

Sex wars: a female genital spine forces male damselflies to shorten copulation duration

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Abstract

In some species, males use weapons to harm females, increasing their short-term fitness. Here we show that females can use genital adaptations against males. Females of the damselfly *Enallagma cyathigerum* have a conspicuous vulvar spine on the eighth abdominal segment, which contacts with the male during copulation. We tested 3 hypotheses for its function: it (a) inflicts damage to the male during copulation; (b) facilitates endophytic oviposition; and (c) stimulates males during copulation to increase their investment. We found that males mated on average for 54 min with control females, but increased copulation to 99 min with females without spine. There was no evidence of physical harm of the spine on the male's seminal vesicle, which shows 8- to 18-folds, exactly where the spine contacts during copulation. Females with and without spine exhibited the same egg-laying rates and showed similar fecundity and fertility. Longevity was also similar in males mated to control and spineless females. In contrast to many species where females resist male harassment by behavioral responses, the morphological adaptation observed in *E. cyathigerum* appears to act as a sexual weapon, allowing females to control copulation duration. We suggest that the spine has evolved because of sexual conflict over mating duration.

Keywords: sexual selection, sexual conflict, copulatory wounding, vulvar spine, Odonata

Introduction

As hypothesized by Darwin, sexual selection has two main components: inter-sexual selection (choosiness, usually by females) and intrasexual selection (competition, generally among males; Hoquet, 2015). Although both sexes collaborate to produce offspring, the fact is that the goals in terms of fitness are different: male reproductive success generally increases with the number of mates, while females are more limited by the number of eggs they produce (often referred to as the “Bateman’s Principle”; Bateman, 1948). However, females’ fitness might increase with multiple matings, if this produces cumulative benefits (Fincke, 1997), such as access to superior habitat resources defended by males, nuptial gifts used to produce more eggs, increased genetic variability in the offspring (Arnqvist & Nilsson, 2000), or good genes. This will generate postmating sexual selection on newly developed male adaptations (by cryptic female choice or sperm competition), by rewarding those males that are able to elicit responses and/or manipulate female reproductive outcome the most (Simmons, 2005). Therefore, mating can be considered as a “battle of sexes” (Dawkins, 1976) and as such, it involves weapons with which to penalize the opposite sex, typically, females (Clutton-Brock & Parker, 1995). One of such cases is the copulatory wounding imposed by males on females (Reinhardt et al., 2014). In some species, males have apparently won the conflict over mating frequency, by evolving sexual coercion as their reproductive strategy, and

imposing high costs on females (Singh et al., 2022). But even under sexual coercion, females can exert some control of their reproductive decisions by varying their resistance to males with different phenotype, like in some fishes (Bisazza et al., 2001). Furthermore, in predatory animals, females can seriously damage approaching males, or even kill them before or during copulation (sexual cannibalism; Schneider, 2014). For instance, in a widow spider 70% of males are killed by females during the first copulation (Baruffaldi & Andrade, 2021), imposing high costs on males. However, even in a sexually cannibalistic mantis, males adopt a coercive strategy or damage females before mating, and reduce in this way the risk of being cannibalized (Burke & Holwell, 2021). Therefore, males may damage females during copulation (e.g., copulatory wounding), and females may kill males (sexual cannibalism), being two extreme cases of sexual conflict. Surprisingly, no cases are known of females harming their mate during copulation in noncannibalistic species, despite this being expected in species with reversed sex roles (see Yoshizawa et al. (2014), for a suggestive example in a Brazilian cave insect).

Females of several damselfly genera within the Coenagrionidae family, have a vulvar spine at the base of the ovipositor located at the ventral side of the eighth abdominal segment (Figure 1; May, 2002). While the function of the ovipositor is broadly known (Emeljanov, 2014; Matushkina & Lambret, 2011) nothing is known about the function of the vulvar spine. It is clearly not used to facilitate or difficult

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mating, because it does not contact with the male before copulation (see Figure 2).

Here, we performed a series of experiments to test three hypotheses that might explain the presence of this structure, using a laboratory colony of the damselfly *Enallagma cyathigerum*. Our first hypothesis derives from the observation of mating in this species (Figure 2, Video 1). During copulation, the female's vulvar spine comes into contact with the male's seminal vesicle located at the second abdominal

segment, and it pushes on the ventral surface of the male's vesicle (A.C.-R., personal observations), every time the male makes the "pumping" movements of stage I and stage II of copulation (Miller & Miller, 1981). It is important to consider that in damselflies the start and end of copulation is under male control (Andrés & Cordero-Rivera, 2000; Miller & Miller, 1981), and mating consists of a first phase which involves in-copula guarding and rivals' sperm removal, and a final phase of insemination (Miller, 1987; Miller & Miller

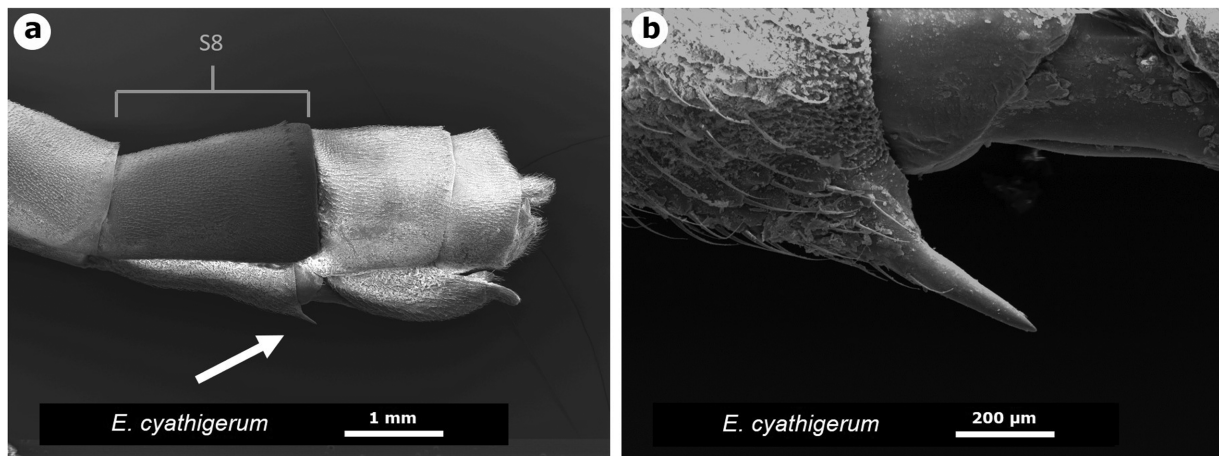


Figure 1. Lateral view of the eight abdominal segment (S8) of an *Enallagma cyathigerum* female observed under a scanning electron microscope (SEM) showing the location of the vulvar spine (A). A magnification of the robust vulvar spine found in this species is shown in (B). Pictures by ACR.

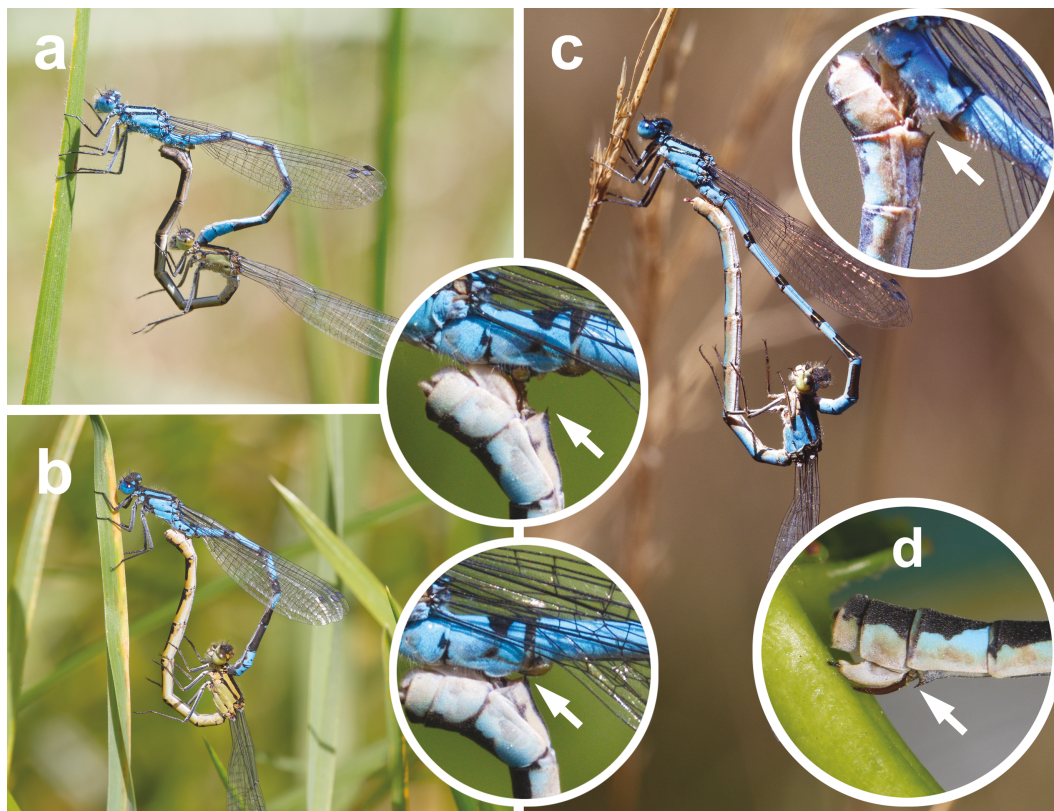


Figure 2. Copulation in *Enallagma cyathigerum* is characterized by a first phase (stage I, A and B) in which the male's abdomen is held in a curved position and shows rhythmic active flexions of the second and third segments, contacting in this way with the vulvar spine (arrows in the inserts). Then, during stage II (C), the male's abdomen is pressed to transfer the sperm to the female, and the spine is fully inserted into the ventral side of the vesicle. During oviposition, the vulvar spine does not come into contact with the plant tissue where the eggs are being inserted (D). Pictures by ACR.

1981). From our observations, we hypothesized that the vulvar spine in *E. cyathigerum* could damage the secondary genitalia of males, and make copulation costly for males, or even induce copulatory wounding. To test this idea, we mated males to intact and spineless females, and compared their copulation duration and their survivorship. We predicted that removing the spine would allow males to increase copulation duration, possibly because they need time to remove sperm from rivals (Miller, 1987) and also because they can guard females by remaining in copula for long periods (Andrés & Cordero-Rivera, 2000). We therefore hypothesize that the spine has evolved in the context of sexual conflict over mating duration, allowing females to shorten copulations in an indirect way. If the spine induces significant damage to males, we expected higher survival for males mated to spineless females and unmated males, compared to males mating with females with the spine. We also studied the seminal vesicle under scanning electron microscopy (SEM), to assess the possible physical damage inflicted by the female's spine to the male's seminal vesicle.

A second hypothesis that could explain the presence of the vulvar spine is that this structure could be an adaptation for greater efficiency at the time of oviposition (egg laying): *Enallagma* females lay eggs endophytically, and the vulvar spine could provide them with a firm anchorage to cut the epidermis of the leaves used as oviposition substrate (Figure 2D). In this scenario, the spine would not be related to sexual conflict, but rather evolved due to natural selection pressures.

The last hypothesis for the presence of the vulvar spine is that this structure could serve as a "stimulatory device" during copulation, increasing male's investment (i.e., inseminate more sperm or transfer nutritional fluids to females). Under this hypothesis, we would predict that females with spine should have higher fecundity and/or fertility rates than spineless females (Bonduriansky, 2001).

Methods

Study species, specimen collection, and laboratory rearing

Enallagma cyathigerum (Charpentier 1840) is a common damselfly species, with a widespread distribution in Europe. Within the genus *Enallagma* females possess a robust vulvar spine on the eighth abdominal segment (May, 2002).

Newly emerged individuals of *E. cyathigerum* (in total about 160 specimens) were collected at the coastal lagoon of Doñinos in the NW Iberian Peninsula (43.490501 N, -8.315606 W, 6 m a.s.l.), throughout 5 days in April–May 2018. The size of this population is very large (many thousands of individuals), and therefore collection had no effect on the viability of the population. Permits to work with the species were issued by the regional government of Galicia. There are no ethical requirements to work with these insects, but we housed them in large insectaries with plenty of food, to minimize any stress. Furthermore, to reduce the number of animals used in the experiments, some individuals were included in two experiments. Animals were collected with a hand net and transported to the laboratory, where males and females were placed separately in wooden insectaries (measuring 50 × 50 × 50 cm; housing 15–20 individuals per cage). Sticks were provided within each insectary to allow perching, and individuals were fed with *Drosophila melanogaster* ad libitum. Humidity was maintained daily by nebulizing water

inside the insectary. After five days damselflies reached sexual maturation and they were individually marked on the wings with a permanent marker (Staedtler Lumocolor permanent pen), to distinguish them during the experiments. To mimic natural behavior, mating tests were started at 9:30 a.m. while oviposition tests were done immediately after.

Copulation duration

Females were randomly separated into two groups: the treatment group had their vulvar spine removed with micro dissecting scissors, and the control group had their vulvar spine intact. The removal of the spine was done under a binocular microscope and took a few seconds. Control females lived for 13.06 ± 0.74 days ($N = 31$) and spineless females lived 12.04 ± 0.61 ($N = 28$). A GLM with binomial errors indicate no significant differences in lifespan (deviance ratio = 1.12, $p = .294$), suggesting that the experimental removal of the spine has no effect on female survival. To observe the mating behavior, males were randomly introduced to the control (with spine) or treatment insectary (spineless females), to control for any effect of the insectary, and were allowed to mate up to three times in their life. Males were housed together because male density is known to affect copulation duration in damselflies (Cordero, 1990). In total we recorded 127 matings involving 55 males and 67 females.

Copulation duration was measured to the nearest minute by direct observation on the insectaries. In this species, there are spontaneous disengagements of the genitalia (but maintaining the tandem junction). Total copulation duration (including breaks; Box–Cox transformed to normalize it) was analyzed with a REML model with treatment (spine present/absent), female mating status (virgin/mated), their interaction, and time of start of copulation as fixed terms, and male and female identity as random terms. One mating that lasted only 6 min was excluded from all analyses, because it was clearly an outlier (range of duration of remaining copulations 24.1 to 237.0 min).

The number of breaks during the stage I of copulation (Miller & Miller, 1981) per minute followed a normal distribution. The effect of the treatments and covariables on this variable was tested with a REML analysis with the same model as above.

Seminal vesicle damage

To test if the female vulvar spine caused genital damage to the males, we studied the sperm vesicles of 46 males in four treatments: teneral males (sexually immature and never mated; $N = 10$), mature but never mated males ($N = 12$), mature males that mated three times with intact females ($N = 11$), and mature males that mated three times with females whose spine was removed ($N = 13$). No more than three males were inside the cage at the same time. Individuals of each group were preserved in 96% ethanol immediately after the end of the last copulation. We dissected the seminal vesicles of all males belonging to the different treatments. All the extracted samples were analyzed by scanning electron microscopy (SEM). We found that the ventral side of the seminal vesicle has a smooth surface, except in the region where the vulvar spine contacts during copulation, which shows several folds, which we hypothesized were produced by the spine. Therefore, we counted the number of folds on the ventral surface of the seminal vesicle of each male. To test for repeatability, counts were done by two people independently and

showed high correlation ($r = 0.73$, $N = 46$, $p < .0001$). We also measured the length of the folds twice ($r = 0.53$, $N = 45$, $p < .001$). Therefore, the mean number and the mean length over both observers were taken as the response variate for the analyses.

Male longevity

Male longevity was compared among three treatments: (a) males mating three times (in nonconsecutive days) with females who possessed their intact vulvar spine ($N = 15$); (b) males mating three times with females whose vulvar spine was removed ($N = 16$); and (c) males that never mated during their entire lifespan ($N = 29$). After each mating session, males were brought back to the insectary.

Fecundity, fertility, and efficiency in egg laying

To test if the vulvar spine stimulates the male to transfer more sperm or nutritional fluids (hence increasing female fecundity and/or fertility) or whether this structure plays a functional role increasing the efficiency of oviposition (hence increasing laying egg rate and total fecundity), females with and without the spine were allowed to mate once. They were then placed inside jars for between 15 and 20 min on moist filter paper as oviposition substrate (Cordero, 1990). Females were observed throughout this time to measure oviposition duration and a timer was stopped whenever they stopped laying eggs. Each female was allowed to oviposit once a day on two consecutive days. After oviposition, filter papers containing egg clutches were placed in spring water, oxygenated with aquarium pumps for 30 days to allow eggs to develop. Egg clutches were preserved in 96% ethanol and the eggs were counted under a binocular microscope, distinguishing between fertile (hatched eggs or eggs containing a visible embryo) and non-fertile eggs.

Fertility data were analyzed using a GLM with binomial errors and logit link, using the total number of eggs laid as binomial totals, and corrected for overdispersion. We analyzed the number of fertile eggs as the response variable and the presence/absence of the spine, age at first oviposition, and the number of clutches as predictors. Fecundity and egg laying rate (the number of eggs laid/minute) followed a normal distribution, and therefore were analyzed by means of a GLM with normal errors, including the same predictor variables as for fertility. Means are presented with their standard errors. All statistical analyses were conducted with xlStat (www.xlstat.com) and GenStat 20th edition software (GenStat, 2020).

Results

Copulation duration

The mean duration of copulation for control females (with vulvar spine) was 53.86 ± 3.32 min ($N = 75$), but almost doubled for spineless females (98.77 ± 5.49 min; $N = 52$; Figure 3). The effect was highly significant (predicted means, back-transformed: 49.60 min for control females vs. 85.58 for spineless; $F_{1,56.0} = 44.99$, $p < .001$). The effect of female mating status was also significant (Figure 3; predicted means, back-transformed: 73.76 min for mated females vs. 56.57 for virgins; $F_{1,83.9} = 24.20$, $p < .001$) and a negative effect of time of start of copulation was detected ($F_{1,105.5} = 8.44$, $p = .004$). The interaction between spine and mating status was close to significance ($F_{1,83.5} = 3.57$, $p = .062$).

Copulation in this species is characterized by a series of temporary disengagements of the genitalia, whose function is unknown (Perry & Miller, 1991). To test if the removal of the spine affected the frequency of these stops, the number of stops per minute of copulation (to control for copulation duration) was analyzed with a REML with the same factors as in the previous analysis. The results indicate a lower number of stops with virgin females (predicted mean: 0.22) compared to mated ones (0.26 stops/min; $F_{1,84.1} = 17.70$, $p < .001$), a significant interaction between the presence of the spine and female mating status (virgins, predicted means: 0.22 for spineless and also for control females; mated females, predicted means: 0.23 for spineless vs. 0.29 for control females; $F_{1,89.2} = 7.93$, $p = .020$), but no significant effect of the presence of the spine ($F_{1,50.9} = 2.16$, $p = .148$) nor of time of start of copulation ($F_{1,116.6} = 2.24$, $p = .137$).

Seminal vesicle damage

The ventral side of the vesicle showed no clear evidence of damage, but a series of folds in its central area, ranging in number from 6 to 18 (Figure 4). These folds were present in the vesicles of teneral (mean \pm SE: 11.9 ± 0.81 folds) and mature unmated males (10.8 ± 0.60 folds), and also in mature males mated three times to control females (with intact spine: 11.8 ± 0.72 folds) and those mated three times to females whose spine was removed (12.0 ± 0.57 folds). We found no significant differences among treatments in the mean number of folds (ANOVA on square-root transformed values, $F_{3,42} = 0.698$, $p = .558$). Furthermore, the sum of lengths of these marks was also similar among groups (ANOVA, $F_{3,40} = 0.650$, $p = .588$).

Male longevity

We found no effect of the spine on male longevity. Males that never mated lived for 11.2 ± 0.73 days, those mating with control females 11.9 ± 0.68 days and those mated to spineless females 12.8 ± 0.84 days. These values are not significantly

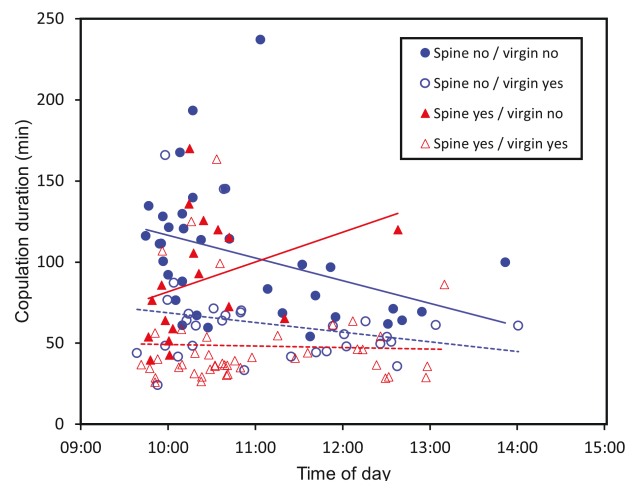


Figure 3. The relationship between time of start of copulation and copulation duration (including spontaneous breaks) for females without (blue circles) and with (red triangles) the vulvar spine, distinguishing virgins (open symbols) and previously mated (closed symbols). Males almost doubled copulation with spineless females, and increased copulation by 30% with mated females compared to virgins.

different (GLM with Poisson errors, deviance ratio = 1.18, $p = .307$).

Egg fertility and efficiency in egg laying

Once-mated females ($N = 8$) with vulvar spine laid an average of 125.3 ± 19.6 (\pm SE) eggs over two days, with a fertility rate of 0.97 ± 0.005 , and an egg laying rate of 4.66 ± 0.49 eggs/min. Females without vulvar spine ($N = 6$) laid an average of 112.0 ± 27.2 eggs, of which 0.96 ± 0.006 were fertile, at a rate of 4.77 ± 0.56 eggs/min. Fertility (GLM with binomial errors, deviance ratio = 0.66, $p = .596$) and fecundity (GLM with normal errors, $F_{3,10} = 0.79$, $p = .526$) were not affected by the presence or absence of the vulvar spine, age at first oviposition or number of clutches. The presence of the spine did not affect egg laying rate ($t_{10} = 0.68$, $p = .512$), nor the number of ovipositions ($t_{10} = -0.90$, $p = 0.390$), but there

was a marginally significant negative effect of age at first oviposition on egg laying rate (coefficient: -0.773 ± 0.414 , $t_{10} = -1.87$, $p = .092$).

Discussion

Our results clearly indicate that males prolong copulation (almost to double its duration) when they mate with spineless females. As occurs in this and other similar species, copulation duration was negatively affected by time of day and was shorter with virgin females (Andrés & Cordero-Rivera, 2000; Cordero, 1990; Uhía & Cordero-Rivera, 2005). The number of spontaneous disengagements of genitalia increased in mated females compared to virgins and was higher when the spine was present, but only in mated females. We found that the seminal vesicle has a series of folds in the area that

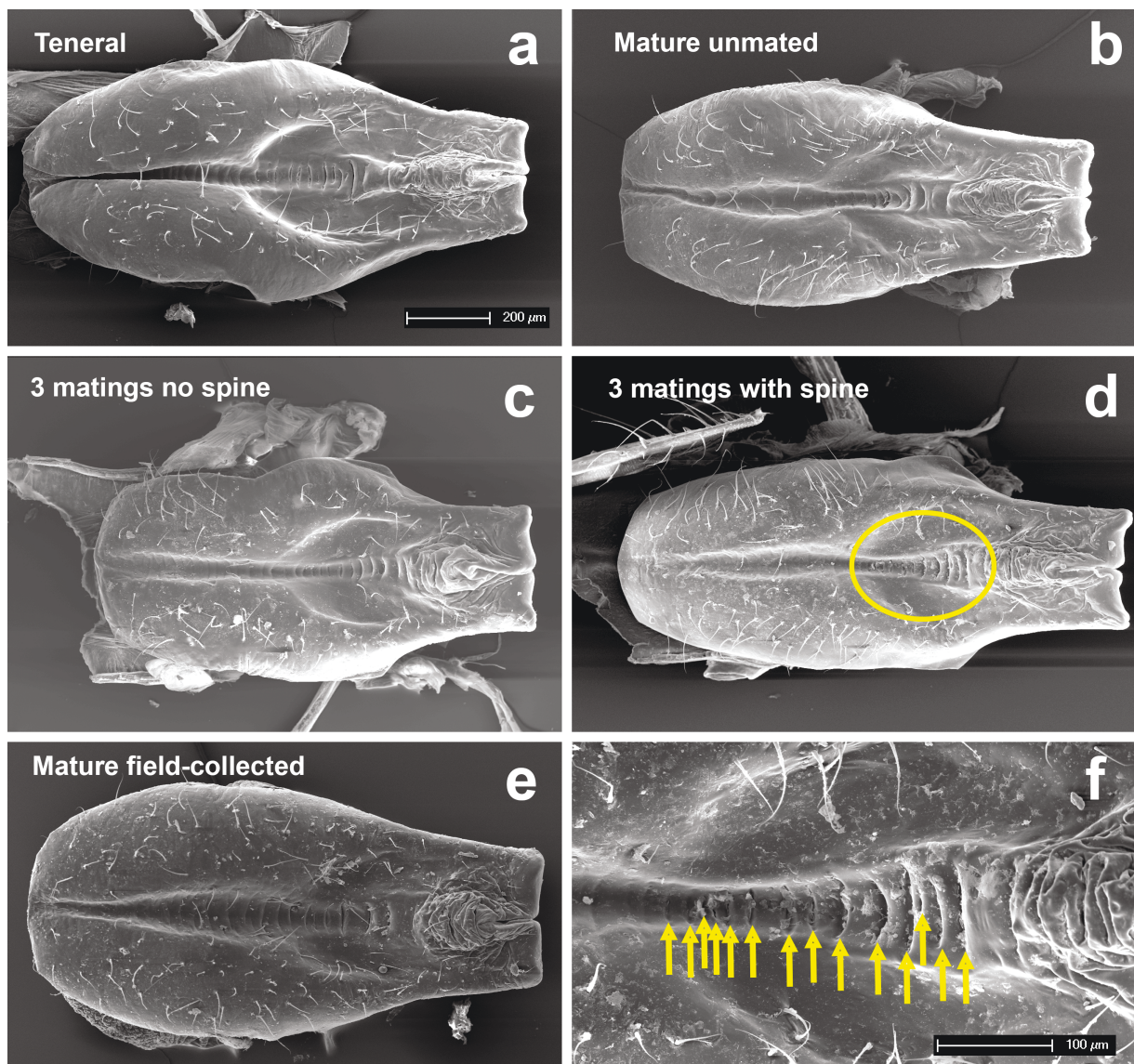


Figure 4. Ventral view of seminal vesicles from five individuals of *Enallagma cyathigerum* observed under SEM, showing the folds found in the place where the female vulvar spine pushes during copulation (indicated by arrows in F). The area where the folds were counted and their length measured is indicated by the ellipse in (D). (A) Teneral individual, sexually immature; (B) mature individual that never mated; (C) mature male mated three times with females whose vulvar spine was removed; (D) mature male mated three times with intact females; (E) field-collected mature individual, whose reproductive history is unknown. All images at the same scale, indicated in (A), except for image F, which shows an amplification of (D). Pictures by ACR.

is contacted by the vulvar spine during copulation, but its number and extension is not affected by the presence of the spine, suggesting that the spine does not harm this structure. Finally, the presence of the vulvar spine did not affect male longevity, female egg laying rate or female fecundity and fertility.

Very long copulations (up to 6 hrs) in damselflies have been shown to be the result of sexual selection on males, which remain in copula to guard the female against rivals (Miller, 1987). This creates a sexual conflict, because females cannot feed during copulation, and may be more exposed to predators during copula (Galicia-Mendoza et al., 2017). Under male-biased sex-ratio mating duration is much longer, creating extreme conflict between the sexes (Cordero, 1990). Sexually antagonistic coevolution may result in the evolution of male structures that grasp or perforate females during copulation but female defensive divergence is very uncommon (Eberhard, 2006), and female “offensive” adaptations, like the spine of *E. cyathigerum*, are unknown. Our results indicate that the vulvar spine allows females to exert some control over copulation duration by producing enough “discomfort” or “pain” to males to reduce copulation duration. It might be questioned whether insects feel something similar to “pain”, but some evidence points to a positive answer (Cordero-Rivera et al., 2021). The seminal vesicle of males is highly elastic, increasing its size several times when full of sperm (Supplementary Video 1). This fact might explain why wounds in the seminal vesicle are not observed, and this elasticity may partly be an adaptive response to the female spine. The folds found on its ventral surface (Figure 4F) might absorb the pressure exerted by the spine, leaving no morphological trace of damage. However, it is clear that the spine affects males, given that they double copulation duration with spineless females.

For males, reducing the time that is invested in copulation might imply a reduction in their ability to remove sperm from rivals, which requires some time in *E. cyathigerum* (Perry & Miller, 1991), and increases the risk of losing the female to rivals after copulation (Cordero-Rivera & Rivas-Torres, 2019). For the females, however, shorter copulations could be advantageous, as they would imply starting ovipositing earlier, and hence, ending laying eggs and to start feeding also earlier. This is important, because in this and other *Enallagma* species, females go underwater to oviposit, and are frequently trapped on the water surface on resurfacing. They are rescued by other males, and mate again with them (Fincke, 1986; Miller, 1990). Given that male reproductive activity decreases over the day, the last females resurfacing from oviposition may not find a “rescuer” male available, increasing the risk of death on the water surface, therefore providing an advantage to those females that mate earlier.

We have not directly tested the fitness costs for females of not having the spine, but the longer copulation duration that males achieve with spineless females is a possible cost. The presence of this spine is rare in damselflies, being recorded in several genera of the Ischnurinae, like *Enallagma*, *Ischnura*, *Mesamphiagrion*, and *Acanthagrion*. In the genus *Ischnura*, some species have a very prominent spine, like for instance *I. elegans* or *I. graellsii*, which are involved in extremely long copulations (up to 6–7 hr; Cordero, 1990; Miller, 1987), and presumably very intense sexual conflict over mating duration. Other species of this genus are likely monogamous (Sánchez-Guillén et al., 2020), and a preliminary survey indicates that these species have a vestigial or no spine at all, like for

instance the monandric females of *Ischnura hastata*, whose mating lasts about 11 min (Cordero-Rivera et al., 2023), suggesting that mating duration and the spine are related, but this needs further study. In some species of water striders, where sexual conflict over mating frequency is intense, females have abdominal spines which thwart harassing males, increasing female control over copulation frequency but not duration (Arnqvist & Rowe, 1995). Females can use behavioral responses to resist male harassment, by biting, kicking or in other way attacking their mates, to the extreme case of sexual cannibalism (Baruffaldi & Andrade, 2021). Teeth, claws, or other structures used by predatory animals can obviously be also used in a sexual conflict scenario, but a morphological adaptation apparently only used to reduce copulation duration, like the spine of *E. cyathigerum*, has not been found in any other taxon as far as we know.

The fact that the spine also affects the frequency of stops during copulation, but only in mated females, suggests that these stops are related to postcopulatory sexual selection (Córdoba-Aguilar & Cordero-Rivera, 2008). Perhaps the spine is affecting male ability to remove sperm, because mated females with the spine had more stops compared to spineless females, but the spine did not affect the number of stops in virgin females. Our results agree with the suggestion that removed sperm is expelled at these breaks (Perry & Miller, 1991), because the stops are less frequent in virgins, and male damselflies are known to be able to detect female mating status (Andrés & Cordero-Rivera, 2000), which is demonstrated by the different duration of copulation between virgin and mated females (Figure 3). The spine contacts with the male seminal vesicle at each movement of the stage I of copulation and might impede that the male fully inserts the genital ligula, and hence results in more breaks. This possibility needs further study.

Having a vulvar spine did not increase the efficiency of oviposition in *E. cyathigerum* females. It could be argued that the filter paper used in our experiments as oviposition substrate did not completely simulate the surface of a natural oviposition substrate, and for this reason females were not laying eggs efficiently. However, the egg-laying rate in nature for *E. cyathigerum* is 4.6–8.25 eggs/min (Doerksen, 1980), a rate similar to the one we observed in our experiment (4.77 eggs/min). Therefore, this explanation is unlikely. Close observation of females laying eggs has shown that the spine is not in contact with the substrate at any moment (Figure 2D), which further refutes the hypothesis of the spine as a naturally selected device to anchor the abdomen on the oviposition substrate.

On the other hand, Odonates are not known to transfer nutrients with the seminal fluid (Fincke, 1997), nor do they increase the amount of sperm inseminated in long copulations (Andrés & Cordero-Rivera, 2000). In agreement with this, fecundity and fertility did not differ between females with and without a vulvar spine, suggesting that the hypothesis of this structure being used as a cryptic female choice device is highly unlikely. However, if the spine affects the ability of males to remove sperm, then it could indirectly increase female ability to cryptically select among males, by increasing sperm mixing.

We hypothesize that the vulvar spine of *Enallagma* serves as a sexually selected weapon, which might have evolved due to the sexual conflict over mating duration in odonates (Córdoba-Aguilar et al., 2003). In favor of this hypothesis is the fact that the removal of the spine “allows” males to

double copulation duration. The logical expectation is therefore that the spine would damage males during copulation. However, we did not find any difference between teneral/mature, mated/unmated, or mated with control/spineless females in the number of folds observed in the male's seminal vesicle. The presence of such marks could be explained if males have evolved tolerance to any potential damage that the vulvar spine could exert on their seminal vesicle. Tolerance would not impose costs to the males, as opposed to evolving a more sophisticated adaptation such as sclerotization of the seminal vesicle, which would be actually costly (Reinhardt et al., 2014). Alternatively, but not mutually exclusive, these folds might have evolved to allow the seminal vesicle to expand when the male translocates sperm to this organ before copulation (the intra-male sperm translocation behavior, see Rivas-Torres et al., 2019). The fact that males mating with intact and spineless females had similar longevity also suggests that the "damage" does not translate into significant costs to males. In a parallel example, skin folds have been found in female marine mammals with the function to reduce the male penis pressure on the female ventral area (Orbach et al., 2017) and sclerotized structures are known in *Drosophila* females which have evolved as a counter adaptation to copulatory wounding (Kamimura & Mitsumoto, 2011; Yassin & Orgogozo, 2013). In the bed bug female (*Cimex lectularius*) an abundance of resilin, an elastic protein, has been found in the cuticle of the female's defense organ, probably evolved to tolerate traumatic penetration (Michels et al., 2015). Conflict over mating rates is strong in odonates, and probably has resulted in color changes from immature to mature females to indicate unreceptivity (Cordero-Rivera et al., 2023; Khan, 2020), and specialized rejection behaviors (Cordero-Rivera et al., 2023; Khelifa, 2021; Xu & Fincke, 2022). It is possible that the ventral side of the seminal vesicle of *Enallagma* and other damselflies whose female has a conspicuous vulvar spine (like some species of *Ischnura* or *Mesamphiagrion*) have also evolved tolerance by resilin accumulation, and this is a priority for future studies. In fact, a preliminary examination of *Ischnura graellsii*, a species whose females also have a conspicuous vulvar spine has shown one large hole in the male's vesicle, which is already present in unmated males. This suggests that the hole is a mechanism of tolerance of the possible damages. If our interpretation is correct, these structures should be absent in species whose females do not have any vulvar spine. We are currently working to test this hypothesis.

Supplementary material

Supplementary material is available online at *Evolution* (<https://academic.oup.com/evolut/qp4073>).

Data availability

All data are provided as Supplementary files.

Author contributions

A.R.-T. and V.D.P. performed the experiments. A.C.-R. designed the study, obtained funding, performed statistical analyses, and supervised the writing. All authors contributed to the final writing.

Conflict of interest: The authors declare no conflict of interest.

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