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Robust mate preferences despite means and opportunity for mate choice copying in an insect

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Abstract:	In mate choice, social learning may take the form of mate choice copying or anti-copying, whereby observed mating decisions are either mimicked or avoided. Alternatively, independent mating decisions may be based on innate preferences or early life social learning. While mate choice copying is widespread among some animal taxa, research in arthropods is limited and results are mixed. We tested these hypotheses using <i>Enchenopa</i> treehoppers (Hemiptera: Membracidae). <i>Enchenopa</i> males produce plant-borne vibrational advertisement signals and females express their mate preferences by selectively duetting with males. Individuals on the plant can monitor these public signals during pair formation. We randomly assigned females to treatment duets consisting of either unattractive or attractive male signals, followed by a long (enthusiastic), short (reserved), or no female treatment responses. We described the test females' mating preferences before and after the treatment duet. We found that female mate preferences were not affected by the treatment duets. Instead, females had consistent individual differences which supports the independent mate choice hypothesis and rejects both social learning hypotheses. Our findings suggest that independent mate choice does not necessarily represent a lack of opportunity for social influences from the immediate social context of mate choice.



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February 27, 2023

Dear Dr. Ron Rutowski,

We would like to thank you and the reviewers for the time it took to thoughtfully review and provide suggestions for our manuscript entitled 'Robust mate preferences despite means and opportunity for mate choice copying in an insect.' We have addressed the suggestions/comments carefully and comprehensively in the response document and ask you to reconsider our manuscript for publication.

We have addressed the reviewers' comments in the attached documents. Specifically, we have added text that clarifies the rationale for our expectation that our treatment would be sufficient to produce an effect and for why we thought social learning might reasonably be expected in this species. We also included information in our statistical analysis section for how we controlled for temperature effects.

We are confident that our manuscript would be of great interest to the audience that *Animal Behaviour* attracts. We declare that there is no conflict of interest to report. We hope you will consider our resubmission for publication in *Animal Behaviour* and look forward to hearing from you. If you have any questions or require further details from us, please do not hesitate to contact us.

Lauren A. Cirino

And on behalf of my co-authors: Ian D. Gallagher, Camille Desjonquères, and Rafael Lucas Rodríguez

Title: Robust mate preferences despite means and opportunity for mate choice copying in an insect

Abbreviated title: No mate choice copying in *Enchenopa* treehoppers

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We really appreciate the suggestions from the reviewers and the time that it took to thoughtfully review our manuscript. We have carefully considered these suggestions and have made changes to improve the manuscript. Below we provide responses to the individual comments, and we provide the line numbers where the changes can be seen in the revised version.

Reviewer #1: In Robust mate preferences despite means and opportunity for mate choice copying in an insect, the authors present their test of three different hypotheses investigating the nature of possible social transmission of mate choice information, or a lack thereof. The study uses *Enchenopa* treehoppers as a model to expand the limited mate choice copying work that has been done in arthropods. Mate preference was assessed in virgin females before and after a single exposure to the mating preference of another female. The authors found support for independent mate choice in this species rather than mate choice copying or anti-copying.

I believe the authors presented a well written and designed study to test independent mate choice, mate choice copying, or mate choice anti-copying. I'm afraid I have little to comment on or suggest. I found that I deleted most of my concerns, as the authors eventually addressed them as I read through the submission. Among them the possibility that the treatment exposure was not efficacious enough to transmit adequate social information and thus a mate choice copying or anti-copying response in focal females. Below I list a handful of issues that should be addressed.

112 — References "X, Y, Z, unpubl." will need to be resolved.

Line 114: This has now been changed to the authors' last names.

192-197 — Perhaps a "(see Methods: Stimulus construction and playback setup)" somewhere in here.

Line 206-207: We have included this at the end of the last sentence in this paragraph.

324 & 350 — why not give the actual p-values?

We agree. This has now been changed on lines 337 and 363.

Figure 4-6 — How do these look in black and white? Is it possible to differentiate the 3 colors in figure 5 and 6?

Figures 4-6 (lines 602-621): We've modified these figures to ensure that they can be read and understood in black and white. We separated all the splines in figure 4 and changed the shape or pattern of the points and lines in figures 5 and 6.

417 — citation needs resolution

Line 433 — we have corrected this in the discussion.

Tables 2 and 3 — I'm wondering why table 3 has estimates and table 2 does not. Why not include estimates (and confidence intervals) throughout?

Lines 557-560: We were interested in repeatability for peak preference and preference selectivity. We have now clarified this in the caption of data table 3 and in the heading of the repeatability column.

Reviewer #2:

This paper tested hypotheses about social learning of mate preferences during the adult stage stemming from brief exposures to female responses to male signals, using a model insect system for studying the evolution of signals and preference. The study design was sound, the hypotheses and predictions clearly laid out in the introduction and then again in the methods. The paper was easy to read and did not seem

to overreach the implications of the results. While the results are negative for social learning, the paper adds a significant advance in our understanding of how to test social learning, and why it might be expressed or not. Finally, the tests of social learning are done in a system where mate preferences are easily quantified along multiple axes using a function-valued approach, which is a significant advance on many past tests of the hypotheses.

1. Were the preferred signal values and preferences corrected for the testing temperature? In lines 256-259 the authors indicate a 2.5 degree C range in temperatures, which can generate significant variation in both male signals and female preferences (Jocson et al 2019). Shifts in the most attractive male signal would therefore need to be corrected for when considered how much the female preference deviated from the most preferred (i.e. females prefer higher frequencies at higher temperatures, so the "most preferred" male signal value would be different at different temperatures). Also, correcting for variation due to thermal differences should also be accounted for in models, as it can generate significant variation in preferences.
Lines 383-384: We originally included temperature in each of the models in our analyses. However, this term was not significant ($p \geq 0.09$) in any model, so we removed it. This is now explained in the stats section in the methods.

2. Line 213: the authors state that the treatment was relatively brief compared to the rounds of playbacks. The explanation of the significance of this actually puts into question the validity of the pre-test experiment. Further explanation would be helpful. Related to this, in lines 189-191: not clear what this means, is this in the same species? Why would the pre-test make copying stronger?

Lines 229-232: We've provided some clarifying details related to our treatment length on these lines.

Lines 198-199: We clarified the details of the Davies et al. 2020 meta-analysis that showed mate choice copying was stronger in a "before-and-after" experimental design.

3. It is unclear whether the selection would actually favor social learning that was tested. Are males sperm-limited? Also, how often do female responses actually end up with mating? Would mating courtship be a better stimuli to use, rather than female preference? In lines 420-421: a wording change to indicate the motive might not be sufficient for switching with the given treatment would be helpful.

Our argument is that the lack of mate choice copying may be due not to the lack of opportunity or capacity, but rather to selection against it. However, the broad literature contains many reasons why mate choice copying could occur (e.g., lower mate search costs). We didn't quite follow other parts of this comment, but can also say that sustained female responses to male signals is what leads to mating in our study species. Lines 101-104, 120-122, & lines 437-438 have now clarified our use of means, opportunity, and motive in the introduction and in the discussion.

Minor comments:

4. When discussing individual differences in preferences, citing past work on the repeatability of mate preferences would be helpful to place the results in the context of the broader literature.

Lines 422-424: We've included a sentence in the discussion about our repeatability results as well as including past work on repeatability (updated citation on lines 692-694).

5. Lines 112 and 417 have X's and Y's in place of what should be names or papers

Lines 114 & 433: These lines have been changed.

6. Lines 167-169: this sentence was a bit difficult to follow

Line 175-176 & line 180: We reduced this sentence to make it easier to follow and included some of the details from this line on a line below (180).

7. Line 147 - what if no variation is present generally in the population, then selection would not favor changes in peak preference (you could cite Fowler-Finn and Rodriguez 2013 to demonstrate variation is expected).

Line 151-154: We have included additional text to this sentence to indicate variation is expected along with the citation.

8. Were the lab-reared insects from different females? If not, then how would genetic similarity among individuals affect the expected outcomes?

Line 182: We have now clarified that the lab-reared insects were obtained from multiple females from the previous breeding season.

Figure 4 would be more useful in terms of demonstrating variation among females in mate preferences if there were a series of panels showing both preference functions per female.

Lines 602-603: Thank you for this suggestion! We have updated this figure and it now can be viewed in black and white. Please note that the current figure is a copy of the original. The original was made in Adobe Illustrator and will be submitted as a high-quality figure if our manuscript is accepted.

Research highlights

- Pair formation in *Enchenopa* treehoppers involves male-female signal duets
- These duets provide information about the mating decisions of nearby individuals
- We tested for mate choice copying in *Enchenopa* with duet playback treatments
- *Enchenopa* mate preferences were unaffected by the playbacks
- No copying may represent selection for fixed preferences, instead of no opportunity

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Abstract

In mate choice, social learning may take the form of mate choice copying or anti-copying, whereby observed mating decisions are either mimicked or avoided. Alternatively, independent mating decisions may be based on innate preferences or early life social learning. While mate choice copying is widespread among some animal taxa, research in arthropods is limited and results are mixed. We tested these hypotheses using *Enchenopa* treehoppers (Hemiptera: Membracidae). *Enchenopa* males produce plant-borne vibrational advertisement signals and females express their mate preferences by selectively duetting with males. Individuals on the plant can monitor these public signals during pair formation. We randomly assigned females to treatment duets consisting of either unattractive or attractive male signals, followed by a long (enthusiastic), short (reserved), or no female treatment responses. We described the test females' mating preferences before and after the treatment duet. We found that female mate preferences were not affected by the treatment duets. Instead, females had consistent individual differences which supports the independent mate choice hypothesis and rejects both social learning hypotheses. Our findings suggest that independent mate choice does not necessarily represent a lack of opportunity for social influences from the immediate social context of mate choice.

Key terms: experience-mediated plasticity, sexual selection, non-independent mate choice, social plasticity, repeatable

Introduction

In social learning, animal decisions are influenced by information provided by the behavior of conspecifics (Danchin et al. 2004). Animals may glean information from conspecifics from their signals or inadvertent cues, or from direct observations of their choices and outcomes (Danchin et al. 2004). Social learning may inform animal decisions in a variety of contexts such as habitat selection, foraging, and mate choice (Danchin et al. 2004).

Social learning may be advantageous in mate choice if it helps acquire information about mate quality and/or decrease the costs of searching and selecting mates (Cotton et al. 2006; Gibson and Hoglund 1992; Jennions and Petrie 1997; Pomiankowski 1987; Pruett-Jones 1992; Vakirtzis 2011; White 2004). In turn, social learning in mate choice may influence the strength and direction of sexual selection with consequences ranging from the maintenance of within-population variation to speciation (Agrawal 2001; Kirkpatrick and Dugatkin 1994; Wade and Pruett-Jones 1990). It is therefore important to understand what determines when and how social learning will influence mate choice.

Social learning in female mate choice may take two forms. Females may engage in mate choice copying, whereby females set their mate preferences to match those of other females whose mating decisions they have observed (Pruett-Jones 1992; Scauzillo and Ferkin 2019; Vakirtzis 2011). Mate choice copying may allow females to favor males that are commonly available or locally of high quality (Cotton et al. 2006; Jennions and Petrie 1997; Pomiankowski 1987; Vakirtzis 2011; White 2004). By contrast, females may engage in “anti-copying,” and set their mate preferences to disfavor mate types that they have observed being chosen by other females (e.g., Loyau et al. 2012). Anti-copying may mitigate competition for mates, help prevent females from mating with males that are sperm depleted, or reduce the risk of losing parental care (Pruett-Jones 1992; Scauzillo and Ferkin 2019). Another possibility is that females may engage in independent mate choice and disregard available social information at the time of mate choice (Pruett-Jones 1992; Scauzillo and Ferkin 2019). Independent mate choice may

reflect mate choice dictated purely by innate mate preferences, private information obtained through females' experiences in prior encounters, and/or through social learning prior to sexual maturity.

Female mate choice copying appears to be taxonomically widespread in vertebrates (Davies et al. 2020; Jones and DuVal 2019; Vakirtzis 2011). The distribution of female mate choice copying in other animal groups, however, remains unclear (Davies et al. 2020). Multiple studies in arthropods, for instance, have examined the use of social information when females make mating decisions (Belkina et al. 2021; Jones and DuVal 2019; Vakirtzis 2011). Results from these studies are mixed – some supporting social learning and others independent mate choice (Jones and DuVal 2019; Vakirtzis 2011), although evidence of anti-copying is rare (Loyau et al. 2012). Most attention regarding female mate choice copying in arthropods has focused on *Drosophila* (Belkina et al. 2021; Jones and DuVal 2019; Vakirtzis 2011). While independent mate choice might be generally expected given arthropods' smaller brains, there is evidence of widespread capabilities for learning and social plasticity in this group (Dion et al. 2019; Dore et al. 2018; Dukas 2008; Hebets and Sullivan-Beckers 2010; Rodriguez et al. 2013b; Verzijden et al. 2012). Indeed, several *Drosophila* studies found evidence for female mate choice copying; however, these results could not be replicated in other studies (Auld et al. 2009; Belkina et al. 2021). Thus, testing across a wider taxonomic range seems necessary to understand whether arthropods use social information to inform their mating decisions, and why.

Here, we test the mate choice copying, anti-copying, and independent mate choice hypotheses in an insect, a member of the *Enchenopa binotata* complex of treehoppers (Hemiptera: Membracidae). Our goal is to broaden the scope of such tests in arthropods, as well as address some potential reasons why use of social information in the immediate context of female mate choice might be rare in arthropods. Enchenopa treehoppers provide a strong advantage in this regard as their mating and communication offers a clear means and opportunity for mate choice copying to occur.

104 *Enchenopa* treehoppers ~~offer two important advantages in this regard. First, their~~ mating
105 system provides a clear opportunity for females to glean social information about other females'
106 mating decisions. As with many plant-feeding insects, *Enchenopa* communicate with plant-
107 borne vibrational signals (Cocroft and Rodriguez 2005; Hill 2008). Pair formation occurs through
108 male-female signal duets (Cocroft et al. 2008; Rodriguez and Cocroft 2006; Rodriguez et al.
109 2006; Rodriguez et al. 2004). Mate-searching males fly from one plant to another and produce
110 advertisement signals. Females that find a male's signal attractive respond with their own
111 signals and establish a duet that helps the male locate the female (Cocroft et al. 2008;
112 Rodriguez and Cocroft 2006; Rodriguez et al. 2012; Rodriguez et al. 2006; Rodriguez et al.
113 2004). These duets often occur in the presence of other reproductively ready females (Cocroft
114 et al. 2008, Little, Cirino, and Rodriguez~~X,Y,Z~~, unpubl.). Thus, duetting provides social
115 information to females as other treehoppers on the same plant can easily detect them (Cocroft
116 and Rodriguez 2005). Further, the duets provide information about females' mating decisions,
117 as *Enchenopa* females express their mate preferences through selective duetting with males –
118 they are more likely to duet and produce more and longer signals in response to males they
119 prefer (Rodriguez et al. 2012; Rodriguez et al. 2004).

120 ~~Second, Besides~~ the natural availability of social information ~~about~~ regarding female
121 mating decisions (the “opportunity” for social learning), the mating system of these treehoppers
122 also provides ample “means” for social learning as there is considerable evidence that sexual
123 communication in *Enchenopa* is socially malleable. The inputs and effects of social experience
124 vary with the life stage at which they occur. Young adult females that experience attractive or
125 mixed mate types become more selective but do not change the mate types they prefer (Fowler-
126 Finn and Rodriguez 2012a; 2012b). By contrast, variation in group density and signaling
127 environment starting at the juvenile stage influences preferred mate types (Desjonquères et al.
128 2019a; Desjonquères et al. 2021; Desjonquères et al. 2019b; Fowler-Finn et al. 2017). Finally,
129 females are more likely to respond to an attractive signal bout when they perceive another

female responding once to it than to the signal without a female response (Escalante et al., under review).

In this study, we experimentally mimicked the experience of females perceiving attractive and unattractive males receiving “enthusiastic,” “reserved,” or no female responses. We randomly assigned females to treatments consisting of playback duets featuring either attractive or unattractive male signals paired with female response signals that were either long, short, or absent (i.e., there were six different treatment combinations of male-female duets) (Figure 2). We manipulated the length of perceived female responses because longer response signals indicate greater attraction (Rodriguez et al. 2012; Rodriguez et al. 2004). We described females’ mate preferences before and after presentation of these treatment duet playbacks, as well as their behavior during the treatments. We described two features of female mate preferences: peak preference (the preferred signal type; Figure 1a) and preference selectivity (how female response decreases with deviation from the preferred signal type; Figure 1b).

In the framework of this experiment, the mate choice copying hypothesis predicts that females will switch their preferences to the perceived male signal that receives a female response. Further, the copying effect should be stronger with longer (more “enthusiastic”) perceived female responses (Dugatkin 1998) (Figure 3a). By contrast, the anti-copying hypothesis predicts that females will switch their preferences away from the perceived male signal that receives a female response. Further, as above, the anti-copying effect should be stronger with longer perceived female responses (Figure 3b). Finally, the independent mate choice hypothesis predicts that females will not switch their preferences according to the treatments, with most variation (if any) due to consistent between-individual differences (Figure 3c) (we would expect some variation given prior evidence of genetic and environmental components influencing mate preferences; (Rodriguez et al. 2013a; Rodriguez et al. 2013b; Desjonquères and Rodríguez 2023).

Preference selectivity has not often been investigated in the context of these hypotheses. However, social learning could also affect selectivity (Figure 1b). If so, the mate choice copying hypothesis predicts that females will become either similarly or *more* selective after perceiving a male signal close to their peak preference that receives a female response. Further, the copying effect should be stronger when females perceive male signals receiving long female responses (Figure 3d). By contrast, the anti-copying hypothesis predicts that females will become less selective after perceiving a male signal close to their peak preference that receives a female response as females are predicted to avoid that male signal and instead respond to other male signals that did not receive female responses. Further, as above, the anti-copying effect should be stronger with longer perceived female responses (Figure 3e). Finally, the independent mate choice hypothesis predicts that there would be no change in selectivity, with most variation (if any), again, due to consistent between-individual differences (Figure 3f).

Methods

Insect collection and rearing

Most species in the *Enchenopa binotata* complex have not yet been described formally (Hamilton and Cocroft 2009). However, they can be easily identified by the host plant they live and feed on, the coloration of the nymphs, and the dominant adult male signal frequency used for mate advertisement (Cocroft et al. 2008; Cocroft et al. 2010). In this experiment, we used the species that lives on ~~nannyberry trees~~, *Viburnum lentago* (Adoxaceae) host plants in Wisconsin, ~~USA~~, has gray nymphs, and has a ~165Hz dominant-frequency male advertisement signal (Rodriguez et al. 2018). We kept voucher specimens in 95% EtOH in the lab collection.

We used both field-collected (N=135) and first-generation lab-reared (N=14) insects. We collected second and third instar nymphs from several trees at two sites in Milwaukee, Wisconsin, USA, in June 2021: Downer Woods Natural Area (43°04'47.4"N 87°52'49.4"W) and

a part of the Oak Leaf Trail (43°04'54.2"N 87°53'26.9"W). Lab-reared nymphs were acquired from eggs that were laid by multiple females collected from the Oak Leaf Trail site in the previous 2020 field season. *E. binotata* juveniles live in aggregations at the terminal end of nannyberry tree branches. Each group is likely comprised of several different females' offspring as many females aggregate on branches to lay egg masses (Wood 1974; Tallamy and Wood 1986; Zink 2003). We collected aggregates of juveniles by clipping the ends of the branches from multiple trees that were several meters apart at each site. We reared all nymphs on potted host plants at the University of Wisconsin-Milwaukee (UWM) Greenhouse. We separated the sexes upon adult eclosion to ensure that females had no experience with male signals or duetting, and that they did not mate, after which they become sexually unreceptive. As they were separated from males, females did not engage in duetting behavior before our trials.

Treatment and testing

Each individual female received three sets of playbacks: 1) a round of playbacks to describe each female's initial mate preference function, so we could establish a baseline mating preference for each female; 2) a treatment male-female duet playback, and 3) a second round of playbacks to describe females' mate preference functions after the treatment to assess any changes in preference due to the treatment (Figure 2). We used this "before-and-after" treatment approach as copying was stronger in animal studies that used this design over those ~~without that had a separate control and social experimental groups a pretest~~ (Davies et al. 2020).

Each round of playbacks to describe females' preference functions consisted of a random sequence of 11 synthetic vibrational stimuli mimicking the structure *Enchenopa* male advertisement signals (each stimulus consisted of a bout of three signals separated by 2 seconds of silence with 12 seconds of silence between bouts) (Cocroft et al. 2010; Rodriguez and Cocroft 2006) (Figure 2). Thus, each round of playbacks to describe females' preference

functions ran for 3 minutes and 22 seconds (see Methods: Stimulus construction and playback setup).

The strongest female mate preference in the *E. binotata* complex is for the dominant frequency of male signals, which are species-specific and represent the most divergent adult phenotype among species in the complex (Cocroft et al. 2008; Cocroft et al. 2010; Rodriguez et al. 2006). We therefore focused on signal frequency in this experiment. The stimuli for the two rounds of playbacks varied in frequency (130, 150, 160, 170, 180, 185, 190, 200, 210, 220, and 240Hz). This range slightly exceeds the natural range in the species, which helps capture the full shape of each preference function (Kilmer et al. 2017).

The treatment male-female duet playbacks were composed of a male signal stimulus component (a bout of three signals as above) that was either attractive (at the peak of the population preference function: frequency at 185Hz) or unattractive (frequency at 150Hz), on the basis of prior work with this population (Desjonquères et al. 2019a; Rodriguez et al. 2013a). This component was followed by a female response signal component that was either long (mean length for the population: 0.86 seconds), short (0.35 seconds, corresponding to the shortest decile in the population), or absent (Figure 2). Treatment duets ran for 8 seconds and were separated by the preceding and following rounds of playbacks to describe female preference functions by 12 seconds (Figure 2). Thus, the treatment was relatively brief compared with the rounds of playbacks for describing the before and after mate preference functions. We chose this design in order to focus on the hypotheses we wished to test – treatments with longer duets would risk confounding the experience of observing attractive-unattractive males receiving enthusiastic-unenthusiastic female responses with the additional experience of the test females not being engaged in their own duetting attempts. Further, ~~even such brief experiences can influence female behavior~~ shorter female response treatments than the ones we describe in this study have been known to influence female engagement with

mates (Escalante et al., in review). Here, we examine if changes in peak preference and preference selectivity are involved.

We randomly assigned females to one of the six treatments: attractive male signal (185Hz) receiving either a long (n=25) or short (n=25) female response or no response (n=25); or unattractive male signal (150Hz) receiving either a long (n=25) or short (n=25) female response or no response (n=24) (Figure 2). We tested females when they became sexually receptive, 3.5-5 weeks after adult eclosion. To start a trial, we placed a single female on a playback plant and assessed their receptivity by playing a primer (a recording of a male signal closely matching the population mean) up to four times. If the female did not respond, we placed the female back onto the rearing plant and tested again up to four days later. If the female responded, we delivered the preference function and treatment playbacks described above (Figure 2).

Stimulus construction and playback setup

We generated the synthetic male signal stimuli by a custom-written program in R. For the female response playback stimuli, we used recordings of duetting females, selected from our library, and played back with the same program in R. We used five replicates (five signals, each from a different female) per treatment length. The male component of these playbacks consisted of a single frequency, but the recordings of female signals have more frequency components (Rodriguez and Cocroft 2006). It was therefore necessary to compensate for the differential filtering of frequency components along the playback plant stem. To this end, we played back and recorded band-limited white noise (90-2000 Hz) through the playback plant stem and generated a digital filter that compensated for the filtering using custom-written software in MATLAB (Nieri et al. 2022). We then standardized all stimuli to an amplitude of 0.15 mm/s using an oscilloscope (Model 72-2580, Tenma Test Equipment, Springboro, OH, USA).

We delivered the playbacks from a Macintosh computer (Mac OS X Version 10.4.11, Apple, Cupertino, CA, USA) through a piezo-electric stack attached to the stem of a potted

257 playback plant by accelerometer wax and regulated by a piezo-controller (MDT694A, Thor labs,
258 Newton, NJ, USA). We recorded the playbacks and female responses by focusing a portable
259 digital laser vibrometer (Polytec PDV100, Polytec Inc., Irvine, CA, USA) on a small piece of
260 reflective tape adhered to the stem of the recording plant. We sent the laser vibrometer output
261 signal through a band-pass filter (40–3000 Hz; Krohn-Hite Corporation, Model 3940, Brockton,
262 MA, USA), and then to an iMac computer (macOS Big Sur Version 11.4, Apple, Cupertino, CA,
263 USA) through a USB audio capture (cakewalk UA-25 EX, Roland Corporation, Hamamatsu,
264 Japan). We recorded the signals on the iMac computer using the program AUDACITY (version
265 3.0.2; <https://www.audacityteam.org/>). We isolated the playback setup from building vibrations
266 by placing it on a ~15-kg epoxy resin tabletop that rested on partially inflated bicycle innertubes
267 on a freestanding table that stood on anti-vibration pads (PneumaticPlus CP6X6, Torrance, CA).
268 The playback plant was further isolated from the tabletop by a sheet of shock absorbing
269 sorbothane (Edmund Scientifics, Tonawanda, NY). We monitored the air temperature by the
270 playback plant at the start of each trial with a digital thermometer (Extech instruments Hygro-
271 Thermometer Clock 445702, Twinsburg, OH, USA; temperatures ranged between 22.9°C-
272 25.4°C ± 0.5).

273 *Describing mate preference functions*

274 Mate preferences are function-valued traits – they are expressed as a function of the
275 features of the signals in which females encounter or interact (Kilmer et al. 2017; Stinchcombe
276 et al. 2012). We therefore used a function-valued approach to describe individual and
277 population mate preferences. Our assay of female response was the number of female
278 responses per male signal stimulus bout (0 – 3 responses). We used the program *PFunc* (v.
279 1.0.2; <https://github.com/Joccalor/PFunc>, Kilmer et al. 2017) to fit cubic spline regressions to the
280 female response data (example shown in Figure 4). This method does not assume preference
281 function shape outside of some smoothness that is determined empirically (Kilmer et al. 2017;
282 Schluter 1988). We generated two preference functions for each female: one before and one

after the treatment playback (Figure 4). We then extracted peak preference and three other preference function traits that together make up preference selectivity with *PFunc* (see below for “preference selectivity” generation).

Ethical Note

All our procedures adhered to the ASAB/ABS Guidelines for the use of animals in research as well as the legal requirements of the U.S.A. and all UWM guidelines.

Statistical Analyses

We first plotted each female’s preference function values against each other (post-treatment versus pre-treatment) and inspected the plots for outliers. We found one female that fell far outside the peak preference data cloud. We ran all statistical analyses described below with and without this female and compared the output. Since, this data point did not qualitatively change the results of the experiment, we removed this female for the final models.

We then tested for correlations between all four preference function traits (peak preference, strength, tolerance, and responsiveness) before and after the treatments. We inspected correlation graphs (using ‘scatterplotMatrix’ function in the car package) and computed Kendall’s Tau correlation coefficients. Peak preference was only weakly correlated with the other traits (in all cases: $\tau \leq 0.08$, $p \geq 0.399$). However, responsiveness, tolerance, and strength were correlated with each other ($\tau > 0.63$, $p \leq 0.001$) as has been found in prior work with *Enchenopa* (Kilmer et al. 2017). To avoid the risk of spurious significance from tests with correlated traits, we loaded these three traits (preference responsiveness, tolerance, and strength) into a principal component analysis to summarize them with a composite trait that we term “selectivity” (Figure 1) (Kilmer et al. 2017).

We analyzed the behavior of the females during the treatment portion of the playbacks. We ran a generalized linear model with a Poisson distribution (log link function) with the number of females’ responses to the treatment playbacks (0-3 responses) as the response variable. We

included male signal treatment (150 or 185Hz), female response treatment (long, short, none), and their interaction as the explanatory variables in this model.

Next, we assessed the homogeneity of variances across pre- and post-treatment preference function traits using Fisher's F-test to ensure that variance differences did not confound our results (Danchin et al. 2020, Cleasby and Nakagawa 2011). Variances did not differ between before and after treatment (peak preference: Fisher's $F = 0.93$, $p=0.68$; preference selectivity: Fisher's $F = 1.00$, $p=0.98$).

To test the predictions of the hypotheses for peak preference, we calculated the absolute value of the difference between the pre- and post-treatment peak preferences for each female and the frequency of the male signal treatment. For example, a female with a pre-treatment peak preference of 205Hz that received the 185Hz male signal treatment would have a pre-treatment difference of $|205-185| = 20$. Similarly, if that same female's post-treatment peak preference was 195Hz, this female would have a post-treatment difference of $|195-185| = 10$.

We then tested for changes in peak preference due to the treatments in terms of changes in the relationship between these pre- and post-treatment differences (Figure 3a-c). In these terms, the mate choice copying hypothesis predicts that females that started *further away* from the male signal treatment value (before the treatment) should come *closer to* that male signal treatment value (after the treatment) (Figure 3a); and this effect should be stronger with longer (more "enthusiastic") female responses (Figure 3a). By contrast, the anti-copying hypothesis predicts that females that started *closer to* the male signal treatment value (before the treatment) should end up *further away* from that male signal treatment value (after the treatment) (Figure 3b); and this opposite effect should also be stronger with longer (more "enthusiastic") female responses (Figure 3b). Finally, the independent mate choice hypothesis predicts that the relationship between the pre- and post-treatment difference values will not be affected by the treatments (Figure 3c).

To test these predictions, we used a linear model with the post-treatment differences as the response variable. We included the pre-treatment differences, male signal treatment, female response treatment, and all two-way interactions as explanatory variables. The three-way interaction was well beyond the level of statistical significance ($p \geq 0.25 = 0.27$), so we removed it from the model. In this model, the pre-treatment value x female response treatment interaction tests for the predicted changes in the steepness of the relationship between the post- and pre-treatment differences. The male treatment x female treatment interaction tests for the predicted stronger effect of the longer female treatment responses between the attractive and unattractive male signal treatments.

To test the predictions of the hypotheses for preference selectivity, we calculated the difference between each female's pre- and post-treatment selectivity. We then tested for changes in preference selectivity due to the treatments in terms of changes in the relationship between this post-pre difference in selectivity and the above absolute value of the difference between pre-treatment peak preference and the frequency of the male signal treatment. In these terms, the mate choice copying hypothesis predicts that females that started *closer to* the male signal treatment in peak preference (before the treatment) should become *more selective* after the treatment (Figure 3d); and this effect should be stronger with longer (more "enthusiastic") female responses (Figure 3d). By contrast, the anti-copying hypothesis predicts that females that started *further away* from the male signal treatment in peak preference (before the treatment) should become *more selective* (after the treatment) (Figure 3e); and this opposite effect should also be stronger with longer (more "enthusiastic") female responses (Figure 3e). Finally, the independent mate choice hypothesis predicts that the relationship between the pre- and post-treatment selectivity will not be affected by either the absolute value of the difference between the pre-treatment for peak preference and the frequency of the male signal treatment nor by the treatments (Figure 3f).

To test these predictions, we used a linear model with the post-pre difference in selectivity as the response variable. As explanatory variables, we included the difference between pre-treatment peak preference and male signal treatment (150 or 185Hz), male signal treatment, female response treatment, and all two-way interactions. The three-way interaction was well beyond the level of statistical significance ($p=0.69 \geq 0.25$), so we removed it from the model. In this model, the pre-treatment difference x female response treatment interaction tests for the predicted changes in the steepness of the relationship between the change in selectivity and the absolute value of the difference between pre-treatment peak preference and the frequency of the male signal treatment. The male signal treatment x female response treatment interaction tests for the predicted stronger effect of the longer female treatment responses between attractive and unattractive male signals.

In both linear models that examined changes in peak preference and preference selectivity, we checked that our response variables had residuals that were normal and homoscedastic (before obtaining the absolute values in the case of peak preferences) by plotting and inspecting a histogram, q-q plot, and the standardized residuals versus fitted values.

We were also interested in the repeatability (Bell et al. 2009) of peak preference and preference selectivity. To estimate repeatability, we used two separate linear models, one for peak preference and one for preference selectivity. We used the post-treatment peak preference or selectivity as the response variable for each model. In each model, we included pre-treatment peak preference or selectivity, respectively, male signal treatment, female response treatment, the male x female treatment interaction. The terms for pre-treatment peak preference or selectivity test for a relationship with the post-treatment values for those preference function traits, and the slope of the terms corresponds to repeatability in these models. We originally included temperature (°C) in all models described above; however, this term was never significant ($p \geq 0.09$), so we removed it from the models.

To help interpret results where we detect no effect through significance testing, we estimated the size of the effects for which we had adequate statistical power ($1 - \beta$) given our sample sizes and standard deviations (Nakagawa and Cuthill 2007). All analyses were performed in R v. 4.0.5 (R Core Team 2021).

Results

Female behavior during treatment

Females' behavior during the treatment playbacks confirmed that the attractive male signal treatment received higher response levels than the unattractive male signal treatment (Table 1, Figure 5). By contrast, female response treatment had no effect on females' behavior during the treatment playbacks (Table 1, Figure 5).

Mate preferences before and after treatment

We show the results separately for each male signal treatment, as the differences between peak preference and male signal treatment frequency were (by definition) overall smaller with the attractive male signal (185Hz) than the unattractive male signal (150Hz) (Figure 6; this difference corresponds to the main term for male treatment in Table 2). We found that neither female peak preference nor preference selectivity were affected by the male-female duet treatments nor any interaction (Table 2, Figure 6). Instead, we found that both peak preference and preference selectivity had significant repeatability (weak for peak preference: $r = 0.28 \pm 0.08$; strong for selectivity: $r = 0.74 \pm 0.06$; Table 3, Figure 6). We also note that the significant term for the difference between pre-treatment peak preference and the frequency of the male signal treatment also tests for consistency in individual differences in peak preference (Table 2).

Although we detected no effect of the male-female duet treatments, with our sample sizes ($n = 24 - 25$ per treatment) and observed data dispersion (peak preference SD = 10.7), these tests had adequate statistical power ($1 - \beta > 0.80$) to detect an 8Hz shift in peak

preference (4%) and high statistical power ($1 - \beta = 0.95$) to detect a 10Hz shift (6%). Such changes would be at the lower end of what we would consider biologically relevant regarding the mate choice copying, anti-copying, and independent mate choice hypotheses.

Discussion

Enchenopa treehoppers find and select mates by using substrate-borne vibrational male-female duetting on their host plants (Cocroft et al. 2008). We capitalized on this natural communication system that publicly advertises signals and mate choice decisions to test hypotheses regarding social learning in the immediate context of mate choice. We found that females did not switch their mate preferences either toward or away from the mate types with which they perceived other females duetting, regardless of whether the perceived response was more or less “enthusiastic” or absent. Instead, females showed stable individual differences in peak preference and preference selectivity before and after the treatments. We also found that repeatability was higher than previously reported for *Enchenopa* likely because we assessed it over a shorter time span (Fowler-Finn and Rodriguez 2013; Bell et al. 2009). Together, these results support the independent mate choice hypothesis and reject the mate choice copying and anti-copying hypotheses.

These results are not due to a total lack of social malleability in *Enchenopa* mate preferences. Social environments and experiences during the juvenile stage influence female peak preferences (Desjonquères et al. 2019a; Desjonquères et al. 2021; Desjonquères et al. 2019b; Fowler-Finn et al. 2017) while social experiences with available mate types during the early adult stage influences female preference selectivity (Fowler-Finn and Rodriguez 2012a; 2012b). However, social experience in the immediate context of mate choice does not seem to influence preferred mate types (~~Speck 2022X and Y, unpubli~~; this paper). We suggest that our results point to an adaptive benefit from not altering mate preferences according to the immediate context of mate choice in the *Enchenopa* mating system. Therefore, a lack of mate choice copying or anti-copying in some animals may not represent a lack of opportunity or

capacity, but instead a lack of “motive” favoring behavioral inflexibility (i.e., no selection favoring the use of social learning in this context) ~~lack advantage or motive.~~

Mate choice can potentially be improved through social learning where females intercept public information to help discriminate between mates. Social learning, such as mate choice copying or anti-copying, is expected to evolve if it facilitates assessment of mate quality, reduces the costs associated with mate choice, and/or enables females to benefit from resources that a male could provide (Gibson and Hoglund 1992; Nordell and Valone 1998). In treehoppers, costs associated with female mate choice may be low since males fly to search for females and produce search vibrations on their host plants to initially find mates (Cocroft et al. 2008; Fowler-Finn et al. 2014). Females only need to respond to a desirable vibration to find a high-quality mate, although some females may produce search vibrations as well (Cocroft et al. 2008; Rodriguez and Cocroft 2006; Rodriguez et al. 2012). Since males become sexually mature before females (Wood and Guttman 1982), there are likely many males to choose from which may reduce the risk involved for females locating and assessing the quality of males. Thus, the cost of mate choice is likely to be low and social learning may be unnecessary at this stage to select a suitable mate. Relying on private information, genetic combined with learned information from earlier life stages, for mating decisions may be most advantageous for mating decisions in this species.

An alternative possibility may be that our single exposure to an 8-second treatment duet may constitute insufficient “dosage” to alter mate preferences. In the experiments which manipulated *Enchenopa* juvenile and early adult social experiences and detected social plasticity in mate preferences, exposures lasted from two to several weeks (Desjonquères et al. 2019a; Desjonquères et al. 2021; Desjonquères et al. 2019b; Fowler-Finn and Rodriguez 2012a; 2012b). Further, dosage may involve not only the time of exposure but also the number of females observed. For instance, in sailfin mollies (*Poecilia latipinna*), mate choice copying did not occur when only one female was observed for 10 minutes but did occur when two females

were observed for 5 minutes each consecutively or when one female was observed for 20 minutes (Witte and Nolte 2002). In nature, *Enchenopa* females may be exposed to a higher number of duets as females begin to engage with males gradually along the mating season (Sullivan-Beckers and Cocroft 2010) and each duet often lasts an hour or longer (Cocroft et al. 2008), although mating pairs can take anywhere from two minutes to three hours from the time they first duet to copulation (Leith et al. 2020). This potential dosage dependence of social learning in the immediate context of mate choice may be an interesting avenue for future research. We note, however, that experiments with such longer dosages will also need to disentangle potential confounds arising from additional social inputs from the experience of the target animals engaging/not engaging with the social treatments.

In summary, it appears that the treehoppers we studied here have no motive to socially adjust mating preferences in the immediate context of mate choice even though they have the means (vibrational communication system) and opportunity (live in aggregations) to do so. Our study contributes to a small number of arthropod studies that demonstrate independent mate choice (Auld et al. 2009; Belkina et al. 2021) including one that could not replicate the results of many *D. melanogaster* social learning studies (Belkina et al. 2021). It is important that we continue to investigate social learning in the context of mate choice in arthropods to help us clarify our understanding among these conflicting studies and inform us of the importance with which social learning plays in shaping the mating preferences in this large group of animals.

Data accessibility: The data and code for all analyses in R are provided in the electronic supplementary materials.

Figures legends:

Figure 1: Traits we used to describe variation in female mate preference functions (Kilmer et al. 2017). (a) Peak preference: the signal trait value that elicits the strongest female response. The black and gray preference functions differ in peak preference (indicated by the corresponding vertical dotted lines) (b) Preference selectivity: the shape of the preference function around the peak. The black preference function indicates a female that is more selective than the female with the gray preference function.

Figure 2: Time course of the experiment we used to test the hypotheses regarding social learning in the immediate context of female mate choice in *Enchenopa* treehoppers. We presented each female with playbacks to describe their mate preferences (a) before and (c) after the (b) treatment playbacks. (b) Treatment playbacks were composed of an unattractive (150Hz) or attractive (185Hz) male signal (3 whines with pulses) paired with either a long, short, or no female response to each of the 3 whine-pulses parts of the signal.

Figure 3: Predictions of the mate choice copying, anti-copying, and independent mate choice hypotheses for peak preference (a-c) and preference selectivity (d-f) in our experiment manipulating social information available in male-female duets.

Figure 4: A sample of the cubic spline regressions that was generated in the program *PFunc* fit to the female response data that was gathered during the preference function playbacks before (left) and after (right) the treatment.

Figure 5: Female responses during the treatment duet portion of the playback. Females responded to one of six different treatment combinations (see Figure 3). Individual female

responses are depicted by small colored ~~circles~~shapes and group means are depicted by colored ~~squares~~diamonds with ± 1 standard error bars around them.

Figure 6: The relationship between pre- and post-treatment peak preferences (a-b) and preference selectivity (c-d) in females that were provided treatment playbacks with an unattractive (150Hz – a, c) or attractive (185Hz – b, d) male signal paired with a female response: long (gray), short (orange), or none (blue). Individuals are represented with open circles and the groups that were provided a female response (long, short, none) are indicated by the lines.

Tables and table legends:

Table 1: Results from a generalized linear model examining female behavior during the treatment duet portion of the playback. Females responded more to the attractive 185Hz male signal frequency than the unattractive 150Hz male signal frequency (significant term is bolded).

Term	df	χ^2	<i>p</i>
Duetting female	2	3.604	0.165
Male signal	1	57.873	<0.001
Duet female x male signal	2	0.160	0.923

Table 2: Results from two separate linear models examining the effect of the male-female duet treatments on peak preference and preference selectivity. Significant terms are indicated in bold.

	Term	df	F-value	<i>p</i>
Peak preference	Duetting female	2	2.095	0.127
	Male signal	1	7.745	0.006
	 Pre-treatment peak preference - treatment frequency 	1	6.314	0.013
	Duet female x male signal	2	1.464	0.235
	Pre-trt peak preference - treatment frequency x duet female	2	0.893	0.412
	Pre-trt peak preference - treatment frequency x male signal	1	2.855	0.093
Selectivity	Duetting female	2	0.146	0.864
	Male signal	1	0.027	0.870
	Pre-treatment peak preference - treatment frequency	1	0.065	0.799
	Duet female x male signal	2	0.059	0.943
	Pre-trt peak preference - treatment frequency x duet female	2	0.155	0.857
	Pre-trt peak preference - treatment frequency x male signal	1	0.251	0.617

Table 3: Results from two separate linear models testing for repeatability in peak preference

and preference selectivity, while accounting for the male-female duet treatments. ~~Both~~

Estimates for repeatability (r) for peak preference and preference selectivity ~~were repeatable are~~

also listed (significant terms are indicated in bold).

	Term	df	F-value	p	Estimate _r	Std. Error
Peak preference	Duetting female	2	2.053	0.132	-	-
	Male signal	1	0.821	0.366	-	-
	Pre-treatment peak preference	1	13.55	<0.001	0.28	0.08
	Duet female x male signal	2	0.869	0.422	-	-
Selectivity (PC1)	Duetting female	2	0.535	0.587	-	-
	Male signal	1	0.626	0.430	-	-
	Pre-treatment selectivity (PC1)	1	156.407	<0.001	0.74	0.06
	Duet female x male signal	2	0.408	0.666	-	-

Figures:

Figure 1:

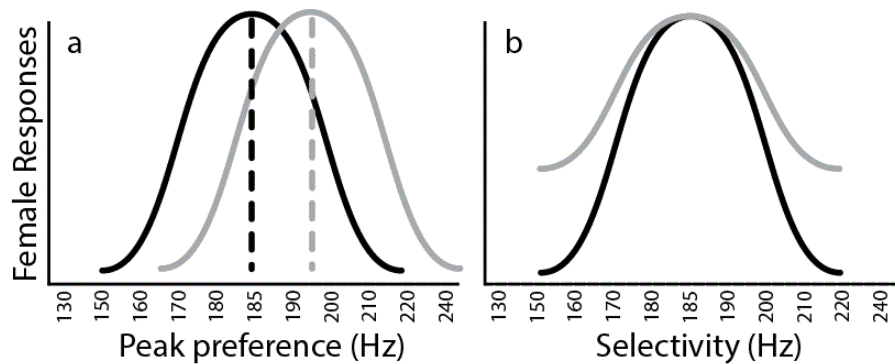


Figure 2:

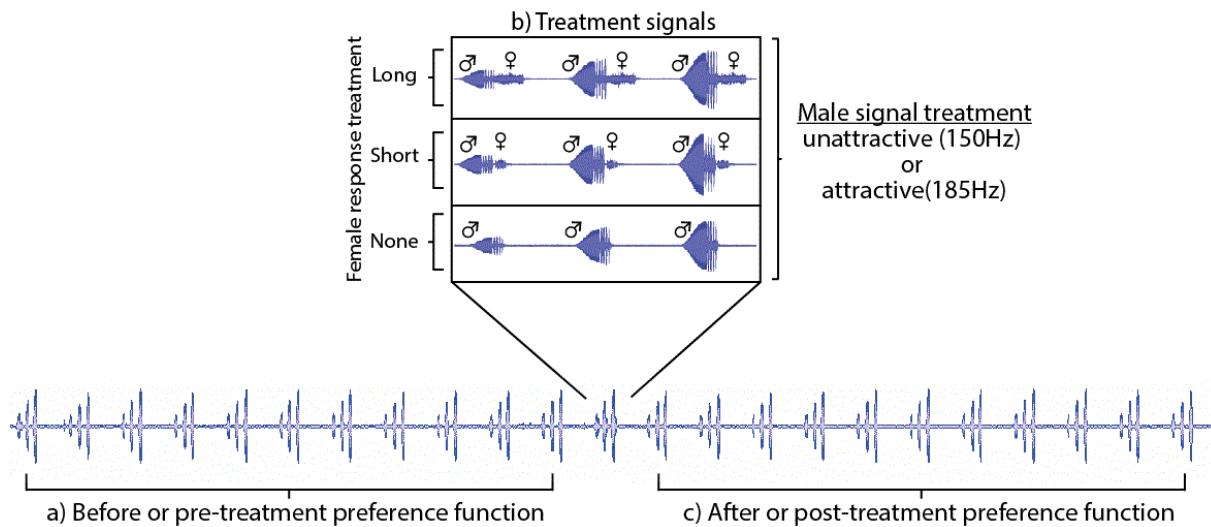
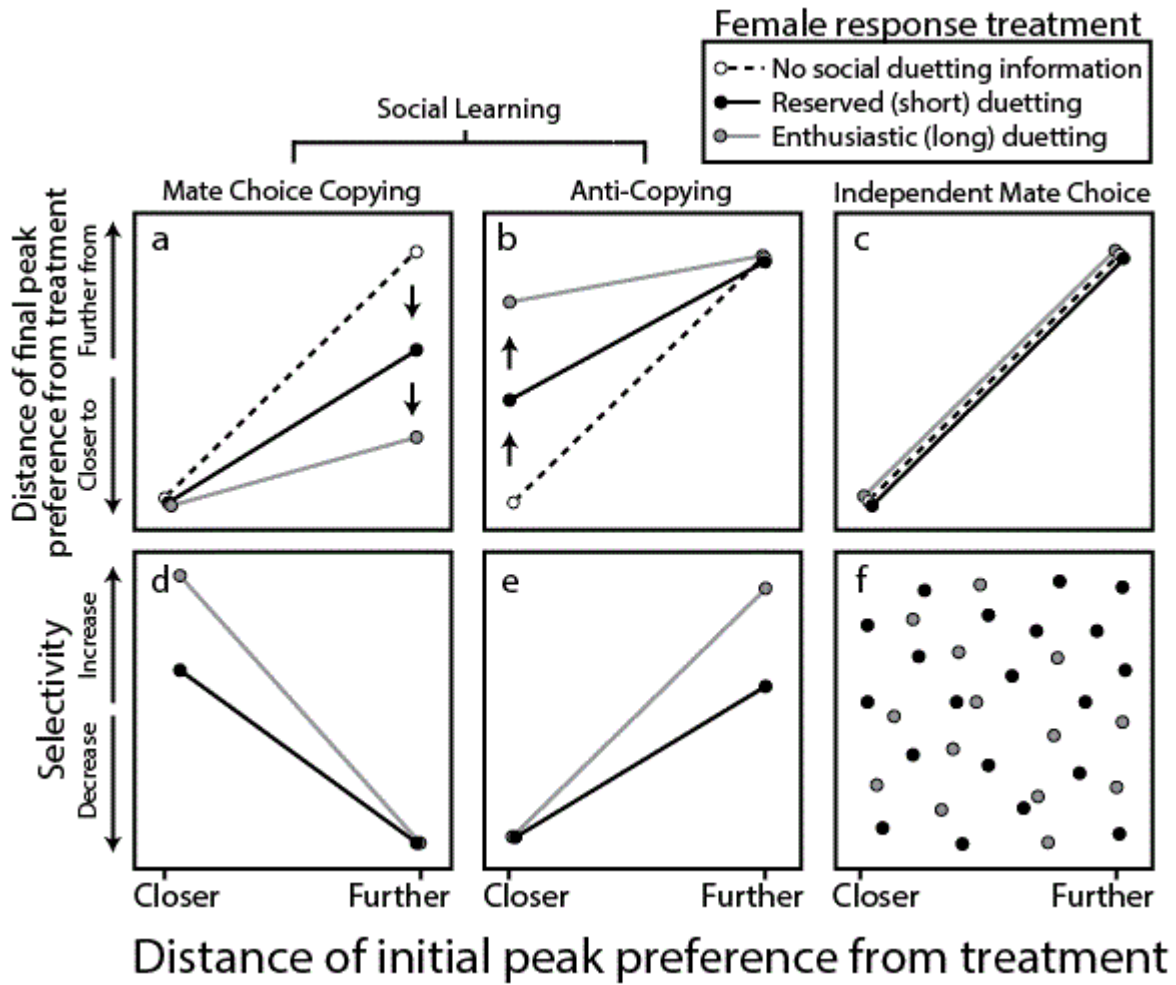
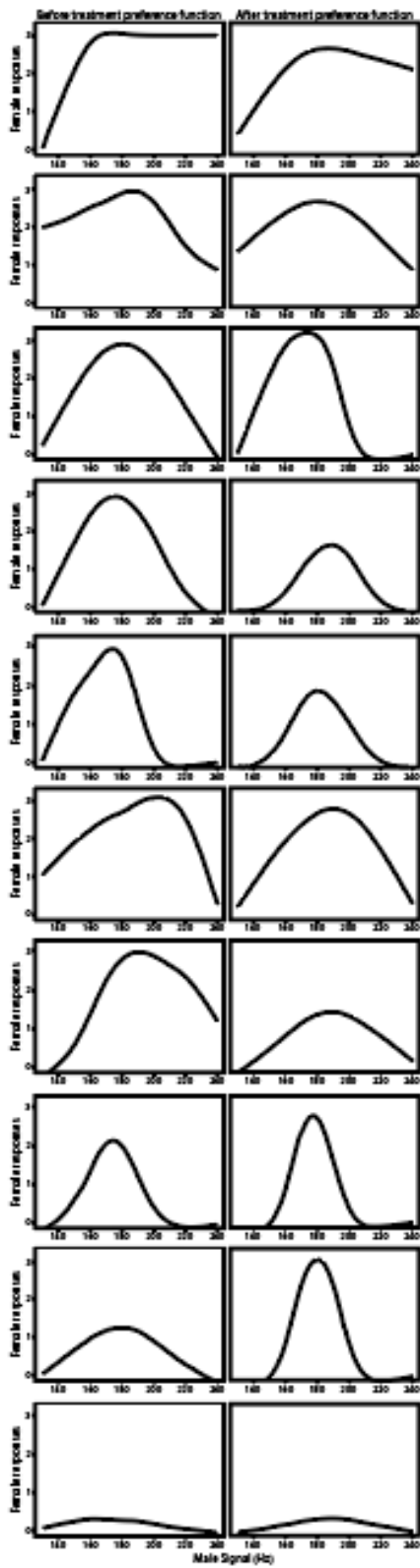


Figure 3:



602 **Figure 4:**



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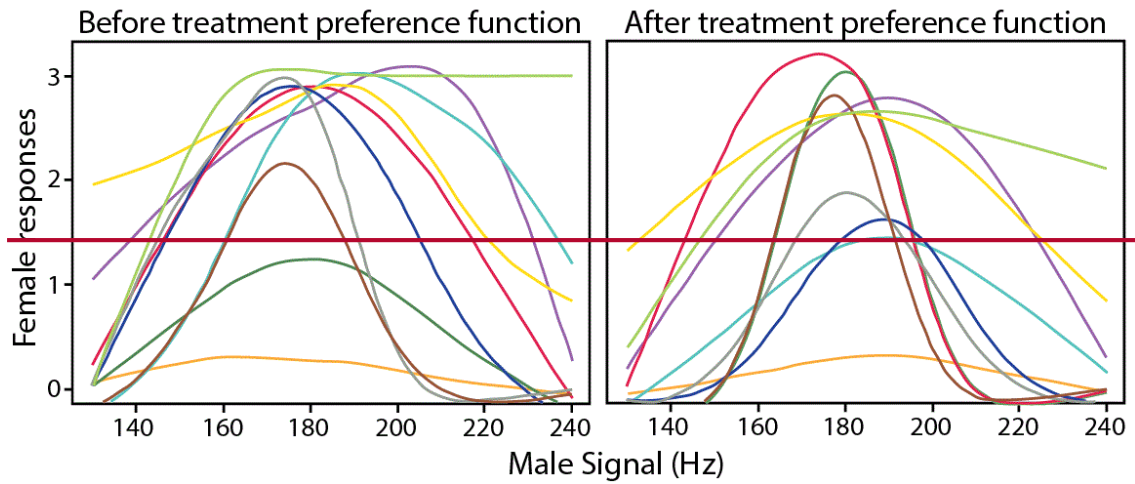
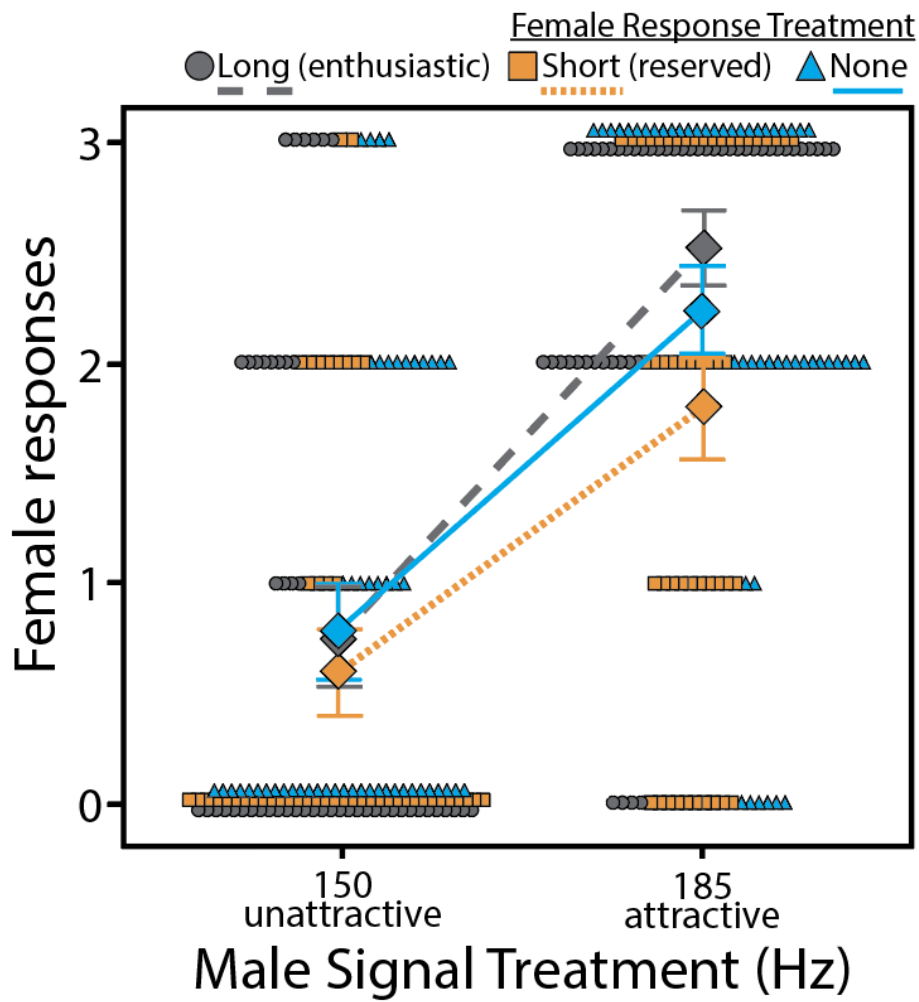
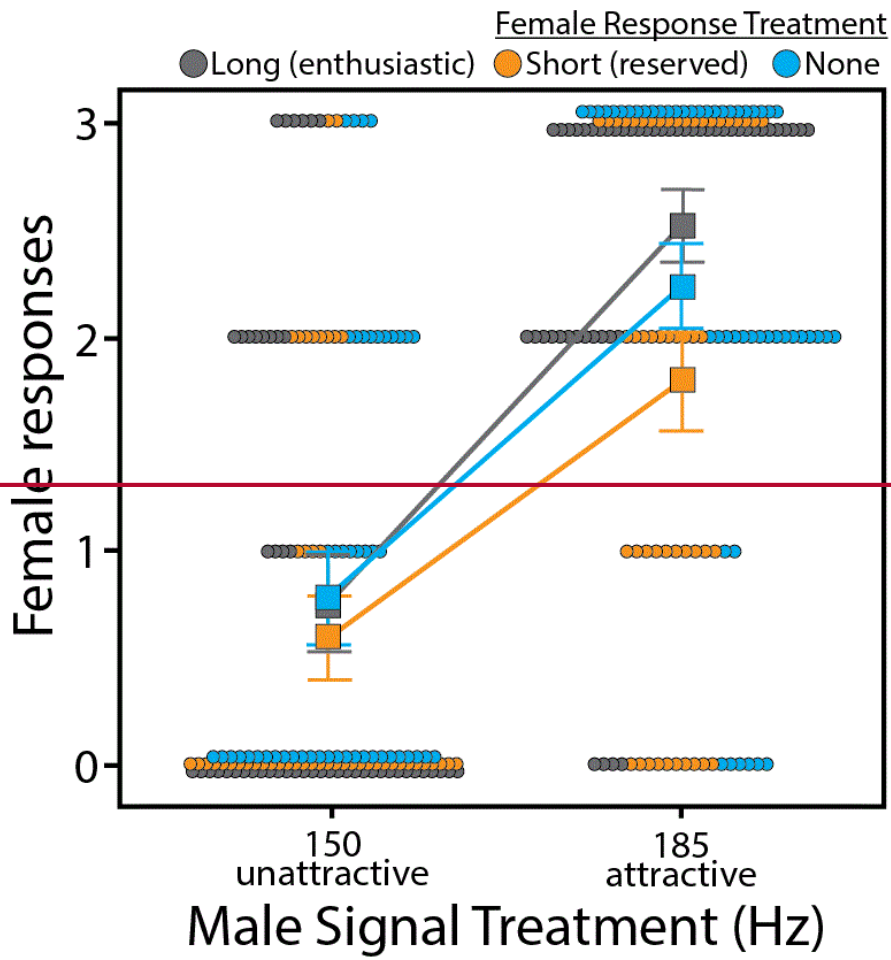
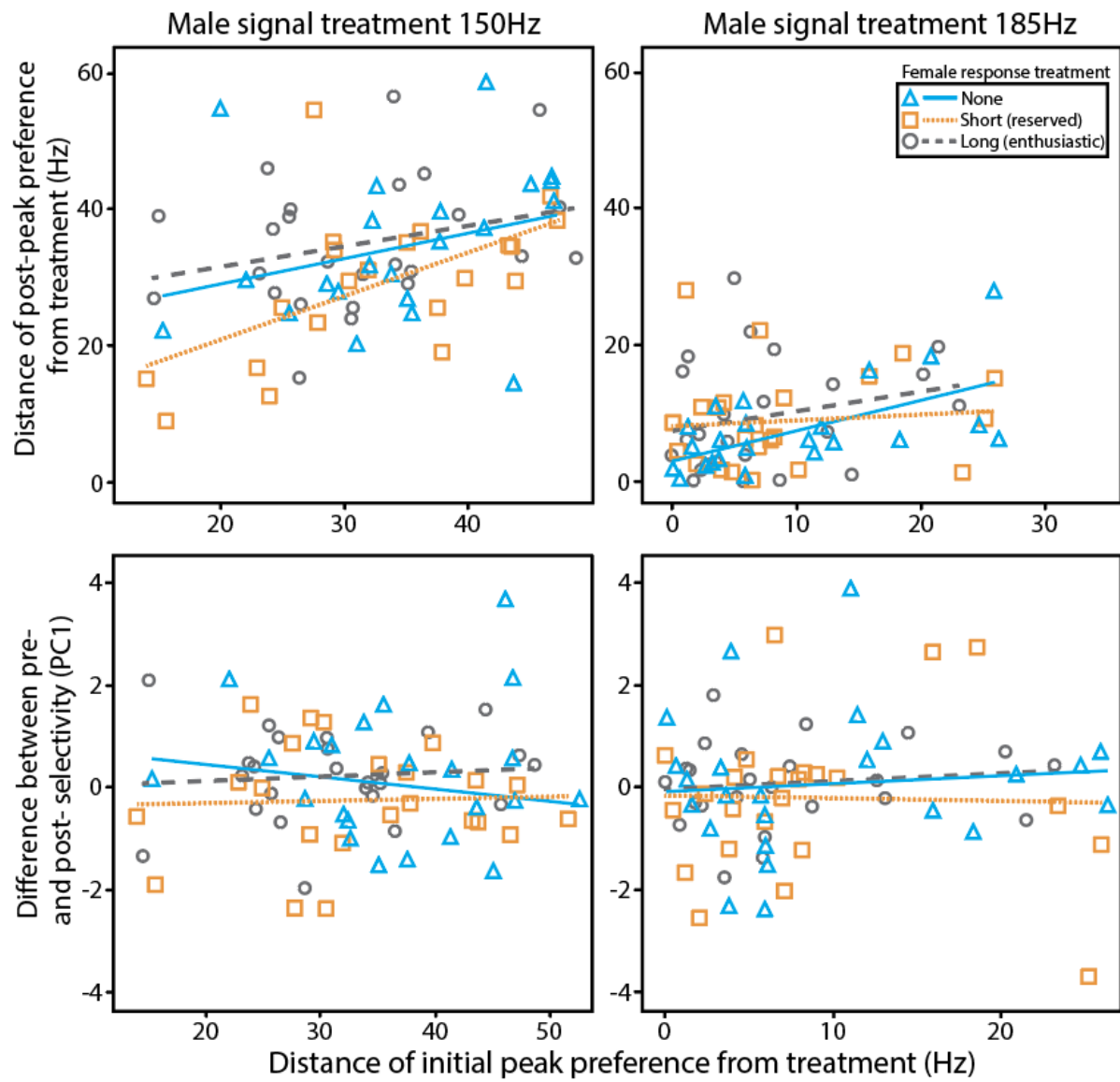


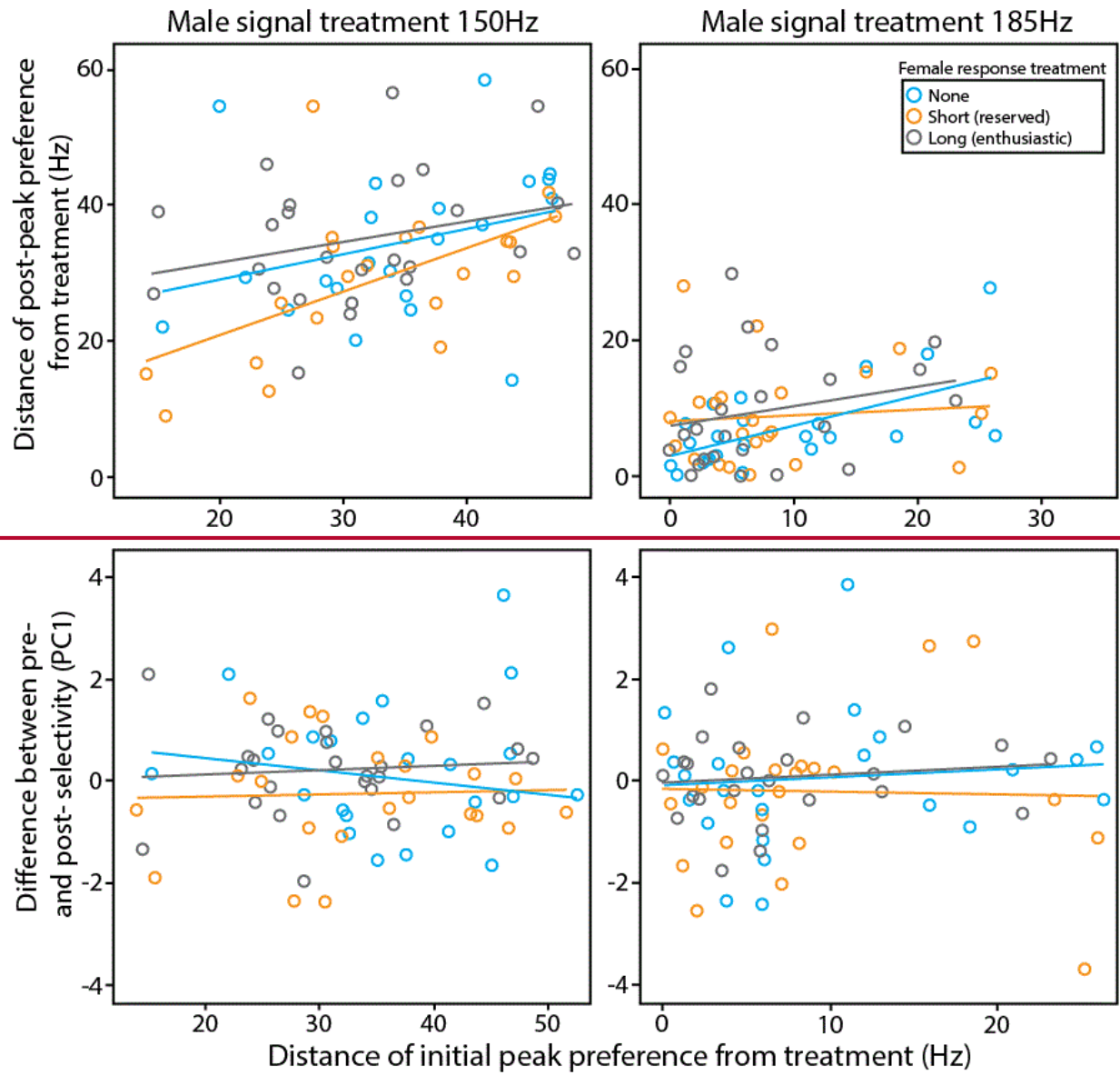
Figure 5:





619 **Figure 6**





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Research highlights

- Pair formation in *Enchenopa* treehoppers involves male-female signal duets
- These duets provide information about the mating decisions of nearby individuals
- We tested for mate choice copying in *Enchenopa* with duet playback treatments
- *Enchenopa* mate preferences were unaffected by the playbacks
- No copying may represent selection for fixed preferences, instead of no opportunity

Abstract

In mate choice, social learning may take the form of mate choice copying or anti-copying, whereby observed mating decisions are either mimicked or avoided. Alternatively, independent mating decisions may be based on innate preferences or early life social learning. While mate choice copying is widespread among some animal taxa, research in arthropods is limited and results are mixed. We tested these hypotheses using *Enchenopa* treehoppers (Hemiptera: Membracidae). *Enchenopa* males produce plant-borne vibrational advertisement signals and females express their mate preferences by selectively duetting with males. Individuals on the plant can monitor these public signals during pair formation. We randomly assigned females to treatment duets consisting of either unattractive or attractive male signals, followed by a long (enthusiastic), short (reserved), or no female treatment responses. We described the test females' mating preferences before and after the treatment duet. We found that female mate preferences were not affected by the treatment duets. Instead, females had consistent individual differences which supports the independent mate choice hypothesis and rejects both social learning hypotheses. Our findings suggest that independent mate choice does not necessarily represent a lack of opportunity for social influences from the immediate social context of mate choice.

Key terms: experience-mediated plasticity, sexual selection, non-independent mate choice, social plasticity, repeatable

Introduction

In social learning, animal decisions are influenced by information provided by the behavior of conspecifics (Danchin et al. 2004). Animals may glean information from conspecifics from their signals or inadvertent cues, or from direct observations of their choices and outcomes (Danchin et al. 2004). Social learning may inform animal decisions in a variety of contexts such as habitat selection, foraging, and mate choice (Danchin et al. 2004).

Social learning may be advantageous in mate choice if it helps acquire information about mate quality and/or decrease the costs of searching and selecting mates (Cotton et al. 2006; Gibson and Hoglund 1992; Jennions and Petrie 1997; Pomiankowski 1987; Pruett-Jones 1992; Vakirtzis 2011; White 2004). In turn, social learning in mate choice may influence the strength and direction of sexual selection with consequences ranging from the maintenance of within-population variation to speciation (Agrawal 2001; Kirkpatrick and Dugatkin 1994; Wade and Pruett-Jones 1990). It is therefore important to understand what determines when and how social learning will influence mate choice.

Social learning in female mate choice may take two forms. Females may engage in mate choice copying, whereby females set their mate preferences to match those of other females whose mating decisions they have observed (Pruett-Jones 1992; Scauzillo and Ferkin 2019; Vakirtzis 2011). Mate choice copying may allow females to favor males that are commonly available or locally of high quality (Cotton et al. 2006; Jennions and Petrie 1997; Pomiankowski 1987; Vakirtzis 2011; White 2004). By contrast, females may engage in “anti-copying,” and set their mate preferences to disfavor mate types that they have observed being chosen by other females (e.g., Loyau et al. 2012). Anti-copying may mitigate competition for mates, help prevent females from mating with males that are sperm depleted, or reduce the risk of losing parental care (Pruett-Jones 1992; Scauzillo and Ferkin 2019). Another possibility is that females may engage in independent mate choice and disregard available social information at the time of mate choice (Pruett-Jones 1992; Scauzillo and Ferkin 2019). Independent mate choice may reflect mate choice dictated purely by innate mate preferences, private information obtained

through females' experiences in prior encounters, and/or through social learning prior to sexual maturity.

Female mate choice copying appears to be taxonomically widespread in vertebrates (Davies et al. 2020; Jones and DuVal 2019; Vakirtzis 2011). The distribution of female mate choice copying in other animal groups, however, remains unclear (Davies et al. 2020). Multiple studies in arthropods, for instance, have examined the use of social information when females make mating decisions (Belkina et al. 2021; Jones and DuVal 2019; Vakirtzis 2011). Results from these studies are mixed – some supporting social learning and others independent mate choice (Jones and DuVal 2019; Vakirtzis 2011), although evidence of anti-copying is rare (Loyau et al. 2012). Most attention regarding female mate choice copying in arthropods has focused on *Drosophila* (Belkina et al. 2021; Jones and DuVal 2019; Vakirtzis 2011). While independent mate choice might be generally expected given arthropods' smaller brains, there is evidence of widespread capabilities for learning and social plasticity in this group (Dion et al. 2019; Dore et al. 2018; Dukas 2008; Hebets and Sullivan-Beckers 2010; Rodriguez et al. 2013b; Verzijden et al. 2012). Indeed, several *Drosophila* studies found evidence for female mate choice copying; however, these results could not be replicated in other studies (Auld et al. 2009; Belkina et al. 2021). Thus, testing across a wider taxonomic range seems necessary to understand whether arthropods use social information to inform their mating decisions, and why.

Here, we test the mate choice copying, anti-copying, and independent mate choice hypotheses in an insect, a member of the *Enchenopa binotata* complex of treehoppers (Hemiptera: Membracidae). Our goal is to broaden the scope of such tests in arthropods, as well as address some potential reasons why use of social information in the immediate context of female mate choice might be rare in arthropods. *Enchenopa* treehoppers provide a strong advantage in this regard as their mating and communication offers a clear means and opportunity for mate choice copying to occur.

Enchenopa treehoppers mating system provides a clear opportunity for females to glean social information about other females' mating decisions. As with many plant-feeding insects, *Enchenopa* communicate with plant-borne vibrational signals (Cocroft and Rodriguez 2005; Hill 2008). Pair formation occurs through male-female signal duets (Cocroft et al. 2008; Rodriguez and Cocroft 2006; Rodriguez et al. 2006; Rodriguez et al. 2004). Mate-searching males fly from one plant to another and produce advertisement signals. Females that find a male's signal attractive respond with their own signals and establish a duet that helps the male locate the female (Cocroft et al. 2008; Rodriguez and Cocroft 2006; Rodriguez et al. 2012; Rodriguez et al. 2006; Rodriguez et al. 2004). These duets often occur in the presence of other reproductively ready females (Cocroft et al. 2008, Little, Cirino, and Rodriguez, unpubl.). Thus, duetting provides social information to females as other treehoppers on the same plant can easily detect them (Cocroft and Rodriguez 2005). Further, the duets provide information about females' mating decisions, as *Enchenopa* females express their mate preferences through selective duetting with males – they are more likely to duet and produce more and longer signals in response to males they prefer (Rodriguez et al. 2012; Rodriguez et al. 2004).

Besides the natural availability of social information regarding female mating decisions (the “opportunity” for social learning), the mating system of these treehoppers also provides ample “means” for social learning as there is considerable evidence that sexual communication in *Enchenopa* is socially malleable. The inputs and effects of social experience vary with the life stage at which they occur. Young adult females that experience attractive or mixed mate types become more selective but do not change the mate types they prefer (Fowler-Finn and Rodriguez 2012a; 2012b). By contrast, variation in group density and signaling environment starting at the juvenile stage influences preferred mate types (Desjonquères et al. 2019a; Desjonquères et al. 2021; Desjonquères et al. 2019b; Fowler-Finn et al. 2017). Finally, females are more likely to respond to an attractive signal bout when they perceive another female

responding once to it than to the signal without a female response (Escalante et al., under review).

In this study, we experimentally mimicked the experience of females perceiving attractive and unattractive males receiving “enthusiastic,” “reserved,” or no female responses. We randomly assigned females to treatments consisting of playback duets featuring either attractive or unattractive male signals paired with female response signals that were either long, short, or absent (i.e., there were six different treatment combinations of male-female duets) (Figure 2). We manipulated the length of perceived female responses because longer response signals indicate greater attraction (Rodriguez et al. 2012; Rodriguez et al. 2004). We described females’ mate preferences before and after presentation of these treatment duet playbacks, as well as their behavior during the treatments. We described two features of female mate preferences: peak preference (the preferred signal type; Figure 1a) and preference selectivity (how female response decreases with deviation from the preferred signal type; Figure 1b).

In the framework of this experiment, the mate choice copying hypothesis predicts that females will switch their preferences to the perceived male signal that receives a female response. Further, the copying effect should be stronger with longer (more “enthusiastic”) perceived female responses (Dugatkin 1998) (Figure 3a). By contrast, the anti-copying hypothesis predicts that females will switch their preferences away from the perceived male signal that receives a female response. Further, as above, the anti-copying effect should be stronger with longer perceived female responses (Figure 3b). Finally, the independent mate choice hypothesis predicts that females will not switch their preferences according to the treatments, with most variation (if any) due to consistent between-individual differences (Figure 3c) (we would expect some variation given prior evidence of genetic and environmental components influencing mate preferences; (Rodriguez et al. 2013a; Rodriguez et al. 2013b; Desjonquères and Rodríguez 2023).

Preference selectivity has not often been investigated in the context of these hypotheses. However, social learning could also affect selectivity (Figure 1b). If so, the mate choice copying hypothesis predicts that females will become either similarly or *more* selective after perceiving a male signal close to their peak preference that receives a female response. Further, the copying effect should be stronger when females perceive male signals receiving long female responses (Figure 3d). By contrast, the anti-copying hypothesis predicts that females will become less selective after perceiving a male signal close to their peak preference that receives a female response as females are predicted to avoid that male signal and instead respond to other male signals that did not receive female responses. Further, as above, the anti-copying effect should be stronger with longer perceived female responses (Figure 3e). Finally, the independent mate choice hypothesis predicts that there would be no change in selectivity, with most variation (if any), again, due to consistent between-individual differences (Figure 3f).

Methods

Insect collection and rearing

Most species in the *Enchenopa binotata* complex have not yet been described formally (Hamilton and Cocroft 2009). However, they can be easily identified by the host plant they live and feed on, the coloration of the nymphs, and the dominant adult male signal frequency used for mate advertisement (Cocroft et al. 2008; Cocroft et al. 2010). In this experiment, we used the species that lives on *Viburnum lentago* (Adoxaceae) host plants in Wisconsin, has gray nymphs, and has a ~165Hz dominant-frequency male advertisement signal (Rodriguez et al. 2018). We kept voucher specimens in 95% EtOH in the lab collection.

We used both field-collected (N=135) and first-generation lab-reared (N=14) insects. We collected second and third instar nymphs from several trees at two sites in Milwaukee, Wisconsin, USA, in June 2021: Downer Woods Natural Area (43°04'47.4"N 87°52'49.4"W) and

a part of the Oak Leaf Trail (43°04'54.2"N 87°53'26.9"W). Lab-reared nymphs were acquired from eggs that were laid by multiple females collected from the Oak Leaf Trail site in the previous 2020 field season. *E. binotata* juveniles live in aggregations at the terminal end of nannyberry tree branches. Each group is likely comprised of several different females' offspring as many females aggregate on branches to lay egg masses (Wood 1974; Tallamy and Wood 1986; Zink 2003). We collected aggregates of juveniles by clipping the ends of the branches from multiple trees that were several meters apart at each site. We reared all nymphs on potted host plants at the University of Wisconsin-Milwaukee (UWM) Greenhouse. We separated the sexes upon adult eclosion to ensure that females had no experience with male signals or duetting, and that they did not mate, after which they become sexually unreceptive. As they were separated from males, females did not engage in duetting behavior before our trials.

Treatment and testing

Each individual female received three sets of playbacks: 1) a round of playbacks to describe each female's initial mate preference function, so we could establish a baseline mating preference for each female; 2) a treatment male-female duet playback, and 3) a second round of playbacks to describe females' mate preference functions after the treatment to assess any changes in preference due to the treatment (Figure 2). We used this "before-and-after" treatment approach as copying was stronger in animal studies that used this design over those that had a separate control and social experimental groups (Davies et al. 2020).

Each round of playbacks to describe females' preference functions consisted of a random sequence of 11 synthetic vibrational stimuli mimicking the structure *Enchenopa* male advertisement signals (each stimulus consisted of a bout of three signals separated by 2 seconds of silence with 12 seconds of silence between bouts) (Cocroft et al. 2010; Rodriguez and Cocroft 2006) (Figure 2). Thus, each round of playbacks to describe females' preference functions ran for 3 minutes and 22 seconds (see Methods: *Stimulus construction and playback setup*).

The strongest female mate preference in the *E. binotata* complex is for the dominant frequency of male signals, which are species-specific and represent the most divergent adult phenotype among species in the complex (Cocroft et al. 2008; Cocroft et al. 2010; Rodriguez et al. 2006). We therefore focused on signal frequency in this experiment. The stimuli for the two rounds of playbacks varied in frequency (130, 150, 160, 170, 180, 185, 190, 200, 210, 220, and 240Hz). This range slightly exceeds the natural range in the species, which helps capture the full shape of each preference function (Kilmer et al. 2017).

The treatment male-female duet playbacks were composed of a male signal stimulus component (a bout of three signals as above) that was either attractive (at the peak of the population preference function: frequency at 185Hz) or unattractive (frequency at 150Hz), on the basis of prior work with this population (Desjonquères et al. 2019a; Rodriguez et al. 2013a). This component was followed by a female response signal component that was either long (mean length for the population: 0.86 seconds), short (0.35 seconds, corresponding to the shortest decile in the population), or absent (Figure 2). Treatment duets ran for 8 seconds and were separated by the preceding and following rounds of playbacks to describe female preference functions by 12 seconds (Figure 2). Thus, the treatment was relatively brief compared with the rounds of playbacks for describing the before and after mate preference functions. We chose this design in order to focus on the hypotheses we wished to test – treatments with longer duets would risk confounding the experience of observing attractive-unattractive males receiving enthusiastic-unenthusiastic female responses with the additional experience of the test females not being engaged in their own duetting attempts. Further, shorter female response treatments than the ones we describe in this study have been known to influence female engagement with mates (Escalante et al., in review). Here, we examine if changes in peak preference and preference selectivity are involved.

We randomly assigned females to one of the six treatments: attractive male signal (185Hz) receiving either a long (n=25) or short (n=25) female response or no response (n=25);

or unattractive male signal (150Hz) receiving either a long (n=25) or short (n=25) female response or no response (n=24) (Figure 2). We tested females when they became sexually receptive, 3.5-5 weeks after adult eclosion. To start a trial, we placed a single female on a playback plant and assessed their receptivity by playing a primer (a recording of a male signal closely matching the population mean) up to four times. If the female did not respond, we placed the female back onto the rearing plant and tested again up to four days later. If the female responded, we delivered the preference function and treatment playbacks described above (Figure 2).

Stimulus construction and playback setup

We generated the synthetic male signal stimuli by a custom-written program in R. For the female response playback stimuli, we used recordings of duetting females, selected from our library, and played back with the same program in R. We used five replicates (five signals, each from a different female) per treatment length. The male component of these playbacks consisted of a single frequency, but the recordings of female signals have more frequency components (Rodriguez and Coccoft 2006). It was therefore necessary to compensate for the differential filtering of frequency components along the playback plant stem. To this end, we played back and recorded band-limited white noise (90-2000 Hz) through the playback plant stem and generated a digital filter that compensated for the filtering using custom-written software in MATLAB (Nieri et al. 2022). We then standardized all stimuli to an amplitude of 0.15 mm/s using an oscilloscope (Model 72-2580, Tenma Test Equipment, Springboro, OH, USA).

We delivered the playbacks from a Macintosh computer (Mac OS X Version 10.4.11, Apple, Cupertino, CA, USA) through a piezo-electric stack attached to the stem of a potted playback plant by accelerometer wax and regulated by a piezo-controller (MDT694A, Thor labs, Newton, NJ, USA). We recorded the playbacks and female responses by focusing a portable digital laser vibrometer (Polytec PDV100, Polytec Inc., Irvine, CA, USA) on a small piece of reflective tape adhered to the stem of the recording plant. We sent the laser vibrometer output

signal through a band-pass filter (40–3000 Hz; Krohn-Hite Corporation, Model 3940, Brockton, MA, USA), and then to an iMac computer (macOS Big Sur Version 11.4, Apple, Cupertino, CA, USA) through a USB audio capture (cakewalk UA-25 EX, Roland Corporation, Hamamatsu, Japan). We recorded the signals on the iMac computer using the program AUDACITY (version 3.0.2; <https://www.audacityteam.org/>). We isolated the playback setup from building vibrations by placing it on a ~15-kg epoxy resin tabletop that rested on partially inflated bicycle innertubes on a freestanding table that stood on anti-vibration pads (PneumaticPlus CP6X6, Torrance, CA). The playback plant was further isolated from the tabletop by a sheet of shock absorbing sorbothane (Edmund Scientifics, Tonawanda, NY). We monitored the air temperature by the playback plant at the start of each trial with a digital thermometer (Extech instruments Hygro-Thermometer Clock 445702, Twinsburg, OH, USA; temperatures ranged between 22.9°C–25.4°C ± 0.5).

Describing mate preference functions

Mate preferences are function-valued traits – they are expressed as a function of the features of the signals in which females encounter or interact (Kilmer et al. 2017; Stinchcombe et al. 2012). We therefore used a function-valued approach to describe individual and population mate preferences. Our assay of female response was the number of female responses per male signal stimulus bout (0 – 3 responses). We used the program *PFunc* (v. 1.0.2; <https://github.com/Joccalor/PFunc>, Kilmer et al. 2017) to fit cubic spline regressions to the female response data (example shown in Figure 4). This method does not assume preference function shape outside of some smoothness that is determined empirically (Kilmer et al. 2017; Schluter 1988). We generated two preference functions for each female: one before and one after the treatment playback (Figure 4). We then extracted peak preference and three other preference function traits that together make up preference selectivity with *PFunc* (see below for “preference selectivity” generation).

Ethical Note

All our procedures adhered to the ASAB/ABS Guidelines for the use of animals in research as well as the legal requirements of the U.S.A. and all UWM guidelines.

Statistical Analyses

We first plotted each female's preference function values against each other (post-treatment versus pre-treatment) and inspected the plots for outliers. We found one female that fell far outside the peak preference data cloud. We ran all statistical analyses described below with and without this female and compared the output. Since, this data point did not qualitatively change the results of the experiment, we removed this female for the final models.

We then tested for correlations between all four preference function traits (peak preference, strength, tolerance, and responsiveness) before and after the treatments. We inspected correlation graphs (using 'scatterplotMatrix' function in the car package) and computed Kendall's Tau correlation coefficients. Peak preference was only weakly correlated with the other traits (in all cases: $\tau \leq 0.08$, $p \geq 0.399$). However, responsiveness, tolerance, and strength were correlated with each other ($\tau > 0.63$, $p \leq 0.001$) as has been found in prior work with *Enchenopa* (Kilmer et al. 2017). To avoid the risk of spurious significance from tests with correlated traits, we loaded these three traits (preference responsiveness, tolerance, and strength) into a principal component analysis to summarize them with a composite trait that we term "selectivity" (Figure 1) (Kilmer et al. 2017).

We analyzed the behavior of the females during the treatment portion of the playbacks. We ran a generalized linear model with a Poisson distribution (log link function) with the number of females' responses to the treatment playbacks (0-3 responses) as the response variable. We included male signal treatment (150 or 185Hz), female response treatment (long, short, none), and their interaction as the explanatory variables in this model.

Next, we assessed the homogeneity of variances across pre- and post-treatment preference function traits using Fisher's F-test to ensure that variance differences did not

confound our results (Danchin et al. 2020, Cleasby and Nakagawa 2011). Variances did not differ between before and after treatment (peak preference: Fisher's $F = 0.93$, $p=0.68$; preference selectivity: Fisher's $F = 1.00$, $p=0.98$).

To test the predictions of the hypotheses for peak preference, we calculated the absolute value of the difference between the pre- and post-treatment peak preferences for each female and the frequency of the male signal treatment. For example, a female with a pre-treatment peak preference of 205Hz that received the 185Hz male signal treatment would have a pre-treatment difference of $|205-185| = 20$. Similarly, if that same female's post-treatment peak preference was 195Hz, this female would have a post-treatment difference of $|195-185| = 10$.

We then tested for changes in peak preference due to the treatments in terms of changes in the relationship between these pre- and post-treatment differences (Figure 3a-c). In these terms, the mate choice copying hypothesis predicts that females that started *further away* from the male signal treatment value (before the treatment) should come *closer to* that male signal treatment value (after the treatment) (Figure 3a); and this effect should be stronger with longer (more "enthusiastic") female responses (Figure 3a). By contrast, the anti-copying hypothesis predicts that females that started *closer to* the male signal treatment value (before the treatment) should end up *further away* from that male signal treatment value (after the treatment) (Figure 3b); and this opposite effect should also be stronger with longer (more "enthusiastic") female responses (Figure 3b). Finally, the independent mate choice hypothesis predicts that the relationship between the pre- and post-treatment difference values will not be affected by the treatments (Figure 3c).

To test these predictions, we used a linear model with the post-treatment differences as the response variable. We included the pre-treatment differences, male signal treatment, female response treatment, and all two-way interactions as explanatory variables. The three-way interaction was well beyond the level of statistical significance ($p=0.27$), so we removed it from

the model. In this model, the pre-treatment value x female response treatment interaction tests for the predicted changes in the steepness of the relationship between the post- and pre-treatment differences. The male treatment x female treatment interaction tests for the predicted stronger effect of the longer female treatment responses between the attractive and unattractive male signal treatments.

To test the predictions of the hypotheses for preference selectivity, we calculated the difference between each female's pre- and post-treatment selectivity. We then tested for changes in preference selectivity due to the treatments in terms of changes in the relationship between this post-pre difference in selectivity and the above absolute value of the difference between pre-treatment peak preference and the frequency of the male signal treatment. In these terms, the mate choice copying hypothesis predicts that females that started *closer to* the male signal treatment in peak preference (before the treatment) should become *more selective* after the treatment (Figure 3d); and this effect should be stronger with longer (more "enthusiastic") female responses (Figure 3d). By contrast, the anti-copying hypothesis predicts that females that started *further away* from the male signal treatment in peak preference (before the treatment) should become *more selective* (after the treatment) (Figure 3e); and this opposite effect should also be stronger with longer (more "enthusiastic") female responses (Figure 3e). Finally, the independent mate choice hypothesis predicts that the relationship between the pre- and post-treatment selectivity will not be affected by either the absolute value of the difference between the pre-treatment for peak preference and the frequency of the male signal treatment nor by the treatments (Figure 3f).

To test these predictions, we used a linear model with the post-pre difference in selectivity as the response variable. As explanatory variables, we included the difference between pre-treatment peak preference and male signal treatment (150 or 185Hz), male signal treatment, female response treatment, and all two-way interactions. The three-way interaction was well beyond the level of statistical significance ($p=0.69$), so we removed it from the model.

In this model, the pre-treatment difference x female response treatment interaction tests for the predicted changes in the steepness of the relationship between the change in selectivity and the absolute value of the difference between pre-treatment peak preference and the frequency of the male signal treatment. The male signal treatment x female response treatment interaction tests for the predicted stronger effect of the longer female treatment responses between attractive and unattractive male signals.

In both linear models that examined changes in peak preference and preference selectivity, we checked that our response variables had residuals that were normal and homoscedastic (before obtaining the absolute values in the case of peak preferences) by plotting and inspecting a histogram, q-q plot, and the standardized residuals versus fitted values.

We were also interested in the repeatability (Bell et al. 2009) of peak preference and preference selectivity. To estimate repeatability, we used two separate linear models, one for peak preference and one for preference selectivity. We used the post-treatment peak preference or selectivity as the response variable for each model. In each model, we included pre-treatment peak preference or selectivity, respectively, male signal treatment, female response treatment, the male x female treatment interaction. The terms for pre-treatment peak preference or selectivity test for a relationship with the post-treatment values for those preference function traits, and the slope of the terms corresponds to repeatability in these models. We originally included temperature (°C) in all models described above; however, this term was never significant ($p \geq 0.09$), so we removed it from the models.

To help interpret results where we detect no effect through significance testing, we estimated the size of the effects for which we had adequate statistical power ($1 - \beta$) given our sample sizes and standard deviations (Nakagawa and Cuthill 2007). All analyses were performed in R v. 4.0.5 (R Core Team 2021).

Results

Female behavior during treatment

Females' behavior during the treatment playbacks confirmed that the attractive male signal treatment received higher response levels than the unattractive male signal treatment (Table 1, Figure 5). By contrast, female response treatment had no effect on females' behavior during the treatment playbacks (Table 1, Figure 5).

Mate preferences before and after treatment

We show the results separately for each male signal treatment, as the differences between peak preference and male signal treatment frequency were (by definition) overall smaller with the attractive male signal (185Hz) than the unattractive male signal (150Hz) (Figure 6; this difference corresponds to the main term for male treatment in Table 2). We found that neither female peak preference nor preference selectivity were affected by the male-female duet treatments nor any interaction (Table 2, Figure 6). Instead, we found that both peak preference and preference selectivity had significant repeatability (weak for peak preference: $r = 0.28 \pm 0.08$; strong for selectivity: $r = 0.74 \pm 0.06$; Table 3, Figure 6). We also note that the significant term for the difference between pre-treatment peak preference and the frequency of the male signal treatment also tests for consistency in individual differences in peak preference (Table 2).

Although we detected no effect of the male-female duet treatments, with our sample sizes ($n = 24 - 25$ per treatment) and observed data dispersion (peak preference SD = 10.7), these tests had adequate statistical power ($1 - \beta > 0.80$) to detect an 8Hz shift in peak preference (4%) and high statistical power ($1 - \beta = 0.95$) to detect a 10Hz shift (6%). Such changes would be at the lower end of what we would consider biologically relevant regarding the mate choice copying, anti-copying, and independent mate choice hypotheses.

Discussion

Enchenopa treehoppers find and select mates by using substrate-borne vibrational male-female duetting on their host plants (Cocroft et al. 2008). We capitalized on this natural communication system that publicly advertises signals and mate choice decisions to test hypotheses regarding social learning in the immediate context of mate choice. We found that females did not switch their mate preferences either toward or away from the mate types with which they perceived other females duetting, regardless of whether the perceived response was more or less “enthusiastic” or absent. Instead, females showed stable individual differences in peak preference and preference selectivity before and after the treatments. We also found that repeatability was higher than previously reported for *Enchenopa* likely because we assessed it over a shorter time span (Fowler-Finn and Rodriguez 2013; Bell et al. 2009). Together, these results support the independent mate choice hypothesis and reject the mate choice copying and anti-copying hypotheses.

These results are not due to a total lack of social malleability in *Enchenopa* mate preferences. Social environments and experiences during the juvenile stage influence female peak preferences (Desjonquères et al. 2019a; Desjonquères et al. 2021; Desjonquères et al. 2019b; Fowler-Finn et al. 2017) while social experiences with available mate types during the early adult stage influences female preference selectivity (Fowler-Finn and Rodriguez 2012a; 2012b). However, social experience in the immediate context of mate choice does not seem to influence preferred mate types (Speck 2022; this paper). We suggest that our results point to an adaptive benefit from not altering mate preferences according to the immediate context of mate choice in the *Enchenopa* mating system. Therefore, a lack of mate choice copying or anti-copying in some animals may not represent a lack of opportunity or capacity, but instead a lack of “motive” favoring behavioral inflexibility (i.e., no selection favoring the use of social learning in this context) .

Mate choice can potentially be improved through social learning where females intercept public information to help discriminate between mates. Social learning, such as mate choice

copying or anti-copying, is expected to evolve if it facilitates assessment of mate quality, reduces the costs associated with mate choice, and/or enables females to benefit from resources that a male could provide (Gibson and Hoglund 1992; Nordell and Valone 1998). In treehoppers, costs associated with female mate choice may be low since males fly to search for females and produce search vibrations on their host plants to initially find mates (Cocroft et al. 2008; Fowler-Finn et al. 2014). Females only need to respond to a desirable vibration to find a high-quality mate, although some females may produce search vibrations as well (Cocroft et al. 2008; Rodriguez and Cocroft 2006; Rodriguez et al. 2012). Since males become sexually mature before females (Wood and Guttman 1982), there are likely many males to choose from which may reduce the risk involved for females locating and assessing the quality of males. Thus, the cost of mate choice is likely to be low and social learning may be unnecessary at this stage to select a suitable mate. Relying on private information, genetic combined with learned information from earlier life stages, for mating decisions may be most advantageous for mating decisions in this species.

An alternative possibility may be that our single exposure to an 8-second treatment duet may constitute insufficient “dosage” to alter mate preferences. In the experiments which manipulated *Enchenopa* juvenile and early adult social experiences and detected social plasticity in mate preferences, exposures lasted from two to several weeks (Desjonquères et al. 2019a; Desjonquères et al. 2021; Desjonquères et al. 2019b; Fowler-Finn and Rodriguez 2012a; 2012b). Further, dosage may involve not only the time of exposure but also the number of females observed. For instance, in sailfin mollies (*Poecilia latipinna*), mate choice copying did not occur when only one female was observed for 10 minutes but did occur when two females were observed for 5 minutes each consecutively or when one female was observed for 20 minutes (Witte and Noltemeier 2002). In nature, *Enchenopa* females may be exposed to a higher number of duets as females begin to engage with males gradually along the mating season (Sullivan-Beckers and Cocroft 2010) and each duet often lasts an hour or longer

(Cocroft et al. 2008), although mating pairs can take anywhere from two minutes to three hours from the time they first duet to copulation (Leith et al. 2020). This potential dosage dependence of social learning in the immediate context of mate choice may be an interesting avenue for future research. We note, however, that experiments with such longer dosages will also need to disentangle potential confounds arising from additional social inputs from the experience of the target animals engaging/not engaging with the social treatments.

In summary, it appears that the treehoppers we studied here have no motive to socially adjust mating preferences in the immediate context of mate choice even though they have the means (vibrational communication system) and opportunity (live in aggregations) to do so. Our study contributes to a small number of arthropod studies that demonstrate independent mate choice (Auld et al. 2009; Belkina et al. 2021) including one that could not replicate the results of many *D. melanogaster* social learning studies (Belkina et al. 2021). It is important that we continue to investigate social learning in the context of mate choice in arthropods to help us clarify our understanding among these conflicting studies and inform us of the importance with which social learning plays in shaping the mating preferences in this large group of animals.

Data accessibility: The data and code for all analyses in R are provided in the electronic supplementary materials.

Figures legends:

Figure 1: Traits we used to describe variation in female mate preference functions (Kilmer et al. 2017). (a) Peak preference: the signal trait value that elicits the strongest female response. The black and gray preference functions differ in peak preference (indicated by the corresponding vertical dotted lines) (b) Preference selectivity: the shape of the preference function around the peak. The black preference function indicates a female that is more selective than the female with the gray preference function.

Figure 2: Time course of the experiment we used to test the hypotheses regarding social learning in the immediate context of female mate choice in *Enchenopa* treehoppers. We presented each female with playbacks to describe their mate preferences (a) before and (c) after the (b) treatment playbacks. (b) Treatment playbacks were composed of an unattractive (150Hz) or attractive (185Hz) male signal (3 whines with pulses) paired with either a long, short, or no female response to each of the 3 whine-pulses parts of the signal.

Figure 3: Predictions of the mate choice copying, anti-copying, and independent mate choice hypotheses for peak preference (a-c) and preference selectivity (d-f) in our experiment manipulating social information available in male-female duets.

Figure 4: A sample of the cubic spline regressions that was generated in the program *PFunc* fit to the female response data that was gathered during the preference function playbacks before (left) and after (right) the treatment.

Figure 5: Female responses during the treatment duet portion of the playback. Females responded to one of six different treatment combinations (see Figure 3). Individual female

responses are depicted by small colored shapes and group means are depicted by colored diamonds with ± 1 standard error bars around them.

Figure 6: The relationship between pre- and post-treatment peak preferences (a-b) and preference selectivity (c-d) in females that were provided treatment playbacks with an unattractive (150Hz – a, c) or attractive (185Hz – b, d) male signal paired with a female response: long (gray), short (orange), or none (blue). Individuals are represented with open circles and the groups that were provided a female response (long, short, none) are indicated by the lines.

Tables and table legends:

Table 1: Results from a generalized linear model examining female behavior during the treatment duet portion of the playback. Females responded more to the attractive 185Hz male signal frequency than the unattractive 150Hz male signal frequency (significant term is bolded).

Term	df	χ^2	<i>p</i>
Duetting female	2	3.604	0.165
Male signal	1	57.873	<0.001
Duet female x male signal	2	0.160	0.923

Table 2: Results from two separate linear models examining the effect of the male-female duet treatments on peak preference and preference selectivity. Significant terms are indicated in bold.

	Term	df	F-value	<i>p</i>
Peak preference	Duetting female	2	2.095	0.127
	Male signal	1	7.745	0.006
	 Pre-treatment peak preference - treatment frequency 	1	6.314	0.013
	Duet female x male signal	2	1.464	0.235
	Pre-trt peak preference - treatment frequency x duet female	2	0.893	0.412
	Pre-trt peak preference - treatment frequency x male signal	1	2.855	0.093
Selectivity	Duetting female	2	0.146	0.864
	Male signal	1	0.027	0.870
	Pre-treatment peak preference - treatment frequency	1	0.065	0.799
	Duet female x male signal	2	0.059	0.943
	Pre-trt peak preference - treatment frequency x duet female	2	0.155	0.857
	Pre-trt peak preference - treatment frequency x male signal	1	0.251	0.617

Table 3: Results from two separate linear models testing for repeatability in peak preference and preference selectivity, while accounting for the male-female duet treatments. Estimates for repeatability (r) for peak preference and preference selectivity are also listed (significant terms are indicated in bold).

	Term	df	F-value	p	r	Std. Error
Peak preference	Duetting female	2	2.053	0.132	-	-
	Male signal	1	0.821	0.366	-	-
	Pre-treatment peak preference	1	13.55	<0.001	0.28	0.08
	Duet female x male signal	2	0.869	0.422	-	-
Selectivity (PC1)	Duetting female	2	0.535	0.587	-	-
	Male signal	1	0.626	0.430	-	-
	Pre-treatment selectivity (PC1)	1	156.407	<0.001	0.74	0.06
	Duet female x male signal	2	0.408	0.666	-	-

Figures:

Figure 1:

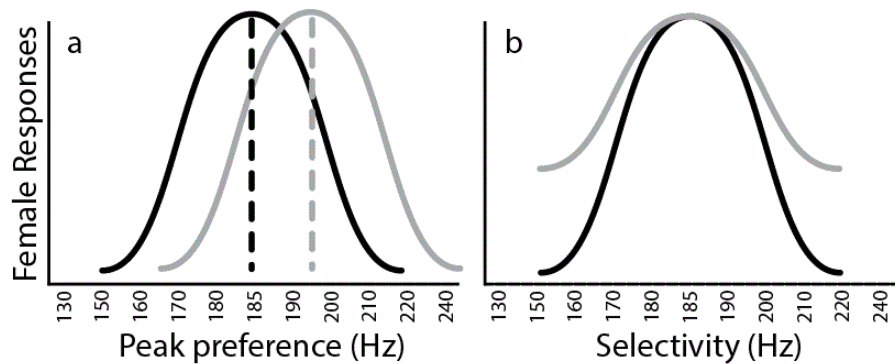


Figure 2:

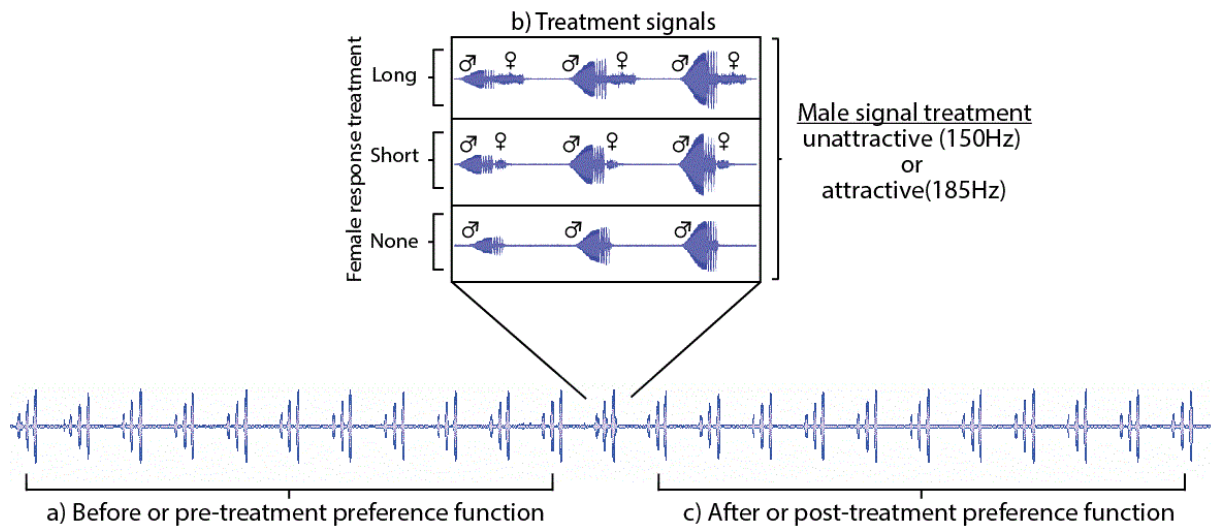
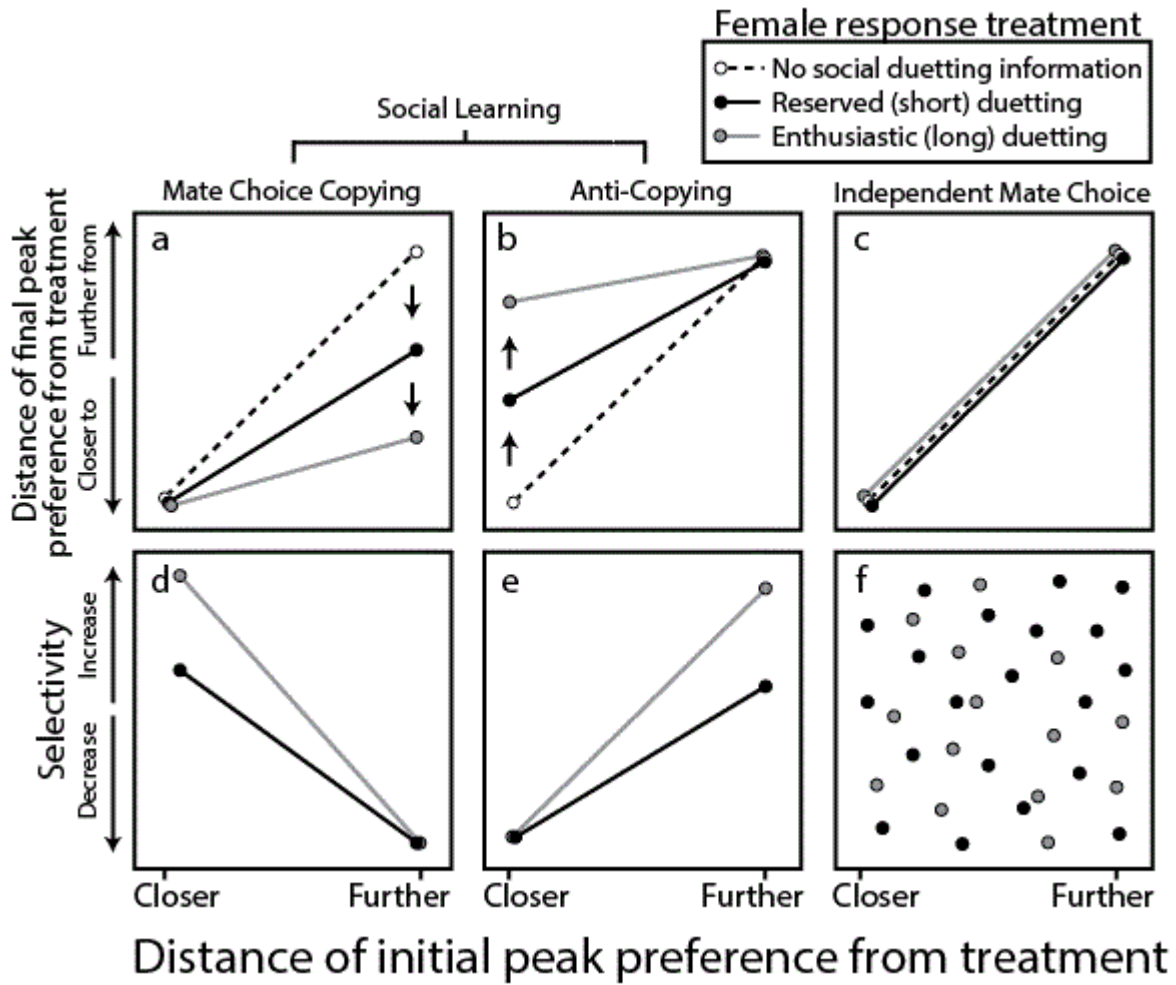
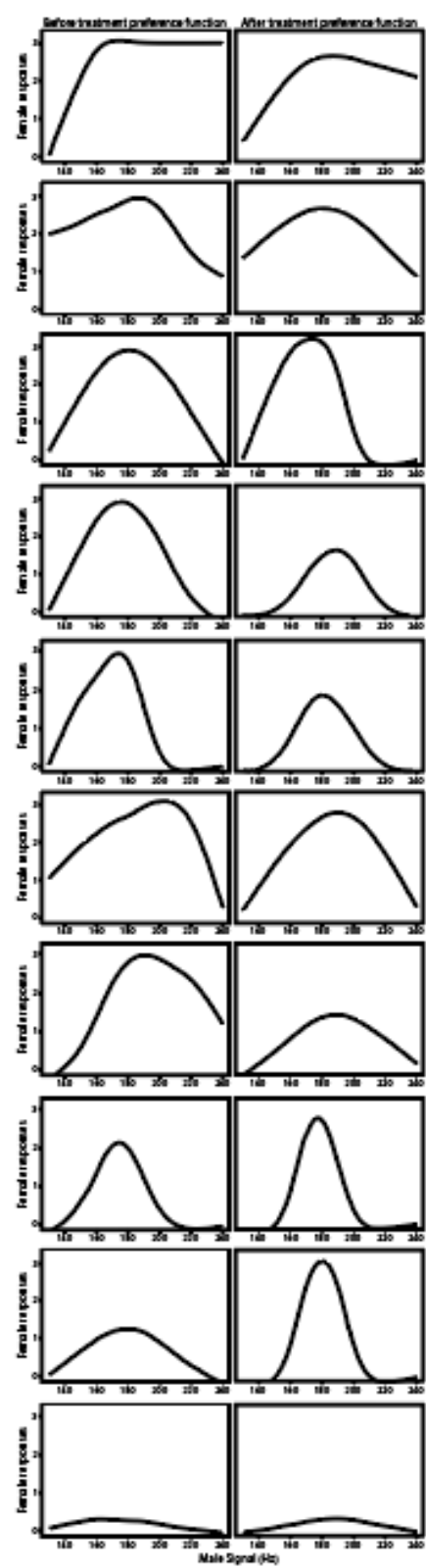


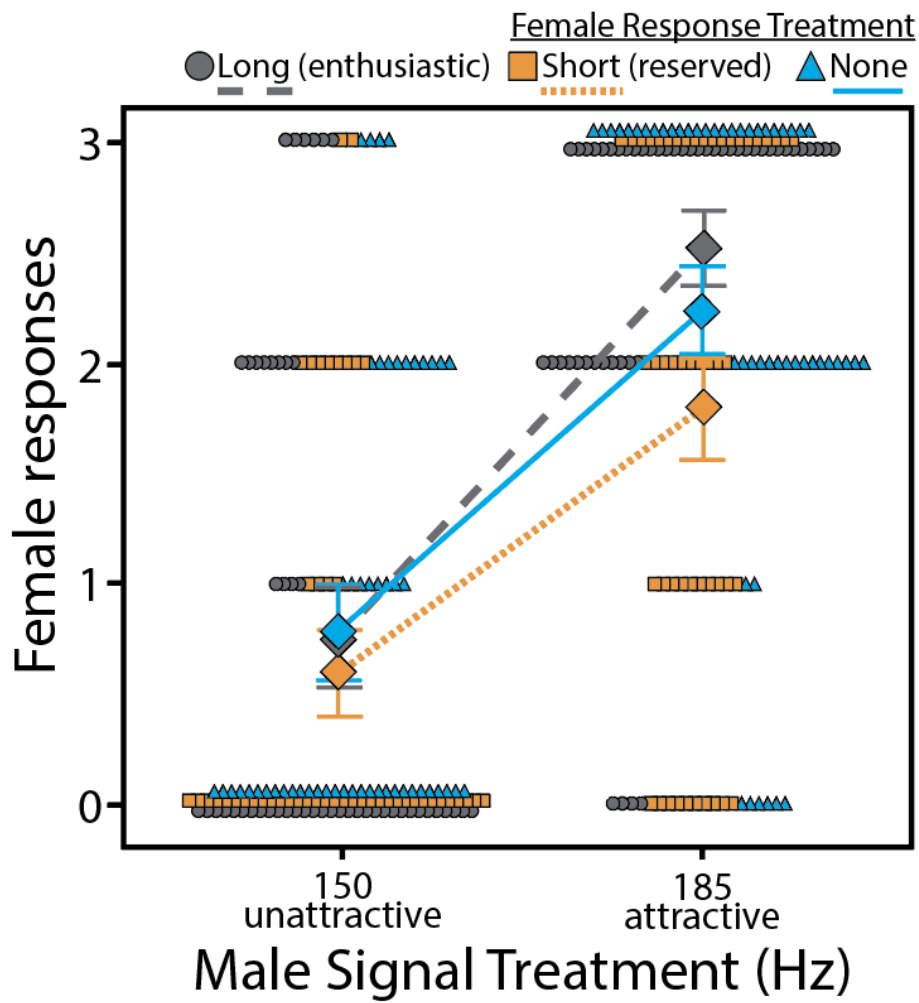
Figure 3:



602 **Figure 4:**

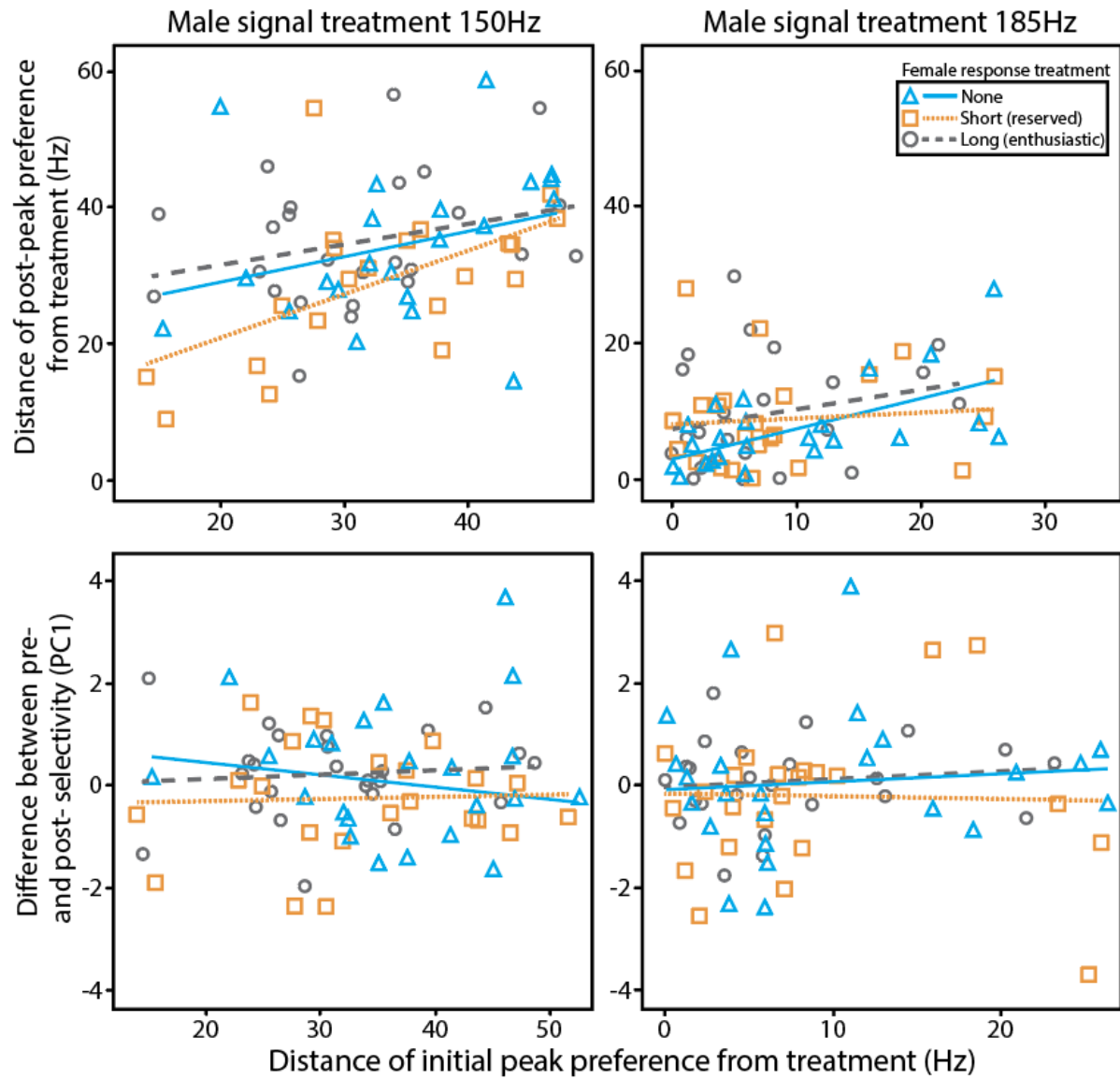


604 **Figure 5:**



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Figure 6



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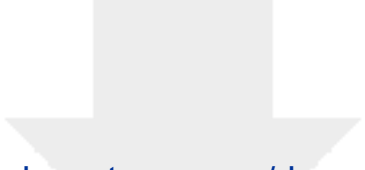
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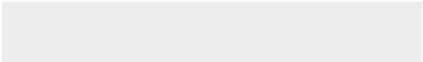




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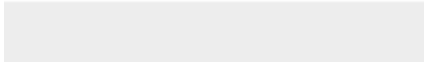




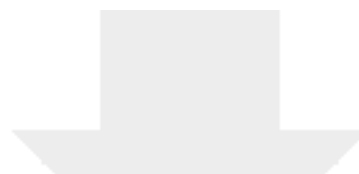
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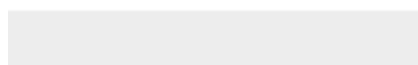
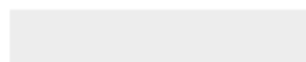






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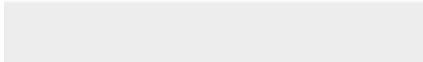
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Supplementary Interactive Plot Data (CSV)
MateCopyDat1.csv



1 *Ethical Note*

2 All our procedures adhered to the ASAB/ABS Guidelines for the use of animals in research as
3 well as the legal requirements of the U.S.A. and all UWM guidelines.

4

Author Contributions Statement:

Lauren A. Cirino: conceptualization, data curation, formal analysis, investigation, methodology, project administration, supervision, validation, visualization, writing – original draft, writing – review & editing; **Ian D. Gallagher**: data curation, investigation, writing – review & editing; **Camille Desjonquères**: data curation, funding acquisition, software, validation, writing – review & editing; **Rafael L. Rodriguez**: conceptualization, formal analysis, funding acquisition, methodology, project administration, resources, supervision, validation, writing – review & editing