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Pair formation in *Enchenopa* treehoppers (Hemiptera: Membracidae) involves complex male–female duetting, and three stages of female mate choice

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Abstract

We explore the complexity of the signal repertoire and sequences of behavioural 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 signalling: 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 females; 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 required to sustain signalling interactions and proceed along the decision-making stages of mate choice. Using attention to detail as an aid to discovery, we aim to promote research on how such animals express such capabilities.

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Keywords
Enchenopa treehoppers, Hemiptera, Membracidae, vibrational signalling.

1. Introduction

In the study of animal behaviour, there is a tradition that favours explanations that posit minimal cognitive abilities (Morgan, 1894; Searle, 1994; Zentall, 2019). 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 behaviour 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 behaviours. 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 behavioural 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, in press), we aim to provide behavioural evidence of the level of signal processing and interaction regulation that these duetting insects are capable of, in order to provide a foundation for research on how they may attain them (Frégnac, 2017; Krakauer et al., 2017).

2. Methods

2.1. *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 behaviour 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).

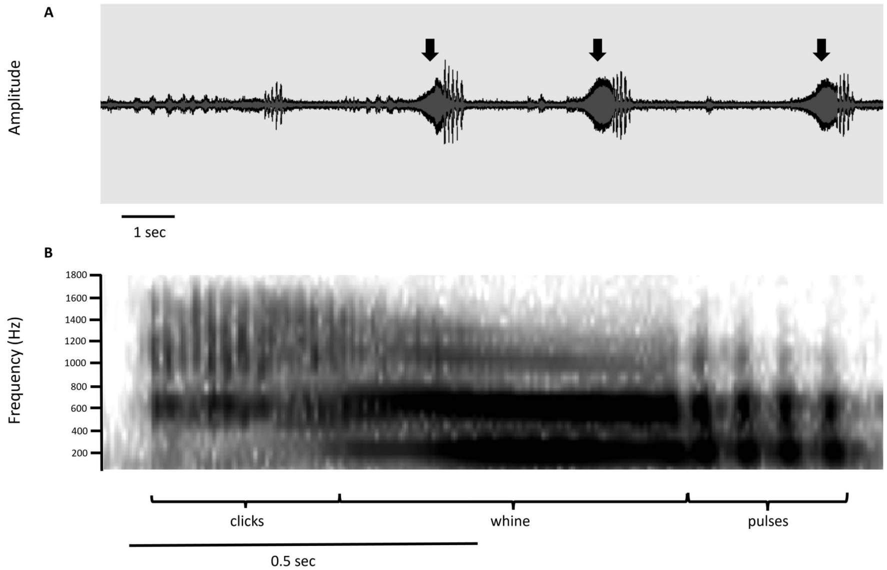

Figure 1. Example of *E. binotata* male advertisement signals. (A) Waveform of the bout of advertisement signals produced by a male. Arrows point to the individual advertisement signals that comprise the bout. (B) Spectrogram of an advertisement signal from A. The broadband clicks, whine, and pulses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

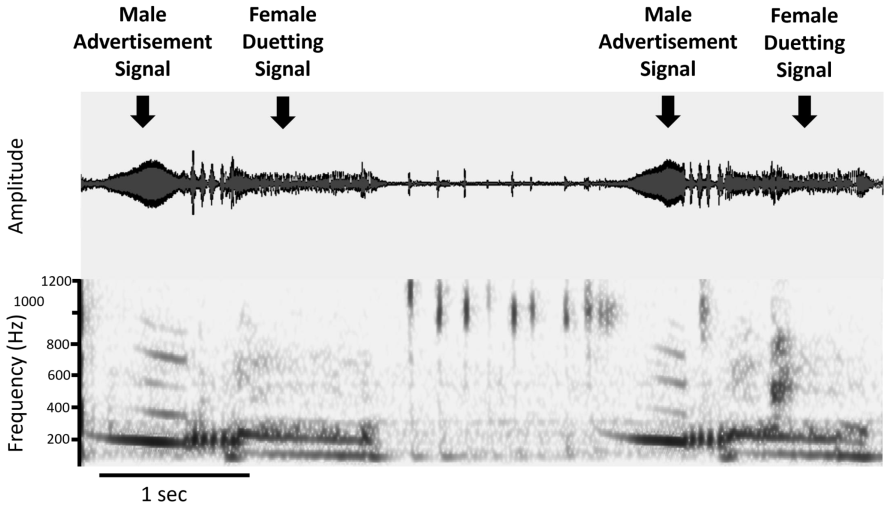

Figure 2. Example of *E. binotata* male-female duet signals with fireworks in between. (Top) Waveform; (bottom) spectrogram.

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 (Rodríguez et al., 2004; Rodríguez & Cocroft, 2006; Cocroft et al., 2008, 2010). 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. 185 Hz (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, USA), Waubedonia Park (Fredonia, WI, USA), Warnimont Park (Cudahy, WI, USA), Oak Leaf Trail (Milwaukee, WI, USA), Kletzsch Park (Glendale, WI, USA) and Lion's Den Park (Grafton, WI, USA). 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 moulted 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 Oak Leaf Trail, Kletzsch Park and Minooka Park. 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 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.

2.2. *Experimental treatments*

To help us capture the breadth of the details of behavioural 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.).

2.3. Recording treehopper behaviour

We monitored the treehoppers' behaviour on a potted *V. lentago* plant (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' behaviour 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 (catalogue No. 14-675AQ, Thermo Fisher Scientific, Waltham, MA, USA) with an extension clamp (catalogue No. 05-769-6Q, Thermo Fisher Scientific). The webcam was plugged into a MacBook Pro laptop computer (Apple, Cupertino, CA, USA) and we saved videos through the program QuickTime Player (v. 10.4; http://apple.com/macosx/apps/all.htmlNo.quicktime).

We simultaneously recorded the treehoppers' plant-borne vibrational signals using a laser doppler vibrometer (Polytec CLV 2534; Polytec, Auburn, MA, USA). We sent the output from the vibrometer through a frequency filter (40–4000 Hz; Krohn-Hite 3202; Krohn-Hite, Brockton, MA, USA) and oscilloscope (1 MB 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 (catalogue No. 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, USA) on top of a 135 kg iron plank resting on partially inflated inner tubes on a lab table. The legs of the table were on rubber pads.

2.4. Annotating behaviour

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

2.5. Inclusion criteria for signals and behaviours

Describing the details of the treehoppers' pair formation interactions required deciding what to include as different signal types, signal elements, or meaningful behaviours, 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 behaviours 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 behaviours/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, overlap another male's advertisement signal or a female's response

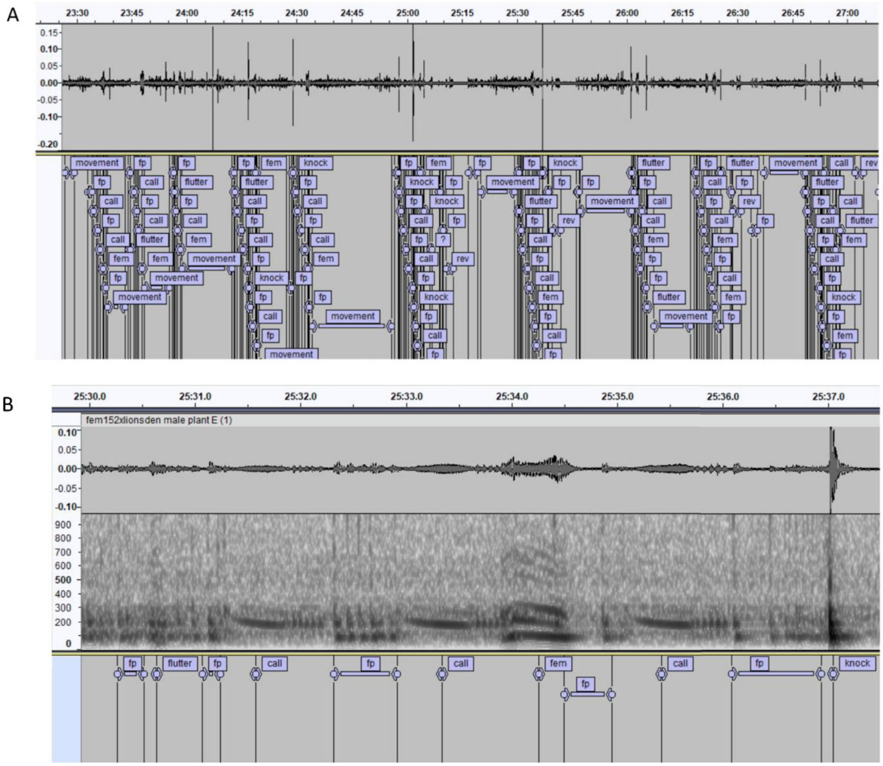

Figure 3. Example of an annotated recording of a 1 male–1 female trial with *E. binotata* treehoppers. We labelled recordings with the program AUDACITY. (A) A 3.5 min clip of a male courting a female (actual courtship lasted over an hour). (B) A 7-s portion of the clip showing both the waveform and spectrogram 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).

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 behaviours as signals or elements, but we did not use conspecific response alone to distinguish behaviours 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 behaviour as unique from others.

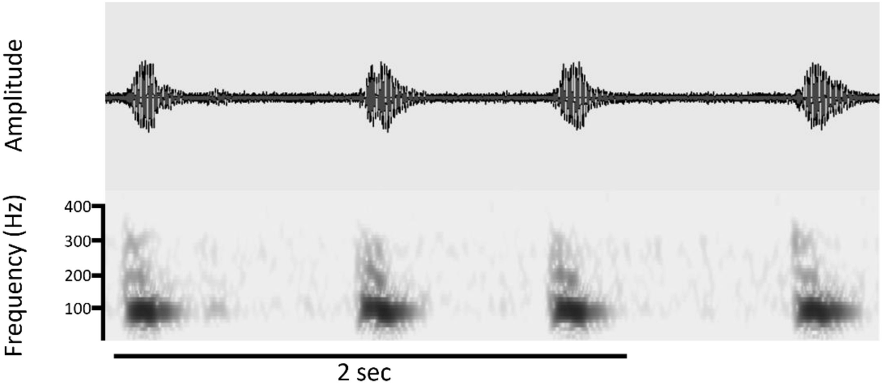

Figure 4. Example of fireworks produced by an *E. binotata* male. (Top) Waveform; (bottom) spectrogram.

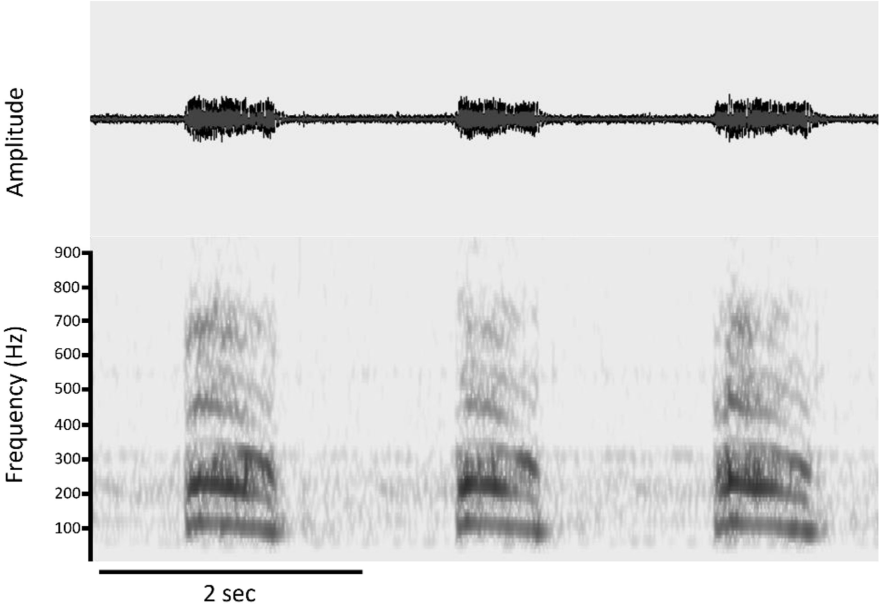

Figure 5. Example of spontaneous female duetting signals produced by an *E. binotata* female. (Top) waveform; (bottom) spectrogram. In this example, the female produced three spontaneous signals in a row.

3. Results

The description below 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.

3.1. First stage of female choice: male–female signalling 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" (Figures 1 and 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 and Table 1). Females sometimes signalled spontaneously (Figure 5), either before a male had signalled 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 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 Video 1 at 10.6084/m9.figshare.27225822). Males produced knocks by hitting the host plant with their head via a forceful and rapid forward tilt of the body (see Video 2 at 10.6084/m9.figshare.27225822). Knocks had greater

Table 1.
A synthesis of *E. binotata* signals, signal elements, and behaviours described in this paper and their prevalence across treatments.

Behaviour	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 followed by a mating attempt by the male.	4/13 (31%)	11/13 (85%)	2/10 (20%)
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 behaviour 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%)

Table 1.
(Continued.)

Behaviour	Description	1 M–1 F	1 M–2 F	2 M–1 F
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 signalling amongst all individuals on the plant.	6/13 (46%)	12/13 (92%)	7/10 (70%)

Results are reported as No. trials in which we observed the behaviour/numbers of trials and percentage.

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 Video 3 at 10.6084/m9.figshare.27225822). In our species, males most commonly produced revs shortly after a bout. We also observed males incorporating other signal elements into revving behaviour, 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.

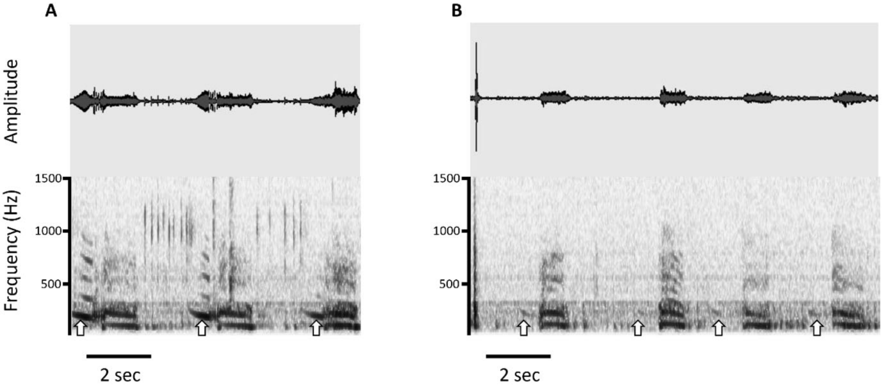

Figure 6. Example of adjustment of male advertisement signal amplitude according to engagement by a female in *E. binotata*. Both panels show signals produced by the same male and female in the same pair-formation interaction. (A) Initial "call-fly" bout produced by the male following and interspersed 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.

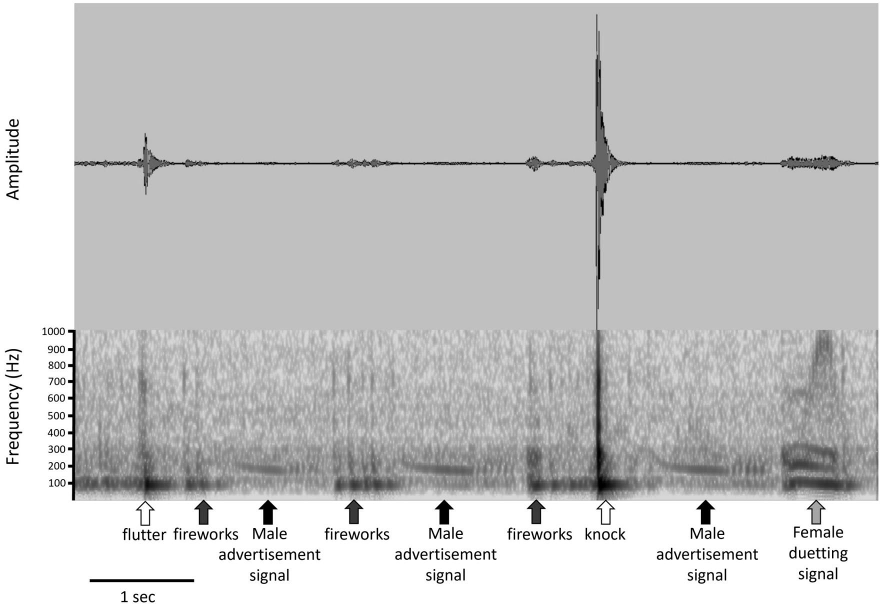

Figure 7. An example of an *E. binotata* male using both flutters and knocks in their bouts. The first bout is intiated with a flutter while the second bout is intitiated by a knock. (Top) Waveform; (bottom) spectrogram. Arrows label signals of note in the duet.

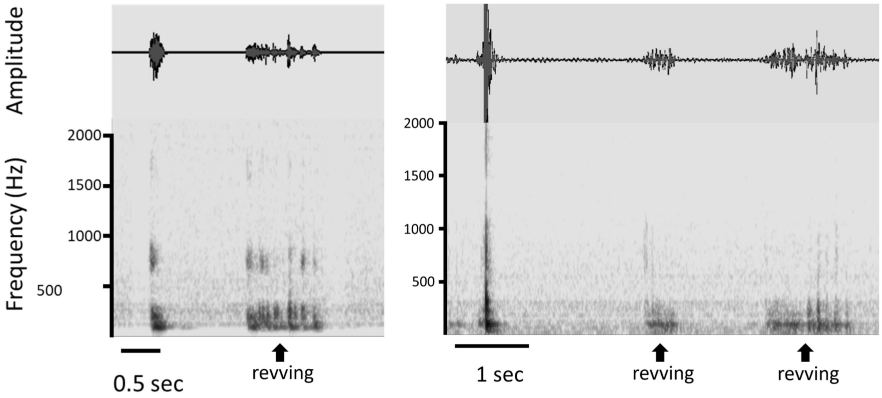

Figure 8. Examples of different sequences of revving behaviours; the left depicts a firework followed by a single rev, and the right depicts a knock followed by back to back revs. (Top) Waveform; (bottom) spectrogram.

3.2. *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

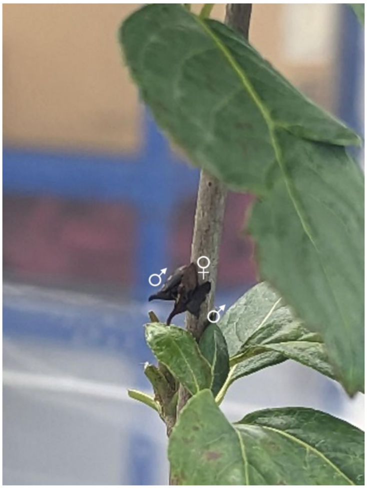

Figure 9. Image of two males mounting a female in *E. binotata*. The two males and the female have been labeled with white symbols for clarity. Photo credit: Dr Lauren A. Cirino.

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 signalling.

We discovered an additional female signal type: ‘advancing signals’ (Figure 10). Females produced this signal repeatedly for approx. 5 s 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.

3.3. Third stage of female choice: successful copulation, and male rejection behaviour

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 signalling 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.

3.4. Male–male signalling interactions

In 2m–1f trials, males seemed to take turns signalling 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 unit 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

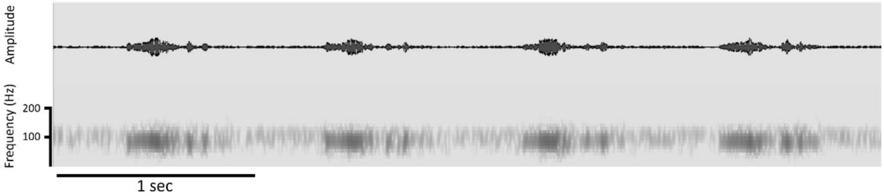

Figure 10. A section of a female advancing signal in *E. binotata* (see example of complete signal in supplemental). (Top) Waveform; (bottom) spectrogram.

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.

3.5. 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 s but one went on for 90 s. 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

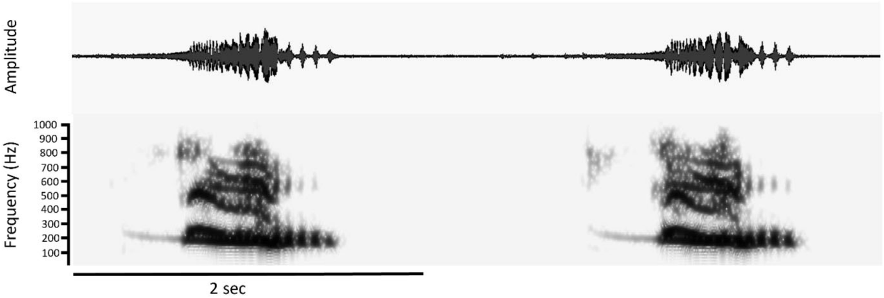

Figure 11. Example of *E. binotata* jamming signals from a 2 male–1 female trial. One male produced two advertisement signals (bottom traces on the spectrogram). The other male produced two jamming signals that overlapped the whine component of the first male's advertisement signals (top traces on the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

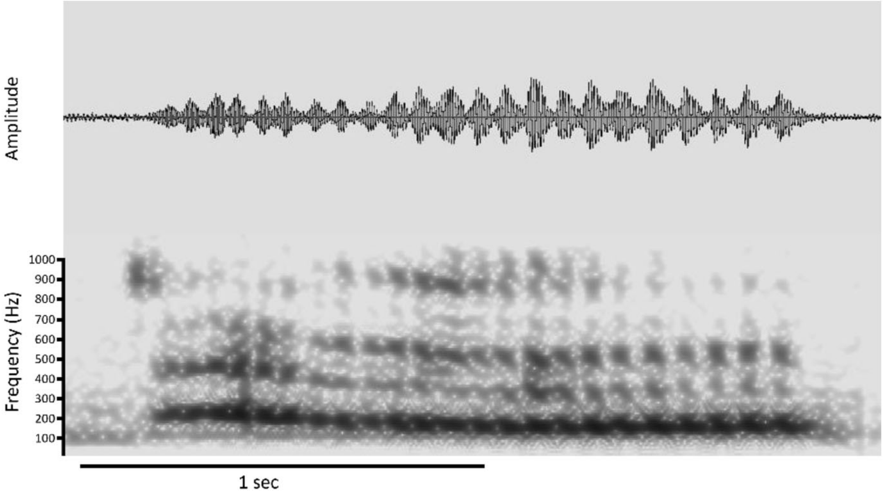

Figure 12. Example of an *E. binotata* vibrato signal from a 2 male–1 female trial. See Figure 1 for comparison with a typical advertisment signal. (Top) Waveform; (bottom) spectrogram.

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, signalling often stopped for ca. 2–5 s (and for 19 s in the case of the 90 s-buzz). There were a few instances in which females produced wing buzzes when a male was walking

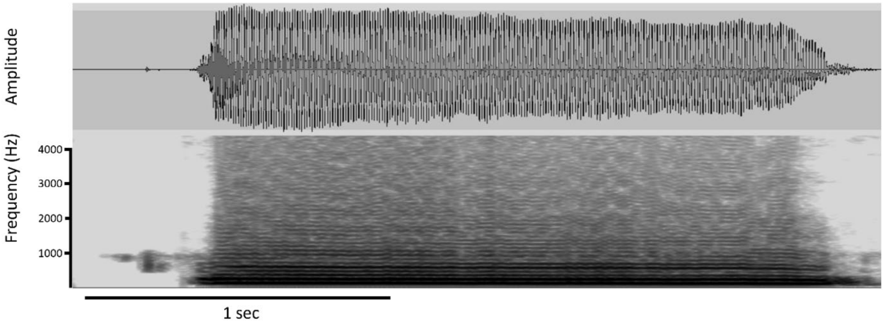

Figure 13. Example of an *E. binotata* female wing buzz. (Top) Waveform; (bottom) spectrogram.

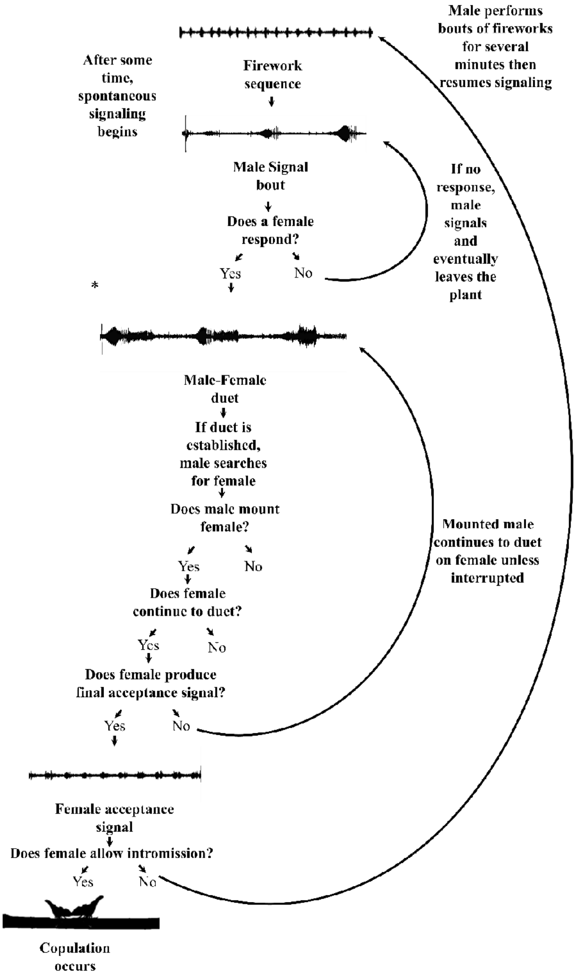

Figure 14. Sketch of the sequence of behaviours observed in pair formation and mating in *E. binotata*. The asterisk (*) denotes where duetting behaviour begins.

and not signalling. In some of these instances, males began producing bouts of advertisement signals within a few seconds after the buzz.

4. Discussion

Here we attempt a comprehensive description of the signal repertoire and behavioural 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.

4.1. 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” behaviour 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 behaviour, 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.).

4.2. 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, data not shown). 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 cause 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).

4.3. Signal repertoires in duetting insects

The behavioural 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 behaviour 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 (Elias et al., 2012), suggesting convergent neural abilities (Farris, 2008).

4.4. Implications for E. binotata cognitive abilities

Our results provide several suggestions regarding these insects' abilities to process complex information. To sustain their signalling 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). They are capable of sustained goal-directed behaviour (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 behaviours that unfolds along a suite of stages of active female selective cooperation with males required for mating in an insect. Regulation of these interactions may require more processing and cognitive sophistication than currently appreciated. Examples of similar or even higher behavioural repertoire richness in other insects and spiders (Miranda, 2006; Elias et al., 2012; Cossio-Rodriguez et al., 2019) suggest that such capabilities may be widespread across animals (Mendelson et al., 2016; Krakauer et al., 2017). Investigating their distribution and expression in brains of different sizes and architectures will be highly illuminative.

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