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When sex becomes a wrestling game in a dragonfly: female refusal behavior to male harassers

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Dragonflies never cease surprising me. I thought the most astonishing observation was the apparent “death feigning” (tonic immobility) of females to avoid male harassment. I observed this behavior during my Ph.D. studies in the Swiss Alps in the holarctic moorland hawker (sedge darner) *Aeshna juncea* (Linnaeus) (Khelifa 2017). Ever since then, I have wondered whether this behavior occurs in other closely related dragonfly species. During my postdoctoral studies at the University of British Columbia (Vancouver, Canada), I had the opportunity to study dragonflies on the south coast of British Columbia (Appendix S1; Fig. S1). Among this community, I focused on a related species to the moorland hawker, the blue-eyed darner (*Rhionaeschna multicolor*), a large dragonfly widespread in western North America. The blue-eyed darner behaved similarly to the moorland hawker during reproduction and showed high harassment pressure of males to single females. Thus, I suspected I might observe the same escape strategy in this species. Instead, I noted a different defense mechanism that involved a female “wrestling” with her harasser, often “sabotaging” their linked mating flight.

Clutton-Brock and Parker (1995) argued that “forced copulation can generate arms races between males and females that may have substantial costs to both sexes.” Sexual conflict, the divergence of fitness optima between males and females, explains this prediction, as males often tend to mate with as many females as they can to

increase their reproductive success, whereas females need to mate with a single or very few males to fertilize all eggs (Parker 1979). This creates an arm’s race between sexes where the male tries to increase his ability to mate with multiple females whereas females evolve mechanisms to counter male harassment.

Understanding sexual conflict is fundamental to unravelling the behavioral adaptation of sexes. In insects, males of many species are aggressive in their pursuit for fertilizing eggs, probably resulting from what Darwin described as “a struggle between the males for possession of the females” (Darwin 1859). While females have evolved different strategies to escape male harassment (Fincke 1997), it is particularly interesting that, often, females have not evolved retaliatory tactics that incite the male to cease harassment. There are some cases, however, where females can show aggression towards males. For instance, a female earwig chases the male partner out of the burrow prior to oviposition (Lamb 1976). It is likely that the low frequency of female retaliation to male harassment is the result of favoring low-risk defense strategies that maximize fitness (Smith and Price 1973). Nevertheless, in some species and under high population density the costs of harassment and forced copulation outweigh the benefits of passivity or acceptance.

Certain mating systems are prone to the evolution of retaliatory behavioral responses to harassment. Such an evolutionary trajectory can be fueled by high male-biased sex ratio at reproductive sites, absence of male-guarding, long exposure to male harassment (high spatiotemporal overlap), lack of shelter in oviposition sites, and potential fitness costs of multiple matings. One model organism with such a mating system is the Order Odonata (dragonflies and damselflies; Fincke 1997). Here, I report female blue-eyed darners using retaliatory tactics against male harassers. Specifically, the reproductive behavior of the species was surveyed using both direct observations and high-speed video field monitoring in seminatural ponds in Vancouver (British Columbia, Canada) with a particular focus on the behavioral response of females to male harassment.

Sex ratio was male-biased at the reproductive sites, but not at emergence, which is typical in odonates (Cordero-Rivera and Stoks 2008) (Appendix S1; Fig. S2). After copulation (Fig. 1A), a male left the female alone laying eggs (Fig. 1B) in ponds, which were constantly patrolled by males. When the female was detected by the male, she used a series of behavioral displays to avoid harassment (Fig. 2). Of 82 harassments (42 females, 21 marked), 48 (58.5%) resulted in forced copulation, and 34 (41.5%) resulted in females successfully rejecting the males. To initiate copulation, the male

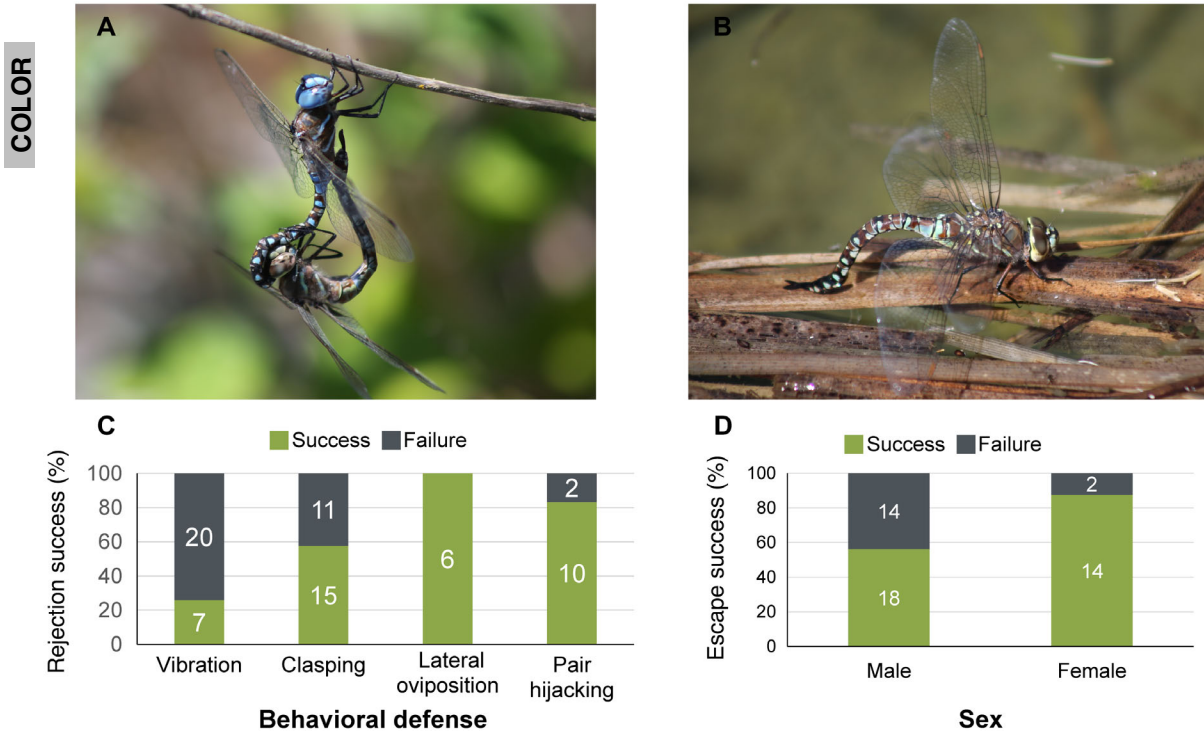


FIG. 1. Behavioral response of female to male harassers of blue-eyed damselfly (*Rhionaeschna multicolor*). (A) Copulation. (B) Typical oviposition. Oviposition is performed without male guarding. (C) Success rate of male rejection by females using four behavioral defense strategies: wing vibration, clasping the substrate, lateral oviposition, and pair hijacking (HWS, dropping to the water with the male). The plot shows that lateral oviposition and HWS gave the highest success in rejecting males. (D) Flight success after artificial exposure of the insect (body and wings) to the water.

must grasp the female's head with his terminal appendages (cerci and epiproct), and form the copulatory wheel. Some females initially refused mating by tilting, vibrating, and flapping wings (first defense strategy; Video S1), which impeded physical contact with the male. This display was successful in 7 out of 27 (26%) cases (Fig. 1C) and it suggests that males could be receptive to refusal (Utzeri 1988). In 26 cases, the male was able to grab the female by the head to attempt copulation but the female clasped the oviposition substrate tightly to avoid flying with the male, which is necessary to form a copulatory wheel (Video S2). This second defense strategy was successful in 15 cases (58%) (Fig. 1C). After repeated male harassment (head grasping and attempting to fly with the female), a third defense strategy was to lie on the side and minimize movement while ovipositing eggs (lateral oviposition; Video S3), which made the back of her head inaccessible to the males. This strategy was successful in 100% of cases (six cases, Fig. 1C).

When the male was successful in flying into the air with an unwilling female, he often failed to make the rotation that leads to wheel formation. High-speed videos showed that what often appeared as a fully

locked wheel (male secondary genitalia at the base of the abdomen in the female genital aperture at the tip of the abdomen) is actually nothing but a male holding the female's abdomen tightly with his legs. An unwilling female can break the wheel at this stage in which case the male will try again to make a rotation in the air with difficulty. The pair often hit the water surface or other obstacles such as plants when attempting wheel formation (Video S4). It is likely that the female has a role in the instability of the flight (Rüppell and Hilfert-Rüppell 2014) because wheel formation is most likely a cooperative act where both sexes coordinate movement to achieve it (Corbet 1999). Thus, the unstable flights after forced pairing attempts are either the result of female non-cooperation or female active maneuvering to sabotage the flight. Two observations suggest that both could be true. First, it appears that a female wiggles her head repeatedly in flight, probably to detach the male (Video S5). Second, the female was observed flying in a different direction than the male, suggesting that she has some control over the pair flight (Video S6) (Rüppell and Hilfert-Rüppell 2014). Out of 12 occasions, 10 (83%) led to the splitting of the pair, and 9 (75%) ended with the male floating on

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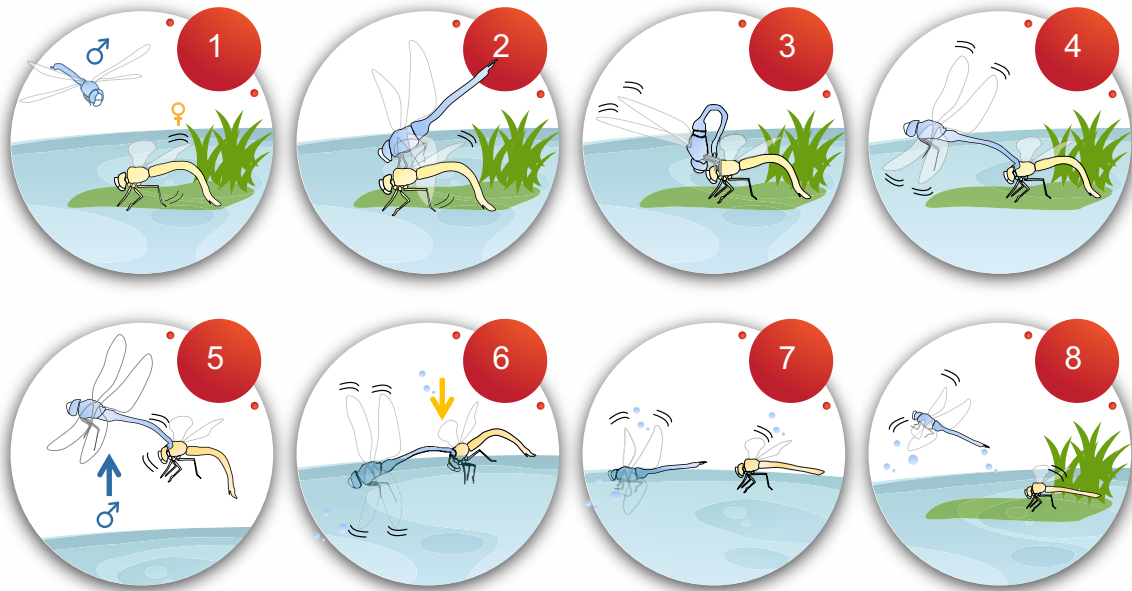


FIG. 2. Defensive mechanism of females against male coercion during oviposition in the blue-eyed damselfly (*Rhionaeschna multicolor*). (1) Female vibrates wings to signal refusal; (2) male attempts to mount the female; (3) male grabs the female by the head; (4) male tries to fly to form the copulatory wheel while the female claps the plant substrate; (5) male takes off with the female and tries to form the copulatory wheel; (6) the female drops to the water with the male attached to her; (7) the pair split and flap wings on the water surface; (8) the male takes off and stops harassment while the female continues to lay eggs.

the water (eventually taking off a few seconds after) while the female was free. The entire sequence of dropping rapidly with the male onto the water surface looks like “pair hijacking” (Figs. 1D, 2). These unsuccessful males did not further harass after pair splitting, and the female returned to the water to continue laying eggs.

After pair hijacking, the male took off, performed a “spin-dry” behavior (Walker 2016), which consists of spinning at $>1,200$ rpm six to nine times to remove water from its body (Video S7). The spin-dry behavior occurred whenever the male has been in contact with the water. This behavior appears to be important to avoid potential wetting-related fitness costs. Wing wetting increases the mass load, particle contamination (dust, algae, fungal spores, and bacteria), as well as the energy required to fly, and consequently may decrease aerial competitive ability, reduce foraging success, and increase the predation risk (Webb et al. 2014). The interesting aspect of pair hijacking is that it may have fitness consequences to the male as well as the female. However, females might be better adapted to wetting than males given their more frequent contact with water during oviposition (Kuitunen et al. 2014).

To understand the potential costs of wetting, a field experiment simulating the wetting that occurs during pair hijacking was carried out where 32 males and 16 females were captured, marked, gently immersed in the

water for 1–2 s, then released (Video S8). We recorded whether the male and the female were able to fly after getting wet. The percentage success of escape from the water was 56% in males and 87% in females (Fig. 1D), but the difference was not significant (Fisher’s exact test, $P = 0.35$). Those that did not fly fell on the water surface (44% of males and 13% of females), flapped wings repeatedly but could not take off. Although the wetting simulation was not ideal due to touching wings using hands, nevertheless it suggests that pair hijacking has the potential to trap the male (and potentially the female) in the water, which could lead to death by fish or frog predation.

The observed reproductive behavior in these ponds should also occur in natural habitats. It is unlikely that the artificial aspect of the ponds has fundamentally changed the behavior of the dragonflies. For instance, sex ratio at emergence was not significantly different from 1:1 (Appendix S1: Fig. S2), thus the studied habitat did not favor male over female density. Although low density of vegetation might have exacerbated the frequency of harassment to some extent, the species reproduces naturally in open ponds with low vegetation cover and the males search the vegetation thoroughly regardless of its density.

This female response fits the prediction of Clutton-Brock and Parker (1995) who stated that forced copulation can lead to the evolution of traits that may have

major costs to both sexes. The big question that remains is why would females prefer to endure physical stress rather than submit to convenience polyandry (Cordero and Andrés 2002). The accumulation of evidence of behavior to avoid sex in odonates (Cordero-Rivera and Rivas-Torres 2019) highlights the costs of multiple matings for females, which could involve both apparent and cryptic costs that merit further investigation (copulatory wounding, sexually transmissible disease, or seminal toxins) (Johnstone and Keller 2000, Reinhardt et al. 2015).

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LITERATURE CITED

- Clutton-Brock, T. H., and G. A. Parker. 1995. Sexual coercion in animal societies. *Animal Behaviour* 49:1345–1365.
- Corbet, P. S. 1999. *Dragonflies: behaviour and ecology of Odonata*. Harley Books, Colchester, UK.
- Cordero, A., and J. A. Andrés. 2002. Male coercion and convenience polyandry in a calopterygid damselfly. *Journal of Insect Science* 2:14.
- Cordero-Rivera, A., and A. Rivas-Torres. 2019. Sexual conflict in water striders, dragonflies and diving beetles. Pages 295–320 in *Aquatic insects*. Springer.
- Cordero-Rivera, A., and R. Stoks. 2008. Mark-recapture studies and demography. Pages 7–20 in A. Córdoba-Aguilar, editor. *Dragonflies and damselflies: Model organisms for ecological and evolutionary research*. Oxford University Press, Oxford, UK.
- Darwin, C. 1859. *The origin of species*. John Murray, London, UK.
- Fincke, O. M. 1997. Conflict resolution in the Odonata: implications for understanding female mating patterns and female choice. *Biological Journal of the Linnean Society* 60:201–220.
- Johnstone, R. A., and L. Keller. 2000. How males can gain by harming their mates: sexual conflict, seminal toxins, and the cost of mating. *American Naturalist* 156:368–377.
- Khelifa, R. 2017. Faking death to avoid male coercion: extreme sexual conflict resolution in a dragonfly. *Ecology* 98:1724–1726.
- Kuitunen, K., A. Kovalev, and S. N. Gorb. 2014. Sex-related effects in the superhydrophobic properties of damselfly wings in young and old *Calopteryx splendens*. *PLoS ONE* 9:e88627.
- Lamb, R. J. 1976. Parental behavior in the Dermaptera with special reference to *Forficula auricularia* (Dermaptera: Forficulidae). *Canadian Entomologist* 108:609–619.
- Parker, G. 1979. Sexual selection and sexual conflict. Page 166 in M. S. Blum, and N. A. Blum, editors. *Sexual selection and reproductive competition in insects*. Academic Press, New York, New York, USA.
- Reinhardt, K., N. Anthes, and R. Lange. 2015. Copulatory wounding and traumatic insemination. *Cold Spring Harbor Perspectives in Biology* 7:a017582.
- Ruppell, G., and D. Hilfert-Ruppell. 2014. Slow-motion analysis of female refusal behaviour in dragonflies. *International Journal of Odonatology* 17:199–215.
- Smith, J. M., and G. R. Price. 1973. The logic of animal conflict. *Nature* 246:15–18.
- Utzeri, C. 1988. Female “refusal display” versus male “threat display” in Zygoptera: is it a case of intraspecific imitation? *Odonatologica* 17:45–54.
- Walker, J. S. 2016. Splash-dunk/spin-dry analysis for 2011–2016. *Argia* 28:41–44.
- Webb, H. K., R. J. Crawford, and E. P. Ivanova. 2014. Wettability of natural superhydrophobic surfaces. *Advances in Colloid and Interface Science* 210:58–64.

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