirely melanized wings in *C. virgo* (Svensson and Friberg 2007; Svensson and Waller 2013; Svensson et al. 2018).

In natural populations of these two species, male mating harassment is frequent during the reproductive season, and can be observed along the riversides where the adults emerge. In the area of this study, *C. splendens* is only weakly territorial, and the reproductive behavior involves chases of females by one or more males, followed by a male restraining the female using clasping structures of the last segment of the abdomen, clasping the female by the prothorax (Corbet 1999). During clasping, males and females form the tandem position, after which females must bend their abdomen to reach male genitalia, forming the mating wheel (Corbet 1999; Svensson et al. 2016). After mating, females move to submerged vegetation along the river where they oviposit. Males and females can mate repeatedly, and in conjunction with each mating event males remove the sperm from previous matings by male competitors (Waage 1979; Hooper and Siva-Jothy 1996). Males compete with each other during chasing, clasping and mating, and such male–male reproductive interference within or between species can break up tandem pairs and can hence reduce male mating success (Corbet 1999).

Males in the other species (*C. virgo*) are more strongly territorial, and chase away other males from their territory along the riverside. Male *C. virgo* are aggressive toward the subdominant

*C. splendens* males, particularly those with large wing patches, as the extent of wing pigmentation gives them a similar phenotype to *C. virgo* males (Tynkkynen et al. 2005, Svensson et al., 2018). Male *C. virgo* also discriminate strongly against *C. splendens* females, and the reproductive behavior is also different: *C. virgo* males court females with a hovering flight, whereas *C. splendens* males have a less pronounced courtship and employ more chasing behavior. These courtship differences between the two species make heterospecific mating attempts rare and highly asymmetric, with *C. splendens* males more often mating with *C. virgo* females than vice versa (Svensson et al. 2007, 2016).

### GENERAL FIELD WORK PROCEDURES

This study was performed during the reproductive seasons of *C. virgo* and *C. splendens*, during June and July 2015 and 2016 at four different *Calopteryx* localities in the province of Scania, southern Sweden. We collected animals from two localities for each of the two species. *C. virgo* were captured at the sympatric sites “Klingavälsån Naturreservat” and “Omma at Åsumsån,” whereas *C. splendens* were captured from the allopatric sites “Kävlingeån at Harlösa” and “Höje Å at Värpinge.” In *C. splendens*, species discrimination and mate preference gradually develop by learning and through reproductive interactions during ontogeny (Svensson et al. 2007, 2010; Verzijden and Svensson 2016). We therefore captured *C. splendens* from allopatric populations that have no experience with heterospecifics. Geographic locations and other ecological information about these sites are provided in detail elsewhere (Svensson et al. 2007, 2010, 2014, 2016). One hundred sixty-eight individuals were captured and used in our experiments in this study, 60 of which were females (48 *C. splendens* and 12 *C. virgo*) and 108 of which were males (72 *C. splendens* and 36 *C. virgo*).

All captured individuals were transported to Stensoffa Field Station (55.695145, 13.447076) in netting containers (“Port a bug” #T-11-232, 10.2 cm diameter × 22.9 cm height), a maximum of 10 individuals per container and separated by sex. Morphological measurements were taken from all these individuals, including hind- and forewing length and width, thorax width, and total body length (measured in mm).

### MESOCOSM EXPERIMENTS

In June and July 2015, we performed mesocosm experiments where we simultaneously manipulated male density and species composition in a series of large outdoor cages (3 × 3 × 3 m = 27 m<sup>3</sup>) at Stensoffa Field Station. Each cage contained two water containers and natural vegetation to resemble natural conditions as closely as possible. These cages were covered with mesh, with mesh size small enough to keep the damselflies in but wide enough to let smaller insects in, thus allowing natural prey items (small insects like flies and moths) to enter the cages, further mimicking

natural environmental settings (Takahashi et al. 2014; Svensson et al. 2018).

In each cage we had six *C. splendens* females and manipulated the density of males: either low density (six conspecific males) or high density (12 conspecific males). We also manipulated the species composition (“allopatric”; *C. splendens* alone, and “sympatric”; *C. splendens* mixed with *C. virgo*). Therefore, in the low male density environments, we had six *C. splendens* males in the allopatric treatment and six males of each species in the sympatric treatment. In the high male density environments, we had 12 *C. splendens* males in the allopatric treatment and 12 of each species in the sympatric treatment. We thus have four different unique treatments: (1) sympatric and low density, (2) sympatric and high density, (3) allopatric and low density, and (4) allopatric and high density (Table S1), with two replicates of each treatment. Thus, we had a complete factorial design, where we manipulated both male density and species composition simultaneously.

In the sympatric treatment, we placed three *C. virgo* females to stimulate *C. virgo* male territoriality and interspecific male–female interference competition between *C. splendens* and *C. virgo* males. In each cage, we individually color marked all males and females with a unique randomized three-color code on the last three abdominal segments for identification. These color markings allowed us to measure the survival and longevity of all individuals, as we visited these cages on a daily basis.

Note that our experimental design affects absolute number of males, as well as male and female species composition in the cages. Female–female interactions are relatively weak or even absent in these species of damselflies and are therefore likely to be negligible, compared to stronger and more pronounced male–male interactions. Also, our previous experimental work has shown that *C. virgo* males discriminate strongly against *C. splendens* females (Svensson et al. 2007, 2016), suggesting that such heterospecific males should not affect *C. splendens* female survival directly, but only possibly indirectly through these heterospecific males interactions with *C. splendens* males, which we aimed to investigate in this study. Based on these features of the natural history and ecology of these damselfly species, we therefore feel confident in our assumption that conspecific male density and species composition are the two most important factors that are likely to affect *C. splendens* female survival, with little or no effect of female–female interactions or heterospecific male–female interactions.

The mesh in these cages is wide enough to allow a constant supply of prey to enter the cages, such that survival is unlikely to be strongly affected by a limited food supply (see Takahashi et al. 2014). Nevertheless, to investigate if there was any strong density-effect in these cages, we analyzed the effect of total individual density on males and female survival. However, we found no such survival effects of the total number of individuals (Table S2).

These results are consistent with our previous experimental studies in these cages, which showed only limited density effects on various fitness components, compared to frequency effects (Takahashi et al. 2014, Svensson et al., 2018).

## STATISTICAL ANALYSES

To analyze female survival, we recorded the number of females that were alive after two days in the cages. The number of individuals alive after two days have been used previously to study male survival in a different species of damselflies (Gering 2017), and it represents a large fraction of the natural life span for *C. splendens* (Svensson et al. 2006; Waller and Svensson 2016). We used each female as an observation with two possible values (alive = 1, dead = 0), and analyzed the data using a model with a binomial error structure. Conspecific male density (high or low), male species composition (allopatric or sympatric), and the interaction between these two experimental factors were used as fixed factors and were thus the predictor variables. We included cage ID as a random factor to account for subtle differences between cages unrelated to these fixed treatment effects. We then selected the best-fit model using the lowest Akaike information criterion (AIC). AIC values of the different models are shown in Table S3. Tukey post hoc tests of the interaction between male density and species composition were also carried out. All these analyses were performed using the R-packages “lme4” (Bates et al. 2014), “car” (Fox et al. 2009) and “lsmeans” (Lenth 2016) in the R statistical environment (R Development Core Team 2015).

In the present study, we were interested in the effect of intra- and interspecific competition in female survival, as we have presented data on male survival in relation to species density and frequency elsewhere (Svensson et al., 2018). However, in the Supplementary Information, we present new data on the effects on male survival. We could not find an effect of either species composition or density, nor the interaction between these two variables (Table S4). These results broadly agree with previous findings of how male survival is affected by density and species composition (Svensson et al. 2018).

## MATING TRIALS

In June 2016, a series of smaller cages were set up to record more detailed and quantitative data on mating interactions in the different types of environments. We used cylindrical netting cages (IKEA “Laundry basket” #800.992.24, 45 cm in diameter × 50 cm in height). Each cage contained vegetation and a 100 mL water container. In each mating cage, we placed one *C. splendens* female and manipulated male species composition (“allopatric”; *C. splendens* alone or “sympatric”; *C. splendens* mixed with *C. virgo*). We also manipulated the male density: low male density (one conspecific male) or high (three conspecific males). Thus, there were three males of each species in the high-density

sympatric treatment and three *C. splendens* males in the high-density allopatric treatment, and like in the mesocosm experiments, we used a fully factorial experimental design (Table S5). Males were marked with one of four different fluorescent color dust in the genital area and the claspers (Gosden and Svensson 2009). This experimental set-up made it possible for us to identify males that mated or attempted to mate by recording the color dust traces on females (Gosden and Svensson 2007, 2009). All *C. virgo* males were marked with the same color and the *C. splendens* males with an individual easily distinguishable color, which made it possible to identify multiple males mating one female. After 24 h, the females were checked for traces of fluorescent color dust under UV-light (Gosden and Svensson 2009). Matings were recorded if the female had traces of the marker dust on her genitalia. If the female had color dust traces on the thorax only, then this indicated an unsuccessful mating event (i.e., the male clasped the female, but did not copulate with her) (Gosden and Svensson 2007, 2009). Morphometric measures of females were taken at the termination of the experiment.

### STATISTICAL ANALYSES

Two separate models were carried out, and in both models we assumed that the response variables (clasping or mating) were binomially distributed with only two different outcomes (i.e., 1 = clasp or 0 = no clasp; and 1 = successful or 0 = unsuccessful mating attempt). In the first model, we analyzed the proportion of successful male mating attempts (i.e., clasplings that were followed by a copulation) and in the second model, we analyzed only male clasplings (i.e., males that clasped a female). Male density, species composition, female size (i.e., female forewing length) and all the two- and three-way interactions between these variables were used as initial predictor variables in the original (full) models. We then employed a standard model reduction procedure using the lowest AIC to arrive to the final model. AIC values of alternative models of successful matings and claspling probability are shown in Tables S6 and S7, respectively. All analyses were performed using R-package “lme4” (Bates et al. 2014) and “car” (Fox et al. 2009).

## Results

We recorded survival of 48 females (six in each of the four treatments, with two replicates of each treatment) in the mesocosm experiments. We found a significant effect of the interaction between species composition and conspecific male density ( $\chi^2 = 7.82$ ,  $P = 0.0051$ , Table 1). Tukey post hoc analysis revealed that in the low-density treatment, female survival was higher under sympatric compared to allopatric conditions (estimate = 3.49,  $Z = 2.82$ ,  $P = 0.023$ ; Fig. 1, Table S8). Interestingly, we found no evidence of a main effect of species composition ( $\chi^2 = 0.13$ ,

**Table 1.** Binomial model of *Calopteryx splendens* female survival in the mesocosm experiments.

Factor	$\chi^2$	df	<i>P</i>
Male density	0.8176	1	0.3658
Species composition	0.1316	1	0.7168
Species composition $\times$ density	7.8234	1	0.0051**

We included conspecific male density and species composition as fixed factors and cage ID as a random factor to control for intrinsic differences between cages that were not related to the experimental treatments.

All data come from 2015. Total number of *C. splendens* females = 48.

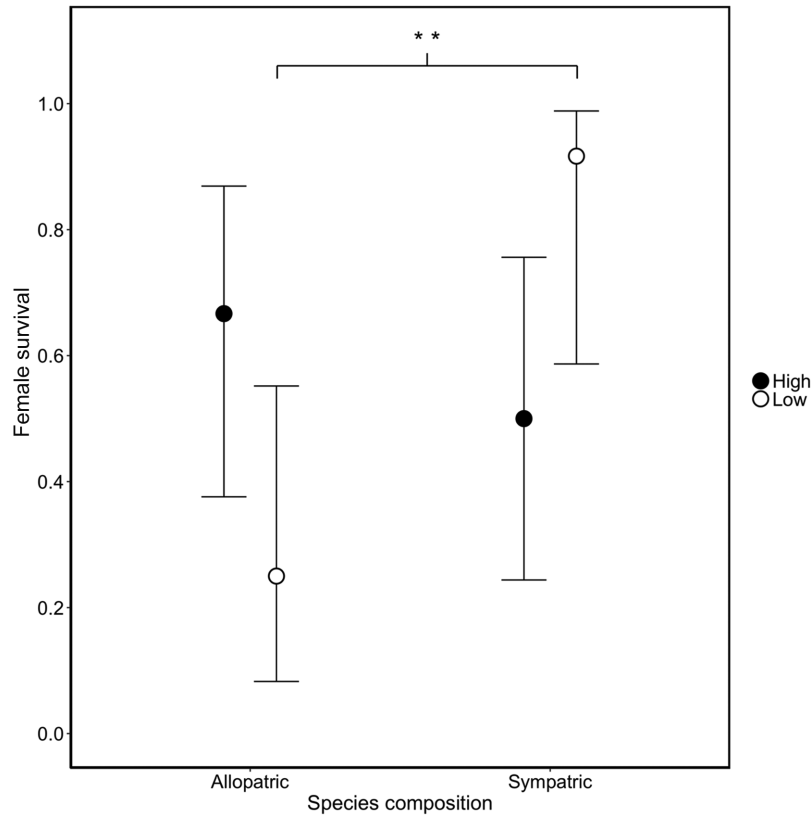
$P = 0.71$ ) or male density ( $\chi^2 = 0.81$ ,  $P = 0.36$ ) alone, but only this strong interaction effect (Table 1; Fig. 1).

We carried out a total of 85 mating cage experimental trials, where we measured female size and recorded mating attempts (i.e., clasplings,  $N = 54$ ) and copulations (i.e., successful mating attempts,  $N = 28$ ). We found evidence for a significant interaction between species composition and female size ( $\chi^2 = 6.05$ ,  $P = 0.013$ ). Male *C. splendens* was more likely to mate with large females in the allopatric treatment compared to the sympatric treatment, where the *C. splendens* males instead mated with smaller females (Fig. 2A; Table 2). We found no effect of female size (i.e., forewing length;  $\chi^2 = 0.26$ ,  $P = 0.61$ ) or species composition ( $\chi^2 = 0.58$ ,  $P = 0.44$ ) on the number of successful mating attempts. We used a total of 71 *C. virgo* males in the mating trial experiment, and we found only three heterospecific mating attempts (*C. virgo* males mating with *C. splendens* females), of which two were successful. All heterospecific mating attempts were with relatively large females (forewing length > 17.1 mm).

Male mating attempts (i.e., clasplings) were significantly affected by male density ( $\chi^2 = 6.04$ ,  $P = 0.013$ ). We found a higher proportion of clasplings in low density than in high density (Fig. 2B). We found a strong trend of species composition, with higher proportion of clasplings in the sympatric than in the allopatric treatments ( $\chi^2 = 3.82$ ,  $P = 0.051$ ; Table 3). There was no significant interaction between male density and species composition ( $\chi^2 = 2.17$ ,  $P = 0.13$ ).

## Discussion

The results in this study suggest a role of heterospecific male-male interference competition in mitigating sexual conflict, with consequences for female survival. Under low male density, *C. splendens* females survived poorly in allopatry, whereas when heterospecific *C. virgo* males were present (sympatry), *C. splendens* female survival increased (Fig. 1). Interestingly, high male density (as a main effect) had no effect on female survival. The follow-up mating trials experiments suggest that intra- and



**Figure 1.** *Calopteryx splendens* female survival in mesocosm experiments. Species composition (“Allopatry”: *C. splendens* alone or “Sympatry”: *C. splendens* and *C. virgo* males) and male density (high and low) were simultaneously manipulated in a fully factorial design in large outdoor cages (mesocosm experiments). Female survival was higher in the sympatric than in the allopatric treatment in the low-density environment. However, in the high male density environment, female survival was higher under allopatric than under sympatric conditions (Tables 1 and S3). Results of post hoc tests between significant pairwise comparisons are shown (\*\* $P = 0.0051$ ). Shown are model predictions and 95% confidence limits around the estimates.

**Table 2.** Binomial model of *Calopteryx splendens* successful mating attempts.

Factor	Estimate	SE	$z$	$P$
Intercept	0.2892	0.4218	0.686	0.4929
Species composition	0.8	0.8112	0.986	0.3241
Female size	-1.6822	0.9108	-1.847	0.0647
Species composition $\times$ female size	3.3302	1.5314	2.175	0.0297*

We included species composition, female size, and their interaction as fixed factors.

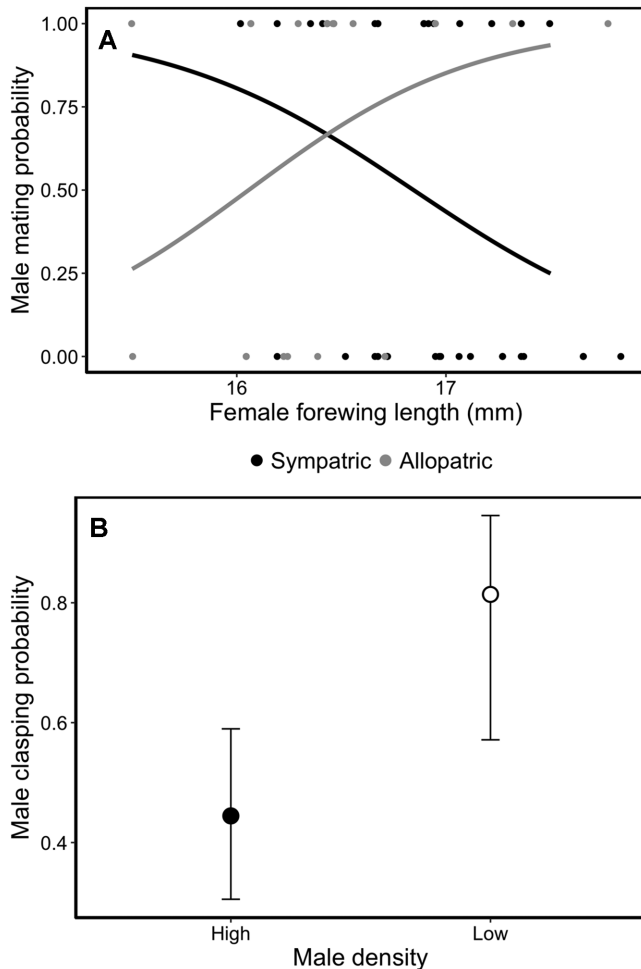
All data comes from 2016. Total number of *C. splendens* males = 53.

**Table 3.** Binomial model of *Calopteryx splendens* clasplings.

Factor	Estimate	SE	$z$	$P$
Intercept	-0.2231	0.3	-0.744	0.457
Male density	1.4759	0.6414	2.301	0.0214*
Species composition	-0.8459	0.4396	-1.932	0.0533
Male density $\times$ Species composition	-1.2506	0.8676	-1.441	0.1495

We used male density, species composition and their interaction as fixed factors.

All data come from 2016. Total number of *C. splendens* males = 134.



**Figure 2.** *Calopteryx splendens* mating behavior experiments. (A) Successful mating attempts. In the sympatric treatments males were more likely to mate with small females, whereas in the allopatric treatments males mated to a higher extent with larger females (Table 2). Shown are observations and model predictions (slopes). (B) Male clasplings. Males were significantly more likely to clasp females in low than in high-density treatments (Table 3). Shown are model predictions and 95% confidence limits.

interspecific male–male reproductive interference competition is likely to be the main cause behind the differential female survival under these different ecological conditions. These results suggest reduced harassment by *C. splendens* males on their conspecific females when heterospecific males are present, at least under low density conditions. The reversal of this female survival effect under high density (Fig. 1) suggests that at high conspecific male density, intraspecific male reproductive interference can have a similar beneficial effect on female survival as the interspecific male reproductive interference has under low density conditions. This is partially consistent with a nearly neutral model, where these two species are ecologically close to equivalent, consistent

with previous studies on odonates (McPeck and Brown 2000; Siepielski et al. 2010; Svensson et al. 2018).

Male mating harassment has been shown to be costly for females and can reduce their fitness substantially (Magurran and Seghers 1994a; Arnqvist and Rowe 2005; Long et al. 2009). However, our results show no general difference in female survival between high and low male densities as a main effect and itself in either sympatric or allopatric treatments (Fig. 1). This suggests that species composition, rather than male density, is most important factor affecting female fitness. It is possible that females might actually benefit under some conditions of high male density, if males become more involved in aggressive intra-sexual interactions, leading to lower mating harassment and reduced pressure on females. In support of this, we found that male *C. splendens* clasping probabilities were reduced in the high-density treatments (Fig. 2B). This suggest that intense intraspecific male–male competition can reduce male clasping and mating success, and thereby, also reduce harassment on females to similar levels as the low-density treatments. Previous studies on *Calopteryx* and other damselfly species have indeed shown that high male density and intensive male–male aggressive interactions can reduce male condition (Plaistow and Siva-Jothy 1996; Opaev and Panov 2016; Gering 2017). Decreased male condition can translate into less time, opportunities, and energy to invest in mating (competition, chasing, and clasping).

In contrast to our empirical findings in this study, previous research investigating speciation mediated by sexual conflict have sometimes assumed that high male densities increase the intensity of sexual conflict and that this in turn promote the emergence of reproductive barriers (Gavrilets 2000; Gavrilets and Waxman 2002; Martin and Hosken 2003). The experimental results in this study suggest that this assumption that high male density leads to stronger sexual conflict might not always hold. We suggest that intense male–male competition can actually reduce the strength of sexual conflict, particularly if males have to trade mating attempts against agonistic male–male interactions. Another possibility is that density-dependent effects are nonlinear, and male density could be beneficial for females (due to increased male–male competition) at extreme densities and negative (due to increased harassment) at moderate densities, or vice versa. The possibility of such nonlinear effects of male density, female survival, and male–female mating interactions should be investigated in future studies across a wider range of densities. Our results call for an expansion of previous theoretical work, exploring alternative scenarios of how population density shapes sexual conflict.

In the low-density treatments, *C. splendens* females had higher survival in the presence of *C. virgo* males (Fig. 1). This supports our original hypothesis that females indirectly benefit from the presence of heterospecific males (Fig. 1). Male aggression from the dominant species *C. virgo* toward *C. splendens* males

has been experimentally demonstrated before (Tynkkynen et al. 2005). Our results on female survival suggest that such interspecific male–male interference competition reduces *C. splendens* male mating harassment toward their own conspecific females. This shows that community composition might indeed reduce costly conspecific male mating harassment on females, raising the question of how relevant previous single-species studies in laboratory settings are for our general understanding of the importance of sexual conflict in more natural settings. Another general implication of our study is that the presence of other species cannot and should never be taken a priori as evidence for interspecific competition and as something that must be negative for fitness. On the contrary, sympatric species could coexist under the constraints of conditional neutrality and ecological drift (Svensson et al. 2018) and in some cases species might even coexist through facilitation or other positive interactions (Stachowicz 2001; Day and Young 2004).

Female *C. splendens* can mate repeatedly, and males remove the sperm from previous copulations. Repeated mating with sperm removal can therefore make matings highly costly, as benefits are gained only in the last copulation, while costs (from chasing, clasping and possible physical damage of sperm removal) occur in every mating event (Waage 1979; Hooper and Siva-Jothy 1996). However, the degree of male mating harassment can also vary depending on female phenotypic quality (Long et al. 2009; Chenoweth et al. 2015). In our study, we found that under sympatric conditions, *C. splendens* males were less likely to mate with large (presumably highly fecund) conspecific females compared to allopatric conditions (Fig. 2A).

Heterospecific interference competition between males can reduce male mating success with high-quality females, indirectly increasing female survival. In allopatry, harassment directed toward large females could reduce the longevity of high-quality females and reduce overall female survival. Previous research on fruit flies (*Drosophila melanogaster*) have shown that male mating harassment is differentially directed toward large females, and the costs of such sexual harassment can offset the intrinsically higher fitness of large, high-fecundity females (Long et al. 2009; Chenoweth et al. 2015). Previous work on reproductive interference has mainly focused on female costs due to forced heterospecific matings (Gröning and Hochkirch 2008; Manzano-Winkler et al. 2017), but little work has been done on the effect of interspecific competition, where recent interest has grown on the evolutionary consequences of interspecific interference (Grether et al. 2017). Here, we have presented evidence for a novel outcome from reproductive interference competition, in which heterospecific competition reduces conspecific male mating harassment, and indirectly facilitates female survival.

Previous work has revealed that *C. virgo* males are dominant over *C. splendens* males, and behave more aggressive toward

*C. splendens* males with large wing patches (i.e., high-quality *C. splendens* males with similar wing phenotype as *C. virgo*) (Tynkkynen et al. 2005; Honkavaara et al. 2011). These previous studies and the results in the present study suggest an important role of community composition in the modulation of sexual conflict and its effects on both male and female fitness (see also Kyogoku and Sota 2017 for another recent study in this area using an experimental evolution approach). Indirect ecological effects and changes in selection associated with community composition is currently a very active research area, although sexual selection remains to be integrated in this rapidly growing field (Walsh 2013; terHorst et al. 2015).

In many insects, including *Calopteryx* damselflies, sexual conflict has led to a coevolution of male and female genitalia (Waage 1979; Arnqvist 1998; Córdoba-Aguilar et al. 2003; Rivera et al. 2004). Specifically in the context of *Calopteryx*, the potential evolutionary effects of future changes in sexual selection regimes due to increased encounter rates with heterospecifics should be further explored (Svensson et al. 2010, 2014; Verzijden et al. 2014). This question is also of urgent practical and concern for conservation biology, as human activities and climate change are expected to increase the future rate of secondary contact of formerly allopatric species (Walther et al. 2002; Post et al. 2013; Chunco 2014).

Although sexual conflict has been intensively studied previously (Arnqvist and Rowe 2005), little is known about how ecological factors like conspecific densities and presence of heterospecifics shape such sexual conflict (Magurran and Seghers 1994b; Kyogoku and Sota 2017). The results in this study, in combination with the findings in previous studies on *Calopteryx* damselflies (Tynkkynen et al. 2004, 2005, Svensson et al. 2007, 2010, 2014, 2016, 2018) strongly suggest that interspecific interactions can modify the outcome of sexual conflict through interspecific reproductive interference between males. Our results call for the incorporation of more ecological realism in the study of sexual conflict, particularly how local community composition interspecific interactions could have different fitness consequences for the two sexes.

#### AUTHOR CONTRIBUTIONS

MAG-L and EIS conceived the idea and planned the experiments. EIS performed preparatory fieldwork and located suitable sampling populations. MAG-L and HMB collected the data for the cage experiments. MAG-L performed the statistical analysis and wrote the paper. EIS and HMB. read and contributed to the writing of the paper.

#### ACKNOWLEDGMENTS

We are thankful to E. Fjellstedt for his help in the field and T. Gilman for critical comments on earlier drafts. Funding for this project are provided as follow: MAG-L is funded by the Natural Environmental Research Council (NERC: grant no. NE/K500859/1), EIS is funded by The Swedish



Research Council (VR: grant no. 621-2012-3768) and Carl Tryggers Foundation (CTS).

## DATA ARCHIVING

The doi of our data is: <https://doi.org/10.5061/dryad.g1qg040>.

## LITERATURE CITED

- Abbott, R., D. Albach, S. Ansell, J. W. Arntzen, S. J. E. Baird, N. Bierne, J. Boughman, A. Brelsford, C. A. Buerkle, R. Buggs, et al. 2013. Hybridization and speciation. *J. Evol. Biol.* 26:229–246.
- Arnqvist, G. 1998. Comparative evidence for the evolution of genitalia by sexual selection. *Nat. Wkly. J. Sci.* 393:784–786.
- Arnqvist, G., and T. Nilsson. 2000. The evolution of polyandry: multiple mating and female fitness in insects. *Anim. Behav.* 60:145–164.
- Arnqvist, G., and L. Rowe. 2005. *Sexual conflict*. Princeton Univ. Press, Princeton, NJ.
- Arnqvist, G., M. Edvardsson, U. Friberg, and T. Nilsson. 2000. Sexual conflict promotes speciation in insects. *Proc. Natl. Acad. Sci.* 97:10460–10464.
- Bates, D., M. Maechler, B. Bolker, and S. Walker. 2014. lme4: Linear mixed-effects models using Eigen and S4. R Packag. version 1.
- Chapman, T. 2006. Evolutionary conflicts of interest between males and females. *Curr. Biol.* 16:R744–R754.
- Chenoweth, S. F., N. C. Appleton, S. L. Allen, and H. D. Rundle. 2015. Genomic evidence that sexual selection impedes adaptation to a novel environment. *Curr. Biol.* 25:1860–1866. Elsevier Ltd.
- Chunco, A. J. 2014. Hybridization in a warmer world. *Ecol. Evol.* 4:2019–2031.
- Corbet, P. S. 1999. *Dragonflies: behaviour and ecology of Odonata*. Harley Books, Essex, England.
- Córdoba-Aguilar, A., E. Uhía, and A. C. Rivera. 2003. Sperm competition in Odonata (Insecta): the evolution of female sperm storage and rivals' sperm displacement. *J. Zool.* 261:381–398.
- Day, T., and K. A. Young. 2004. Competitive and facilitative evolutionary diversification. *Bioscience* 54:101.
- Fox, J., D. Bates, D. Firth, M. Friendly, G. Gorjanc, S. Graves, R. Heiberger, G. Monette, H. Nilsson, D. Ogle, et al. 2009. CAR: companion to applied regression, R Package version 1.2-16.
- Gavrilets, S. 2000. Rapid evolution of reproductive barriers driven by sexual conflict. *Nature* 403:886–889.
- Gavrilets, S., and D. Waxman. 2002. Sympatric speciation by sexual conflict. *Proc. Natl. Acad. Sci.* 99:10533–10538.
- Gavrilets, S., G. Arnqvist, and U. Friberg. 2001. The evolution of female mate choice by sexual conflict. *Proc. R. Soc. B Biol. Sci.* 268:531–539.
- 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. *Am. Nat.* 173:709–721.
- Grether, G. F., K. S. Peiman, J. A. Tobias, and B. W. Robinson. 2017. Causes and Consequences of Behavioral Interference between Species. *Trends Ecol. Evol.* 32:760–772.
- Grönning, J., and A. Hochkirch. 2008. Reproductive interference between animal species. *Q. Rev. Biol.* 83:257–282.
- Honkavaara, J., D. W. Dunn, S. Ilvonen, and J. Suhonen. 2011. Sympatric shift in a male sexual ornament in the damselfly *Calopteryx splendens*. *J. Evol. Biol.* 24:139–145.
- Hooper, R. E., and M. T. Siva-Jothy. 1996. Last male sperm precedence in a damselfly demonstrated by RAPD profiling. *Mol. Ecol.* 5: 449–452.
- Kyogoku, D., and T. Sota. 2017. The evolution of between-species reproductive interference capability under different within-species mating regimes. *Evolution* 71:1–16.
- Lenth, R. 2016. lsmeans: Least-squares means. R package version 2.22.
- Long, T. A. F., A. Pischedda, A. D. Stewart, and W. R. Rice. 2009. A cost of sexual attractiveness to high-fitness females. *PLoS Biol.* 7:e1000254.
- Magurran, A. E., and B. H. Seghers. 1994a. A cost of sexual harassment in the guppy, *Poecilia reticulata*. *Proc. R. Soc. B Biol. Sci.* 258:89–92.
- . 1994b. Sexual conflict as a consequence of ecology: evidence from guppy, *Poecilia reticulata*, populations in Trinidad. *Proc. R. Soc. B Biol. Sci.* 255:31–36.
- Manzano-Winkler, B., A. J. Hish, E. K. Aarons, and M. A. F. Noor. 2017. Reproductive interference by male *Drosophila subobscura* on female *D. persimilis*: a laboratory experiment. *Ecol. Evol.* 7:2268–2272.
- Martin, O. Y., and D. J. Hosken. 2003. The evolution of reproductive isolation through sexual conflict. *Nature* 423:979–982.
- McPeck, M. A., and J. M. Brown. 2000. Building a regional species pool: Diversification of the Enallagma damselflies in eastern North America. *Ecology* 81:904.
- Opaev, A. S., and E. N. Panov. 2016. Variations of space use in males of the banded demoiselle (*Calopteryx splendens*, Zygoptera, Odonata): alternative tactics or an age-dependent trend? *Entomol. Rev.* 96:525–536.
- Parker, G. A. 1979. Sexual selection and sexual conflict. Pp. 123–166 in M. S. Murray and N. A. Blum, eds. *Sexual selection and reproductive competition in insects*. New York Academic Press, New York.
- Pfennig, K. S., and D. W. Pfennig. 2009. Character displacement: ecological and reproductive responses to a common evolutionary problem. *Q. Rev. Biol.* 84:253–276.
- Plaistow, S., and M. T. Siva-Jothy. 1996. Energetic constraints and male mate-securing tactics in the damselfly *Calopteryx splendens xanthostoma* (Charpentier). *Proc. R. Soc. B Biol. Sci.* 263:1233–1239.
- Post, E., U. S. Bhatt, C. M. Bitz, J. F. Brodie, T. L. Fulton, M. Hebblewhite, J. Kerby, S. J. Kutz, I. Stirling, and D. A. Walker. 2013. Ecological consequences of sea-ice decline. *Science* 341:519–524.
- R Development Core Team. 2015. R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.
- Rice, W. R. 1996. Sexually antagonistic male adaptation triggered by experimental arrest of female evolution. *Nature* 381:232–234.
- Rivera, A. C., J. A. Andrés, A. Córdoba-Aguilar, and C. Utzeri. 2004. Postmating sexual selection: Allopatric evolution of sperm competition mechanisms and genital morphology in calopterygid damselflies (Insecta: Odonata). *Evolution* 58:349.
- Siepielski, A. M., K. Hung, E. E. B. Bein, and M. A. McPeck. 2010. Experimental evidence for neutral community dynamics governing an insect assemblage. *Ecology* 91:847–857.
- Stachowicz, J. J. 2001. Mutualism, facilitation, and the structure of ecological communities. *Bioscience* 51:235–246.
- Svensson, E. I. 2013. Beyond hybridization: diversity of interactions with heterospecifics, direct fitness consequences and the effects on mate preferences. *J. Evol. Biol.* 26:270–273.
- Svensson, E. I., and M. Friberg. 2007. Selective predation on wing morphology in sympatric damselflies. *Am. Nat.* 170:101–112.
- Svensson, E. I., L. Kristoffersen, K. Oskarsson, and S. Bensch. 2004. Molecular population divergence and sexual selection on morphology in the banded demoiselle (*Calopteryx splendens*). *Heredity* (Edinb). 93:423–433.

- Svensson, E. I., J. Abbott, and R. Härdling. 2005. Female polymorphism, frequency dependence, and rapid evolutionary dynamics in natural populations. *Am. Nat.* 165:567–576.
- Svensson, E. I., F. Eroukhmanoff, and M. Friberg. 2006. Effects of natural and sexual selection on adaptive population divergence and premating isolation in a damselfly. *Evolution* 60:1242–1253.
- Svensson, E. I., K. Karlsson, M. Friberg, and F. Eroukhmanoff. 2007. Gender differences in species recognition and the evolution of asymmetric sexual isolation. *Curr. Biol.* 17:1943–1947.
- Svensson, E. I., F. Eroukhmanoff, K. Karlsson, A. Runemark, and A. Brodin. 2010. A role for learning in population divergence of mate preferences. *Evolution* 64:3101–3113.
- Svensson, E. I., and J. T. Waller. 2013. Ecology and sexual selection: evolution of wing pigmentation in Calopterygid damselflies in relation to latitude, sexual dimorphism, and speciation. *Am. Nat.* 182:E174–E195.
- Svensson, E. I., A. Runemark, M. N. Verzijden, and M. Wellenreuther. 2014. Sex differences in developmental plasticity and canalization shape population divergence in mate preferences. *Proc. R. Soc. B Biol. Sci.* 281:20141636–20141636.
- Svensson, E. I., A. Nordén, J. T. Waller, and A. Runemark. 2016. Linking intra- and interspecific assortative mating: consequences for asymmetric sexual isolation. *Evolution* 70:1165–1179.
- Svensson, E. I., M. Gómez-Llano, A. Rivas, and H. M. Bensch. 2018. Frequency-dependence and ecological drift shape coexistence of species with similar niches. *Am. Nat.* *In press*.
- Takahashi, Y., K. Kagawa, E. I. Svensson, and M. Kawata. 2014. Evolution of increased phenotypic diversity enhances population performance by reducing sexual harassment in damselflies. *Nat. Commun.* 5:1–7.
- terHorst, C. P., J. A. Lau, I. A. Cooper, K. R. Keller, R. J. La Rosa, A. M. Royer, E. H. Schultheis, T. Suwa, and J. K. Conner. 2015. Quantifying nonadditive selection caused by indirect ecological effects. *Ecology* 96:2360–2369.
- Tynkkynen, K., M. J. Rantala, and J. Suhonen. 2004. Interspecific aggression and character displacement in the damselfly *Calopteryx splendens*. *J. Evol. Biol.* 17:759–767.
- Tynkkynen, K., J. S. Kotiaho, M. Luojumäki, and J. Suhonen. 2005. Interspecific aggression causes negative selection on sexual characters. *Evolution* 59:1838–1843.
- Verzijden, M. N., and E. I. Svensson. 2016. Interspecific interactions and learning variability jointly drive geographic differences in mate preferences. *Evolution* 70:1896–1903.
- Verzijden, M. N., S. K. Scobell, and E. I. Svensson. 2014. The effects of experience on the development of sexual behaviour of males and females of the banded demoiselle (*Calopteryx splendens*). *Behav. Processes* 109:180–189.
- Waage, J. K. 1979. Dual function of the damselfly penis: sperm removal and transfer. *Science*. 203:916–918.
- Waller, J., and E. I. Svensson. 2016. The measurement of selection when detection is imperfect: how good are naïve methods? *Methods Ecol. Evol.* 7:538–548.
- Walsh, M. R. 2013. The evolutionary consequences of indirect effects. *Trends Ecol. Evol.* 28:23–29.
- Walther, G.-R., E. Post, P. Convey, A. Menzel, C. Parmesan, T. J. C. Beebee, J.-M. Fromentin, O. Hoegh-Guldberg, and F. Bairlein. 2002. Ecological responses to recent climate change. *Nature* 416:389–395.

Associate Editor: D. Roff  
Handling Editor: M. Servedio

## Supporting Information

Additional Supporting Information may be found in the online version of this article at the publisher's website:

**Table S1.** In June–July 2015, we conducted mesocosm experiments in large outdoor cages.

**Table S2.** To test whether the total density of individuals affected male and female survival, we performed a binomial model using number of individuals alive after two days as response variable.

**Table S3.** AIC values showing the fit of alternative models affecting female survival.

**Table S4.** Analysis of male survival in mesocosm experiments.

**Table S5.** In June–July 2016, we conducted a behavioral experiment consisting on a series of small mating cages.

**Table S6.** AIC values alternative models of successful mating attempts.

**Table S7.** AIC values of alternative models of mating attempts (i.e., clasplings).

**Table S8.** Tukey post hoc analysis of *Calopteryx splendens* female longevity in the mesocosm experiments.