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Females “assist” sneaker males to dupe dominant males in a rare endemic damselfly: sexual conflict at its finest

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Sneaky mating tactics have fascinated scientists for decades, and the mechanisms behind its evolution remain unclear. In many taxa, sneaker males are thought to outsmart the dominant males because they can secure fertilization of eggs either through pre-copulatory or post-copulatory processes (Shuster and Wade 2003). For instance, sneaker males of a fish mimic the female’s appearance to dupe the dominant male (Todd et al. 2017) whereas, in some insects, sneaker males steal females from male’s territories and force copulation (Cordero and Andrés 2002). Unlike the commonly assumed theory that sneaker males do all the work to outsmart dominant males (Oliveira et al. 2008), here I show in a rare endemic riverine damselfly species, *Calopteryx exul* Selys (Fig. 1), that females contribute to the success of sneaker males by actively avoiding dominant males and thus securing sneaker’s paternity.

Sperm precedence was first discovered in *Calopteryx* damselfly (Waage 1979) and since then the evolutionary significance of post-copulatory guarding has been widely recognized in many taxa. A dominant male guards females in a territory because his sperm inside the females could be displaced and replaced by other competing males (Cordero-Rivera 2017). A sneaker male does not possess a territory and thereby his mating success depends not only on his ability to steal a female but also on the probability that a female secure access to the oviposition site without copulating with the dominant male (Siva-Jothy and Hooper 1995). Although post-copulatory selection of the sneaker male has been suggested (Siva-Jothy and Hooper 1996), little attention has been devoted to the potential nonintuitive role of female in determining the pre-copulatory selection of sneaker male.

I conducted a behavioral survey in 2010 and 2011 in northeast Algeria in the largest ever recorded population of *C. exul* worldwide (Khelifa et al. 2016). The harem-like mating system of *C. exul* consists of a male guarding a territory (patches of floating leaves) where females come to copulate and lay eggs (Mellal et al. 2018). A few dominant males (two to four) guarded all females (20–30 females) while other males are perched nearby. Dominant males chased all conspecific males that flew near the territory, but some sneaker males were successful at stealing females and copulating a few meters away. Interestingly, when the female finished copulating with the sneaker male, she returned to the same territory where the dominant male copulated again with her. Given that species of *Calopteryx* are known to have a penis structure that removes almost all sperm from the reproductive tract of the female (last male sperm precedence) (Waage 1979), the success of the sneaky attempt seems very negligible, if not null. However, the existence of sneaky behavior in this species and other congenerics (Cordero 1999) suggests that sneakers are not only able to remove the sperm of the territorial male, but also to ensure the fertilization of the eggs without having the necessary resource (Taborsky 1994). To decipher the potential fitness benefits of sneaker behavior, I marked individuals and assessed the roles of dominant males and females in shaping the success of sneaker males (Appendix S1).

I carried out focal observations on 275 ovipositing females (63 in 2010 and 212 in 2011) and 28 territorial males (10 in 2010 and 18 in 2011) in two different territories. Sneaking attempts were recorded 110 times (48 in 2010 and 62 in 2011), of which 66.6% in 2010 and 74.2% in 2011 were successful. The number of sneaking attempts depended positively on population density (Poisson GLM, slope = 0.027, SE = 0.005, $N = 58$, $z = 5.157$, $P < 0.0001$, Appendix S1: Fig. S1, Table S1), suggesting that sneaker behavior is density dependent (Cordero and Andrés 2002). It is likely that mate and/or resource (territory) limitation shortage induced by territory monopolization were the trigger of the expression of sneaker tactic, as it has been suggested for another calopterygid (Waage 1973) and other insects (Thornhill 1981).

I took advantage of the marked individuals to assess how sneaker males ensure the fertilization of the eggs

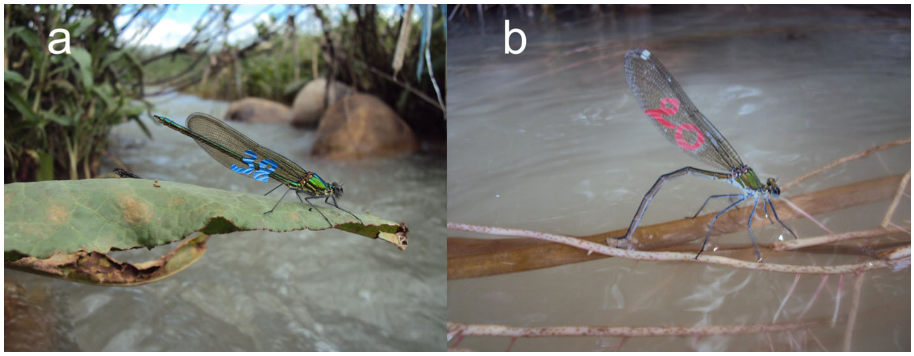

FIG. 1. Mature adult of *Calopteryx exul* (a) male and (b) female. This damselfly is endemic to North Africa (Morocco, Algeria, and Tunisia) and is listed as endangered in the IUCN red list. The species is territorial where males guard patches of floating plant substrates that attract females for egg laying (Video S1).

without having the necessary resource (territory). This leads to a more general question: what are the evolutionary mechanisms that allow sneaky behavior to exist in species with sperm precedence? After forced copulation (a copulation performed without courtship by a male that does not have a territory on a female that generally shows refusal behaviors) with a sneaker male, which lasts around 2 min, the female returns to the territory where she will potentially recopulate with the dominant male and have almost all of the sperm removed from the reproductive tract. Given that the dominant male might misrecognize the sneaked female and copulate with another female (Waage 1987), I analyzed the relationship between the probability of misrecognition of newly landed female in the territory by the focal territorial males (Appendix S1: Fig. S2), the number of females already ovipositing in the territory and focal female aggregation behavior (clumping near conspecifics despite the availability of alternative oviposition supports) (Fig. 2; Appendix S1: Table S2). The results showed that the probability of misrecognition of the female increased with the number of females present in the territory (Poisson GLMM, slope = 0.682, SE = 0.152, $N = 169$, $z = 4.479$, $P < 0.0001$) and its aggregation behavior (Poisson GLMM, $\Delta = 0.682$, SE = 0.493, $N = 169$, $z = 3.684$, $P = 0.0002$), revealing that the fitness of sneakers relies on the probability of female recognition by the territorial males, which is mainly dependent on the number of females in the territory (Alcock 1983) and the aggregation behavior of the female. This, together with the previous finding, suggests that sneaky behavior is expressed when population density is high because its probability of success becomes higher.

The second important question to answer was: does harassment (forced copulation) lead to female aggregation? In damselflies, sneaky behavior is costly to females because it can lead to (1) external damages on the wings that affect flight ability (Combes et al. 2010), (2) internal damages in the reproductive tract due to repeated copulations (Crudgington and Siva-Jothy 2000), or (3) energy loss due to copulation avoidance (Watson et al. 1998). I assessed whether sneaky copulation affects the aggregation behavior of the sneaked female using observations on 41 different females (11 in 2010 and 30 in 2011) that underwent forced copulation by sneakers. McNemar's test shows that forced sneaky copulation leads to female aggregation ($\chi^2 = 20.16$, $P < 0.0001$). Among the surveyed females that did not aggregate before forced sneaky copulation, 79.3% ($n = 29$) aggregated after sneaky copulation. Given that aggregation affects the recognition of the female (Video S1), it can be suggested

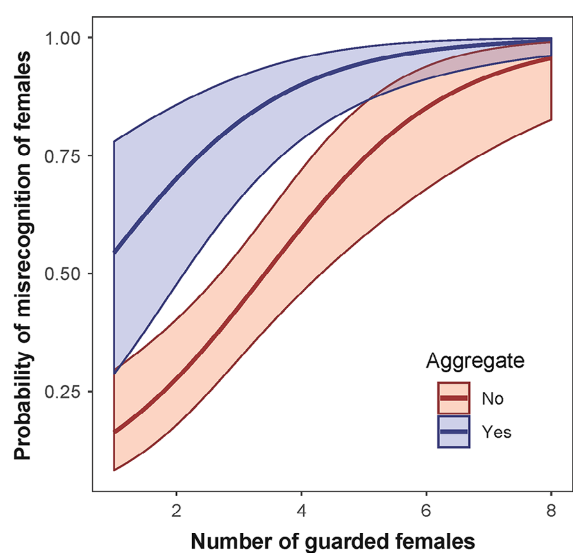

FIG. 2. Probability of misrecognition of females by territorial males of *Calopteryx exul* depending on the aggregation and the number of ovipositing females. The results show that the probability of misrecognition increased both with the number of females ovipositing in a territory and aggregation behavior. Lines show logistic regressions; shaded areas show confidence intervals.

that females participate in deceiving the territorial male, and hence, indirectly plays a role in securing the fertilization of the eggs by the sneaker's sperm. The female aggregation behavior can serve as a prevention process against subsequent sneaky attempts and recopulation with the territorial male, which can affect her fitness (Crudgington and Siva-Jothy 2000). Odonates' females use different behavioral strategies including death feigning to avoid such costs (Khelifa 2017).

Regarding the proportion of females that was last inseminated by the territorial male, our data showed that the apparent lifetime mating success (LMS; summing all females present in the territory) was 6.10 ± 4.77 in 2010 (mean ± SD; $n = 10$) and 11.64 ± 9.42 ($n = 17$) in 2011. However, this LMS can be partitioned into three categories based on the last copulation: (1) the realized LMS for the territorial male represented only 69.2% and 73.4%, (2) sneakers represented 10.5% and 8.9%, and (3) the remaining 20.2% and 17.6% are of unknown origin (females securing oviposition without copulation with the dominant or sneaker male) in 2010 and 2011, respectively (Appendix S1: Fig. S3). One behavioral observation that strengthens the hypothesis that females tend to avoid multiple copulations is the fact that many females (forced or not) tend to start oviposition without copulation (Siva-Jothy and Hooper 1996). Those females probably had sperm stored in their spermathecae from previous matings, which might be of mixed paternity (Siva-Jothy and Hooper 1995).

This study highlights the importance of natural history to understand sexual selection. Although genetic analyses can reveal the paternity of sneakers in the next generation (Siva-Jothy and Hooper 1995, 1996), it is insufficient per se to understand the mechanisms that secure paternity. For instance, without assessing the reproductive behavior of *C. exul*, one might conclude through genetic analysis that the high paternity of sneaker males is mainly due to post-copulatory sperm selection, excluding the possibility of pre-copulatory mechanisms of selection by the female. This theory does not exclude the potential contribution of post-copulatory mechanisms when sperm is stored in spermathecae.

This study is, to my knowledge, the first to suggest that females might play a role in deceiving the dominant male and helping (indirectly) the sneaker male to secure fertilization of eggs, which implies that the evolution of alternative mating tactics can result from a complex sexual conflict in which the female "selects" the less fit male over the fitter male to prevent fitness costs. The results might increase our knowledge of the role of sexual conflict in the evolution and maintenance of phenotypic diversity in insects and animals with sperm precedence.

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