Pair formation in *Enchenopa* treehoppers (Hempitera: Membracidae) involves complex male-female duetting signal exchanges, and three stages of female mate choice

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Abstract

We explore the complexity of the signal repertoire and sequences of behavioral interactions involved in pair formation in *Enchenopa binotata* treehoppers, which communicate via plant-borne vibrational signals, and whose pair formation involves prolonged male-female duetting interactions. We recorded these interactions using laser vibrometry and video assays. In males, we report two phases of signaling: a searching phase in which males use a basic repertoire to solicit engagement from females; and a more complex phase incorporating additional signal types and elements males used once engaged by females. In females, we report a novel three-stage process of selective cooperation with males, as well as a novel signal type that was necessary but not sufficient for copulation to occur. These three stages include active duetting with a male that was necessary for him to locate and mount her; the novel signal that females produce after continued mounted duetting that prompts the male to attempt genital coupling; and the female actively allowing coupling. We discuss implications of our observations for these insects' cognitive abilities in terms of the memory and selective attention~~, and mental model construction~~ required to sustain signaling interactions and proceed along the decision-making stages of mate choice. Using attention to detail as an aid to discovery, we aim to promote ~~neurobiological~~ research on how they express such capabilities.

Keywords:

~~mental processing,~~ vibrational signaling, *Enchenopa* treehoppers, Hemiptera, Membracidae

Introduction

In the study of animal behavior, there is a tradition that favors explanations that posit minimal cognitive abilities (Morgan 1894; Zentall 2019; Searle 1994). Whether involving hard-wired circuits or learned stimulus-response associations, this approach privileges a view of animals as simple decision-making machines. This school of thought has had considerable success in animal communication. For example, simple reflex-like circuits are indeed involved in important aspects of the behavior of some animals, such as selective phonotaxis in crickets (Hedwig 2004; Kostakaros & Hedwig 2012; Schöneich et al. 2015; Göpfert & Hennig 2016; Gray 2022).

There is evidence, however, that even animals like arthropods are capable of more complex cognitive processing. Comparative neuroanatomy suggests that arthropods navigate the world as many vertebrates do, by constructing models of their environment and their position in it (Barron & Klein 2016; Feinberg & Mallatt 2016). Although relatively small, their brains are elegantly structured and capable of tasks like forming conceptual relationships; learning from observing conspecifics; spatial planning; recognizing objects across multiple sensory modalities; and keeping track of time in decision making (Avarguès-Weber & Giurfa 2013; Alem et al. 2016; Parent et al. 2017; Gallo & Chittka 2018; Cross & Jackson, 2017, 2019; Solvi et al., 2020; Chittka 2022).

Examples of cognitive sophistication can even be found in contexts such as insect communication, where simple-circuit explanations have been triumphant. For example, some insects locate sound sources by using sequential stimulus comparison involving memory (Greenfield et al. 2002). An interesting line of evidence regarding the capabilities of arthropods comes from the size of their repertoires of signals and behaviors. For instance, pair formation in many insects involves signal exchanges (duets) between males and females, sometimes across different modalities (Henry 1994; Bailey 2003; Virant-Doberlet & Cokl 2004; Cocroft & Rodríguez 2005; Cocroft et al. 2008; Villareal & Gilbert 2013; Rodríguez & Barbosa 2014; Saha et al. 2023). Some duetting species use several signal types, beyond one male and one female signal, and over considerable spans (Hunt & Nault 1991; Hunt 1994; Cocroft 2003; Nuhardiyati & Bailey 2005; Bailey et al. 2006; Percy et al., 2006; Miranda 2006; Sullivan-Beckers 2008; Kuhelj et al., 2015; Kuhelj & Virant-Doberlet, 2017; Cossio-Rodriguez et al. 2019; Escalante et al. 2022, 2024). For example, in the treehopper *Ennya chrysura*, male advertisement signals are comprised of two 'verses', each with different signal elements (Miranda 2006). Such observations point to processes yet to be understood, which allow males and females to keep track of each other and sustain their interactions.

Here we attempt a fairly complete description of the signal repertoire and sequences of behavioral interactions involved in pair formation in a duetting insect, a member of the *Enchenopa binotata* species complex of treehoppers (Cocroft et al. 2008). Using attention to detail as an aid to discovery (Rodríguez & Soley 2024), we aim to provide behavioral evidence of the level of signal processing and interaction regulation that these duetting insects are capable of—in order to provide a foundation for ~~promote neurobiological~~ research on how they may attain them (Frégnac 2017; Krakauer et al. 2017).

Methods

Basic outline of pair formation in E. binotata

The basic form of communication that *E. binotata* use for pair formation has been described in prior work. These phloem-feeding insects live in groups on their host plants, both as juveniles and as adults, and communicate with plant-borne vibrational signals (Cocroft & Rodríguez 2005; Cocroft et al. 2008). Sexually mature males fly from plant to plant and produce bouts of several advertisement signals, each consisting of three main components: (i) a brief series of broadband clicks, (ii) a frequency modulated pure tone "whine"; and (iii), a series of pulses (Hunt 1994; Cocroft et al. 2008, 2010; Holan et al. 2010) (figure 1). If a receptive female finds a male's advertisement signals attractive, she responds with her own signal and alerts him of her presence; the male and female then duet while he walks on the plant searching for her and until copulation begins (Rodríguez et al. 2004, 2006; Rodríguez & Cocroft 2006; Cocroft et al. 2008; see below) (figure 2). This behavior of selective duetting has revealed strong mate preferences in *E. binotata* females for the features of male advertisement signals, mainly according to dominant frequency (Rodríguez et al. 2004, 2006, 2013a; Cirino et al. 2023).

Most members of the *E. binotata* complex are not yet described (Hamilton & Cocroft 2009). However, they can be identified by their host plant, nymph coloration, and their adult signal frequencies (Cocroft et al., 2008, 2010; Rodríguez et al., 2004; Rodríguez & Cocroft 2006). We worked with the species that lives on the host plant *Viburnum lentago* (Adoxaceae) in Wisconsin (USA), has grey nymphs, an average male dominant signal frequency of ca 165 Hz, and an average female peak preference for signals of ca 185Hz (Rodríguez et al. 2013a, 2018; Desjonquères et al. 2023).

We ran this study over two years. In 2022, we collected nymphs from the following sites: Minooka Park (Waukesha, WI), Waubedonia Park (Fredonia, WI), Warnimont Park (Cudahy, WI), Oak Leaf Trail (Milwaukee, WI), Kletzsch Park (Glendale, WI) and Lion's Den Park (Grafton, WI). We reared the nymphs on potted exemplars of their host plant at the University of Wisconsin- Milwaukee greenhouse, 30 nymphs per plant, and keeping nymphs from different sites separate. When the nymphs molted to adults, we sorted them by sex to prevent any instances of courtship experience or mating. We kept all adults on these plants for the remainder of the trials, only removing them to conduct assays.

In 2023, we reared the treehoppers from eggs laid by mated females that we had collected in the late summer of 2022 from the following sites: Oak Leaf Trail (Milwaukee, WI), Kletzsch Park (Glendale, WI), and Minooka Park (Waukesha, WI). We placed the females on potted host plants and allowed them to lay eggs on the plants. Once egg-laying had ceased for two weeks, we placed the plants outside to expose to cooler temperatures and to initiate dormancy to mimic the natural life cycle of the treehoppers, which overwinter as eggs (Cocroft et al. 2008). We then placed egg-bearing plants into cold storage at 3-4°C. In February of 2023, we moved the dormant plants to the greenhouse and gradually increased the temperature to trigger phloem movement and hatching. We then reared the treehoppers as above and tested them.

Experimental Treatments

To help us capture the breadth of the details of behavioral interactions, we conducted observations under experimental treatments that varied the immediate social context of communication. We observed interactions under three different social context treatments: 1 male-1 female (n = 13 pairs); 1 male-2 females (n = 13 trios); and 2 males-1 female (n = 10 trios). To start each trial, we placed the female(s) on the recording plant and allowed them to settle for two minutes. We then placed the corresponding number of males for the treatment on the recording plant with the female(s). Each trial lasted one hour, unless a mating started or one of the treehoppers jumped off the plant. We conducted the 1m-1f and 1m-2f trials in 2022 (assigning individuals randomly to one of the treatments), and we conducted the 2m-1f trials in 2023. We report the effect of these treatments on female mate choice decisions in a forthcoming paper (Little et al. in prep.).

Recording treehopper behavior

We monitored the treehoppers' behavior on a potted *V. lentago* plant each year (henceforth, the recording plant). We recorded all treehoppers on a single plant each year to minimize any differences in plant signal transmission features across vibrational recordings (Cocroft & Rodríguez 2005; McNett & Cocroft 2008).

We monitored the treehoppers' behavior with video and sound recording starting when we placed the male(s) on the recording plant. We recorded video using a webcam (model N5, XPCAM, xplore technology, Shenzhen, China) held by a chemical stand (catalog # 14-675AQ, Thermo Fisher Scientific, Waltham, MA, USA) with an extension clamp (catalog # 05-769-6Q, Thermo Fisher Scientific, Waltham, MA, USA). The webcam was plugged into a MacBook Pro laptop computer (Apple; Cupertino, California) and we saved videos through the program QuickTime Player (v. 10.4; http://apple.com/macosx/apps/all.html#quicktime).

We simultaneously recorded the treehoppers' plant-borne vibrational signals using a laser doppler vibrometer (Polytec CLV 2534; Polytec Inc., Auburn, MA, USA). We sent the output from the vibrometer through a frequency filter (40–4000 Hz; Krohn-Hite 3202; Krohn-Hite Corporation, Brockton, MA, USA) and oscilloscope (1MB mixed signal oscilloscope; HMO 1002; Rohde and Schwarz; Munich, Germany) to the MacBook Pro laptop computer through a USB audio interface (Edirol USB Audio Capture UA-25; Roland, Hamamatsu, Japan). We recorded the signals on this computer with the program AUDACITY (v. 2.1.2; http://audacity.sourceforge.net/) at a sampling rate of 44.1 Hz.

We thus recorded both audio and video simultaneously on the same computer. To ensure that audio and video could be synced together after (if need be), we clapped three times at the beginning of the recording as a sound marker. During recordings we monitored the air temperature near the position of the plant (within 40 cm) with a thermometer (catalog number 14-648-26, Fisher Scientific, Hampton, NH, USA).

We isolated the above recording set up from building vibrations by placing the recording plant on a pad of shock-absorbing sorbothane (Edmund Scientifics, Tonawanda, NY) on top of a 135kg iron plank resting on partially inflated inner tubes on a lab table. The legs of the table were on rubber pads.

Annotating behavior

We completed all annotations in AUDACITY, reviewing each trial twice. First, we labeled each signal observed in the audio recording (figure 3). Next, we turned to the video, noting any movements or observable behaviors, and adding them to the previous label. This allowed us to create a single comprehensive label containing both visual and substrate-borne behaviors.

Inclusion criteria for signals and behaviors

Describing the details of the treehoppers' pair formation interactions required deciding what to include as different signal types, signal elements, or meaningful behaviors, and what to exclude as incidental sounds or movements. We adapted the criteria used by Eberhard (1994) to consider movements performed during copulation as courtship. Specifically, we only included sounds and behaviors that were: (i) stereotyped and repeated within and across individuals (i.e., within and across trials); (ii) produced in circumstances that were consistent across individuals (e.g., sounds produced by males only when another male was courting the same female); (iii) not incidental to other movements (e.g., not caused by walking); (iv) mechanically irrelevant to staying on the female (in the case of mating attempts by males); and (v) had a distinctive temporal and spectral features and/or were produced by distinct behaviors/mechanisms. Further, we defined signals as distinct sounds that may elicit a conspecific response and/or appeared to have an established function. For instance, below we discuss male advertisement and jamming signals as different signal types. Besides having distinct temporal and spectral features, these signal types differ in that advertisement signals are aimed at females and elicit female responses when successful, whereas jamming signals do not appear to be used to elicit a female response, but instead, overlaps another male's advertisement signal or a female's response to it. We defined signal elements as distinct sounds or movements added in the context of producing a signal (e.g., added to advertisement signals) that did not seem to elicit a direct conspecific response by themselves.

Note that we used the presence of stereotyped conspecific responses to classify behaviors as signals or elements, but we did not use conspecific response alone to distinguish behaviors as distinct from one another. This is because individuals may choose not to respond or to respond in different ways (e.g., to advertisement signals). Thus, we did not entirely rely on the reaction of the receiver to classify a behavior as unique from others.

Results

The below description follows the general sequence of pair formation events we observed, starting when we placed a male on the recording plant. For most signals, there was no visible body movement associated with their production. This is because most signals are produced by subtle movements of the thorax muscles and abdomen (cf. Miles et al. 2017), and in our treehoppers the abdomen is fully covered by the wings (but see Hunt 1994 for observations with a different member of the *E. binotata* complex). We only mention body movements associated with signals in the cases in which the former were visible.

First stage of female choice: male-female signaling interactions during pair formation

As expected from prior work (see above), males spontaneously produced whine-pulse advertisement signals when placed on the recording plant stem (figure 1). Before bouts of advertisement signals, males often produced a percussive signal element that we term "fireworks" (figure 1, 4). In all trials we observed males producing advertisement signals and females responding with a duetting signal, although some females became less receptive or stopped duetting completely later in courtship (figure 2, table 1). Females sometimes signaled spontaneously (figure 5), either before a male had signaled or while a male walked between bouts of advertisement signals.

Once engaged in duetting by a female, males reduced the amplitude of their signals (figure 6). Females, by contrast, did not change the amplitude of their signals (figure 6).

Once males were duetting with a female, they also incorporated additional elements into their bouts. They started to produce either a "flutter" or a "knock" (figure 7) before each signal bout (figure 7). Males added these elements regardless of whether duetting was started by them or by females producing spontaneous signals. We also observed that males sometimes produced fireworks before a flutter or knock (see the knock featured in figure 7).

Males produced flutters by rapidly and briefly moving their wings (see supplemental for video). Males produced knocks by hitting the host plant with their head via a forceful and rapid forward tilt of the body (see supplemental for video). Knocks had greater amplitude but were overall less common than flutters: knocks were observed in some males, while flutters were observed in every male (table 1).

Another signal element that males produced while duetting with a female was "revving". Males revved by tilting forward and rapidly moving their abdomen up and down (see supplemental for video). In our species, males most commonly produced revs shortly after a bout. We also observed males incorporating other signal elements into revving behavior, with males “announcing” the rev with some other element. These elements included a single firework, knock, flutter, or a shorter rev which would then be immediately followed by revving (figure 8). The context in which most revs occurred seemed to be when the number of female responses to male advertisement signals had diminished.

Second stage of female choice: male-female signal exchanges during mounts

Male-female duetting continued while the male moved up and down the plant (often walking directly past and even over the female multiple times) until he found and mounted her. Duetting often led to mounting (see table 1 for counts). Once mounted, males never performed knocks, but continued with flutters at the beginning of each signal bout. Duetting continued until either the female produced an advancing signal (see below) or stopped responding to the male. If the female became unresponsive, she sometimes resumed walking along the plant stem, with the male still on her. In two instances this seemed to dislodge the male. Once females had ceased responding to the male and started walking, they never resumed duetting even if the male had remained on her and continued signaling.

We discovered an additional female signal type: 'advancing signals' (figure 10). Females produced this signal repeatedly for ~ 5 sec only after a male had mounted a female and produced several bouts of advertisement signals while mounted. Of the males who mounted a female during their trial, 4 of 7 males in 1m-1f trials and 11 of 12 of males in 1m-2f trials received an advancing signal (see table 1). Thus, females seemed to use this signal type selectively, as with their duetting signals. When the female finished producing the advancing signals, the male immediately attempted genital coupling. Males only attempted this if the female had produced an advancing signal.

Third stage of female choice: successful copulation, and male rejection behavior

Following the advancing signal, males attempted genital coupling. To do so, they lifted and held both wings up while attempting to make genital contact from the mounted position. Once in intromission, males lowered the wings to their normal resting position, moved backwards along the side of the female, dismounted, and turned to face slightly away from her. If the pair maintained genital coupling after these movements, the male then further turned until he was facing ca. 180 degrees away from the female. We recorded for five minutes after genital coupling and observed no further signaling or movements. (With ca. 95% of females in the *E. binotata* complex mating only once and no species distinctive divergence in male genitalia, we would not expect further courtship interactions after this point; Wood & Guttman 1982; Sullivan-Beckers 2008).

In 13 of 26 of trials with one male (1m-1f and 1m-2f) trials, males received an advancing signal from the mounted female, attempted genital intromission, and succeeded (table 1). Failures were likely due to the female not lifting her abdomen, which is required for the male to be able to achieve intromission (Cocroft et al. 2008). Thus, even after giving an advancing signal, the female still possessed the ability to reject a male by simply not lifting her abdomen. In cases of failure, males usually dismounted the female and produced a series of fireworks, usually for several minutes, sometimes also revving. After some minutes, males often started producing bouts of advertisement signals again. In some cases, the female resumed duetting with the male and the male re-mounted her. Some females produced another series of advancing signals, and some males achieved genital intromission. In multiple trials, there were two or three such rounds before successful copulation occurred.

Male-male signaling interactions

In 2m-1f trials, males seemed to take turns signaling and walking/searching for the female. One male would signal and then walk along the plant. While that male was moving, the other male would signal and then walk along the stem as well. The first male would then stop walking to signal again, and so on, resulting in a staggered duetting with the female. Females were at least sometimes responsive to both males, suggesting that they could assess multiple suitors in this format.

Sometimes, one male produced a "jamming" signal while the other produced advertisement signals (table 1). The jamming signal consisted of a short frequency modulated whine and pulses with higher frequency components than those in advertisement signals (figure 11). Males often produced these jamming signals so that they overlapped the other male's advertisement signals and/or the female's responses to that male. Males produced jamming signals not only while the other male duetted and searched for the female, but also when the other male had mounted the female and even in instances where both males mounted the same female (see below). The jamming signal itself did not elicit a response from females.

In 5/10 of 2m-1f trials, males produced what seemed to be a modified advertisement signal (table 1). This "vibrato" signal type consisted of a shortened whine and a prolonged series of pulses (figure 12). Males produced this signal type while duetting, either as they searched for the female or when they had mounted her. Females duetted with vibrato signals as with the "main" advertisement signals.

In 2/10 of 2m-1f trials, both males mounted the female (figure 8). When the first male mounted the female, the second male either jumped off the plant or quickly mounted her from the other side. In our trials, we observed duetting during the double mount, but we did not observe males voluntarily dismounting. In one trial, the female began walking, making it seemingly more difficult for the males to hold on and ultimately dislodging both of them.

Wing buzzing

There was another signal type that both males and females produced in the context of duetting. Individuals of both sexes sometimes buzzed their wings. Wing buzzes typically lasted for ca. <1-8 sec but one went on for 90 sec. Buzzes produced a high amplitude vibration that had both plant-borne (figure 13) and airborne components (we could hear the latter without the aid of the vibrometer).

We consider wing buzzes to be a type of signal for the following reasons: they produced a distinct soundwave and spectrogram; many different individuals produced them in different trials; they were not associated with any mechanical function (e.g., they did not precede the individual jumping off the plant); and in our trials they were mainly produced by males when females had ceased duetting with them and by females in the middle of male bouts of advertisement signals. While we do not know the function for the wing buzz signal, it would appear this signal is used commonly within the species (table 1).

After a male or a female produced a wing buzz, signaling often stopped for ca. 2-5 sec (and for 19 sec in the case of the 90 sec-buzz). There were a few instances in which females produced wing buzzes when a male was walking and not signaling. In some of these instances, males began producing bouts of advertisement signals within a few seconds after the buzz.

Discussion

Here we attempt a comprehensive description of the signal repertoire and behavioral interactions involved in pair formation for one species in the *E. binotata* complex of treehoppers. We find surprising levels of complexity in the signal repertoires and interactions leading to mating, including novel signal elements and signal types for males and females. We also find that pair formation in these insects involves a remarkable three-stage process of active female mate choice decisions involving not only duetting signals but also a novel ' advancing ' signal type.

Repertoire

In males we found a dynamic and diverse repertoire which incorporated nine different signal types or elements, deployed differently in courting females or countering other males. Males switched between knocks and flutters to initiate their bouts of advertisement signals, and used revs seemingly according to the immediate receptiveness of females. One remarkable adjustment males made was to *lower* the amplitude of their advertisement signals once they had been engaged in duetting by a female, whereas females did not change the amplitude of their signals along duetting interactions. This differs from typical male "call fly" behavior prior to engagement by a female, whereby males arrive at a plant and produce bouts of advertisement signals that increase gradually in amplitude along the bout (Cocroft et al. 2008, 2010). This amplitude reduction has also been observed in the member of the *E. binotata* complex that lives on *Celastrus scandens* (Celastraceae) host plants (RB Cocroft & RL Rodríguez, unpubl.). These contrasting amplitude profiles along duets may achieve different functions for males and females. We speculate for future work that males may seek to avoid eavesdropping by other males, whilst females may seek to recruit other nearby suitors.

In trials with 2 males and 1 female, males made several changes in their behavior, from modifying their own advertisement signals to jamming the signals of competitors, and from giving up a mating attempt to disrupting mounting by another male. Jamming signals have also been noted for another species in the *E. binotata* complex, but without pulses as in our species (Sullivan-Beckers, 2008). We do not have experimental evidence that *E. binotata* jamming signals actually interfere with the other male's duetting—a matter which needs further investigation. However, this function has been demonstrated for a similar signal in *Tylopelta gibbera* treehoppers (Legendre et al. 2012). Unlike with other members of the *E. binotata* complex (Cocroft et al. 2008), we did not observe male-male chorusing with only two males. This may be due to species differences in population density during the mating season, with our species being on the low end across the complex (Cocroft et al. 2008; pers. obs.).

Three stages of mate choice

We also observed a remarkable set of stages of female mate choice. First is the decision of a female of whether to engage in duetting with a signaling male, and whether to sustain duetting through the male searching for her and while he has mounted her. Through this decision, *E. binotata* females can decide whether to inform a particular male about their presence on the plant and allow them to court them. Females selectively duet with individual males to express strong mate preferences for male signal features (Rodríguez et al. 2004, 2006, 2013a; Cocroft et al. 2008). There is thus an element of selective cooperation with males at play in this decision. Females also produced spontaneuous duetting signals, which have been shown to increase the likelihood of signaling by males (Rodríguez et al., 2012) and may help establish or sustain duetting (Rodríguez et al. 2012; Seidita & Rodríguez in prep.). However, with males lowering their signal amplitude but females sustaining theirs, we speculate that there is also some tension between males seeking to secure the female for themselves and females perhaps seeking other suitors.

Second is the decision of a female of whether to produce an 'advancing signal' to prompt the male to attempt genital coupling. Remarkably, males never attempted this until the female had produced an advancing signals. A female signal that may have a similar function and is produced when the male has mounted the female has been reported in *Ennya maculicornis* treehoppers (Cossio-Rodriguez et al. 2019).

Third is the decision of whether to actually allow the male to achieve genital coupling. Our videos were zoomed out to observe the entire recording plant, so we were unable to determine the causes of these failures to couple. However, prior observations have shown that females have to actively raise the tip of their abdomen to allow the male to achieve intromission (Cocroft et al. 2008). Further work will be required to ask whether these second and third female decisions express mate preferences and whether they are related to male signal features or other aspects. We consider, however, that females likely made these decisions selectively, as duetting was observed in all trials but only some males received an advancing signal and even fewer achieved intromission (table 1).

Signal repertoires in duetting insects

The behavioral and signal repertoires we find in *Enchenopa* may not be unusual among treehoppers and other duetting insects. For instance, the signal elements that accompany advertisement signals and duetting that we report here—flutters, revving, and knocks—have also been observed in other members of the *E. binotata* complex as well as double mountings, and jamming signals (Sullivan-Beckers 2008). A behavior similar to knocks has also been described in *Ennya* treehoppers (Miranda 2006). Comparable diversity of signal repertoires occurs in other vibrational Hemiptera such as psyllids and cicadellids (e.g., Percy et al 2006; De Groot et al. 2012; Kuhelj et al. 2015; Kuhelj & Virant-Doberlet 2017). Even the signal repertoires of some non-duetting arthropods such as jumping spiders are as rich and complex as to be comparable to those of birds, suggesting convergent neural abilities (Elias et al 2012; Farris 2008).

Implications for E. binotata *cognitive abilities*

Our results provide several suggestions regarding these insects' abilities to process complex information. To sustain their signaling interactions and proceed along the decision-making stages of pair formation and mate choice that we have described, these insects may be capable of using memory over much longer intervals than moment to moment or minute to minute (cf. Greenfield et al. 2002; Parent et al. 2017). ~~Their sustained goal-directed behavior (searching, continuing to~~

~~duet, waiting for an advancing signal, providing an advancing signal) further suggests they may construct mental models of their place in the plant physical and social context, including expectations about the outcomes of their actions (cf. Mendl & Paul 2020).~~ They are capable of sustained goal-directed behavior (searching, continuing to duet, waiting for an advancing signal, providing an advancing signal) in their physical plant and social contexts.

As females only responded to male advertisement and vibrato signals, it is not clear what the function of the other signal elements may be. However, in the cognitive landscape of courting and mate choice, incorporating signal elements like revs and interchanging flutters and knocks may help sustain the attention of the female and her interaction with the male by ameliorating habituation and/or sensory adaptation (Eberhard 2024). The lowering of signal amplitude by males once engaged in duetting by females may serve this habituation-preventing function, and perhaps also activate other aspects of the females' cognition such as perception of temporal contrasts and curiosity biases (MacGillavry et al. 2023). Additionally, signal elements like knocks and flutters, which "announce" the immediate coming of a signal bout, may draw female attention prior to the advertisement signals to ensure her duetting signals are ‘in time’ to prevent overlapping of male and female signals (cf. Hebets & Papaj 2005).

In conclusion, we report a flexible and involved repertoire of signals and behaviors in an insect that unfolds along a suite of stages of active female selective cooperation with males required for mating. Regulation of these interactions may require more processing and cognitive sophistication than currently appreciated. Examples of similar or even higher behavioral repertoire richness in other insects and spiders (Miranda 2006; Elias et al. 2012; Cossio-Rodriguez et al. 2019) suggest that such capabilities may apply broadly be widespread across animals (Mendelson et al. 2016; Krakauer et al. 2017). Investigating their distribution and expression in brain of different sizes and architectures will be highly illuminative.

Acknowledgments

We thank Paul Engevold for his expertise and help in rearing our host plants at the UWM greenhouse. We thank Lauren Cirino and Sara Seidita for their help in both maintaining the plants in the greenhouse as well as in insect collection and assaying. We thank Peter O'Dunn, Linda Whittingham, Gerlinde Höbel, Emily Latch, Filipe Alberto, Madi Rittinger, Sara Seidita, Hossein Asgari, and Sage Delong for comments and feedback during the design, execution, and writing of this project.

Funding

This study was funded in part by NSF Grant IOS–1855962 to RLR and C. Desjonquères. KL was supported by a UWM Stipends for Undergraduate Research (SURF) fellowship.

References

Alem S, Perry CJ, Zhu X, Loukola OJ, Ingraham T, Søvik E & Chittka L. (2016). Associative mechanisms allow for social learning and cultural transmission of string pulling in an insect. PLoS Biol 14: e1002564.

Avarguès-Weber A & Giurfa M. (2013). Conceptual learning by miniature brains. Proc R Soc B 280: 20131907.

Bailey WJ. (2003). Insect duets: underlying mechanisms and their evolution. Physiological Entomology 28, 157-174.

Bailey WJ, MacLeay C & Gordon T. (2006). Acoustic mimicry and disruptive alternative calling tactics in an Australian bushcricket (Caedicia; Phaneropterinae; Tettigoniidae; Orthoptera): does mating influence male calling tactic? Physiological Entomology 31, 201-210.

Barron, A. B., & Klein, C. (2016). What insects can tell us about the origins of consciousness. *Proceedings of the National Academy of Sciences*, *113*(18), 4900-4908.

Cirino L, Gallagher I, Desjonquères C & Rodríguez RL. (2023). Robust mate preferences despite means and opportunity for mate choice copying in an insect. Animal Behaviour 200, 137-146.

Cocroft RB. (2003). The social environment of an aggregating, ant-tended treehopper (Hemiptera: Membracidae: *Vanduzea arquata*). Journal of Insect Behavior 16, 79-95.

Cocroft RB & Rodríguez RL. (2005). The behavioral ecology of insect vibrational communication. BioScience 55, 323–334.

Cocroft RB, Rodríguez RL & Hunt RE. (2008). Host shifts, the evolution of communication and speciation in the *Enchenopa binotata* complex of treehoppers. In: Specialization, speciation, and radiation: the evolutionary biology of herbivorous insects. Univ. of California Press (K Tilmon, ed), p 88-100.

Chittka L. (2022). The mind of a bee. Princeton University Press.

Cossio-Rodriguez R, Cocroft RB, Niemeyer HM & Pinto CF. (2019). Mate searching in *Ennya maculicornis* (Membracidae: Polyglyptini) initiated by females: behavioural and acoustic descriptions. Ecological Entomology 44, 406-412.

Cross, F. & Jackson, R. (2017). Representation of different exact numbers of prey by a spider-eating predator. Interface Focus 7: 20160035.

Cross, F. R., & Jackson, R. R. (2019). Portia's capacity to decide whether a detour is necessary. *Journal of Experimental Biology*, *222*(15), jeb203463.

De Groot, M., Derlink, M., Pavlovčič, P., Prešern, J., Čokl, A., & Virant-Doberlet, M. (2012). Duetting behaviour in the leafhopper Aphrodes makarovi (Hemiptera: Cicadellidae). *Journal of Insect Behavior*, *25*, 419-440.

Desjonquères C, Holt RB, Speck B & Rodríguez RL. (2020). The relationship between a combinatorial rule and a continuous preference function in an insect. Proceedings of the Royal Society B 287, 20201278

Desjonquères C, Maliszewski J, Lewandowski EN, Speck B & Rodríguez RL. (2019a). Social ontogeny in the communication system of an insect. Animal Behaviour 148, 93-103.

Desjonquères C, Maliszewski J & Rodríguez RL. (2021). Juvenile social experience and practice have a switch-like influence on adult mate preferences in an insect. Evolution 75, 1106-1116.

Desjonquères C & Rodríguez RL. (2023). The direction and strength of social plasticity in mating signals and mate preferences vary with the life stage of induction. Animal Behaviour 200, 255-261.

Desjonquères C, Speck B & Rodríguez RL. (2019b). Signalling interactions during ontogeny are a cause of social plasticity in *Enchenopa* treehoppers (Hemiptera: Membracidae). Behavioural Processes 266, 103887.

Desjonquères C, Speck B, Seidita S, Cirino L, Escalante I, Sergi C, Maliszewski J, Wiese C, Höbel G, Bailey NW & Rodríguez RL. (2023). Social plasticity enhances signal-preference co-divergence. American Naturalist 202, 818-829.

Eberhard WG. (1994). Evidence for widespread courtship during copulation in 131 species of insects and spiders, and implications for cryptic female choice. Evolution 48, 711-733.

Eberhard, W. G. (2024). A Bridge between Animal Psychology and Sexual Selection: Possible Effects of Habituation and Neural Adaptation on Mate Choice Signals. *The Quarterly Review of*

Biology, *99*(1), 23-49.

Elias DO, Maddison WP, Peckmezian C, Girard MB & Mason AC. (2012). Orchestrating the score: complex multimodal courtship in the *Habronattus coecatus* group of *Habronattus* jumping spiders (Araneae: Salticidae). Biological Journal of the Linnean Society 105, 522-547.

Escalante I, Kellner JR, Rodríguez RL & Desjonquères C. (2022). A female mimic signal type in the vibrational repertoire of male *Enchenopa* treehoppers. Behaviour 159, 1319-1340.

Escalante I, Kellner JR, Desjonquères C & Noffsinger GM, Cirino LA, Rodríguez AN, DeLong SA & Rodríguez RL. (2024). On the function of a female-like signal type in the vibrational repertoire of *Enchenopa* male treehoppers (Hemiptera: Membracidae). Journal of Evolutionary Biology 37, 110-122.

Farris, S. M. (2008). Evolutionary convergence of higher brain centers spanning the protostome-deuterostome boundary. *Brain Behavior and Evolution*, *72*(2), 106-122.

Feinberg, T. E., & Mallatt, J. (2016). *The ancient origins of consciousness: How the brain created experience*. MIT Press.

Fowler–Finn KD & Rodríguez RL. (2012a). Experience–mediated plasticity in mate preferences: mating assurance in a variable environment. Evolution 66, 459-468.

Fowler–Finn KD & Rodríguez RL. (2012b). The evolution of experience–mediated plasticity in mate preferences. Journal of Evolutionary Biology 25, 1855-1863.

Frégnac, Y. (2017). Big data and the industrialization of neuroscience: A safe roadmap for understanding the brain?. *Science*, *358*(6362), 470-477.

Gallo V & Chittka L. (2018). Cognitive Aspects of Comb-Building in the Honeybee? Front Psychol 9: 900.

Göpfert, M. C., & Hennig, R. M. (2016). Hearing in insects. *Annual Review of Entomology*, *61*(1), 257-276.

Gerhardt HC & Huber F. (2002). Acoustic Communication in Insects and Anurans: Common Problems and Diverse Solutions. University of Chicago Press, Chicago.

Gray, D. A. (2022). Sexual selection and 'species recognition'revisited: serial processing and order-of-operations in mate choice. *Proceedings of the Royal Society B*, *289*(1971), 20212687.

Greenfield MD. (1994). Synchronous and alternating choruses in insects and anurans: common mechanisms and diverse functions. Am. Zool. 34, 605-615.

Greenfield MD. (2002). Signalers and Receivers: Mechanisms and Evolution of Arthropod Communication. Oxford University Press, Oxford, UK.

Greenfield MD & Merker B. (2023). Coordinated rhythms in animal species, including humans: entrainment from bushcricket chorushing to the philharmonic orchestra. Neuroscience and Biobehavioral Reviews 153, 105382.

Greenfield MD, Tourtellot MK & Snedden WA. (1997). Precedence effects and the evolution of chorusing. Proc. R. Soc. Lond. B 264, 1355-1361.

Greenfield MD, Tourtellot MK, Tillberg C, Bell WJ & Prins N. (2002). Acoustic orientation via sequential comparison in an ultrasonic moth. Naturwissenchaften 89, 376-380.

Hamilton, K. G. A., & Cocroft, R. B. (2009). Establishing the identity of existing names in the North American Enchenopa binotata species complex of treehoppers (Hemiptera: Membracidae). *Entomological News*, *120*(5), 554-565.

Hebets, E. A., & Papaj, D. R. (2005). Complex signal function: developing a framework of testable hypotheses. Behavioral Ecology and Sociobiology, 57, 197-214.

Hedwig, B., & Poulet, J. F. (2004). Complex auditory behaviour emerges from simple reactive steering. Nature, 430(7001), 781-785.

Henry CS. (1994). Singing and cyptic speciation in insects. Trends in Ecology & Evolution 9, 388-392.

Holan SH, Wikle CK, Sullivan-Beckers LE & Cocroft RB. (2010). Modeling complex phenotypes: generalized linear models using spectrogram predictors of animal communication signals. Biometrics 66, 914-924.

Hunt RE. (1994). Vibrational signals associated with mating behavior in the treehopper *Enchenopa binotata* Say (Hemiptera: Membracidae). Journal of the New York Entomological Society 102, 266-270.

Hunt RE & Nault LR. (1991). Roles of interplant movement, acoustic communication, and phototaxis in mate-location behavior of the leafhopper *Graminella nigrifrons*. Behavioral Ecology and Sociobiology 28, 315-320.

Kostarakos K & Hedwig B. (2012). Calling song recognition in female crickets: temporal tuning of identified brain neurons matches behavior. Journal of Neuroscience 32, 9601-9612.

Krakauer, J. W., Ghazanfar, A. A., Gomez-Marin, A., MacIver, M. A., & Poeppel, D. (2017). Neuroscience needs behavior: correcting a reductionist bias. Neuron, 93(3), 480-490.

Kuhelj, A., de Groot, M., Pajk, F., Simčič, T. & Virant-Doberlet, M. (2015). Energentic cost of vibrational signalling in a leafhopper. —Behav. Ecol. Sociobiol. 69: 815-828.

Kuhelj, A. & Virant-Doberlet, M. (2017). Male-male interactions and male mating success in the leafhopper *Aphrodes makarovi*. —Ethology 123: 425-433.

Legendre F, Marting PR & Cocroft RB. (2012). Competitive masking of vibrational signals during mate searching in a treehopper. Animal Behaviour 83, 361-368.

MacGillavry T, Spezie G & Fusani L. (2023). When less is more: coy display behaviours and the temporal dynamics of animal courtship. Proceedings of the Royal Society B 290, 20231684.

McNett, G. D., & Cocroft, R. B. (2008). Host shifts favor vibrational signal divergence in Enchenopa binotata treehoppers. *Behavioral Ecology*, *19*(3), 650-656.

Mendelson, T. C., Fitzpatrick, C. L., Hauber, M. E., Pence, C. H., Rodríguez, R. L., Safran, R. J., ... & Stevens, J. R. (2016). Cognitive phenotypes and the evolution of animal decisions. *Trends in Ecology & Evolution*, *31*(11), 850-859.

Mendl M & Paul ES. (2020). Animal affect and decision-making. Neuroscience and Biobehavioral Reviews 112, 144-163.

Miles, C. I., Allison, B. E., Losinger, M. J., Su, Q. T., & Miles, R. N. (2017). Motor and mechanical bases of the courtship call of the male treehopper Umbonia crassicornis. *Journal of Experimental Biology*, *220*(10), 1915-1924.

Miranda X. (2006). Substrate-borned signal repertoire and courtship jamming by adults of *Ennya chrysura* (Hemiptera: Membracidae). Annals of the Entomological Society of America 99, 374-386.

Morgan CL. (1894). An introduction to comparative psychology. Walter Scott, London.

Nuhardiyati M & Bailey WJ. (2005). Calling and duetting behavior in the leafhopper *Balclutha* *incisa* (Hemiptera: Cicadellidae: Deltocephalinae): opportunity for female choice? Journal of Insect Behavior 18, 259-280.

Parent J-P, Takasu K, Brodeur J & Boivin G. (2017). Time perception-based decision making in a parasitoid wasp. Behavioral Ecology 28, 640-644.

Percy, D.M., Taylor, G.S. & Kennedy, M. (2006). Psyllid communication: acoustic diversity, mate recognition and phylogenetic signal. —Invertebr. Syst. 20: 431-445.

Pratt G & Wood TK. (1993). Genitalic analysis of males and females in the *Enchenopa binotata* (Say) complex (Membracidae: Homoptera). Proceedings of the Entomological Society of Washington 95, 574-582.

Rodríguez RL & Barbosa F. (2014). Mutual behavioural adjusment in vibrational duetting. In: Studying vibrational communication. Animal signals and communication 3. Springer (RB Cocroft, M Gogala, PSM Hill, A Wessel, eds), p 147-169.

Rodríguez RL & Soley F. 2024. Special Issue in honour of William G. Eberhard and Mary Jane West-Eberhard: The importance of detailed observations of behaviour and natural history for generating novel questions and answering them. Animal Behaviour (in press).

Rodríguez RL, Haen C, Cocroft RB & Fowler–Finn KD. (2012). Males adjust signaling effort based on female mate–preference cues. Behavioral Ecology 23, 1218-1225.

Rodríguez RL, Hallet, AC, Kilmer JT & Fowler–Finn KD. (2013a). Curves as traits: genetic and environmental variation in mate preference functions. Journal of Evolutionary Biology 26, 434-442.

Rodríguez RL, Rebar D & Fowler–Finn KD. (2013b). The evolution of social plasticity in mate preferences. Animal Behaviour 85, 1041–1047

Saha, K., Joshi, K., & Balakrishnan, R. (2023). Multimodal duetting in katydids under bat predation risk: a winning strategy for both sexes. *Biology letters*, *19*(5), 20230110.

Schöneich S Kostarakos K & Hedwig B. (2015). An auditory feature detection circuit for sound pattern recognition. Science Advances 1:e1500325.

Searle, J. R. (1994). Animal minds. Midwest studies in philosophy, 19(1), 206-219.

Solvi, C., Gutierrez Al-Khudhairy, S., & Chittka, L. (2020). Bumble bees display cross-modal object recognition between visual and tactile senses. *Science*, *367*(6480), 910-912.

Speck B, Seidita S, Belo S, Johnson S, Conley C, Desjonquères C & Rodríguez RL. (2020). Combinatorial signal processing in an insect. American Naturalist 196, 406-413.

Sullivan-Beckers, L. (2008). The ecology of mate choice: identifying the agents of sexual selection on mating signals in Enchenopa treehoppers. Unpublished PhD thesis, University of Missouri–Columbia.

Villareal SM & Gilbert C. (2013). Female acoustic reply to variation in the male call in a duetting katydid, *Scudderia pistillata*. Behaviour 150, 525-546.

Virant-Doberlet M & Cokl A. (2004). Vibrational communication in insects. Neotropical Entomology 33, 121-134.

Wood TK & Guttman SI. (1982). Ecological and Behavioral Basis for Reproductive Isolation in the Sympatric *Enchenopa binotata* Complex (Homoptera: Membracidae). Evolution 36, 233-242.

Zentall TR. (2018). Morgan's Canon: is it still a useful rule of thumb? Ethology 124, 449-457.

Table 1. A synthesis of E. binotata *signals, signal elements, and behaviors described in this paper and their prevalence across treatments (reported as # trials in which we observed the behavior / trial* N*, and percentage)*

Behavior	**Description**	**1 M – 1 F**	**1 M – 2 F**	**2 M – 1 F**
Advancing signal	A series of low frequency whines produced by the female after a male had mounted and continued to duet. This signal was always immediately	4/13 (31%)	11/13 (85%)	2/10 (20%)

	followed by a mating attempt by the male.			
Advertisement signal	The primary signal of males composed of brief fireworks, a pure tone whine which decreases slightly in frequency, and a series of pulses. These signals elicit the duetting signals in females.	13/13 (100%)	13/13 (100%)	10/10 (100%)
Duetting signal	Performed both spontaneously and in response to male advertisement signals. This signal is comprised of a single low frequency tone and elicits males to adjust from call-fly behavior to true duetting, locate and mount females, and communicate receptiveness.	13/13 (100%)	13/13 (100%)	10/10 (100%)
flutter	Produced by males at the beginning of an established bout with a female via a brief and rapid movement of the wings	13/13 (100%)	13/13 (100%)	10/10 (100%)
fireworks	Brief percussive cues often produced in a series, these signals can either crescendo (as has been observed leading up to the first male bout on the plant) or at a semi-regular tempo. These elements were produced right before advertisement signals, between bouts, and following a failed mating attempt with a female.	13/13 (100%)	13/13 (100%)	10/10 (100%)
Jamming	A high frequency whine produced by males when a competitor male was present. These signals often overlapped either the competitor advertisement signal or the female response to said signal. These signals were observed to be produced spontaneously in the presence of another male as well.	0/13 (0%)	0/13 (0%)	3/10 (30%)
knock	Produced by males at the beginning of an established bout with a female via the rapid forward tilting and thereby slamming of the body into the plant stem.	8/13 (62%)	3/13 (23%)	4/10 (40%)
revving	Male signal element produced by rapidly “see-sawing” (moving their abdomen and head up and down) while simultaneously producing vibrational signals. Typically produced when female receptiveness has decreased.	8/13 (62%)	8/13 (62%)	3/10 (30%)
Vibrato signal	A type of advertisement signal. Rather than a separate whine and pulse, both are combined into one component. This signal was only observed when a competitor male was present.	0/13 (0%)	0/13 (0%)	5/10 (50%)
mounting	Male mounted a female from behind and continued to duet by sending vibrations directly into the female	7/13 (54%)	12/13 (92%)	5/10 (50%)

Wing buzz	A prolonged and rapid movement of the wings. These signals were produced by males and females and often interrupted signaling amongst all individuals on the plant.	6/13 (46%)	12/13 (92%)	7/10 (70%)

Figure 1. Example of *E. binotata* male advertisement signals. A. Waveform of the bout of advertisement signals produced by a male. Arrows represent the individual advertisement signals that comprise the bout. B. Spectrogram of an advertisement signal A. The broadband clicks, whine, and pulses are labeled for clarity

Figure 2. Example of *E. binotata* male-female duet signals with fireworks in between Top: waveform; bottom: spectrogram.

Figure 3. Example of an annotated recording of a 1 male-1 female trial with *E. binotata* treehoppers. We labeled recordings with the program AUDACITY. **A.** A 3.5 min clip of a male courting a female (actual courtship lasted over an hour). **B.** A seven second portion of the clip showing both the waveform and spectrogram which were applied to identify signals in AUDACITY. Each label corresponds to a signal type (flutter: refers to the flutter signal; knock: refers to the knock signal; fem: refers to a female response; fp: refers to fireworks; see text or table 1 for signal type explanations).

Figure 4. Example of fireworks produced by an *E. binotata* male. Top: waveform; bottom: spectrogram

Figure 5. Example of spontaneous female duetting signals produced by an *E. binotata* female. Top: waveform; bottom: spectrogram. In this example, the female produced three spontaneous signals in a row.

Figure 6. Example of adjustment of male advertisement signal amplitude according to engagement by a female in *E. binotata*. Both panels show signals produced by the same male and female in the same pair-formation interaction. **A.** Initial "call-fly" bout produced by the male following and interspersed with fireworks. The female responded to each of the signals in the bout. **B.** Duetting 10 min later. Note the much lower amplitude of the male's signals. Again, the duetting female responded to each of the advertisement signals in the bout. Arrows on the spectrogram indicate the male advertisement signals.

Figure 7. An example of an *E. binotata* male using both flutters and knocks in their bouts. The first bout is intiated with a flutter while the second bout is intitiated by a knock. Top: waveform; bottom: spectrogram. Arrows label signals of note in the duet.

Figure 8. Examples of different sequences of revving behaviors; the left depicts a firework followed by a single rev, and the right depicts a knock followed by back to back revs. Top: waveform; bottom: spectrogram

Figure 9. Image of two males mounting a female in *E. binotata*. The two males and the female have been labeled with white symbols for clarity. Photo credit: Dr Lauren A. Cirino.

Figure 10. A section of a female advancing signal in *E. binotata* (see example of complete signal in supplemental). Top: waveform; bottom: spectrogram

Figure 11. Example of *E. binotata* jamming signals from a 2 male-1 female trial. One male produced two advertisement signals (bottom traces on the spectrogram). The other male produced two jamming signals that overlapped the whine component of the first male's advertisement signals (top traces on the spectrogram). See Figure 1 for comparison with an advertisement signal. Top: waveform; bottom: spectrogram

Figure 12. Example of an *E. binotata* vibrato signal from a 2 male-1 female trial. See Figure 1 for comparison with a typical advertisment signal. Top: waveform; bottom: spectrogram

Figure 13. Example of an *E. binotata* female wing buzz. Top: waveform; bottom: spectrogram

Figure 14. Sketch of the sequence of behaviors observed in pair formation and mating in *E. binotata*. The asterisk (*) denotes where duetting behavior begins.