



A female mimic signal type in the vibrational repertoire of male *Enchenopa* treehoppers

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Received 20 April 2022; initial decision 15 June 2022; revised 4 July 2022;
accepted 13 July 2022; published online 21 October 2022

Abstract

Animals vary in the complexity and size of the signal repertoire used in communication. Often, these behavioural repertoires include multiple signal types for the same process, for instance, courtship. In *Enchenopa* treehoppers (Hemiptera: Membracidae) mate-searching males produce plant-borne vibrational advertisement signals. Receptive females then respond to males with their own signals. Here we describe an additional signal type in the repertoire of these males. We collected nymphs in Wisconsin, USA, and recorded the spontaneous signalling bouts of adult males and duetting signals of females using laser vibrometry. Two-thirds of males produced the additional signal type, which differed in temporal and spectral features from the main male advertisement signals, whilst resembling female duetting signals in placement and acoustic features. Our findings suggest that this might be a female mimic signal. Overall, our findings highlight the diversity in the behavioural repertoire that animals may use for reproduction.

Keywords

behavioural repertoire, *Enchenopa binotata*, Membracidae, vibratory signal, seismic signal.

1. Introduction

Animals vary in the number and types of signals they perform, which results in a wide diversity of signal repertoires. An animal's repertoire can include dozens of signal types that may vary in modality, structure, composition, duration, and/or frequency modulation (Hebets et al., 2016; Raboin & Elias, 2019; Odom et al., 2021). Bottlenose dolphins, for instance, use up to 12

signal types with different structures (Boisseau, 2005). Pale spear-nosed bats have eight main signals used in social contexts, and four other less-frequent signals (Lattenkamp et al., 2019). Diverse signalling repertoires also occur in geckos (Kabir & Thaker, 2021), jewel bugs (Mukai et al., 2022), fruit flies (Belkina et al., 2021), songbirds (Searcy, 1992; Odum et al., 2021), and tungara frogs (Akre & Ryan, 2010). Even animals that might be expected to have limited communication systems produce multiple signal types. For instance, arthropods such as wolf spiders (Rosenthal et al., 2021), jumping spiders (Rivera et al., 2021), and katydids (Korsunovskaya, 2008, 2009) have diverse signalling repertoire.

Signal repertoires often include signal types with different functions. For instance, different signals function to maintain group cohesion, establish dominance, engage in contests, or compete for mates (Akre & Ryan, 2010; Prat et al., 2016; Lattenkamp et al., 2019). In addition, behavioural repertoires may include multiple signal types for the same process. One of the processes in which animals show extensive signalling repertoires is courtship. Males of *Quiscalus quiscula* Common Grackle that use four signal types in courtship have higher mating success than males that use only one (Searcy, 1992). Variation in temporal and spectral signal features also occurs between individuals in a population, as found in male birds (Fitzsimmons et al., 2008; Sandoval & Escalante, 2011; Garcia et al., 2012).

Exploring animal signal types and the range of contexts in which they function can help understand the ways that animals have evolved to navigate their competitive and cooperative interactions using communication. Here we add a signal type to the repertoire of males in the *Enchenopa binotata* species complex of treehoppers (Hemiptera: Membracidae). As many other plant-feeding insects, *Enchenopa* communicate using plant-borne vibrational signals (Cocroft & Rodríguez, 2005; Cocroft et al., 2008; Hill, 2008; Rodríguez & Desjonquères, 2019). These treehoppers have diverse repertoires as juveniles and adults (Cocroft et al., 2008; Rodríguez et al., 2018; Desjonquères et al., 2019; Michael & Cocroft, unpubl.). Mate-searching adult males (Figure 1A) produce bouts of advertisement signals composed of two elements: a near pure-tone whine followed immediately by a series of pulses (Figure 1C, D) (Rodríguez et al., 2004; Cocroft et al., 2008, 2010). Performing the elements in that combination is crucial to obtaining a female response (Desjonquères et al., 2020; Speck et al., 2020). A receptive female (Figure 1B) responds to signals of males that it finds attractive by producing

her own response signals and engaging in a duet that helps the male locate her on the plant (Rodríguez et al., 2004; Rodríguez & Cocroft, 2006; Cocroft et al., 2008; Gibson & Cocroft, 2018).

In addition to the signals used in this ‘main’ system of duetting for pair formation, *Enchenopa* males also produce other signal types. One of them is, for example, a signal that overlaps with the end of the advertisement signal of another male in the plant, a potential rival (Sullivan-Beckers, 2008). Such ‘masking’ signals have been documented in other treehoppers, and have been shown to reduce the directional information provided by female responses and lower the likelihood of further female responses (Miranda, 2006; Legendre et al., 2012).

We recently discovered that males in several members of the *E. binotata* treehopper complex sometimes produce another signal type at the beginning of advertisement signal bouts (RB Cocroft & RL Rodríguez, pers. obs.; and see below). Our goal here was to investigate the features of this additional signal type in the repertoire of males. We describe the acoustic features of this signal type for two species in the *E. binotata* complex and compare it with other signals in the repertoire of males and females: the ‘main’ male advertisement signals and female duetting response signals (Figure 1B). We found that the additional signal differs from a male advertisement signal and resembles a female response signal in several acoustic features. Hence, we refer to it here as the ‘female mimic’ signal type.

2. Methods

2.1. Collecting and rearing treehoppers

Our two study species, as most members of the *E. binotata* complex, have not yet been formally described (Hamilton & Cocroft, 2009). Nevertheless, species in the complex can be readily distinguished by their host plant species, nymph coloration, and the dominant frequency of adult male advertisement signals (Cocroft et al., 2008, 2010). Our study species are both found on *Viburnum lentago* (Adoxaceae) host plants in Wisconsin, USA. They differ markedly in nymph coloration (grey versus black with waxy white stripes) and the dominant frequency of male signals (185 Hz versus 315 Hz, respectively) (Rodríguez et al., 2018). Hence, we refer to these species as sp_{low} and sp_{high} , respectively.

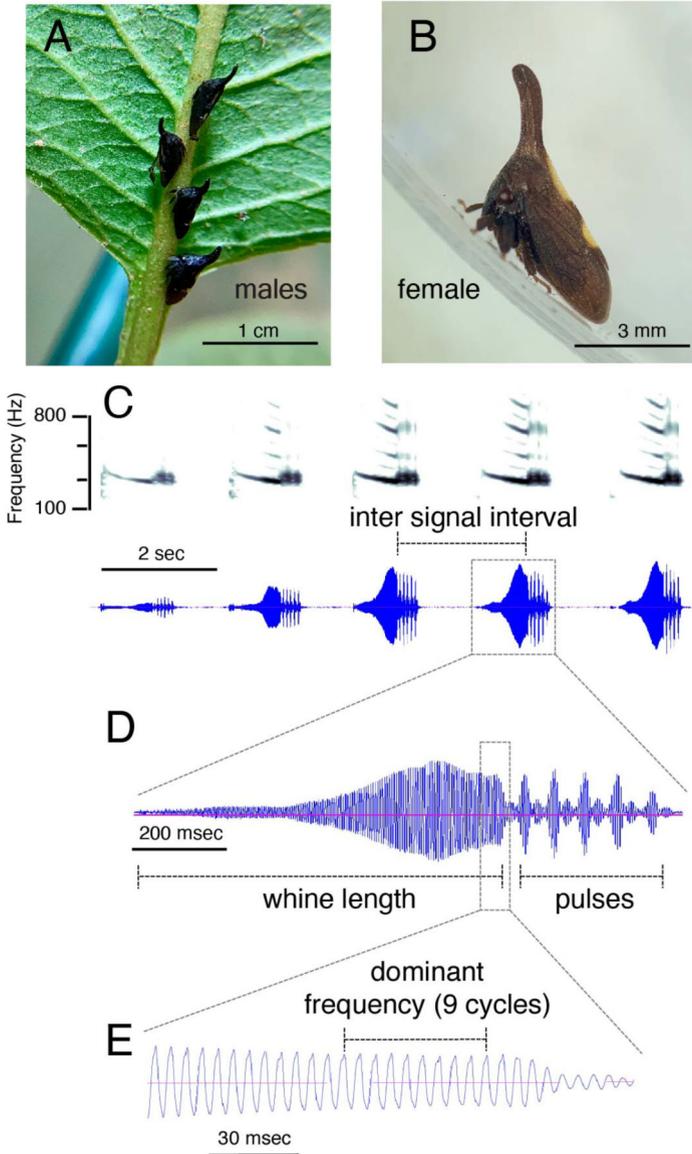


Figure 1. Signalling displays of *Enchenopa binotata* treehoppers (Hemiptera: Membracidae) and the temporal and frequency features explored. Sample (A) males and (B) a female of the *sp_{low}* species. (C) Bout of five main signals of a male and a visual description of the inter signal interval (see description in Methods). (D) Zoom of the fourth main signal, showing the whine and pulses. (E) Zoom at the end of the signal depicts the nine cycles measured to calculate the dominant frequency (in Hz) of the signal.

We collected third instar individuals on June 16, 2021, in meadows and forest edges at the Cedarburg Bog State Natural Area, in Saukville, Ozaukee County, WI, USA (43.403349° N, 87.998840° W, 404 m in elevation). We reared the nymphs to maturity at the University of Wisconsin–Milwaukee Greenhouse on potted *V. lentago* plants of 30–50 cm in height. Plants were netted with mesh in enclosures of 30 cm in diameter and 70 cm in height. Each plant housed a maximum of 40 nymphs. We separated recently moulted males and females on different plants (with 20 individuals/plant maximum) to ensure that our recordings were from males and females that had not engaged in courtship or mating (Figure 1B).

We analysed recordings of a total of 74 males and 40 females of both species from the abovementioned main field site. We also included recordings of 46 males of both species collected as nymphs in four other nearby meadow and forest edge field sites during 2018, 2019 and 2020 (range 6–15 individuals per site). Three of these sites are within 4 km of the main site, and the other is located 38 km southeast of it. These males were collected and reared in the same conditions as described above (Desjonquères et al., data not shown here, for further information and details on the sites).

2.2. Recording vibrational signalling displays

To record male and female signals, we transferred one individual at a time from its rearing plant to a recording plant in the laboratory using an Eppendorf tube opened on both ends. We allowed the treehoppers to walk on their own onto the recording plant, avoiding handling and stress. We used only one recording plant to control for potential confounds of morphological structure and to avoid variation in the vibration transmission on the plant.

We used a portable laser Doppler vibrometer (Polytec PDV-100; Polytec, Auburn, MA, USA) to record male vibrational signals. We focused the beam of the laser on a 0.5 cm² square of adhesive reflective tape on the recording plant's stem. We band-pass filtered the output of the laser vibrometer (40–3000 Hz) using an electronic filter (Krohn-Hite model 3202; Brockton, MA, USA) and sent the signal through a USB audio interface (Edirol USB Audio Capture UA-25; Roland, Hamamatsu, Japan) to a MacBook Pro computer (Apple, Cupertino, CA, USA). We recorded the signals at a sampling rate of 44.1 Hz using the program Audacity version 2.1.2 (<http://audacity.sourceforge.net/>).

We recorded the spontaneous signalling bout produced by each male. If a male did not signal within 5 min, we placed him back on the rearing plant and

tried again once every three days. Of all males, 46% signalled the first time we attempted to record, and 47% did between the second and fourth time they were tested (range 1–7 tries). For each recording, we noted the time of day and the air temperature using a hygro-thermometer (445 702, Extech Instruments, Nashua, NH, USA) placed on a table next to the recording computer. After the males signalled, we weighted them to the nearest 0.001 mg in a Mettler Toledo X6 analytical balance (Mettler Toledo, Greifensee, Switzerland).

To record female vibrational duetting signals (Figure 2D, also the Supplementary audio file that can be accessed at [10.6084/m9.figshare.20712457](https://doi.org/10.6084/m9.figshare.20712457)), we placed them on the recording plant as described above, and presented an artificial playback designed to mimic an advertisement signal bout of males of the same population (all features were set to the population mean; see below). We sent the playbacks from an Intel Core Duo iMac computer using MATLAB to a piezoelectric stack attached to the stem of the plant, driven by a piezo-controller (Thorlabs MDT694A; Thorlabs, Newton, NJ, USA). We coupled the piezo-electric stack to the stem of the playback plant with accelerometer wax. We calibrated the amplitude of the playback stimuli at 0.15 mm/s using a Tenma 72-2580 digital oscilloscope (Newark Element 14; Tenma, Chicago, IL, USA). We recorded the playbacks and female duetting responses with Audacity as well.

We isolated the recording and playback setups from building vibrations by placing them on a ca. 135-kg iron plank that rested on partially inflated bicycle inner tubes on a table. The plant was isolated from the iron plank by shock-absorbing Sorbothane (Edmund Scientifics, Tonawanda, NY, USA). We preserved treehoppers in individual vials with 95% ethanol, which are available upon contact with the authors.

2.3. Describing signals

We conducted bioacoustics analyses with Audacity, using a 100–2000 Hz bandpass filter. First, we qualitatively scored the recordings noting whether the signal bout had the female mimic signal type, and if so, whether it had a series of pulses or not (Fig. 2B and 2C, respectively), and its location in the main signals in the bout (e.g., after the first). On average, males from our study species produce 4 ‘main’ advertisement signals per bout (R.L. Rodríguez, unpublished). Hence, we use the fourth signal in a bout as a landmark for comparisons. As the female mimic signal type was often placed after the first signal (see below) we also compared the features of the latter.

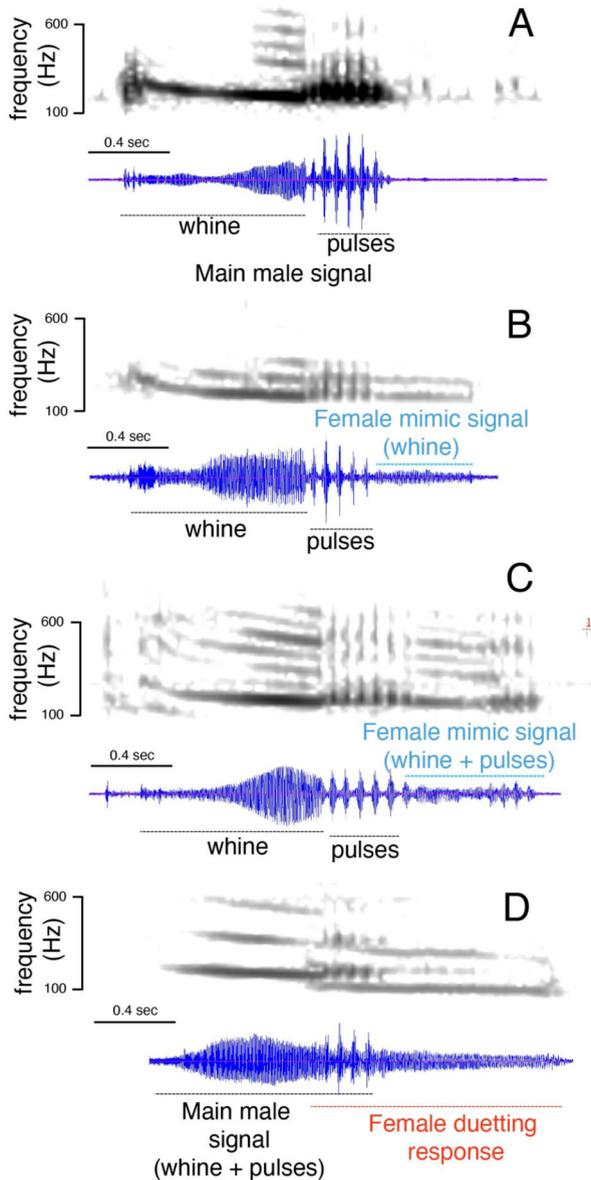


Figure 2. (A–C) Types of signalling displays of males of two species of the *Enchenopa bino-tata* (Hemiptera: Membracidae) treehoppers complex. (D) Sample male–female duet depicting the male signal and its elements as well as a female response. The sounds of these signals are presented in the Supplementary audio file that can be accessed at [10.6084/m9.figshare.20712457](https://doi.org/10.6084/m9.figshare.20712457).

We then quantitatively described seven temporal and spectral features of the first and fourth main advertisement signals, and the female mimic signal (Figure 1C–E): (1) length of the whine (Figure 1D); (2) dominant frequency at the beginning and end of the whine (calculated from the length of 9 cycles of the sine wave on the waveform (note that the dominant frequency at the end of the whine corresponds to the dominant frequency of the signal because of the higher amplitude at this portion of the signal) (Figure 1E); (4) frequency modulation, by subtracting the frequency at the end of the whine from the frequency at the start of the whine; (5) number of pulses (Figure 1D); (6) pulse rate, calculated as the number of pulses divided by the time interval from the peak of the first pulse to the peak of the last pulse (Figure 1D); and (7) inter signal interval (between signal 1 and the female mimic, between signal 1 and 2 for the first main signal, and between signal 3 and 4) (Figure 1C). We measured these features from the recordings visualized in Audacity using a custom-written code in R (R Core Team, 2021).

We measured four features in 20 recordings of female duetting responses: (1) signal length; (2) signal delay (duration between the end of the whine of a male's main signal and the start of the female response; Rodríguez & Cocroft, 2006); (3) dominant frequency; and (4) frequency modulation, both measured in the same way as described for male signals.

To compare male and female signals, we accounted for the fact that the male data had a repeated measures design (we extracted information for multiple signals from each male's recording), whereas the female data did not. We randomly divided the data from the males of each species in two. For each species, we included the features of the female mimic signal of half the males and the features of the fourth of the other half. This way, we compared all individuals from each species (20 females and 60 males).

2.4. Statistical analysis

We used a contingency Chi-square test to compare the proportions of males that produced the different signal types between the two species. The signal types were: advertisement signal bout without female mimic (Figure 2A), bout with female mimic without pulses (Figure 2B), or bout with female mimic with pulses (Figure 2C).

To compare the features of the different signal types (female mimic signal and main first and fourth advertisement signals), we first analysed values generated by a principal component analysis (PCA). This allowed us to avoid

the risk of spurious significance due to correlations between traits (Rice, 1989). We used the seven features measured to construct the PCA with the *prcomp* function in R. We included data from 61 males of sp_{low} and 59 males of sp_{high} (for a total of 120 males) that produced a female mimic signal (with or without pulses, see Figs. 2B, C). Each individual contributed three sets of values, one for each signal type (first, fourth, and female mimic). We pooled the female mimic signals with and without pulses for this analysis, as well as in all the models described below. This pooling accounted for the fact that the first and fourth main advertisement signals always have pulses, whereas the female mimic can be performed with or without pulses. Hence, pooling allowed accurately comparing the temporal and spectral features of the three signal types.

We then used linear mixed-effects models with PC1 and PC2 as the dependent variables (fit separately), as these were the only ones with an eigenvalue higher than 1.0, and together explained 71% of the variation in the dataset (Table 1). The models had the following fixed explanatory terms: signal type (first, fourth, and female mimic), species (sp_{low} and sp_{high}), recording temperature, and the interactions between signal type and species and between species and temperature. The main terms for signal type and species test for overall differences among them, and their interaction tests for species differences in the way the signal types vary. Our focus was therefore on the main term for signal type and its interaction with species. (Overall species differences in the main male advertisement signals have previously been described across the *E. binotata* complex and for our two study species: Cocroft et al., 2010; Rodríguez et al., 2018). The main term for temperature accounts for variation in temperature during recording, which has been found to influence *E. binotata* signalling and other reproductive behaviours (Leith et al., 2020). The interaction between species and temperature allows for species differences in that effect. The models also included male ID as a random term (as we extracted information for three signals from each individual). We ran these models using the *lmer* function in the *lme4* package (Bates et al., 2015) in R, and calculated *F* and *p* values using the ANOVA function in the *car* package (Fox & Weisberg, 2019).

This preliminary analysis, free of the risk of spurious significance, revealed substantial differences between signal types and species (see Results). We then explored those differences with the original signal features (except the frequency at the start of the whine, as this was gathered to calculate the frequency modulation).

We also compared the female mimic male signal type with female duetting response signals (Figure 2D, Supplementary Audio S1), and with the fourth main male signal for reference. We used models with the following explanatory terms: signal type (female mimic, female, and male main), species (sp_{low} , sp_{high}), recording temperature, and the interactions between signal type and species and species and temperature. The dependent variables and models were as follows (we fit each dependent variable separately). The residuals of the signal length were normally distributed, so we fit a linear model (lm) using the function lm of the *lme4* package. However, the residuals of the dominant frequency, frequency modulation, and signal delay were not normally distributed. Therefore, we fit general linear models (GLM) with Gamma distributions using the glm function in the *lme4* package. The Gamma distribution is particularly robust to fitting non-negative data that deviates from normality, like those three signal features.

For all the models we ran we first included the individual's weight as a continuous predictor. However, we removed it from the models we are presenting here as this term was not significant in any model. Additionally, we did not include field site or year as predictor variables in the models as those variables showed great variation in sample sizes (see available dataset). The displays of males are similar between field sites and years in the features we explored. Hence, we pooled all data as our focus was to explore the between-signal differences. The dataset is available on Dryad (URL: <https://doi.org/10.6078/D1RM6K>).

3. Results

3.1. Production of the female mimic signal type

Most males (67%) of the two study species produced signalling bouts that contained the female mimic signal type (sp_{low} : 71 of 107 males; sp_{high} : 70 of 103 males) (Figure 3). Of all the 141 males that produced the female mimic signal, 90% produced only one in the bout (immediately after the first main signal). The remaining 10% produced two female mimic signals: one after the first main signal and another after the second (8.6% of males) or fourth (1.4%) main signals in the bout. Of the males that produced the female mimic signal, most — particularly in sp_{high} — produced it with pulses (Figure 2B) rather than without (Figure 2C) ($\chi^2 = 14.52$, $df = 2$, $p = 0.001$; Figure 3, also the Supplementary audio file that can be accessed at [10.6084/m9.figshare.20712457](https://doi.org/10.6084/m9.figshare.20712457)).

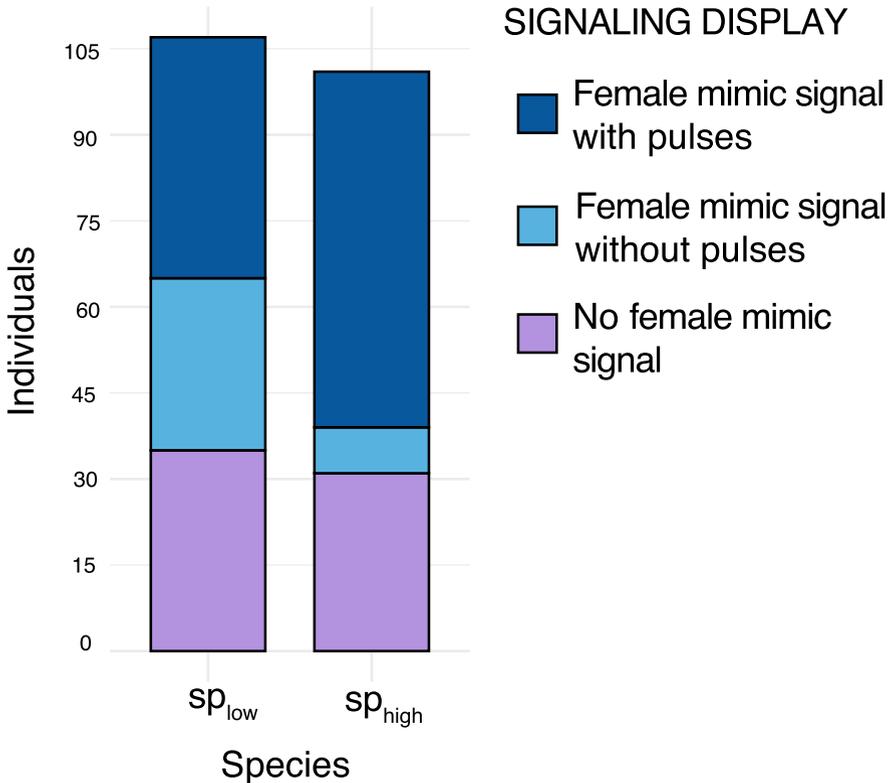


Figure 3. Variation in the type of signalling display of males of two species of treehoppers in the *Enchenopa binotata* complex. 67% of males produced a female mimic signal, and 75% of those males performed it with pulses. Additionally, the sp_{high} males performed female mimic signals without pulses less frequently than sp_{low} males (see Results for further statistical information). Recordings from Southeastern Wisconsin, USA, Summers of 2018, 2019, 2020 and 2021.

3.2. The female mimic signal type differed from the main male signal type

The analysis with the PC signal traits indicated that the main advertisement and female mimic signal types differed in both species (Figure 4, Table 1). Therefore, we explored the patterns in each signal trait and found differences in all temporal and spectral features. In both species, female mimic signals were on average shorter (whine length), had a shorter inter signal interval, lower dominant frequency, shallower frequency modulation, fewer pulses, and slower pulse rate than the first and fourth main advertisement signals (values in Table 2 and significant signal terms in Table 3). These differences

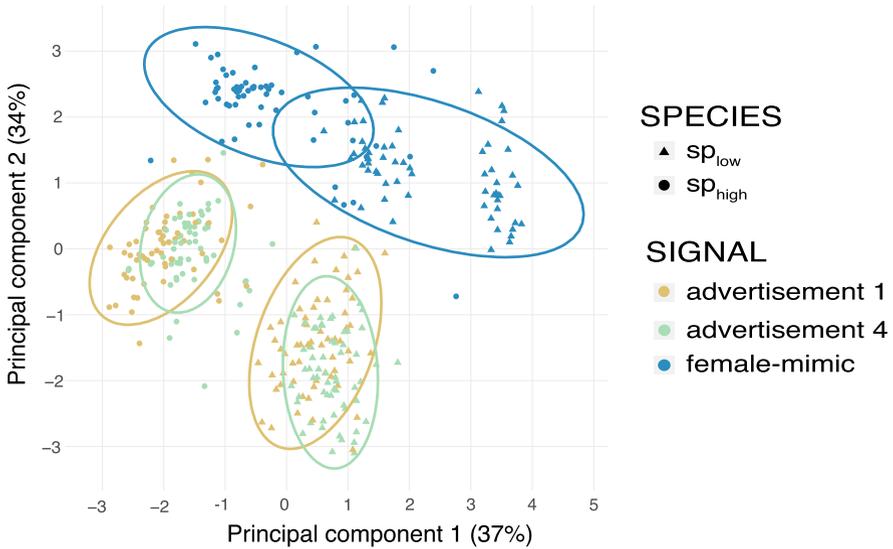


Figure 4. Quantitative variation in three signals of males of two species of treehoppers in the *Enchenopa binotata* complex. The two first principal components together summarize 71% of the variation on seven temporal and spectral features of the signals (specific variation shown in the x - and y -axes). The female mimic signal was significantly different to the main signals (which did not differ statistically) in the PC1 (linear mixed-effect models: signal term; $\chi^2_{2/352} = 615.8$, $p < 0.001$), the species differ in the PC1 scores (species term; $\chi^2_{1/352} = 612.9$, $p < 0.001$), but the pattern of differences between signals did not differ between species (signal \times species interaction term; $\chi^2_{2/352} = 3.2$, $p = 0.20$). The PC2 showed similar patterns (signal term; $\chi^2_{2/352} = 1840.2$, $p < 0.001$, species term; $\chi^2_{1/352} = 204.1$, $p < 0.001$), but the pattern of differences between signals differed between species (signal \times species interaction term; $\chi^2_{2/352} = 39.5$, $p < 0.001$). The female mimic signals shown here (and in Figure 5) include both the signals with and without pulses. The dispersion ellipses surround the position of each signal type for each species, providing an index of the dispersion around the centroid (95% of the signal type occurrences are expected to fall into the associated ellipse).

were more pronounced in sp_{high} , particularly for whine length, inter signal interval, dominant frequency, and the number of pulses (significant signal type \times species interaction terms in Table 3).

3.3. The female mimic signal type resembled female signals

In both species, the female mimic signal type resembled female duetting response signals in two of the four features we assessed. First, female mimic

Table 1. Principal component analysis loadings derived from seven temporal and spectral features of male signals in two species of treehoppers in the *Enchenopa binotata* complex.

| Signal feature | Principal component | | | | | | |
|--|---------------------|--------|-------|-------|-------|-------|-----------|
| | PC1 | PC2 | PC3 | PC4 | PC5 | PC6 | PC7 |
| Loadings of each signal feature | | | | | | | |
| Whine length (s) | 0.11 | -0.56* | 0.22 | -0.34 | 0.48 | -0.54 | 0.000004 |
| Start frequency (Hz) | -0.55* | 0.17 | 0.37 | 0.03 | 0.13 | -0.04 | -0.72 |
| Dominant frequency (Hz) | -0.51* | 0.27 | 0.32 | 0.10 | 0.29 | -0.06 | 0.68 |
| Frequency modulation (Hz) | -0.30 | -0.42* | 0.31 | -0.29 | -0.72 | 0.04 | 0.15 |
| Number of pulses | -0.38* | -0.27 | -0.51 | 0.53 | -0.14 | -0.47 | -0.000001 |
| Pulse period (Hz) | -0.39* | 0.12 | -0.59 | -0.68 | 0.11 | 0.08 | -0.000001 |
| Inter signal interval (s) | -0.18 | -0.57* | -0.07 | 0.20 | 0.34 | 0.69 | -0.000002 |
| Parameters of each principal component | | | | | | | |
| Eigenvalue | 1.62* | 1.53* | 0.99* | 0.69 | 0.62 | 0.42 | 0.000029 |
| Percentage of variance explained | 37* | 34* | 14* | 7 | 5 | 3 | 0 |
| Cumulative proportion | 0.37 | 0.71 | 0.85 | 0.92 | 0.97 | 1.00 | 1.00 |

*Features with higher loadings per principal component (above) and the PCs that explained most of the variation (below).

Table 2. Temporal and spectral features of two main advertisement signals and the female mimic signal type produced by males of two species of treehoppers in the *Enchenopa binotata* complex.

| Species | Feature | First main signal | | | Fourth main signal | | | Female mimic signal | | |
|------------------------------------|---------------------------|-------------------|-------|---------------|--------------------|-------|---------------|---------------------|-------|---------------|
| | | Mean | SD | Range | Mean | SD | Range | Mean | SD | Range |
| <i>sp</i> _{low} (N = 61) | Whine duration (s) | 0.67 | 0.14 | 0.26–1.13 | 0.82 | 0.12 | 0.45–1.12 | 0.46 | 0.16 | 0.18–0.79 |
| | Start frequency (Hz) | 207.55 | 14.76 | 170.44–236.4 | 210.11 | 14.26 | 176–237.74 | 177.75 | 8.68 | 160.02–199.85 |
| | Dominant frequency (Hz) | 177.88 | 9.25 | 161.18–195.57 | 178.10 | 9.01 | 155.73–196.61 | 167.82 | 10.22 | 147.89–198.72 |
| | Frequency modulation (Hz) | 29.66 | 11.84 | 1.47–53.66 | 32.01 | 10.49 | 5.56–51.58 | 9.93 | 6.17 | –1.85–27.34 |
| | Number of pulses | 5.66 | 1.35 | 3–10 | 4.30 | 0.72 | 3–6 | 3.94 | 1.25 | 2–7 |
| | Pulse rate (Hz) | 18.05 | 2.66 | 14.22–26.91 | 17.81 | 2.04 | 14.22–22.69 | 23.67 | 5.52 | 16.9–39.87 |
| | Inter-signal interval (s) | 2.60 | 0.42 | 1.82–4.07 | 2.85 | 0.65 | 1.9–4.52 | 0.43 | 0.11 | 0.21–0.8 |
| | Whine duration (s) | 0.50 | 0.08 | 0.23–0.74 | 0.60 | 0.07 | 0.47–0.79 | 0.39 | 0.16 | 0.22–1.42 |
| | Start frequency (Hz) | 345.42 | 21.02 | 281.76–374.69 | 347.82 | 18.93 | 292.65–391.16 | 301.79 | 24.47 | 204.8–348.23 |
| | Dominant frequency (Hz) | 312.45 | 14.89 | 266.95–335.36 | 313.35 | 15.98 | 270.66–337.74 | 289.76 | 25.22 | 169.33–321.38 |
| <i>sp</i> _{high} (N = 59) | Frequency modulation (Hz) | 32.98 | 11.62 | 6.74–59.12 | 34.48 | 12.52 | 6.06–75.45 | 12.03 | 13.65 | –16.18–56.95 |
| | Number of pulses | 6.07 | 1.35 | 2–8 | 4.32 | 0.71 | 3–6 | 3.31 | 1.23 | 1–6 |
| | Pulse rate (Hz) | 21.68 | 2.58 | 17.06–31.35 | 20.99 | 1.59 | 17.65–25.7 | 26.00 | 10.23 | 0–42.61 |
| | Inter-signal interval (s) | 2.25 | 0.27 | 1.85–3.17 | 2.14 | 0.42 | 1.64–4.34 | 0.36 | 0.06 | 0.17–0.47 |

See Figures 1 and 2 for further description.

Table 3.

Variation in the signal temporal and spectral features between the signal types (first and fourth main advertisement signals, and the female mimic signal) in males of two treehoppers species in the *Enchenopa binotata* complex (*sp*_{low}, *sp*_{high}).

| | χ^2 | df | <i>p</i> |
|---------------------------|----------|------|----------|
| Whine length (s) | | | |
| Signal type | 401.24 | 2352 | <0.001* |
| Species | 44.12 | 1352 | <0.001* |
| Temperature | 34.95 | 1352 | <0.001* |
| Signal type × species | 28.36 | 2352 | <0.001* |
| Species × temperature | 0.26 | 1352 | 0.61 |
| Inter signal interval (s) | | | |
| Signal type | 3294.44 | 2352 | <0.001* |
| Species | 22.42 | 1352 | <0.001* |
| Temperature | 131.09 | 1352 | <0.001* |
| Signal type × species | 59.25 | 2352 | <0.001* |
| Species × temperature | 5.99 | 1352 | 0.01* |
| Dominant frequency (Hz) | | | |
| Signal | 201.76 | 2352 | <0.001* |
| Species | 4540.53 | 1352 | <0.001* |
| Temperature | 100.10 | 1352 | <0.001* |
| Signal × species | 30.99 | 2352 | <0.001* |
| Species × temperature | 15.64 | 1352 | <0.001* |
| Frequency modulation (Hz) | | | |
| Signal | 363.11 | 2352 | <0.001* |
| Species | 0.01 | 1352 | 0.90 |
| Temperature | 13.25 | 1352 | <0.001* |
| Signal × species | 0.23 | 2352 | 0.89 |
| Species × temperature | 1.04 | 1352 | 0.31 |
| Number of pulses | | | |
| Signal | 327.17 | 2352 | <0.001* |
| Species | 0.08 | 1352 | 0.78 |
| Temperature | 0.04 | 1352 | 0.84 |
| Signal × species | 13.05 | 2352 | 0.001* |
| Species × temperature | 2.11 | 1352 | 0.15 |
| Pulse period (Hz) | | | |
| Signal | 76.83 | 2352 | <0.001* |
| Species | 10.52 | 1352 | 0.001* |
| Temperature | 17.45 | 1352 | <0.001* |
| Signal × species | 1.04 | 2352 | 0.60 |
| Species × temperature | 7.18 | 1352 | 0.007* |

Results of the linear mixed-effects models. ×, interaction terms.

*Significant terms at the $p < 0.05$ level.

signals and female duetting signals had shorter delays than the male main advertisement signal (Figure 5B; significant signal type term in Table 4). Thus, the relative timing, or placement, of female mimic signals and female duetting signals within male signal bouts was similar. Second, female mimic

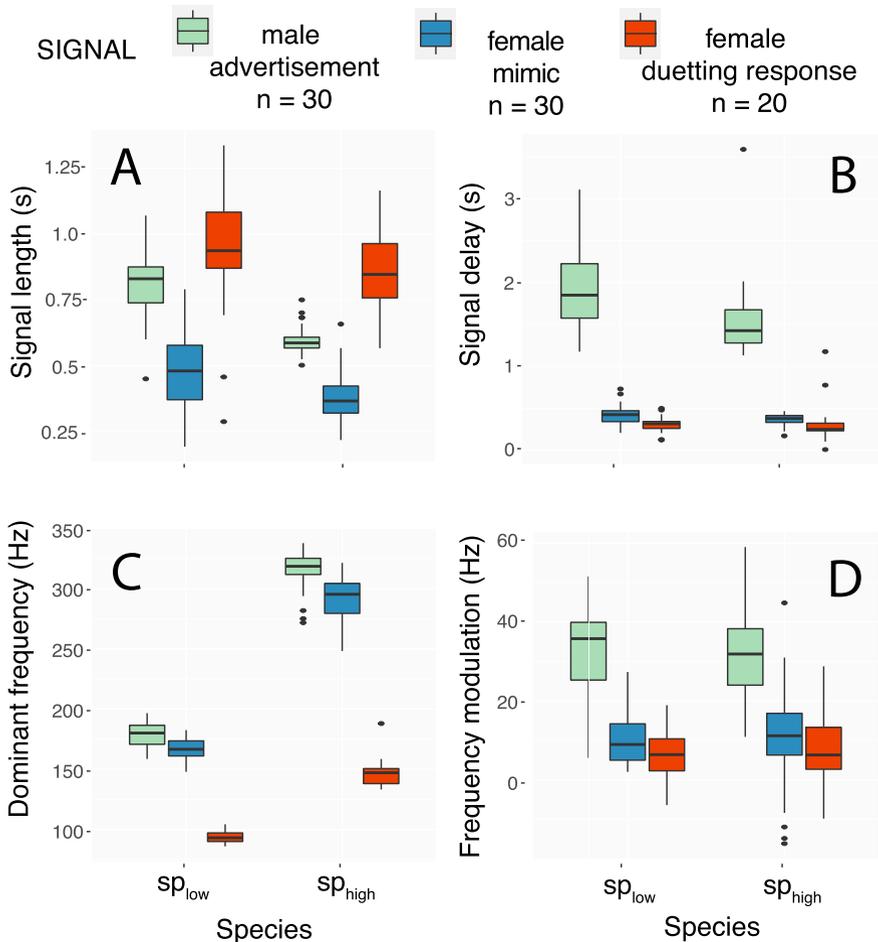


Figure 5. The female mimic signal resembles the female responses in two of four signal temporal and spectral features (B and D) in two species of treehoppers in the *Enchenopa binotata* complex. Boxplots show the median, and the 25% and 75% percentiles, vertical lines show the value range, and values beyond the lines are outliers. The sample sizes are shown in the legend. See Methods for the description of each feature. The female mimic signal was significantly different from the female response in A or C (see Results and Table 4 for statistical results).

Table 4.

Variation in temporal and spectral features between signal types (fourth main male signal, female mimic signal, and female response signal) in two *Enchenopa binotata* species (sp_{low} , sp_{high}).

| | <i>F</i> | df | <i>p</i> |
|---------------------------|----------|------|----------|
| Signal length (s) | | | |
| Signal type | 142.28 | 2152 | <0.0001* |
| Species | 0.84 | 1152 | 0.41 |
| Temperature | −2.07 | 1152 | 0.04* |
| Signal type × species | 2.30 | 2152 | 0.02* |
| Species × temperature | −0.76 | 1252 | 0.45 |
| Signal delay (s) | | | |
| Signal type | 427.21 | 2152 | <0.0001* |
| Species | 1.41 | 1152 | 0.72 |
| Temperature | 41.15 | 1152 | 0.02* |
| Signal type × species | 1.62 | 2152 | 0.27 |
| Species × temperature | 0.24 | 1252 | 0.97 |
| Dominant frequency (Hz) | | | |
| Signal type | 1677.55 | 2152 | <0.0001* |
| Species | 3999.18 | 1152 | <0.0001* |
| Temperature | 103.47 | 1152 | <0.0001* |
| Signal type × species | 1.60 | 2152 | 0.09 |
| Species × temperature | 2.51 | 1252 | 0.11 |
| Frequency modulation (Hz) | | | |
| Signal type | 105.38 | 2152 | <0.0001* |
| Species | 0.10 | 1152 | 0.24 |
| Temperature | 5.36 | 1152 | 0.05* |
| Signal type × species | 0.19 | 2152 | 0.86 |
| Species × temperature | 1.72 | 1252 | 0.24 |

signals and female duetting signals had shallower frequency modulation than the male main advertisement signal (Figure 5D; significant signal type term in Table 4). By contrast, female mimic signals had higher dominant frequencies than female duetting signals (Figure 5A, C; Table 4). Further in sp_{high} , female mimic signals were shorter than female duetting signals (Figure 5A, significant signal × species interaction term in signal length in Table 4).

4. Discussion

Here we add a signal type to the repertoire of males in the *Enchenopa binotata* complex of treehoppers: a female mimic signal. This signal type

was common (ca. two-thirds of males produced it) and differed from male advertisement signals in all the temporal and spectral features we explored. Additionally, it resembled the duetting responses of *E. binotata* females in that it was placed after the male advertisement signals with similar timing to how females place their duetting signals; its shallow frequency modulation was similar to those of the female duetting signals, and it sometimes lacked pulses. Consequently, we suggest that this signal type may function to mimic the female duetting response signals.

Sexual mimicry in acoustic signals usually occurs when males produce female mimic signals with similar timing as the female signals (Bailey et al., 2006). For example, male bushcrickets mimic a female response with similar latency as when females usually respond during a duet (Bailey et al., 2006). Beyond these similarities in timing, similarities in spectral features have not been explored in the context of acoustic communication in invertebrates. Hence, we provide novel findings regarding the similarity between the female mimic signal and the female duetting response in having a lower frequency modulation, compared with the high frequency modulation of the main male advertisement signals.

The males of our two species differed mainly in the dominant frequency of their main advertisement signals, as is typical in the *E. binotata* complex (Cocroft et al., 2010; Rodríguez et al., 2018; this paper). Female mimic signals also reflected this difference in their dominant frequency. The only differences we found regarding the female mimic signals were that more males of the sp_{high} produced pulses than males of sp_{low} (Figure 3), and that it was shorter in length than the main male advertisement signal only in the sp_{high} males. Despite this, the female mimic signal was consistently different from the main advertisement males and similar to the female duetting response in both species.

Males may mimic female behaviours and signals for various adaptive reasons. For example, male cuttlefishes using the ‘sneaker’ alternative mating strategy mimic the behaviour, body shape, and coloration of females (Norman et al., 1999). Male scorpionflies mimic female behaviour to steal nuptial gifts from other males and mate with females (Thornhill, 1979). In the context of species that use male-female duetting in pair formation, males may mimic female signals for several reasons. The female mimic signal might inhibit other males from signalling (cf. Bailey et al., 2006), as they could

interpret that a female has initiated a duet with the signalling male. Additionally, this signal could mimic competition for males, thus inducing females to respond to them (cf. Jones & DuVal, 2019; Davies et al., 2020). We experimentally address the function of the female mimic signal in a forthcoming paper (Escalante et al., data not shown).

In conclusion, we found that most of the males of two species of *Enchenopa* treehoppers performed a female mimic signal in their courtship displays. Our findings add a signal type to the repertoire produced by adult treehoppers during courtship (Cocroft & Rodríguez, 2005; Miranda, 2006; Legendre et al., 2012). These findings highlight the diversity of signals that male treehoppers produce, and their potential importance for reproduction. Additionally, diverse signaling repertoires challenge the view of insects having simple signal repertoires. Overall, describing variation in signals allows us to explore the diversity of these phenotypes in animals, the functions of signals, and more broadly better understand communication in animals.

Acknowledgements

We are grateful to P. Engevoold and the UWM Greenhouse, as well as to G. Meyer and the UWM Field Station for their support to this project. Additional support in the data collection, insect rearing, and plant care was provided by S. Seidita, L. Cirino, B. Speck, C. Sergi, D. Little, I. Gallager, M. Rittinger, C. Wiese, J. Maliszewski, V. Melnikov, N. Abuomar, B. Muran-sky, and N.C. Wagner. Constructive feedback during the data analyses and writing stages was provided by G. Höebel, L. Whittingham, P. Dunn, and E. Latch. Two anonymous reviewers provided useful feedback. We thank R. Cocroft for the MATLAB code. This project was funded by a UWM Support for Undergraduate Research Fellows (SURF) award to JRK, a UWM Discovery and Innovation (DIG) grant to RLR, and a National Science Foundation grant (IOS-1855962) to RLR and CD.

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Supplementary audio file. Types of signals of males and females of two species of the *Enchenopa binotata* (Hemiptera: Membracidae) treehoppers complex, which are shown in Figure 2. The sounds are: (1) the main advertisement male signal without the female mimic signal; (2) the main signal followed by a female mimic signal without pulses; (3) the main signal followed by a female mimic signal with pulses; and (4) a male–female duet of the male signal followed by a female response. Each of the four signal groups are separated by 1.5 s of silence. This file can be accessed at [10.6084/m9.figshare.20712457](https://doi.org/10.6084/m9.figshare.20712457).