

THE IMPORTANCE OF FEMALE CHOICE, MALE–MALE COMPETITION, AND SIGNAL TRANSMISSION AS CAUSES OF SELECTION ON MALE MATING SIGNALS

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Selection on advertisement signals arises from interacting sources including female choice, male–male competition, and the communication channel (i.e., the signaling environment). To identify the contribution of individual sources of selection, we used previously quantified relationships between signal traits and each putative source to predict relationships between signal variation and fitness in *Enchenopa binotata* treehoppers (Hemiptera: Membracidae). We then measured phenotypic selection on signals and compared predicted and realized relationships between signal traits and mating success. We recorded male signals, then measured lifetime mating success at two population densities in a realistic environment in which sources of selection could interact. We identified which sources best predicted the relationship between signal variation and mating success using a multiple regression approach. All signal traits were under selection in at least one of the two breeding seasons measured, and in some cases selection was variable between years. Female preference was the strongest source of selection shaping male signals. The *E. binotata* species complex is a model of ecological speciation initiated by host shifts. Signal and preference divergence contribute to behavioral isolation within the complex, and the finding that female mate preferences drive signal evolution suggests that speciation in this group results from both ecological divergence and sexual selection.

KEY WORDS: Sexual selection, signal evolution, treehopper, vibrational communication.

A central goal of evolutionary biology is to understand how selection acts on traits in natural populations. Although the strength and direction of selection acting on traits have often been quantified, the agents of selection are more often inferred based on the nature of the trait (Endler 1986; Møller et al. 1998; Kingsolver et al. 2001). Testing hypotheses about the sources of selection is challenging because it requires not only measuring variation in fitness associated with specific traits, but also either experimentally manipulating potential sources of selection (Wade and Kalisz 1990) or comparing realized trait values with those pre-

dicted based on a priori characterization of potential sources of selection (Endler 1986).

Secondary sexual traits such as mating signals have several advantages for identifying the agents of selection. Sexual selection is expected to be strong on theoretical grounds (West-Eberhard 1983), and in empirical studies is usually stronger than natural selection (Kingsolver et al. 2001). Additionally, there is a large body of theory about causes of sexual selection on signals, which can be used to generate hypotheses (Andersson 1994). Potential sources of selection include female preferences and male–male

competition, and both sources have been well characterized in many species (Kirkpatrick and Ryan 1991; Gerhardt 1994; Welch et al. 1998; Bee et al. 2002; Kokko et al. 2006). In addition, mating signals evolve in response to natural selection for efficient transmission through the environment (Endler and Basolo 1998; Boughman 2002) and for reduced conspicuousness to predators that use signals to locate prey (Zuk and Kolluru 1998; Haynes and Yeargan 1999). Mating signals are thus potentially the target of multiple abiotic and biotic sources of selection. The challenge is therefore to find a species and methods that allow isolating the contributions of these interdependent sources of selection on signals (Wong and Candolin 2005).

Two general approaches have been used to identify the sources of selection on sexual signals. One is to experimentally manipulate possible sources of selection and compare the relationship of trait and fitness among treatments (e.g., Mitchell-Olds and Shaw 1990; Kelly 2008). Another common approach is to characterize a potential source of selection and compare the expected phenotypic distribution to that observed in natural populations (Endler and Houde 1995; Shaw and Herlihy 2000; Simmons et al. 2001; Bentsen et al. 2006). An extension of this approach is to examine variation in both the source of selection and the trait distribution among populations (Ritchie 1996; Simmons et al. 2001) or species (Bonduriansky 2003; Rodríguez et al. 2006). Although this approach can identify responses to past selection, it does not directly measure selection in contemporary populations.

A recently developed alternative approach is to characterize potential sources of selection in sufficient detail to permit comparison of predicted and observed relationships of trait variation with fitness under natural conditions (Bleay et al. 2007). This approach may be especially powerful for sexually selected traits like mating signals, for which we can characterize not just an optimum value, but also an expected fitness curve based on the range of signal variation. This allows us to make quantitative predictions about how a particular source of selection would shape signal evolution. For example, female preference can be represented as a preference function, a curve that represents signal attractiveness as a function of variation in signal traits (e.g., Ritchie 1996; Brooks et al. 2005; Gerhardt 2005; Rodríguez et al. 2006). Similar curves can be estimated for male competition, where signal variation reflects competitive ability (Gerhardt and Huber 2002; Wyman et al. 2008) and transmission efficiency, where particular signal parameters improve the broadcast distance or reliability of the signal in the communication channel (Endler and Basolo 1998). The relationships between signal variation and attractiveness, competitive success, and transmission through the environment can be used to generate hypotheses about selection on signals (Gerhardt 1994). Because each potential source of selection will have a complex relationship to the range of trait variation, it will often be possible

to distinguish between sources of selection even if they predict the same peak value.

Because different sources of selection can interact and vary in their strength (Hunt et al. 2009), their relative importance must be tested in a context in which all sources can interact. Female preferences or other potential causes of selection measured in the laboratory do not necessarily translate into realized selection in nature. Environmental and social factors may influence the expression of female preferences; for example, male–male interference may limit the scope of female choice (Cremer and Greenfield 1998); females' ability to discriminate among signals may be influenced by the signal transmission medium (Seehausen et al. 1997) or by background noise (Gerhardt and Klump 1988); and the expression of preferences may depend on the particular set of individuals being compared (Morris 1989; Jang and Greenfield 1998; Bateson and Healy 2005). Additionally, female choice depends not only on preferences, but also on the effort females are willing to expend on mating decisions (Jennions and Petrie 1997).

Although identifying the sources of selection in the field is critical for understanding the evolution of sexual signals, there are few cases in which measures of selection on signals have been compared quantitatively with those predicted by female preferences and male competition. In contrast, several studies have revealed a qualitative correspondence between female preferences characterized in the laboratory and male mating success measured under natural conditions (anurans: Ryan 1985; Morris and Yoon 1989; Ryan et al. 1990; Passmore et al. 1992; Wagner and Sullivan 1995; Grafe 1997; fruit flies: Aspi and Hoikkala 1995; Hoikkala and Suvanto 1999; wax moths: Jang and Greenfield 1996, 1998; Cremer and Greenfield 1998). Given the success of these qualitative studies, fine-scale quantitative comparisons are a promising avenue for identifying the importance of female preferences and other sources of selection in contemporary populations. However, we know of no studies that have jointly tested whether independently derived preference functions, functions of male competitive ability, and transmission functions predict the shape of the relationship between signal variation and male mating success under natural conditions.

In this study, we determine the role of three sources of selection on mating signals in an herbivorous insect that communicates using plant-borne vibrations. Our study species is the treehopper *Enchenopa binotata* "Ptelea" (Hemiptera: Membracidae), whose mating signals are likely under selection from multiple sources. Comparative evidence suggests that female preference is an important influence on signal evolution in the *E. binotata* species complex (Rodríguez et al. 2006). Direct male–male competition occurs in the presence of receptive females, and the outcome of this competition is influenced by differences in male signals (Sullivan-Beckers 2008; Supporting Information). In addition, plant stems and leaves act as frequency filters for vibrational

signals, selectively attenuating some frequencies and transmitting others (Cökl and Virant-Doberlet 2003), and there is evidence that in *Enchenopa*, male signal frequency is adapted to the filtering properties of the substrate used for communication (McNett and Cocroft 2008). We expect these sources of selection to act simultaneously on male signals. Males compete largely through exploitation competition, and the interference competition that does occur takes place in close proximity to the female, during the process of mate assessment.

The *E. binotata* species complex is a group of 11 host specialists that are broadly sympatric across eastern North America. Speciation in this clade is hypothesized to have occurred as a result of host shifts, which can cause allochronic shifts in life-history timing and divergent natural selection for adaptation to different host environments (Wood 1993). However, behavioral isolation is stronger than ecological isolation between populations on different hosts (Wood 1980), and this isolation results from differences in mating signals, which have diverged between species on different hosts in parallel with female preferences (Rodríguez et al. 2006). Across the geographic range of the complex, variation in signal traits important for mate recognition (especially signal frequency and duration) is greater between populations on different hosts than between distant populations on a single host (Cocroft et al. 2010). Host shifts can alter the nature of sexual selection through a variety of mechanisms (Cocroft et al. 2008), and if we find direct evidence that sexual selection is important in signal evolution in *Enchenopa*, this would support the hypothesis that host shifts have led to speciation in part through their effects on sexual selection.

To identify agents of selection on signals we first measured selection on male signals at two population densities; if the importance of a given source of selection changes with density, as is likely with processes such as male–male competition (Andersson 1994), this should increase our ability to identify the contribution of different sources of selection. For example, we would expect male–male competition to play a larger role at higher population densities. Then, for three potential sources of selection that cannot be evaluated independently (female choice, male–male competition, and signal transmission), we use quantitative measurements from other studies (Rodríguez et al. 2006; McNett and Cocroft 2008; Supporting Information) to predict the relationship between signal variation and male mating success for each trait. Importantly, females in this species mate only once (Wood 1982), so male mating success is a good proxy for fitness. Next, we use a multiple regression approach to evaluate how well the predicted relationships based on each potential source of selection match the pattern of realized selection for signal variation. This is the first study to generate and test, under natural conditions, quantitative predictions from multiple sources of selection on acoustic (i.e., airborne or vibrational) mating signals.

Materials and Methods

PART I: MEASURING SELECTION UNDER NATURAL CONDITIONS

To measure selection on signals we established males and females on host plants in outdoor enclosures at two levels of population density and then monitored male mating success throughout the breeding season. These insects live less than 1 year and females mate only once, so by counting the number of mates a male obtained, we gained an accurate count of its lifetime mating success. The experiment was conducted for 2 years, to include two breeding seasons.

The experiments were conducted during June–August of 2005 and 2006 at the University of Missouri (MU). *Enchenopa binotata* “Ptelea” juveniles were collected from a site on the MU campus containing a patch of approximately 15 wafer ash trees (*Ptelea trifoliata*). (Note: the species in the *Enchenopa binotata* complex have not yet been formally described, and are currently referred to using the name of their host plant.) At the time of collection, most individuals were in the third nymphal stadium, 2–3 weeks prior to adult eclosion. Juveniles were housed until adult eclosion in a single outdoor screen enclosure (72" × 36" × 30") containing 10 potted host plants.

SIGNAL RECORDINGS

Males began signaling approximately 1 week after adult eclosion, at which point they were brought into the laboratory for signal recording. Males and females of *Enchenopa* treehoppers produce substrate-borne vibrational signals (Hunt 1994) by tremulating their abdomens (L. Sullivan-Beckers and R. B. Cocroft, pers. obs.) The signal traits of males are significantly repeatable during the breeding season (Sattman and Cocroft 2003), so our recordings provide an accurate estimate of male signals throughout the season. Within each year, the signals from the males were recorded on the same individual host plant. We used a different recording plant each year, but the signal traits measured here are not affected by differences between individual host plants (Sattman and Cocroft 2003).

To record male signals, we used a laser Doppler vibrometer (Polytec CLV 1000 with a CLV M030 decoder module; Polytec Inc., Auburn, MA). We placed a male approximately 1 cm from the laser recording location and played back a male–female duet through loudspeakers to prime the male to signal. A small piece of reflective tape (1 mm²) was attached to the plant to increase laser reflectance. All signals were high-pass filtered (Krohn-Hite 3202) with a cutoff frequency of 70 Hz (the lowest frequency in the signal is approximately 300 Hz). Signals were recorded using Raven (v 1.2, Cornell Laboratory of Ornithology, Ithaca, NY) with a sampling rate of 44.1 kHz on a Macintosh G4 computer. Male signals were monitored using a Radio Shack MPA-45

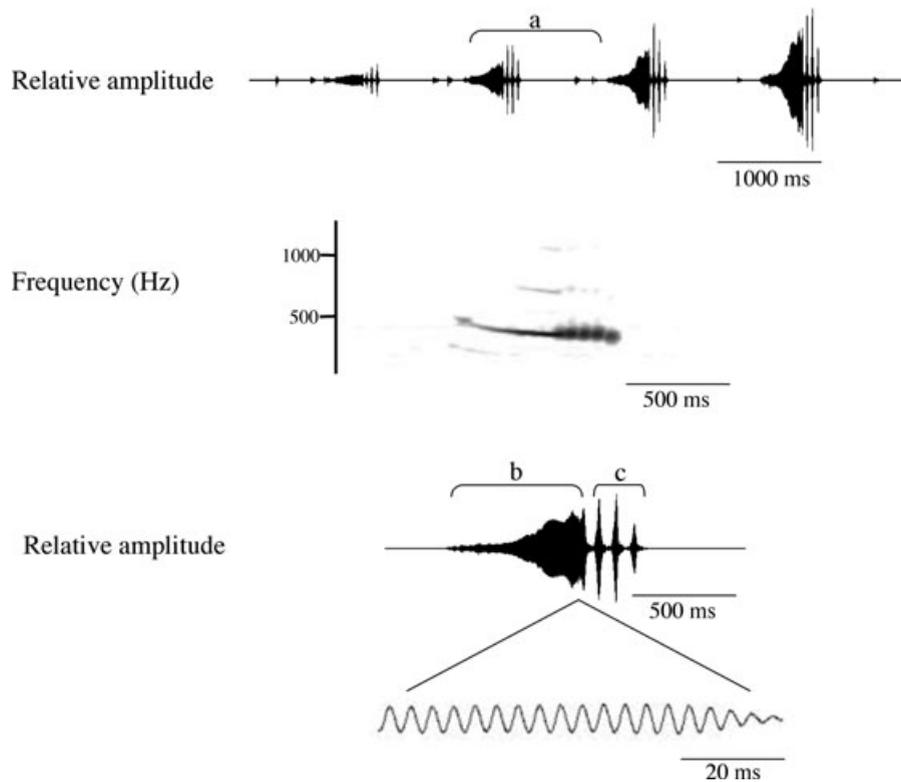


Figure 1. *Enchenopa binotata* "Ptelea" advertisement signal parameters. Upper trace: waveform of a bout of 4 signals. Middle trace: spectrogram of one signal (whine + pulses). Lower trace: waveform of one signal. (a: signal period; b: whine; c: pulses).

amplifier connected to an RCA loudspeaker and a Hameg HM 203–7 20 MHz oscilloscope. Males that did not signal within 10 min were not used; in addition, note that in 2005, logistics did not permit recording of all males before the start of the season (i.e., the signals of some of the males used in the experiment in the first year were not recorded). The recording room was maintained at $26 \pm 1^\circ\text{C}$. Once a male's signal was recorded, the male was weighed with an analytical balance (Ohaus SL Adventurer, Pine Brook, NJ) and marked with Apple Barrel[®] Gloss acrylic paint. All males and females were given unique color combinations by applying a series of dots to the pronotum, to allow us to monitor individual location and mating behavior.

SIGNAL ANALYSIS

Male signals were analyzed using SoundEdit version 2 (Macromedia, Inc., San Francisco, CA). The advertisement signal is a frequency-modulated whine, followed by a series of short pulses (Fig. 1). Males produce vibrational signals in bouts of 2 to 20 signals. Signals early in the bout are lower in amplitude and differ slightly in temporal parameters from later signals. In light of this change in the signals over the course of the bout, as well as extensive variation in bout length among males, we chose to measure the fourth signal of the signaling bout for all males to provide a comparable landmark for all male signals (see Sattman

and Cocroft 2003). Measurements were made as in Cocroft et al. (2010). Temporal parameters were measured from waveforms and included whine length, number of pulses, pulse rate, signal period, and the number of signals per bout (Fig. 1). The signal is dominated by a single pure tone, allowing us to make measurements of the signal frequency from a waveform by counting the number of cycles per unit time. Signal frequency was measured from 10 cycles at the end of the whine, the point of highest amplitude in the signal.

EXPERIMENTAL DESIGN

In both years, 10 outdoor screen enclosures ($6 \times 6 \times 6$ ft) were stocked with the females and recorded males. To generate a comparable range of variation in each treatment, we equalized the number of individuals rather than the number of replicates. The other alternative (to use the same number of replicates per treatment but place more host plants in larger cages to create low densities) would sacrifice control over density because the insects were free to move within the cages. Eight enclosures contained 10 males and 10 females, and two enclosures contained 40 males and 40 females. There were thus 80 males and 80 females assigned to each treatment. Inside each enclosure were three potted host plants (*Ptelea trifoliata*) approximately 50 cm in height. To facilitate observations, plants were elevated 1 m above the ground

using a wire frame. Plants were watered every second day. The resulting density of treehoppers per host plant (6.7 for low density, 26.7 for high density) falls in the range of natural variation (results from one survey of a local population indicated a range of 2–26 individuals/host plant; at other times we have observed >100 individuals on a given plant; L. Sullivan-Beckers, unpubl. data). All individuals were randomly assigned to enclosures, with approximately a third of those assigned to a given enclosure placed on each host plant. Once all cages were stocked, we waited 24 h before beginning observations. Individuals were able to move freely once inside the cages. From the time the juveniles were collected to the end of the experiment, the insects were kept outside under natural conditions, with the exception of the brief period in the laboratory for weighing and recording.

We located each individual in the enclosures every 90 min during daylight hours (approximately 0800–1900 h). Copulations last between 90 and 180 min (L. Sullivan-Beckers, pers. obs.), so our monitoring scheme ensured that most copulations were recorded. During each observation period individuals were located, checked for mating activity, and their position on the plant recorded (the plants were marked with Revlon fingernail polish in 5 cm increments). Occasionally, individuals flew to the wall of the enclosures; if they were found on the cage walls for two consecutive observation periods they were manually replaced on the nearest host plant to avoid desiccation.

The experiment included 160 males in each summer, but males that died before the first mating of the season were not included in the analysis (the first copulations were observed in mid-June, approximately one month after adult eclosion). Also, in 2005, we were only able to record 98 of the 160 males before the start of the experiment; note that this was due to logistical reasons and there were no systematic differences between the males that were recorded and those that were not. Observations were ended once 95% of the surviving females had mated, or when the start of the academic year precluded full-time observations.

CHARACTERIZING SELECTION ON SIGNALS

Selection gradients for both linear (β) and nonlinear (quadratic) selection (γ) were calculated to examine the net and direct selection on the signal traits (Lande and Arnold 1983). Quadratic regression coefficients were doubled to estimate stabilizing/disruptive selection (Stinchcombe et al. 2008). All signal parameters were first log-transformed and standardized within year. The selection gradients were calculated using partial regression coefficients and significance testing was done using a logistic regression, as in Fairbairn and Preziosi (1996) and LeBas et al. (2004). The model included both linear and nonlinear forms of each signal parameter. The number of mates a male obtained over the course of the breeding season was the ordinal-dependent variable. Other terms included in the model were density treatment, enclosure

(nested within treatment), survival (linear and quadratic), and weight (linear and quadratic). Interactions between variables and between variables and treatment were also examined. To qualitatively assess differences in selection over time, we analyzed the data separately by year.

To visualize the relationship between signal traits and male mating success, we used cubic splines (Schluter 1988; Schluter and Nychka 1994), which more accurately reflect the true relationship between trait variation and fitness than simple linear or quadratic coefficients (Brodie III et al. 1995). For each spline the relationship between signal characteristics and the number of mates was determined ± 1 standard error. The standard error is based on 1000 bootstraps with the program created by D. Schluter (www.zoology.ubc.ca/~schluter/lab.html). If a trait did not differ in its relationship to mating success between density treatments, then treatments were pooled within year.

PART II: QUANTITATIVE PREDICTIONS FROM PREVIOUS STUDIES

Potential sources of selection were quantified in separate studies. Female preferences were investigated using playback of synthesized advertisement signals that varied in one signal parameter at a time (Rodríguez et al. 2006). A receptive female alternates her vibrational signals with those of the male, and the presence or absence of this duetting response provides an index of female preference. Most of the male signal traits tested influenced female responses, with preference curves varying in shape and strength (Fig. 2A).

The influence of male–male competition on signal evolution was investigated in experimental mating trials in which two males were placed on a plant with a receptive female (Supporting Information). Males engage in physical contests during which two signaling males will stand on the side of the female and jostle each other. Eventually one of these males will leave the female. The male that remains with the female is considered the winner, as this male usually copulates with the female. The outcome of male–male competition was correlated with variation in some of the same traits that influenced female preferences, although the shape of the relationship usually differed (Fig. 2B).

For animals using plant-borne vibrations to communicate, selection from the signaling environment arises from the properties of host plant tissues. Plant stems act as frequency filters for the vibrational signals traveling along them (Michelsen et al. 1982). McNett and Cocroft (2008) measured frequency attenuation curves for *Ptelea trifoliata*, and their results suggest that across the range of frequencies used by males of *E. binotata* “Ptelea,” higher-frequency signals will transmit more efficiently (Fig. 2C). Accordingly, if filtering by the substrate is a source of selection on male signals, it should impose directional selection favoring higher frequencies. We do not think that substrate

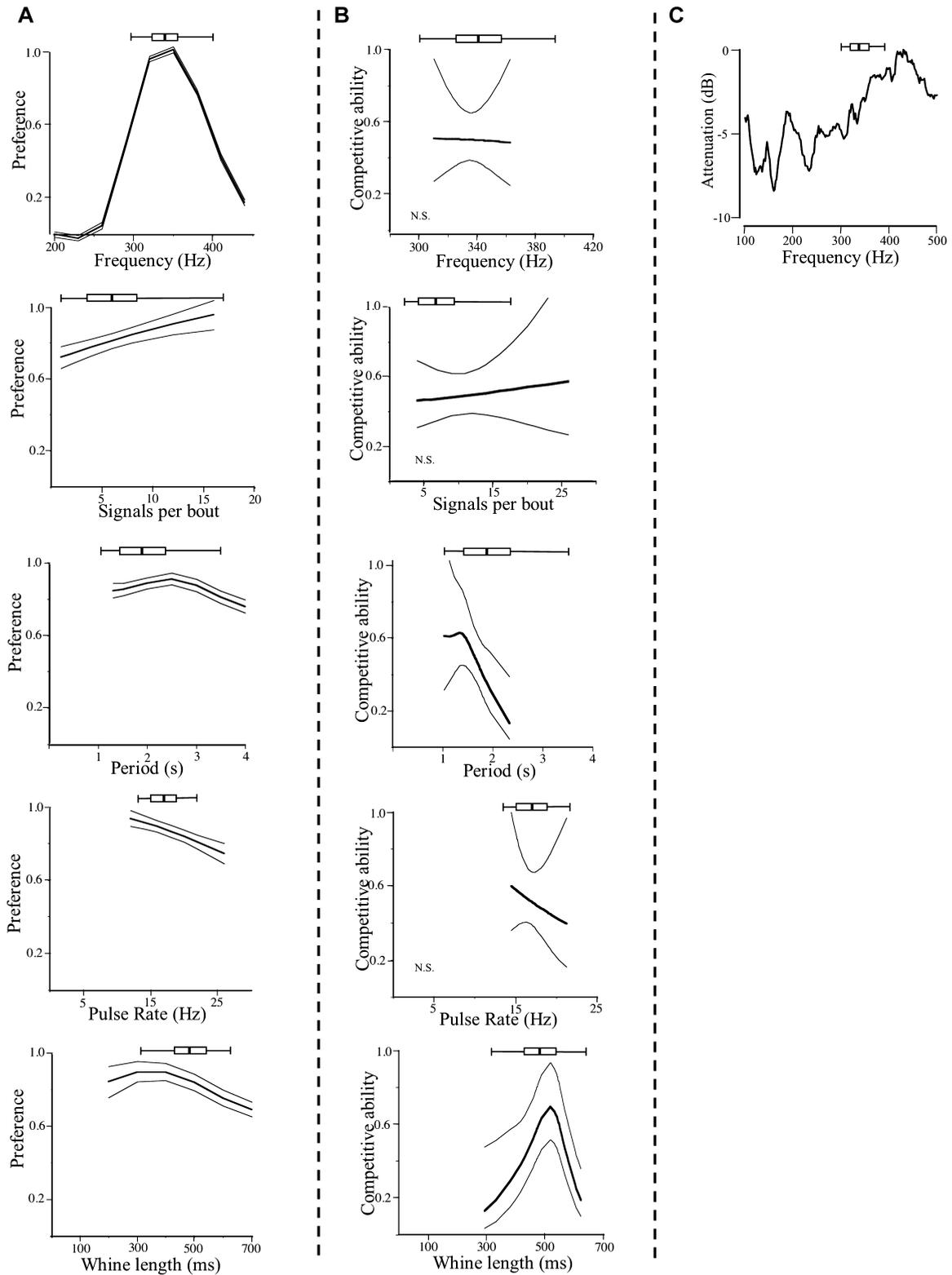


Figure 2. Characterization of three potential sources of selection. Curves show correlation of signal traits with (A) female preferences (shown as cubic splines ± 1 SE modified from Rodríguez et al. 2006), (B) male–male competition (shown as cubic splines ± 1 SE from Supporting Information), and (C) plant filtering properties of signal traits (shown as signal attenuation curve from McNett and Cocroft 2008). Male trait variation is shown above each panel; the horizontal line designates trait range, thick vertical line represents the population mean, and the white box represents ± 1 standard deviation.

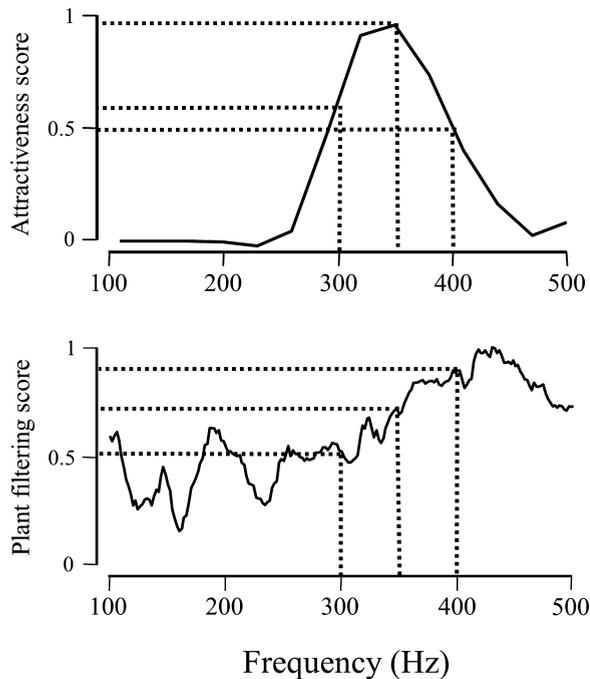


Figure 3. Illustration of how male frequency was scored for two sources of selection. Upper panel: male frequency score for female preference. Lower panel: male frequency score for plant filtering properties. Dashed lines show scores for males with signal frequencies of 300, 350, and 400 Hz.

properties exert strong selection on the temporal features of *Enchenopa* signals, such as signal duration, because our recordings on natural substrates reveal little signal degradation from reflected waves.

We developed a quantitative method to assess the match between the measured relationship of male traits and fitness, and the relationship predicted by each potential source of selection. The rationale of this approach is as follows. If female preference for signal frequency, for example, is exerting selection on male signals, then the relative attractiveness of the frequency of a male's signal should predict his mating success. A male's attractiveness on the basis of his signal frequency can be given a score between 0 and 1, based on the value of the female preference function (scaled to a maximum value of 1) at that frequency. Each male receives an attractiveness score for frequency, and these scores are used to predict mating success in a logistic regression (see Fig. 3 for an illustration). If attractiveness scores predict mating success, this would provide evidence that female preferences are exerting selection on male signal frequency, and similar reasoning applies to the other potential sources of selection.

Signal traits were examined individually, as there was no evidence of correlational selection (see below). As an example, for signal frequency we include two potential sources of selection: female preferences and plant filtering (signal frequency was un-

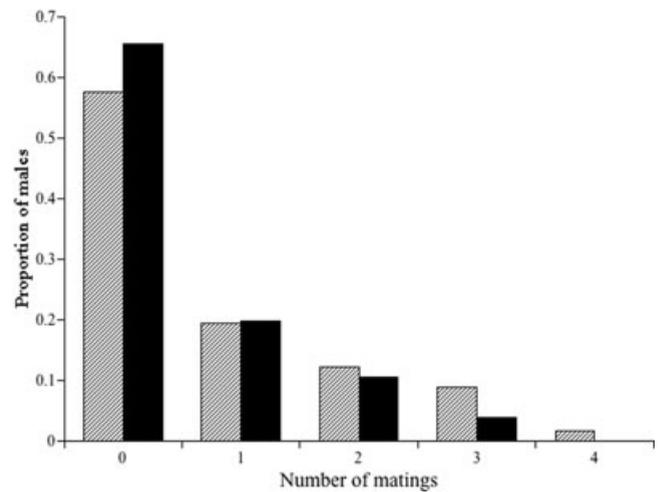


Figure 4. Frequency histogram showing male mating success in each year. (striped bars = 2005, black bars = 2006).

related to the success of males in contests). The model included the control variables in our previous multivariate models (treatment, cage, weight, and survival), as well as an "attractiveness" score and a "transmission efficiency" score. We also examined the statistical interactions between predictors.

Results

MATING BEHAVIOR

In 2005, 101 matings were observed over 45 days. In 2006, 82 matings were observed over 55 days. Of the 183 matings observed, 7 were second matings; thus of the 176 females that were observed mating, 96% mated once and 4% mated twice. The proportion of mated versus unmated females differed between years (Bartlett test, $F = 4.46$, $P < 0.05$), due to a combination of female mortality (both years) and females that did not mate by the end of the study period (2006). An average of 2.27 ± 3.15 (SD) females mated daily in 2005, and 1.47 ± 1.67 (SD) females mated daily in 2006. There was no difference between density treatments in the number of matings (ANOVA, 2005: $F = 0.9029$, $P = 0.345$; 2006: $F = 0.6451$, $P = 0.4232$). Most males never mated (57.3% in 2005 and 63.6% in 2006; see Fig. 4).

CHARACTERIZING SELECTION ON SIGNALS

Each signal trait measured was a direct target of selection in at least 1 year (Tables 1 and 2). The relationships between signal traits and fitness are shown in Figure 5. There was no effect of density on the relationship between signal traits and fitness, except for two traits (signals per bout and pulse rate) in 2005.

There were no significant differences in trait variance between treatments for 2005 (Levene's test for homogeneity of variance: all $P > 0.2$), whereas for 2006 there were significant

Table 1. Logistic regression evaluating the relationship between male traits and mating success. Likelihood ratio test results are displayed with bold font indicating $P < 0.05$. Significant trait interactions with the density treatment were only observed in 2005, and so are not included in the 2006 model.

	Linear	Quadratic
2005		
Survival (days)	45.035	15.768
Weight (g)	4.941	1.101
Signals per bout	8.246	2.418
Period (msec)	0.003	4.637
Whine length (msec)	2.193	1.252
Pulse rate (msec)	0.529	0.002
Frequency (Hz)	8.663	3.226
Signals per bout \times Density	0.05	8.095
Pulse rate \times Density	0.034	11.59
2006		
Survival (days)	38.167	9.789
Weight (g)	1.569	0.005
Signals per bout	0.202	0.469
Period (msec)	0.305	3.828
Whine length (msec)	4.262	0.064
Pulse rate (msec)	5.126	0.104
Frequency (Hz)	26.615	21.529

(or near-significant) differences in trait variance for frequency ($P < 0.05$), whine length ($P < 0.05$), and pulse rate ($P = 0.06$). For 2006, these differences did not translate into differences in the form of selection between the treatments (i.e., there were no density effects on the form of selection); and in 2005, inspection of the data suggests that the shape of the relationship between trait variation and mating success is sensitive to the absolute range of trait variation (see below), even when this difference is not reflected in statistically significant heterogeneity of variance.

Frequency

The relationship between signal frequency (Fig. 5A) and mating success was nearly flat in 2005. In 2006, the relationship was quadratic, with the highest mating success associated with signal frequencies above the population mean, indicating directional, although not open-ended, selection.

Signals per bout

The number of signals in a bout (Fig. 5B) had a positive, linear relationship with fitness in 2005, indicating directional selection for longer signal bouts. Although there was a significant effect of density treatment on the relationship between signals per bout and fitness, this likely arises from differences in the range of variation between treatments (see Fig. 5B; LD range 3–13 signals per bout,

Table 2. Selection gradients; β_1 , univariate linear selection gradients; γ_1 , univariate nonlinear selection gradients; β_2 , multivariate linear selection gradients; γ_2 , multivariate nonlinear selection gradients for male signal traits. Bold font indicates $P < 0.05$.

	β_1	γ_1	β_2	γ_2
2005				
Survival (days)	0.352	0.127	0.078	0.002
Weight (g)	0.004	0.000	0.050	0.003
Signals per bout	0.073	–	–0.114	–
<i>Low density</i>	–	0.006	–	0.004
<i>High density</i>	–	0.019	–	0.041
Period (msec)	0.012	0.001	0.148	0.022
Whine length (msec)	0.005	0.000	0.024	0.001
Pulse rate (msec)	0.012	–	0.009	–
<i>Low density</i>	–	0.002	–	0.049
<i>High density</i>	–	0.006	–	0.086
Frequency (Hz)	0.001	0.002	–0.184	0.033
2006				
Survival (days)	0.174	0.031	0.187	0.005
Weight (g)	0.005	0.000	–0.036	0.001
Signals per bout	0.001	0.000	–0.080	0.004
Period (msec)	0.035	0.001	0.046	0.002
Whine length (msec)	0.000	0.000	–0.019	0.000
Pulse rate (msec)	0.009	0.001	0.033	0.001
Frequency (Hz)	0.077	0.008	0.222	0.049

HD range 3–17 signals per bout). In both treatments, longer signal bouts are associated with higher mating success, and within the range common to both treatments, the curves agree closely.

Signal period

Signal period (Fig. 5C) appeared to be under directional selection for shorter periods in both years.

Pulse rate

There was a quadratic relationship between pulse rate (Fig. 5D) and fitness in the high-density treatment in 2005 and in the pooled treatments in 2006, with a peak at approximately the mean value, indicating stabilizing selection. Although there was a statistical interaction between pulse rate and density treatment in 2005, this may be due to an absence of males with higher pulse rates in the low-density treatment (LD range 14.0–19.9 pulses/sec; HD range 14.1–22.1 pulses/sec).

Whine length

For whine length (Fig. 5E), values above the mean were associated with lower fitness in both years. The relationship for values below the mean was not consistent between years, and the overall picture suggests stabilizing selection in 2006 and directional selection for lower values in 2005.

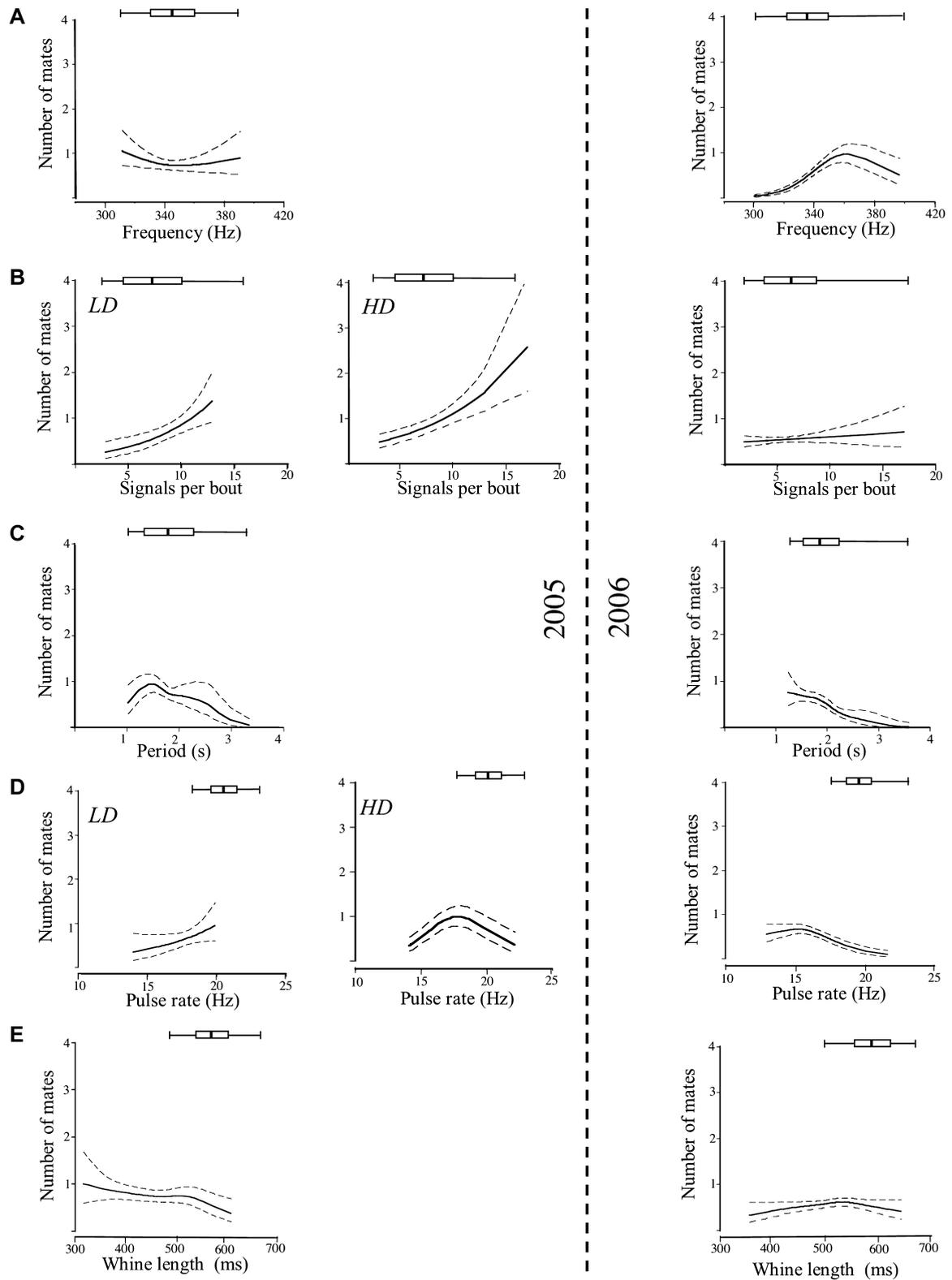


Figure 5. Relationship between male signal traits and mating success, estimated using cubic splines (± 1 SE). Male trait variation is shown above each panel; the horizontal line shows the range, the thick vertical line represents the population mean, and the box represents ± 1 standard deviation. HD, high-density treatment; LD, low-density treatment.

Table 3. Correlation between survival and signal traits. Only frequency showed a significant relationship to survival after correcting for multiple comparisons, and only in 2006 ($P < 0.05$, indicated by bold font). All other coefficients were not statistically significant ($P > 0.1$).

	2005	2006
Signals per bout	0.131	-0.018
Period (msec)	-0.111	-0.108
Whine length (msec)	-0.023	-0.006
Pulse rate (msec)	0.101	-0.072
Frequency (Hz)	0.143	0.161
Weight (g)	-0.047	0.009

SURVIVORSHIP AND SIGNAL VARIATION

Signal frequency was positively correlated with survival in 2006 (Table 3). In 2005, the relationship of frequency and survival was similar in slope, but not statistically significant. No other signal traits were significantly correlated with survival.

IDENTIFYING CAUSES OF OBSERVED SELECTION

Potential sources of selection on male signals are shown in Figure 2. The only potential source of selection that significantly predicted male fitness was female preference. Preference curves largely matched the fitness curves for the number of signals per bout (2005) and signal frequency (2006) (Table 4). Given the range of male trait variation, female preferences are stabilizing or slightly directional for frequency, and directional for the number of signals in a bout. (Table 4). There was a nonsignificant trend suggesting that male–male competition may have contributed to the relationship between whine length and mating success in 2006 (Table 4). For signal traits for which more than one predictor applied (e.g., frequency), there were no significant interactions (all $P > 0.17$).

We found no evidence that male–male competition played a greater role in mating success in high-density enclosures.

Discussion

The vibrational mating signals of *Enchenopa* treehoppers are important in competition for mates: every signal trait measured was significantly correlated with lifetime mating success in at least 1 year of this study. Mate competition was intense, as only one or two females were receptive to mating on any given day of the breeding season, and a large proportion of males did not mate. We used quantitative predictions from three potential sources of selection on signals (female preferences, male–male competition, and the signal environment) to identify the source of realized selection. We identified female preferences as a source of selection on male signals. Many studies have suggested the importance of

Table 4. Prediction of mating success based on different potential sources of selection; female preference curves were a significant predictor of the relationship between mating success and signals per bout (2005) and frequency (2006). There were nonsignificant trends indicating a possible relationship between male–male competitive ability, whine length, and mating success (2006); and female preference, signal period, and mating success (2006). P -values from logistic regression (likelihood ratio tests) shown with bold font indicating statistical significance. Missing values indicate that the source of selection was not predicted to influence that signal trait (see text). There were no significant interactions between potential sources of selection in their effect on the relationship between trait variation and mating success.

	Female preference	Male–male competition	Plant transmission
2005			
Signals per bout	0.017		
Period (msec)	0.608	0.949	
Whine length (msec)	0.870	0.979	
Pulse rate (msec)	0.964		
Frequency (Hz)	0.625		0.918
2006			
Signals per bout	0.782		
Period (msec)	0.081	0.134	
Whine length (msec)	0.242	0.072	
Pulse rate (msec)	0.412		
Frequency (Hz)	<0.001		0.182

female preferences in signal evolution (reviewed in Gerhardt and Huber 2002; Searcy and Nowicki 2005), but few have provided direct evidence that female preferences exert selection on male signals under realistically complex conditions. Male–male competition appeared to predict the pattern of mating success and whine length in 1 year of the study, but the relationship was not significant.

We did not detect selection on male signals arising from host plant transmission properties, alone or via interaction with female preference curves. In the study species, most signaling occurs over short distances and host plant effects on signal transmission are relatively weak (McNett and Cocroft 2008), and this may make detecting selection difficult. In any case, although plant structure ensures that some signals will transmit with less attenuation than others, the efficacy of a given signal will be influenced not only by the filter imposed by the plant, but also by that imposed by female preferences. Ultimately, for male signal frequency to evolve to closely match the frequency that transmits best in their host plant (as occurs in the *E. binotata* species on *Cercis*; McNett and Cocroft 2008), female preferences must also evolve to be “tuned” to the host, perhaps as a consequence of

benefits to females of enhanced detection and assessment of male signals.

Eavesdropping predators can shape signal evolution (reviewed in Zuk and Kolluru 1998; Haynes and Yeorgan 1999), and vibrational signals are likely to be important cues to invertebrate predators (Cocroft and Rodríguez 2005; Roberts et al. 2006; Laumann et al. 2007). However, we have no information about vibrationally orienting predators of *Enchenopa*, and predation was excluded as a source of selection in this study.

The relationship between signals and fitness is sensitive to environmental variation, as reflected in year-to-year differences in the suite of traits under selection. For example, female *E. binotata* have a very strong preference for male signals whose frequency is near the population mean (Rodríguez et al. 2006), and in the second year of the study this preference was an important influence on male mating success, whereas in the first year it was not. The aspect(s) of the environment causing these differences are unknown; one candidate is a prolonged drought that occurred during 1 year of the study (2005). In this year, survivorship may have been particularly important. The only signal trait significantly correlated with survivorship is frequency (males with higher-frequency signals have longer life spans), and during the drought year males with higher-frequency signals had greater mating success than males with average-frequency signals. In contrast, during the other year we found that the relationship of signal frequency to mating success was largely explained by females preferences for signals with frequencies close to the population mean. Temporal variation in the traits that characterize successful males has important implications. In particular, such variation will contribute to the maintenance of additive genetic variation in male signal traits (Lehtonen et al. 2010).

Hunt et al. (2009) consider the likely outcomes when more than one mechanism of sexual selection acts on the same trait. In the present study, there was only one trait (whine length) for which the previous studies (Rodríguez et al. 2006 and Supporting Information) showed that female choice and male–male competition were both significantly influenced by trait variation. Here, there was selection on whine length in 1 year of the study, and a non-significant trend suggesting that male–male competition was the source of that selection. Because the two mechanisms favored different optima, it is possible that the effects of female preferences were countered by male–male competition, although we found no statistical evidence of such an interaction. Detecting the action of potential selective mechanisms with opposing effects will in some instances be impossible without experimental manipulation, because the lack of a relationship between trait variation and fitness could result because both mechanisms were important in nature or because neither was important.

There have been numerous studies, especially in anurans, in which female preferences are the inferred source of selection after

comparing patterns of mating success in the field with results of experimental playbacks to females (e.g., Ryan et al. 1990; Grafe 1997). In these studies the mating signals, or a correlated trait such as body size, and male mating success are quantified in the field. In a separate experiment, female preferences are described under controlled conditions. The two datasets are then qualitatively compared to determine the role of female preferences in signal evolution. These studies (e.g., Wagner and Sullivan 1995) provide some support for the hypothesis that female choice exerts selection on male signals in the field. However, the predictions are qualitative. A quantitative approach like that taken here allows us to take greater advantage of our knowledge of potential sources of selection, because the precise form of, for example, a preference function can be compared to the fitness curve. A close match between the two curves makes it unlikely that some other, unmeasured form of selection determined the relationship. A quantitative approach also allows one to statistically test the influence of multiple sources of selection and their interactions.

Selection on mating signals has recently been estimated using canonical rotation analysis (Brooks et al. 2005; Bentsen et al. 2006; Hall et al. 2008), which estimates the intensity and form of nonlinear selection on a suite of correlated traits (Philips and Arnold 1989; Blows and Brooks 2003). Although this approach increases the likelihood of detecting nonlinear selection by searching for maximum differences in fitness across multivariate character space, it does not allow one to identify the form, direction, or intensity of selection on particular signal parameters of interest. Although selection rarely acts on traits in isolation (Lande and Arnold 1983; Philips and Arnold 1989; Schluter and Nychka 1994), individual signal traits such as frequency are important in divergence and reproductive isolation in the *E. binotata* complex (e.g., Rodríguez et al. 2006; Cocroft et al. 2008). One of the aims of our study, therefore, was to distinguish which sources of selection were acting on specific signal parameters, and whether they were acting in the same or opposite direction.

This is the first study of selection on mating signals to measure lifetime mating success, as opposed to more short-term proxies of fitness. Measuring mating success over an individual's lifetime is difficult for most vertebrate species, as they are typically long-lived and able to move considerable distances. And as noted by Greenfield (2002), there is very little research showing accurate estimates of lifetime mating success of arthropods in natural populations, due to the difficulty of following such small animals over their lifetime. Obtaining a measure of fitness is made possible in *Enchenopa* by the lack of postcopulatory sexual selection (females mate only once); the high host fidelity and low vagility that allows individuals to be observed repeatedly; and the relatively short life span of these small insects, which have only a single breeding season. The most important insight arising from the use of lifetime data is that the strongest predictor of male

mating success is survivorship. There is a single cohort of same-aged individuals in a given season, so the relationship between survivorship and mating success does not lie in age-based mate choice or in an increased competitive ability of older males (see Brooks and Kemp 2001 for a review). A similar pattern is found in many other animals, namely, that individuals that live longer may simply have more opportunities to mate (Andersson 1994). In addition, in the *Enchenopa* system, selection for increased life span likely results in part from a change in the operational sex ratio that contributes to the success of long-lived males: the number of females mating per day remained relatively constant, whereas the number of surviving males decreased. In any case, the mating system of these insects leads to strong, directional selection on male life span.

By measuring lifetime mating success, we also gained insights into the role of male survivorship in signal evolution. Any signal trait correlated with survival would be indirectly favored by selection for longevity. In this study, males with higher frequencies tend to have longer life spans. This is significant because signal frequency is also under selection from female preferences, and survival would select for males with higher frequencies, whereas females select for males with intermediate frequencies.

The method we used to tease apart the relative contribution of sources of selection acting on *Enchenopa* signals represents a useful addition to existing methods for identifying sources of sexual selection. This method will be useful whenever sources of selection cannot be isolated experimentally, or when researchers are interested in allowing the sources of selection to interact. The only requirement is that there be a quantitative characterization of the sources of selection.

The finding that sexual selection is an important cause of signal evolution in a member of the *E. binotata* species complex, in conjunction with comparative evidence of signal and preference divergence (Rodríguez et al. 2006; Cocroft et al. 2010), suggests that we need to revise our understanding of speciation in this group. The *E. binotata* species complex is one of the two most frequently cited examples of speciation initiated by host shifts (Coyne and Orr 2004), with reproductive isolation among populations on different hosts arising from differences in the timing and location of mating (reviewed in Wood 1993). However, the isolation caused by allochrony and host fidelity is incomplete, and interactions do occur between different host-associated species during the breeding season (reviewed in Cocroft et al. 2008). Wood (1980) found that experimentally induced interactions between males and females from different hosts seldom led to mating, for reasons that are now clear: strong sexual selection on male signals, coupled with divergence of signals among host-associated species (Cocroft et al. 2010), leads to female preference for conspecific males (Rodríguez et al. 2004). We therefore suggest that although speciation in the *E. binotata* complex is initiated

by the ecological isolation arising from a host shift, the evolution of mating signals and preferences is a critical component of the process. Signal frequency is a key trait: variation in frequency is strongly associated with host use (Cocroft et al. 2010); the current study shows that it can be under strong selection from female choice, and comparative studies (Rodríguez et al. 2006) suggest the likelihood of divergent selection on signal frequency between populations on different host plants. The evolutionary forces leading to divergence in both male signal frequency and female frequency preference are likely to be complex: differences in host plant transmission properties can favor divergence in signal frequency (McNett and Cocroft 2008), and the current study shows both that evolutionary change in signal frequency could be influenced by its relationship to male longevity, a strong predictor of male mating success, and that selection on signal frequency varies between years. The extended mating period in *E. binotata*, which in this study led to strong selection on male life span, has the potential to facilitate local adaptation in the event of a host shift (Lorch et al. 2003), because males that survive well on the new host will obtain more mates. In sum, studies of *E. binotata* and other insect herbivores (Claridge 1995; Funk et al. 2002; Nosil et al. 2007; Etges and Tripodi 2008) strongly suggest that understanding speciation in specialized phytophagous insects will often require studying ecology and sexual selection in concert.

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Supporting Information

The following supporting information is available for this article:

Appendix S1. Male-male competition as a cause of selection on signals.

Supporting Information may be found in the online version of this article.

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