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Winners wave wings: courtship display increases mating success in Rhagoletis juglandis (Diptera: Tephritidae)

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Abstract

Insects employ a variety of conspicuous traits and courtship behaviors to mediate mate choice. Visual displays are especially common in tephritid flies with patterned wings, but it has long been debated whether courtship display occurs in the Rhagoletis genus. Although recent work demonstrated mutual visual display during courtship in R. pomonella, it is unknown whether such displays are broadly important for mating success across the genus. Here, we tested the functional role of wing display in walnut-infesting R. juglandis by experimentally restricting wing display during courtship. Copulation success was significantly more likely if males were unrestricted and retained full wing display functionality. Among successful matings, copulations with unrestricted males were typically preceded by male and/or female wing displays, whereas restricted males typically initiated copulation without observable courtship. These results suggest that wing displays facilitate the progression of courtship and increase the likelihood of mating success, indicating that sexual selection likely acts on this trait. More broadly, these findings support a functional role for visual signaling in tephritid courtship and suggest that such displays may be more widespread than previously appreciated.

Introduction

Mate choice defines which traits increase an individual's likelihood of mating, and courtship is the arena in which premating choice is on display (Rosenthal 2017). Courtship necessitates a signal produced by a courter to be detected and evaluated by a chooser and these signals are generated through a variety, and often combination, of sensory modalities (Mitoyen et al. 2019). In most cases, both males and females take on roles in this back-and-forth communication that can increase detectability, induce mating receptivity, or provide information about the courting individual (Rosenthal 2017; Mitoyen et al. 2019). However, directly testing the contribution of individual traits to mating success remains challenging. By connecting a signal to mating success, we can begin the first steps towards understanding mate choice.

From dazzling firefly light patterns to the cicada's droning song, insects offer diverse examples of conspicuous traits and behaviors that mediate mate choice (West-Eberhard 1984). In Diptera, many courtship sequences involve some kind of wing display (Burk 1981). *Chrysomyra* blowflies males extend their wings and display wing interference patterns while courting females (Butterworth et al. 2019, 2021), *Ceratitis* flies perform wing buzzing that may fan pheromones towards a prospective mate (Briceño et al. 2005), and *Drosophila* species have a vast repertoire of songs produced by wing vibration (Hoy et al. 1988). Visual displays are especially common in tephritid flies that have patterned wings (Headrick and Goeden 1994), leading many to hypothesize that these spotted, barred, or stellate wings are relevant for species recognition and mate choice (Bush 1966; Sivinski and Pereira 2005; Hood et al. 2012).

It has long been debated whether this common courtship pattern observed in tephritid flies also occurs in *Rhagoletis* flies, especially for the economically-important apple-infesting *R. pomonella* and walnut-infesting *R. completa*. Early descriptions mention variable wing pigmentation patterns between species and that visual cues are especially important to mating decisions (Bush 1966; Prokopy and Bush 1973).

Yet previous work suggested that there is no courtship at all and copulation is forced (Smith and Prokopy 1980, 1982; Benelli et al. 2014). Other studies found that only males in the walnut-infesting species group, and not *R. pomonella*, will fight other males for access to females (Opp et al. 1996) or court females using visual or acoustic signals (Alonso-Pimentel et al. 2000).

Recently, we experimentally tested for sexual selection for the first time in *R. pomonella* and discovered that males and females engage in mutual visual wing displays (Anner et al. in press). Further, higher female display effort significantly predicted copulation success: wing display is a sexually-selected signal (Anner et al. in press). With detailed analysis of video recorded courtship behavior, we were able to redefine our understanding of courtship in *R. pomonella*. Therefore, examining courtship and wing displays in other *Rhagoletis* species can clarify whether courtship is required for mating.

Here, we ask whether restricting courtship display in walnut-infesting *R. juglandis* led to lower mating success than an unrestricted fly. To test this, we paired a female fly with two males: one with unrestricted wing movement and the other with wings glued to the abdomen. Manipulation experiments provide an elegant way to interrupt certain traits and evaluate the behavioral impacts (e.g. Colyott et al. 2016; Belkina et al. 2021). If copulation is forced by males jumping onto females, we would see an equal number of copulations between restricted and unrestricted males. We hypothesize that wing display is a necessary component of courtship and males with restricted wing movements will be significantly less likely to successfully mate.

Methods

Collection and rearing

In August 2023, we collected Arizona walnuts, *Juglans major*, infested with *R. juglandis* from Portal, AZ. We transported these walnuts to the XXXXXXX where they were kept on wire racks in bins under controlled temperature (~23 °C) and light conditions (14:10 hour light:dark). Over the next month, larvae naturally emerged from the fruit and we collected them 3 times a week into dishes of damp vermiculite (up to 200 larvae per dish). We placed these dishes in an environmental chamber (Percival I36VLC9) at 22 °C and a 14:10 light:dark cycle to mimic the natural experience of larvae in which they emerge from infested walnuts that have fallen to the ground and burrow ~10 cm into the soil to pupariate (Lackey et al. 2023). After ~21 days, we sorted through each dish and removed any dead larvae or pupae, as evidenced by very dark coloration or mold growth (Ries, DT 1935). After an additional week (28 days total in 22°C), we transferred the dishes to an incubator set to 4°C and 24 hour darkness to emulate overwintering in the soil following standard rearing conditions (Lackey et al. 2023; Anner et al. 2026).

After 4 months (Sept. 2023-Jan. 2024), we moved half of the pupae to an incubator with 22 °C and 14:10 h light:dark cycles to initiate the end of diapause. We placed each dish into a 32 oz deli container with a mesh panel for air flow and added food (1:3 by mass yeast hydrolysate:sugar) and water (1 oz container with a paper towel wick) *ad libitum*. We moved the remaining dishes out 2 months later (March 2024) so

that adult emergence would be spread out across two time periods and more feasible to manage. Adult flies began emerging about 1 month after we moved them into the 22 °C incubator. We checked for adult flies three times each week and dampened the vermiculite in the dishes of pupae weekly until all flies emerged. Each adult was placed in an individual housing container with a mesh panel for air flow and provisioned with food (1:3 by mass yeast hydrolysate:sugar) and water *ad libitum*.

Mating trials

To test whether display behavior is important to mating, we performed choice trials in which one female chose between an unrestricted male and a male with its wings glued to its abdomen. Once flies were sexually mature (at least 14 days old; Neilson and McAllan 1965), we randomly selected half of the adult male flies and limited their ability to perform wing displays during courtship; hereafter, we refer to these as restricted males. They were sedated for ~30 seconds on a plate that slowly exuded carbon dioxide (Flystuff® Drosophila Ultimate Flypads). Once anesthetized, we placed a small dot of superglue on the thorax, then used forceps to move the wings onto the glue (Fig 1c). We allowed the glue to dry for 15 seconds, then returned the male to its housing and ensured both wings were glued successfully. Restricted flies were still able to walk and hop and showed similar activity levels to unrestricted flies, just with no ability to fly or perform the wing display. *Rhagoletis* always perform simultaneous wing display (both wings at once rather than one at a time) and we confirmed that restricted flies could still attempt to display by observing one instance where only one wing was glued and the fly still performed with the other one. As such we are confident that the gluing process does not hinder the initiation of this behavior, only the execution. We sedated the remaining half of the males and the females in the same manner, then added a dot of Testor's enamel paint to the thorax to aid differentiation during the trial because the sexes are morphologically similar. Both restricted and painted flies were anesthetized for under 2 minutes total and given a minimum of 24 hours to acclimate back in their housing before mating trials to ensure the carbon dioxide would not affect their behavior.

Trials took place between 10am and 4pm when flies are most active (Prokopy and Bush 1973) in a room with ample natural light and consistent room temp (~23 °C). We used 3D printed white plastic mating arenas (12.7cm x 12.7cm x 5.1 cm; Fig 1a) with a transparent plexiglass lid and a slit on one side that allowed us to place a removable divider into the arena. To set up each trial, we brought the mating arena with the divider in place into an enclosed mesh container (BugDorm model 4S3030D) and added the female to one side of the divider and the two males to the other side of the divider. Each arena also contained a green walnut-sized half-sphere as a mating stimulus (Prokopy and Bush 1973; Alonso-Pimentel et al. 1998). The plexiglass lid was taped into place and the arena was placed under a GoPro Hero 8 camera (Fig 1a).

We allowed five minutes of acclimation with the divider in place, then delicately removed the divider through the slit in the arena and began the recording at 60 fps and 1080p. Upon successful copulation, we recorded the time and identity of the copulating male. We defined copulation as the male mounting the female for at least 10 seconds. We included the time minimum because males can attempt

mounting but fail or mount other males, and in both situations, mounting behavior lasts only a few seconds (Prokopy and Bush 1973; Alonso-Pimentel et al. 2000). When copulation was successful, we continued recording until copulation ended or after 30 minutes, whichever occurred first. If copulation was unsuccessful in 30 minutes, we stopped recording and did not include these trials in the analysis.

Statistical analysis

After preliminary visualizations, we noticed that copulation duration showed a bimodal distribution with a distinct split at 10 minutes. In line with previous work on *Rhagoletis* mating, we decided to exclude these trials from the analysis (n=5) as they indicate unsuccessful copulation. Instead, these trials were likely instances in which males would mount another fly for an extended period of time but copulation would be ultimately unsuccessful (i.e. no genital contact; Gullan and Cranston 2014). This could occur if the female rejected the male or, as is common with *Rhagoletis* and other insects, the male attempted to mount the other male (Bush 1966; Monk et al. 2019). The model predicting copulation success that excluded these five trials performed better than the model that included them (Akaike's information criterion corrected for small samples (AICc) model comparison in Supplementary Table 1; Akaike 1974)).

We tested whether the probability of copulation success differed between restricted and unrestricted males in 24 trials using a generalized linear model with a binomial distribution. Then, we ran t-tests to evaluate whether latency to copulation or copulation duration differed between trials in which restricted or unrestricted males were successful. We also ran a linear model to evaluate if copulation duration was impacted by latency to copulation. For the linear models, we assessed model fit to normality with the DHARMa package, which assesses deviation of model residuals and QQ plot residuals (Hartig 2022). For the t-tests, we assessed normality with Shapiro-Wilks tests and equal variances with F tests. All variables met assumptions. We performed all statistics in R version 4.6.0 (R Core Team 2026).

Results

Copulation success was significantly more likely if males were unrestricted and retained full wing display functionality (70.8% change of copulating; likelihood ratio X^2_1 = 12.08, p = 0.0005; Fig. 2; Online Resource 2). Unrestricted males were 5.9 times as likely to copulate, although it was still possible for restricted males to succeed (7 out of 24 trials). Restricted males that were successful initiated copulation by jumping onto females in 85% of trials whereas unrestricted males jumped onto females in only 31% of trials (Fig 3; Online Resource 3). While the shortest latencies to copulation occurred when females mated with unrestricted males, average latency to copulation did not significantly differ for unrestricted and restricted males (Fig. 4; t_{22} = -1.72, p = 0.1). Similarly, copulation duration did not differ between unrestricted and restricted males Supp. Fig. 1; (t_{22} = 0.3, p = 0.6763), and latency to copulation did not affect copulation duration (F_1 = 2.78, p = 0.11).

Discussion

We show that *R. juglandis* males with full wing functionality were nearly six times more likely to copulate than their restricted counterparts. Although some restricted males still successfully copulated, our results indicate that the ability to perform wing displays significantly improves the likelihood of mating success. Latency to copulation did not differ significantly between treatment groups, but notably many unrestricted males mounted females in under 10 minutes, whereas none of the successful restricted males had such short latencies. Once copulation began, copulation duration did not differ between restricted or unrestricted males.

Our results experimentally confirm prior observations that *Rhagoletis* flies perform a wing display during courtship (Bush 1966; Hood et al. 2012) and demonstrate that these displays play a functional role in promoting mating success. Although restricted males were less successful overall, they were still chosen over unrestricted males in seven trials, indicating that multiple pathways to courtship success exist. Females retain ultimate control over mating, as they must exert their aculeus (Headrick and Goeden 1994). However, males can initiate mounting in three ways: by performing a wing display while facing the female and then jumping over her to mount, watching the female perform the wing display then mounting, or by jumping onto her from behind without prior courtship (Anner et al. in press; Prokopy and Bush 1973). Males with restricted wing display could not hold female attention with a wing display or entice females to display and instead more often relied on jumping from behind to mount.

In line with courtship research in other Tephritidae that have conspicuous pigmented wings, the wing display is a visual signal (Headrick and Goeden 1994; Benelli et al. 2014). It is still unclear, however, whether signal reception is focused on the pigmentation patterns, the movement of the wing display, or both (Sivinski and Pereira 2005; Rao et al. 2022). Further, multimodal courtship is common across insects, and many wing displays also carry an auditory or substrate-borne vibrational signal (Mitoyen et al. 2019; Belkina et al. 2021). Previous work in *R. juglandis* revealed a subsonic acoustic signal (14 Hz) performed by males in bouts between five seconds and five minutes (Alonso-Pimentel et al. 2000). *Rhagoletis* also differ in cuticular hydrocarbon profiles between sexes and across species, indicating a potential role for chemical communication (Hood et al. 2022). To experimentally confirm which suite of traits are used during courtship, future research would need to iteratively block signal production or signal evaluation mechanisms for each modality. Here, we limited the production of male visual, and potential acoustic, signals by gluing wings to the abdomen. In a future experiment, we could also blind females with a dot of paint over the eyes or remove aristae to reveal the role of vision and sound reception (Colyott et al. 2016). Recent work in *R. pomonella* also details mutual and reciprocal display behavior in males and females (Anner et al. in press). With this in mind, it would be insightful to repeat the experiment but with restricted female display capability.

Together, these results indicate that wing displays are not strictly required for mating but substantially increase mating success, suggesting they function as courtship signals that influence female acceptance rather than as a mechanical prerequisite for copulation. The ability of wing-restricted males to mate is consistent with the presence of alternative mating pathways, including more opportunistic or less display-mediated tactics, like mounting the female from behind. This pattern implies that while

females retain control over mating, male behaviors can shape how and when that choice is expressed (Fitzgerald et al. 2022). Consequently, sexual selection likely acts on wing displays because they increase the efficiency and likelihood of successful mating. More broadly, these findings demonstrate that courtship signals can enhance mating success without being strictly required for copulation.

Declarations

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Availability of Data and Materials

Data and R code files will be deposited on OSF (link to be added after acceptance).

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Competing Interests

The authors have no relevant financial or non-financial interests to disclose.

Author's contributions

SA: Lead: Conceptualization, Methodology, Formal analysis, Investigation, Data Curation, Writing - original draft, Writing - reviewing and editing, Visualization, Project Administration, Supervision, Funding Acquisition

MM: Lead: Writing - original draft, Investigation; Supporting: Data curation, Visualization, Writing - reviewing and editing

AL: Lead: Resources, Supervision; Supporting: Funding Acquisition, Conceptualization, Methodology, Formal analysis, Data Curation, Writing - original draft, Writing - reviewing and editing, Visualization, Project Administration

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Figures

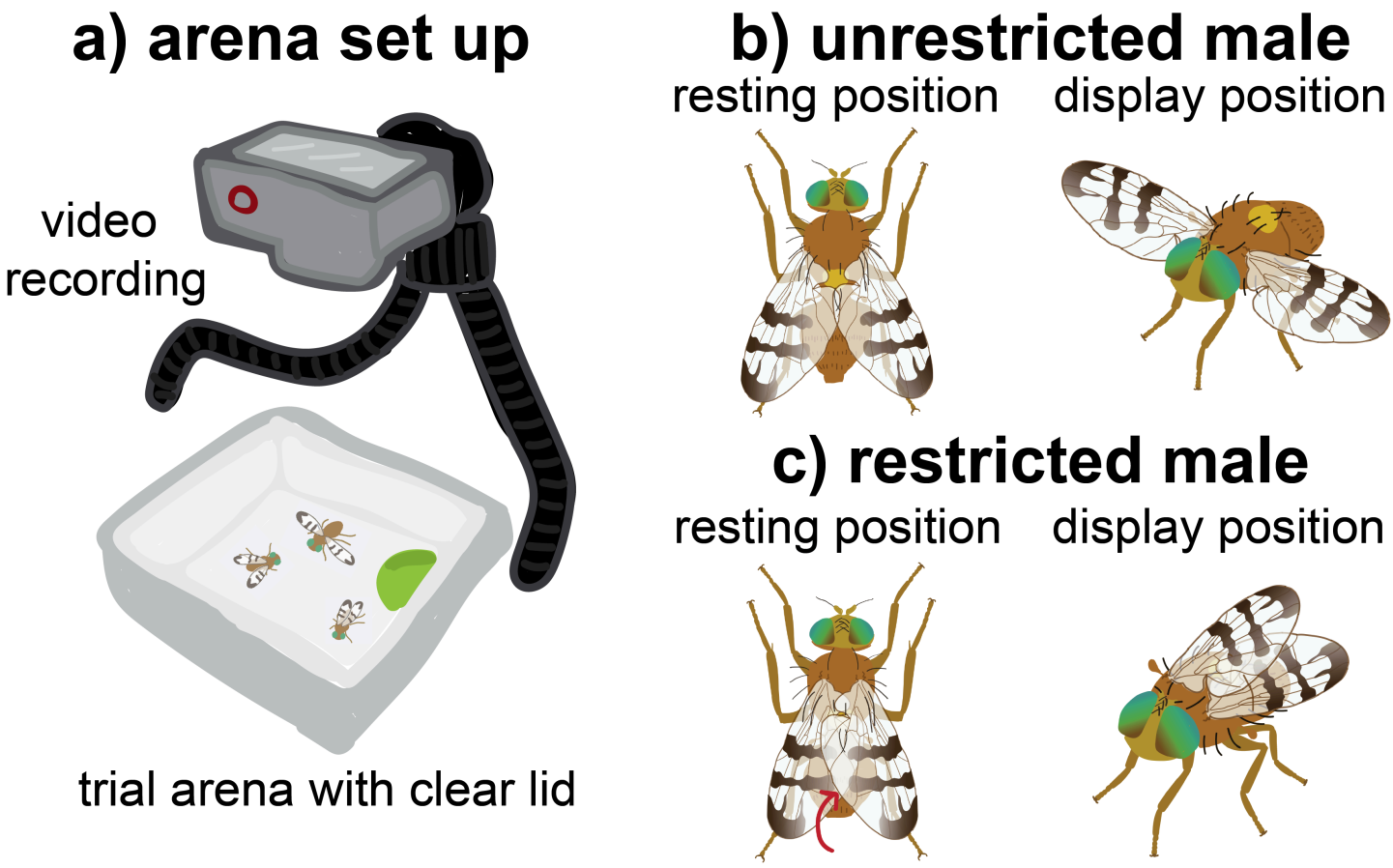


Figure 1

a) The arena set up included a plastic box with a clear plexiglass lid placed under a camera for video recording. b) unrestricted male in resting position and in display position. c) restricted male is resting position (red arrow points to where wings are glued to abdomen) and in display position.

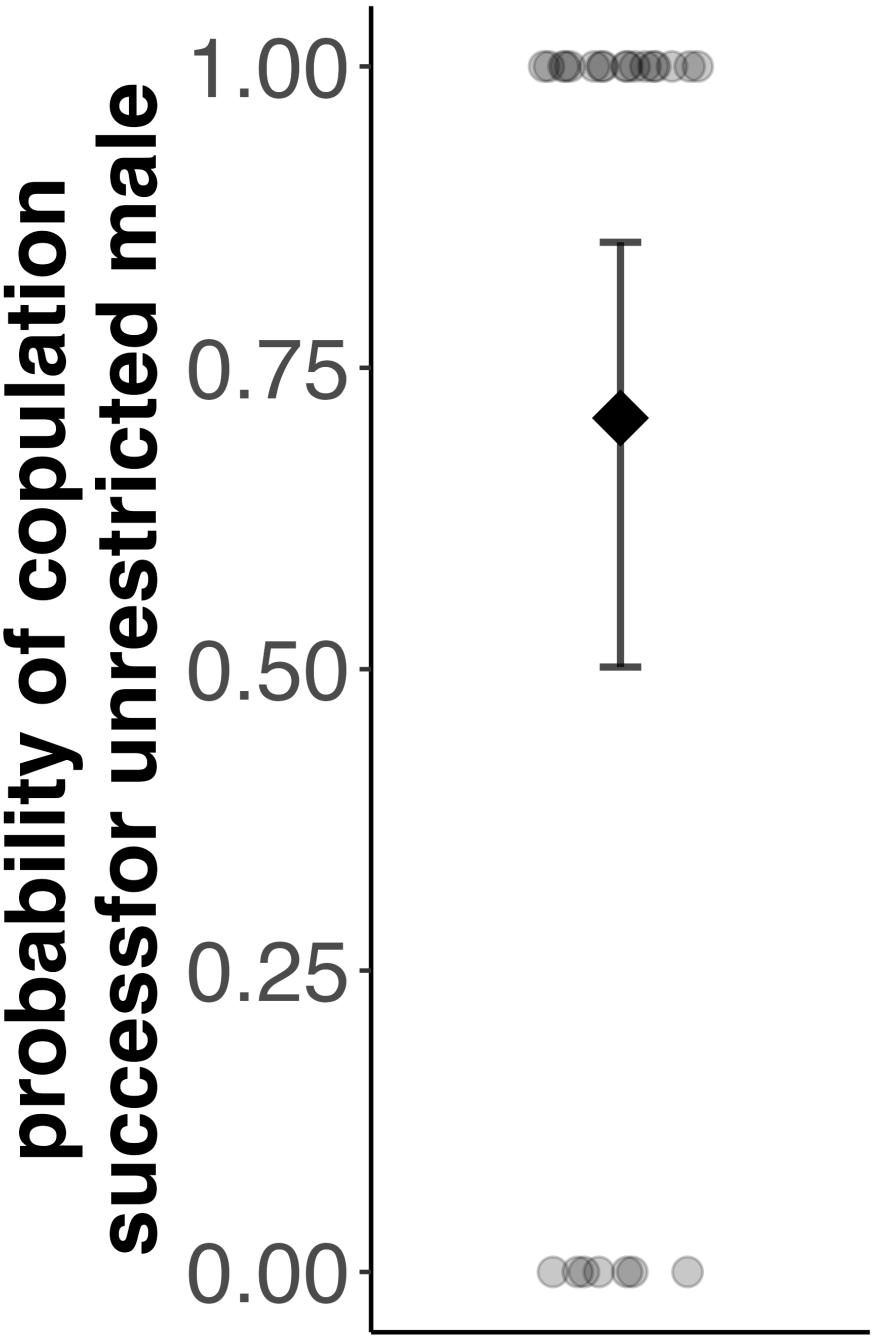


Figure 2

Unrestricted males were more likely to copulate. The diamond shows the estimated marginal mean probability of mating and the error bars show the upper and lower confidence limits. The circles indicate when unrestricted males were successful (1) and unsuccessful (0, i.e. restricted male mated).

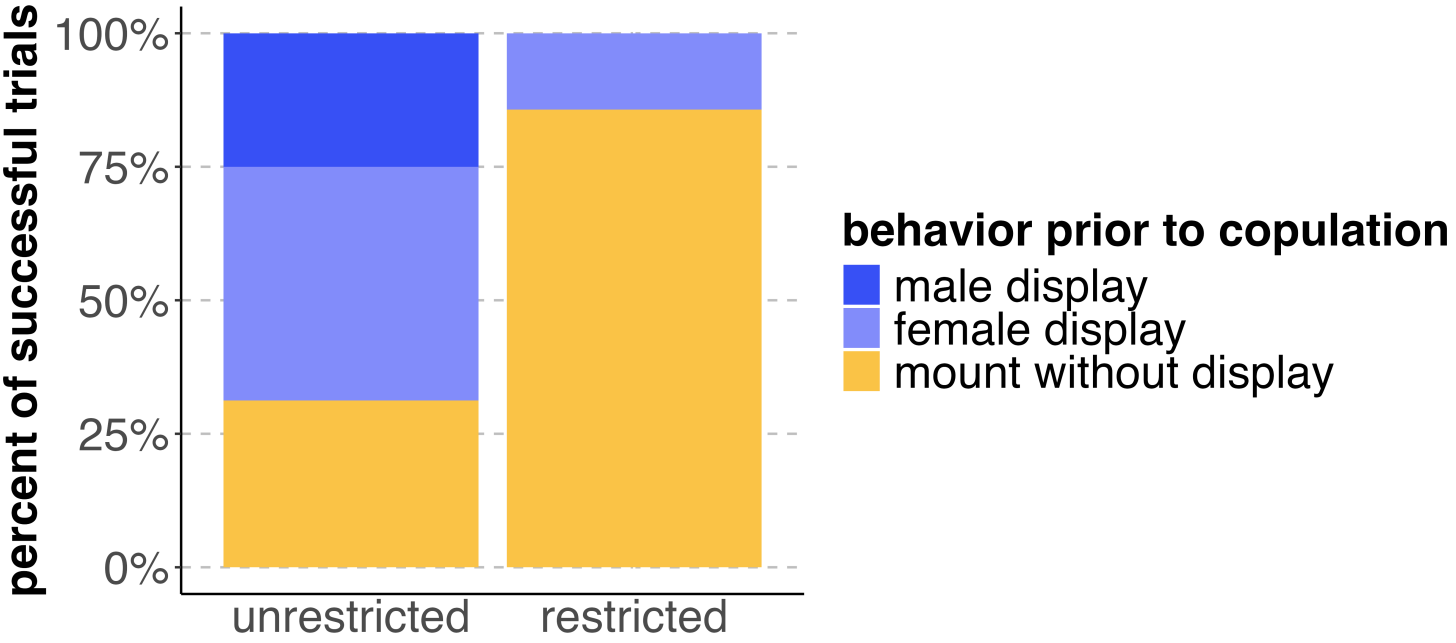


Figure 3

Restricted males typically initiated copulation by jumping onto females whereas unrestricted males typically initiated by performing or responding to a wing display.

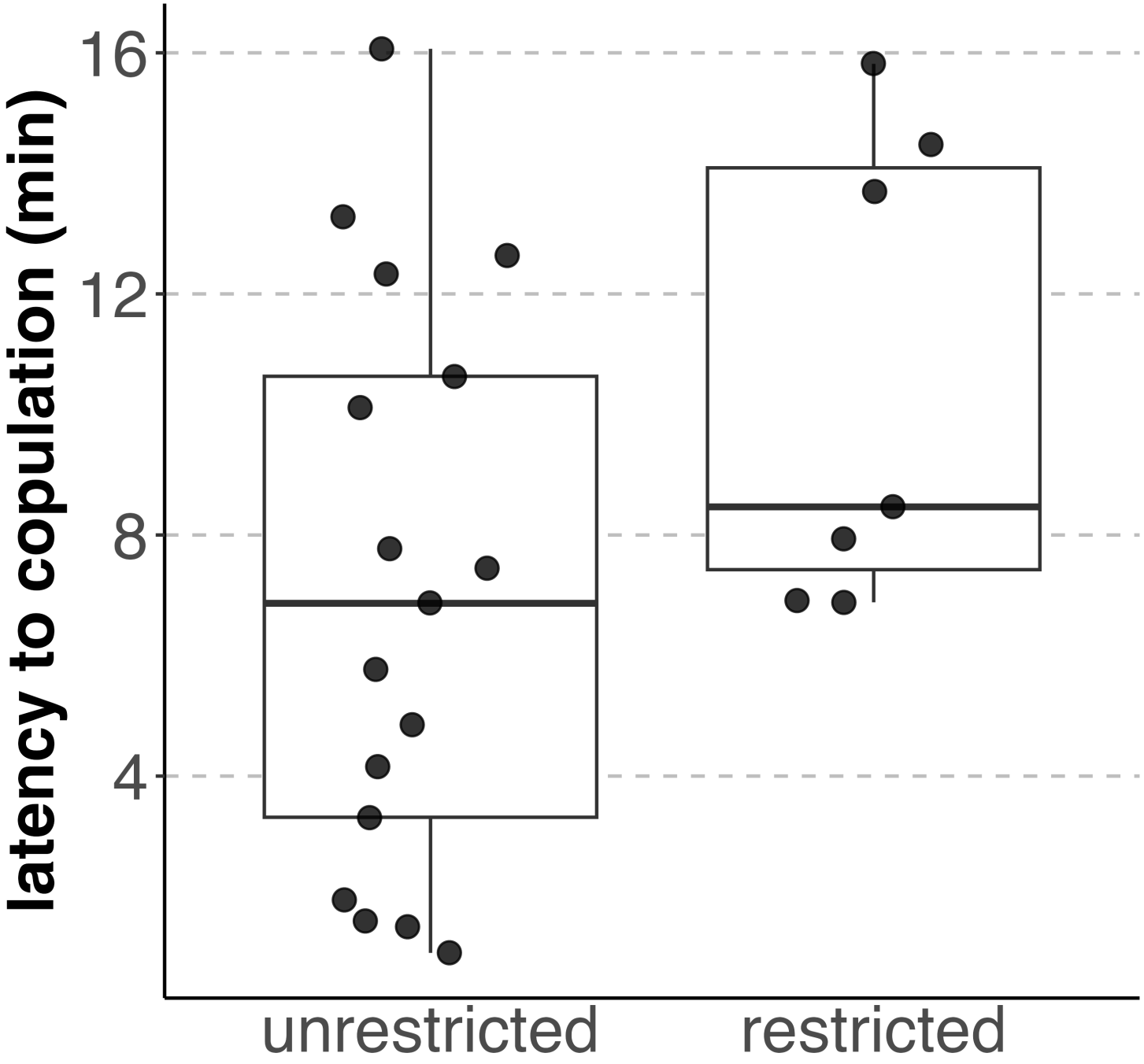


Figure 4

Boxplots of latency to copulation for trials in which successful males were restricted and unable to perform the wing display or unrestricted. Points are data for individual trials

Supplementary Files

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