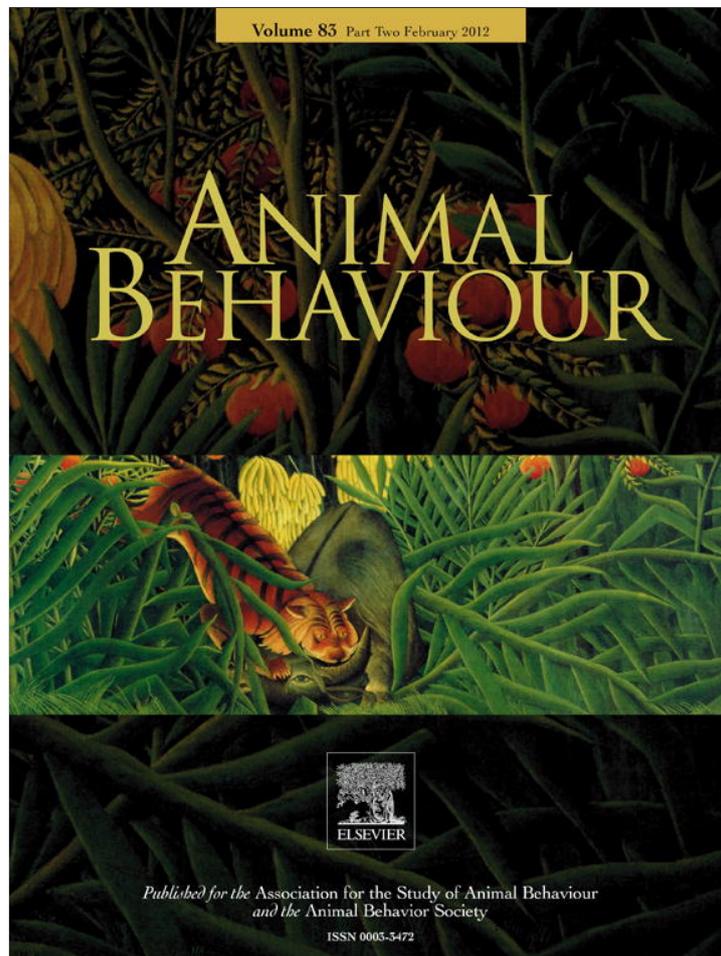


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Competitive masking of vibrational signals during mate searching in a treehopper

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Mate localization in insects that use acoustic, vibrational or visual displays often involves a male–female duet. Female signals provide cues not only to the duetting male, but also to competitors that may attempt to disrupt the duet. Mate localization based on substrate vibrations may be especially vulnerable to such disruption, because localizing vibrations is difficult, and mating success often depends on localization efficiency. We tested the hypothesis that a specialized signal produced by male treehoppers, *Tylopelta gibbera*, disrupts competitors' duets. This hypothesis predicts that the signal occurs during competition to locate a female, and that it decreases the frequency of female responses. We first characterized the search paths of single males duetting with a female. Males walked along host plant stems, frequently stopping and producing signals that elicited female replies. Males made forward/reverse decisions only after the female responded and their accuracy decreased with distance from the female. We then characterized the behaviour of two males duetting with the same female. Single males never produced the putative disruption signal, but pairs of males frequently did, timing it to overlap with the rival's signal. The overlapping signal strongly decreased female responses, both during natural signalling interactions and in response to playbacks. Males took longer to localize the female in the presence of a competitor. The overlapping signal apparently functions to reduce the directional information available to competing males, probably through signal masking. This is one of the few experimental demonstrations of a specialized signal whose function is to disrupt communication.

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In many animal mating systems, males actively search for females (Shuster & Wade 2003). During the search, decisions about the direction of movement may be influenced by environmental cues such as breeding resources, or by direct cues of female location. In some species, receptive females produce 'broadcast' signals such as pheromones, which can influence the behaviour of multiple males over a large area (Svensson 1996). Alternatively, in species in which searching males produce advertisement signals, females may produce more 'narrowcast' signals, following immediately after the male's advertisement signal (e.g. Heller 1990). Such signal-and-response or 'duetting' systems are widespread in arthropods that communicate using acoustic, vibrational and visual modalities (Claridge 1985; Bailey 2003; Virant-Doberlet & Čokl 2004). Response signals of the stationary individual (usually the female) provide a selective localization beacon for individuals whose signals are sufficiently attractive to elicit a reply.

A duetting female's signals provide cues of receptivity and location not only for the duetting male, but also for eavesdropping

competitors (Bailey 2003). Males that detect a duet may parasitize the exchange by silently locating the female (Mazzoni et al. 2009a), or may attempt to initiate a second duet with the female. In addition, males may attempt to prevent or disrupt a duet by producing signals that overlap with either the rival's advertisement signals (Cooley & Marshall 2001) or the female's response signal (Hammond & Bailey 2003; Mazzoni et al. 2009b). Such overlapping signals can apparently mask the target signal (where 'masking' is defined as a change in the likelihood of perception of one signal in the presence of a second signal; Gelfand 2010). We investigated the use of potential masking signals in an insect in which duetting males find the stationary female by homing in on her plant-borne vibrational signals. Locating the source of a plant-borne vibration is a challenging task, especially for small species for which timing and amplitude differences are minimal between receptors in different legs (Michelsen et al. 1982; Virant-Doberlet et al. 2006). As a result, vibration-guided mate searching may be especially vulnerable to disruption by competitive signalling interactions.

Signal masking is a ubiquitous problem in animal communication, because individuals must often communicate in the presence of noise (Brumm & Slabbekoorn 2005; Bee & Micheyl 2008). However, the problems faced by duetting animals subject to competitive signal masking may be especially severe. In contrast to

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incidental masking sources such as wind or signalling by other nearby species, or even the competitive signal-timing interactions of chorusing species in which multiple individuals are producing advertisement signals (Greenfield 1994), competitive masking signals are presumably under selection to maximize the disruption of communication. Such disruption of courtship duets through signal masking has been proposed for a number of acoustically communicating species, but there have been few experimental tests. In some cases, the masking signal functions to prevent females from responding to an advertisement signal. In a periodical cicada (Cooley & Marshall 2001) and a phaneropterine katydid (Bailey et al. 2006), duetting males have a complex signal in which the last part triggers the female response. In both cases, a second male can reduce the female's probability of responding by producing a different signal that overlaps with the 'triggering' section of the duetting male's advertisement signal (see also Miranda 2006 for a possible example of this strategy in a membracid treehopper). In other cases, the masking signal functions not to prevent the female from replying, but to prevent the duetting male from perceiving the female's reply. In a leafhopper (Mazzoni et al. 2009a, b), males that perceive a duet produce a broadband signal that overlaps the female's response signals, effectively disrupting the duet. In a phaneropterine katydid, the duetting male produces a masking signal immediately after the female's reply, preventing eavesdropping males from perceiving and/or locating the female (Hammond & Bailey 2003). Signal masking during duetting has also been reported in a bird (Tobias & Seddon 2009), but duetting in birds does not serve the same role in mate searching as in insects (Hall 2004); as our focus is on competition during mate localization, we do not consider bird duetting further here.

We studied the function of a possible vibrational masking signal in the treehopper *Tylopelta gibbera* (Hemiptera: Membracidae). In the presence of two males, a receptive female will duet with both males, and mate with the first to arrive. When two or more males are interacting with the same female, each male will frequently produce a distinct signal during the advertisement signal of the other. We tested the hypothesis that this overlapping signal functions to mask the advertisement signal, reducing the female's likelihood of responding. We first characterized the search paths of males in the absence of competition, to assess the role of female signals in mate searching. We then experimentally investigated both the context in which the masking signal is produced and the effectiveness of the signal in reducing female responses.

METHODS

Study Species

Tylopelta gibbera is a small treehopper (total length 3–4 mm) occurring in the U.S.A. from the southeast to the southwest, and also in Mexico and Guatemala (Kopp & Yonke 1973; Strümpel 1974). The only known hosts of *T. gibbera* are herbaceous plants in the genus *Desmodium* (Fabaceae; Kopp & Yonke 1973), and in Missouri *T. gibbera* is a common species in open and edge habitats. There are at least two generations of *T. gibbera* per year in Missouri, and after the first individuals appear in late May the insects' phenology is sufficiently asynchronous that adults and nymphs are present throughout the summer and early autumn.

The male advertisement signal of *T. gibbera* consists of a series of low-frequency pulses, a frequency-modulated 'whine', and another series of pulses at a higher carrier frequency (Fig. 1a). Here we focus on the whine and high-frequency pulses, as the initial low-frequency pulses are not always present and have little influence on the female's probability of replying (P. R. Marting & R. B. Ccroft, unpublished data). Both whine and pulses are sinusoidal with most

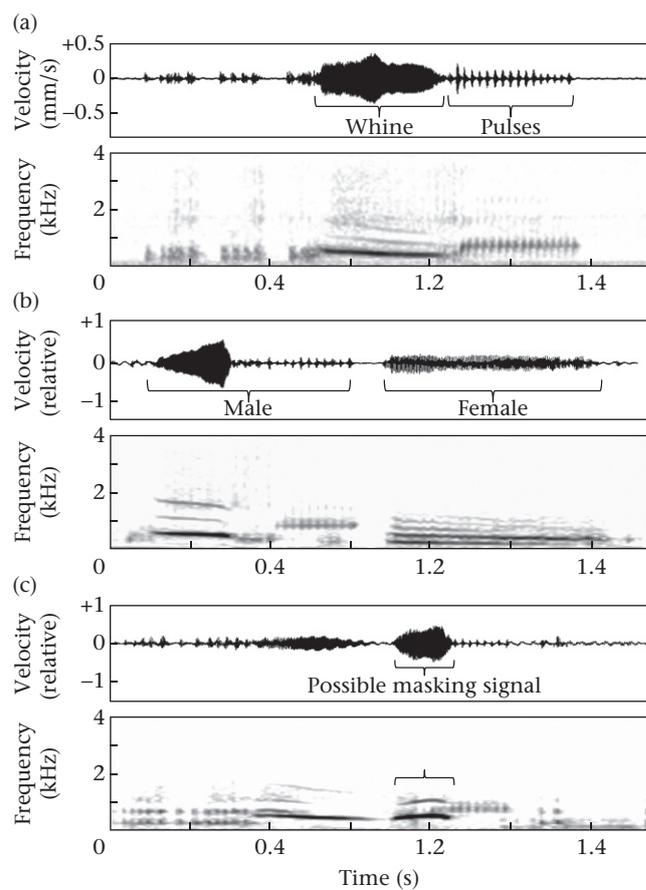


Figure 1. Plant-borne vibrational signals of *Tylopelta gibbera*. (a) Male advertisement signal; (b) male–female duet; (c) male advertisement signal and possible masking signal overlapping with the start of the pulses. Each waveform corresponds to the spectrogram beneath it.

of the energy in the fundamental frequency, and some additional harmonics present at < -10 dB relative to the fundamental. Female response signals are produced within a time window of about 100 ms after the end of the male advertisement signal, and consist of a frequency-modulated harmonic series (Fig. 1b) that is often accompanied by (or followed by) a series of high-pitched clicks. Females will duet with more than one male; males alternate their signalling bouts, and a duetting female will respond immediately after each male's advertisement signals.

The potential masking signal is also tonal, with some frequency modulation (Fig. 1c).

Collection and Maintenance

Individuals used for this study were drawn from a greenhouse colony established with adults and nymphs collected by hand from forest edge and open habitats on and around the University of Missouri campus, Columbia, Missouri in June–August 2008. The experiments described below were conducted from August 2008 to June 2009. The insects were reared on potted host plants (*Desmodium* sp.) enclosed in Bug Dorm rearing cages (BioQuip, Rancho Dominguez, California, U.S.A.), with a day:night cycle of 14:10 h. Nymphs and adults were maintained in separate rearing cages. Teneral adults were moved from the nymph cage every second day, with males and females then maintained in separate cages to ensure that they remained unmated before the experiments (after adult eclosion, sex is easily determined by inspection of the external genitalia). Females will duet with males only during

a window of time preceding their first (and apparently only) mating. Individuals reach sexual maturity about 2 weeks after adult eclosion.

Recording Procedures and Statistical Analyses

Vibrational signals for most experiments were detected with a miniature accelerometer (Knowles BU 1771; 0.28 g; flat frequency response from 20 Hz to 5 kHz) attached with wax to the host plant stem. The accelerometer output was sent to a custom-built operational amplifier, and recorded with Audacity 1.2.6 (<http://audacity.sourceforge.net>) at a sampling rate of 44.1 kHz on a Dell or HP Pavilion dv1000 computer. For the two-males/one-female mating trials described below, signals were recorded with either a laser vibrometer (Polytec CLV 1000 with a CLV M030 decoder module; Polytec Inc., Auburn, MA, U.S.A.) or a shielded piezo film transducer and Laboratory Amplifier (Measurement Specialties, Hampton, VA, U.S.A.). Temperature for the experiments was $25 \pm 2^\circ\text{C}$. Plants were placed on a vibration isolation table to minimize interference from ambient vibrations.

Statistical analyses were conducted using JMP version 5.1 (SAS, Cary, NC, U.S.A.).

One-male/One-female Experiments: Duets and Orientation

To characterize mate-searching behaviour in the absence of competition between males, we placed a male and a receptive female on the same plant. Experiments were conducted using one potted *Desmodium* (approximately 30 cm tall \times 65 cm wide). For each experiment, a receptive female was placed at a position on the plant chosen randomly, as follows: a coin was flipped three times, with the first toss determining whether the female was on the main stem or a side stem, the second determining whether the female was near the top or bottom of the plant, and the third determining (if the female was on a side stem) whether the female was on a stem on the right or left side of the plant. The male was placed at a location on the plant approximately 25 cm from the female (mean male–female distance at the start of the observations = 26.8 ± 5.56 cm). At the beginning of each experiment, a previously recorded duet was played through a loudspeaker to stimulate male calling, and the airborne signals caused sufficient vibrations in the plant stem to elicit signalling. Video recordings were made using a Hi-8 video camera.

Male search paths were characterized in real time by an observer (F.L.) using a 1:1 scale drawing of the plant. Male movement during searches is intermittent: males signal while stationary, and after producing one to five advertisement signals and receiving one or more female responses, males either walk forward or turn around, then stop to signal again. Stopping points were along straight sections of stem rather than at branching points, so choices were one-dimensional (forward/reverse). The observer recorded each sampling location (i.e. the locations where the male stopped walking and produced one or more advertisement signals). Video recordings were consulted as needed to clarify the male's location; however, because *Desmodium* are leafy, branching plants, and the video recording was made from a single direction, the real-time observations provided the most complete data. For each male, we used information on its successive sampling locations to characterize step lengths and accuracy as a function of distance from the female. Movement decisions (forward or reverse after receiving a female's signal) were scored as correct if the male's direction of travel was towards the origin of the female's vibrational signal and incorrect otherwise. Females were stationary while duetting with males. Search paths were described for 14 male–female pairs, with no individual used more than once.

In addition to describing male search behaviour, we recorded all signalling interactions using an accelerometer attached to the stem within 5 cm of the stationary female. Comparison of the signals produced by males in the presence or absence of competition allowed us to determine whether the potential masking signal was produced only when two males were present.

Two-males/One-female Experiments: Competition between Males

Having characterized male searching and signalling behaviour in the absence of competition, we then examined male behaviour when two males were placed on the plant with a female. It was not logistically feasible to obtain the same search path information when there were two males on the plant, so our analysis is focused on production of the masking signals, and on how these influenced female signalling responses. Eight replicates were conducted using the same protocol as that used above in the one-male/one-female experiments, except that two males were placed on the host plant within 1 cm of each other (no individual was used more than once). We used two potted *Desmodium* host plants <50 cm tall; two plants were used because the experiments were conducted over a sufficiently long period that the first plant was no longer suitable.

Male signalling interactions and female response signals were recorded within 5 cm of the female using a laser vibrometer or a piezo film transducer. We estimated the proportion of male signals that were overlapped, and the proportion of overlapped and nonoverlapped signals that elicited a female response.

Male Playbacks: Production of Overlapping Signals

In the experiment with two males and one female, we assumed that the overlapping signals produced during advertisement signals of duetting males were produced by the rival male, and not by the female. However, there are no visual cues available to determine which individual produced which signal (the movements of the abdomen that accompany vibrational signalling in this species are obscured by the opaque wings, and can be observed only from limited angles). Accordingly, we conducted a playback experiment in which a single male was played the signals of a duetting pair. Playbacks were done with 10 males, each receiving a duet constructed using a male advertisement signal and a female response signal; all signals were drawn from our library of recorded signals, and none was used for more than one male. We played back stimuli with a rare earth magnet glued to the stem, opposite an electromagnet driven by a Dell computer and a Radio Shack PA amplifier (as in Rodríguez et al. 2006). Filtering properties of the host plant were estimated by playing a broadband noise stimulus (80–5000 Hz). The filter characteristics of the host plant were then used to create a compensating digital filter using Matlab version 6.5 (Mathworks, Natick, MA, U.S.A.), so that the frequency spectrum of the stimulus matched that of the original recording (see Cocroft & Rodríguez 2005). To obtain an appropriate amplitude, we adjusted the peak acceleration of the signal to that of the same signal when originally recorded. The goal of the playback was to elicit up to 10 overlapping signals, by simulating mate searching under competitive conditions in which the female responds to both males. The male was first played two male–female duets; once the male began producing advertisement signals, it was played a female signal immediately after four or five of its own signals. Thereafter (for up to 10 min), playback of duets alternated with playback of female signals (triggered by the investigator) immediately after the focal male's advertisement signals. We switched between these two kinds of playback every four or five of the male signals.

Female Playbacks: Response to Overlapped Signals

In addition to characterizing female responses to overlapped and nonoverlapped advertisement signals during natural interactions between two males, we conducted a playback experiment with receptive females. For each female ($N = 13$), we used the playback methods described above. As before, exemplars were drawn from our library of recorded signals: each female received one of 13 advertisement signals and one of 13 recorded overlapping signals (with no signal used more than once). To obtain 'pure' overlapping signals (free from any energy from the signal they overlapped), we chose signals that were substantially higher in amplitude than the signal they overlapped. We then used a bandpass filter to remove any energy present in the advertisement signal; this was possible because the energy in the potential masking signal is primarily in the 450–500 Hz range, and it occurs during the pulsed section of the advertisement signal, which has a carrier frequency in the 800–900 Hz range. Each female received 80 signals, half of which were overlapped. Five overlapped signals alternated with five nonoverlapped signals, with the order (overlapped versus non-overlapped first) alternated between females. The timing of signals within a playback series mimicked that of a natural male signal bout structure: signals were grouped into bouts of one or two signals, with 2 s between signals within a bout, 5 s between bouts, and 20 s between each series of 20 signals.

RESULTS

One-male/One-female Experiments: Duets and Orientation

Once a male began signalling and detected the reply of a receptive female (see Fig. 1b), it actively searched within the plant. Males typically walked only a short distance before resampling: 'step lengths' between successive samples had a mean \pm SD of 1.9 ± 0.5 cm (range 0.1–12.0 cm; see Fig. 2 for example search paths). Note that for all descriptive statistics that summarize information for multiple individuals, we first calculated the individual means and then used those means to obtain the overall mean and variation. As is typical for *Desmodium*, the plant was highly branched: as a male traversed a 5 cm section of stem, there was an average of two to four side stems, and males frequently walked onto side stems and signalled.

Most of the males (13/14) located the female, engaged in a brief short-range courtship, and then copulated with the female. One male fell from the stem before locating the female, at which point the trial was ended. Although the initial distance along the stem between male and female was typically less than 30 cm, males travelled up to 180 cm along stems during their searches (mean \pm SD = 114 ± 42.7 cm), stopping to sample 21–93 times (62.6 ± 20.9). It took an average of 10.7 ± 4.7 min for the male to locate the female (range 3.96–17.57 min).

Male directional decisions (i.e. the forward/reverse movement decisions made after sampling) were significantly more accurate than predicted by chance (binomial probabilities, combined using Fisher's method: $\chi^2_{28} = 161.1$, $P < 0.001$); on average, $69.1 \pm 10.8\%$ of movements made after detecting a female signal were in the correct direction. Directional accuracy decreased with distance from the female (logistic regression, likelihood ratio test: $\chi^2_1 = 25.86$, $P < 0.0001$), dropping steeply at distances greater than 30 cm from the female (Fig. 3). There were also significant differences in accuracy between duetting pairs (logistic regression, likelihood ratio test: $\chi^2_{13} = 46.76$, $P < 0.0001$). Accuracy and efficiency (minimum possible distance travelled/observed distance travelled) were positively correlated ($r^2 = 0.81$, $t = 6.96$, $N = 13$, $P < 0.0001$).

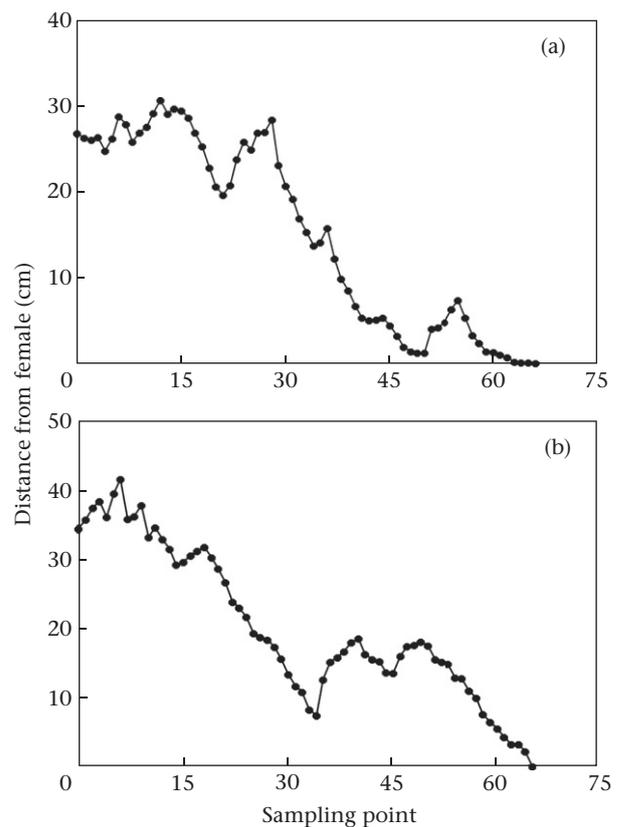


Figure 2. Two representative search paths of duetting *T. gibbera* males searching for a stationary female, in the absence of competition. Movement is intermittent, and 'sampling points' are locations at which males stopped and produced advertisement signals. Both males reached the female after about 10 min; the male in (a) started at a distance of 27 cm from the female, and travelled a total of 85 cm, while the male in (b) started at a distance of 35 cm from the female, and travelled a total of 98 cm.

Two-males/One-female Experiments: Competition between Males

During mating trials with two males and one female on the plant, the female responded to the advertisement signals of both males. It took an average of 18.5 ± 6.1 min for the first male to locate the female, about 1.7 times longer than the time required for single males to locate the female on similarly sized plants. We seldom observed overlapping advertisement signals, but the potential masking signal was produced during up to 82% of advertisement signals (mean \pm SD = $50.4 \pm 24.8\%$). The potential

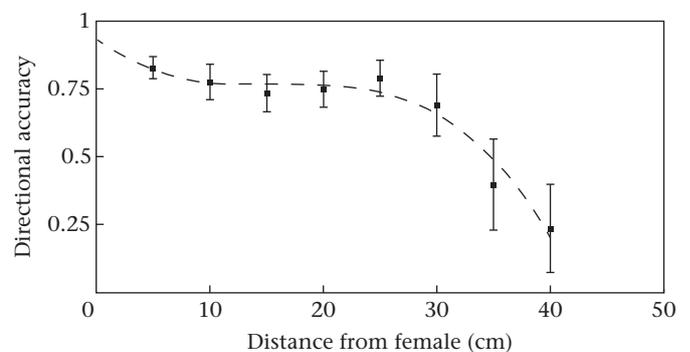


Figure 3. The accuracy of directional decisions by searching *T. gibbera* males in relation to distance from the female (the fitted curve is a third-degree polynomial). Means are shown \pm SD.

masking signal was never detected during the one-male mating trials.

For each two-males trial, we selected 10 overlapped signals and characterized the placement of the potential masking signal in the advertisement signal. Timing was nonrandom, with the masking signal typically produced during the beginning of the pulsed section of the advertisement signal (Fig. 4).

Females responded less frequently to overlapped male advertisement signals than to nonoverlapped signals (Wilcoxon signed-ranks test: $Z = -18.0$, $N = 8$, two-tailed $P < 0.01$; Fig. 5a).

Male Playbacks: Production of Overlapping Signals

Playbacks to males that simulated mate searching in the presence of a competitor (by playing back not only female responses to the male's own signals, but also duets in which the female response followed the signal of another male) elicited overlapping signals from all males ($N = 10$). As in the natural signalling interactions between two males duetting with a female, most ($85.5 \pm 24.5\%$) of the overlapping signals were produced during the pulsed segment of the male advertisement signal.

Female Playbacks: Response to Overlapped Signals

During playback of male advertisement signals, female responses were strongly inhibited by the presence of an overlapping signal (Wilcoxon signed-ranks test: $Z = -45.5$, $N = 13$, $P < 0.01$; Fig. 5b). Females produced $64 \pm 26\%$ fewer responses when signals were overlapped.

DISCUSSION

Mate-searching males of many small insects that communicate using plant-borne vibrations are faced with the difficult problem of locating a vibration source on the complex three-dimensional geometry of a living plant. Determining vibrational directionality is expected to be difficult for small insects, for which the available cues of source direction (amplitude or time differences of the female's vibrational signal at front versus back legs) are minimal (Virant-Doberlet et al. 2006). Our results, which provide the first characterization of the search paths of an insect locating a vibration source on a plant, suggest that vibration localization is indeed a difficult search problem, and one that is made even more complex by the presence of a competitor.

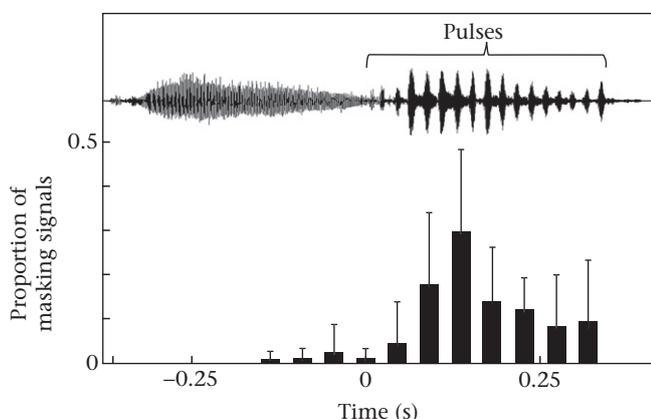


Figure 4. Timing of potential masking signals, relative to the beginning of the pulses (waveform of typical advertisement signal is provided for reference). Means are shown \pm SD.

We first examined the search behaviour of individual male *T. gibbera* duetting with a female, to assess the efficiency of mate searching in the absence of a competitor, and the role of the female's vibrational signals in localization. We do not yet know what cues male *T. gibbera* use to localize female signals. Although amplitude or time differences of the female's signal between front and back legs are negligible (Virant-Doberlet et al. 2006), if the mechanical response of the body to substrate vibration in *T. gibbera* is highly directional, as it is in a related species (Cocroft et al. 2000), then assessment of the direction of wave propagation may be possible. Sequential sampling along an amplitude gradient is another possible localization mechanism. In any case, the difficulty of mate localization for this small species is suggested by the travel distances of males, which walked on average four to five times further along the plant stems than the minimum required to walk directly from the starting point to the female. For example, one male started out 25 cm (ca. 70 body lengths) from the female, but travelled 180 cm (>500 body lengths) before locating her. Our observations suggest three possible sources of inefficiency. First, male search paths reveal a 'hilly' search landscape with multiple local optima, indicated by runs of sequential samples in which the male moves further from the female. We have not detected significant vibrational echoes, so a run of incorrect decisions is unlikely to be based on waves propagating in a misleading direction; possibly males were influenced by local amplitude gradients. Second, localization cues appear to be severely attenuated by distance. The accuracy of male directional decisions (i.e. whether the male travelled towards or away from the source after perceiving the female's signal) is strongly distance dependent: within 30 cm of the female, about 75% of directional decisions were accurate. At distances of 30–50 cm males clearly perceived the female's signals, as evidenced by their duetting behaviour, but their directional accuracy was, on average, no better than random. Note that females responded to virtually all male signals in these single-male trials, so male accuracy was not limited by a lack of female signals. Third, given the male's sampling behaviour, the branching structure of the plant imposes limits on searching efficiency. On host plants of *T. gibbera* (*Desmodium* spp.), a male was likely to encounter two to four side stems for every 5 cm travelled along a stem. Males sampled branching points by walking onto the side stem and signalling, rather than sampling both stems simultaneously. Accordingly, even with perfect directional accuracy, the need to sample multiple branching points requires additional travel beyond the minimum.

Search efficiency and directional accuracy were highly correlated, and varied substantially between male–female pairs: the greater a male's accuracy, the more efficient the search path. We cannot separate out the contributions to this relationship between plant location, female behaviour, male sampling behaviour and male directional accuracy, and so we cannot determine the causes of this variation in search efficiency. However, given the decrease in directional accuracy with increasing distance from the source, it is likely that early decisions can have a large impact on overall search efficiency; for example, if a male happens to begin searching (or to stray) further than 30 or 40 cm from the female, the unreliability of directional cues at that distance is likely to make searching inefficient.

Several lines of evidence underscore the importance of vibrational information for directional decisions during mate localization. First, vibrational duetting between male and female continued throughout the search process. Such exchanges may have multiple functions (e.g. both courtship and localization), but their importance in localization is suggested by the observation that actively searching males walked an average of less than 2 cm (five to six body lengths) between signal exchanges with the

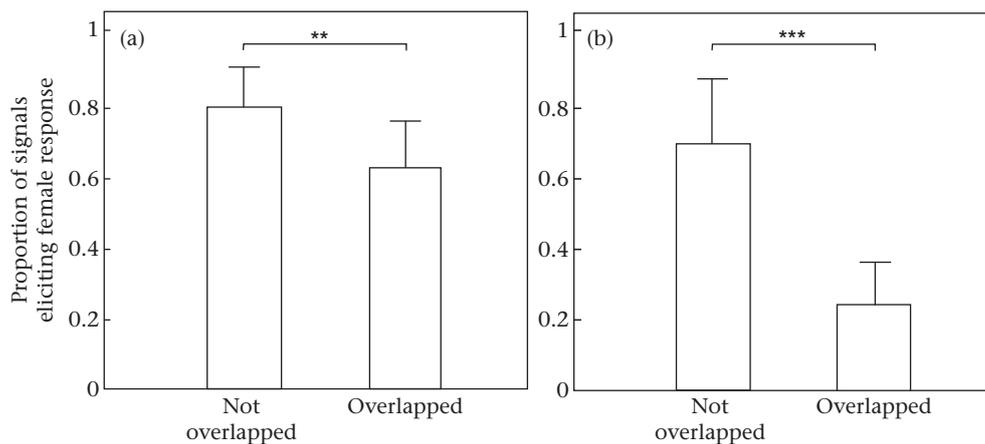


Figure 5. Female responses to advertisement signals that were overlapped by the potential masking signal or were not overlapped, during both (a) natural signalling interactions and (b) playbacks. Means are shown + SD. ** $P < 0.01$; *** $P < 0.001$.

female, and that virtually all changes of direction were made immediately after such signal exchanges. Second, males made more errors when further from the source, where changes in the signal will be greater because of propagation through the plant (e.g. decreases in amplitude, filtering out of some signal frequencies). An increase in accuracy as males draw close to the female could also be explained by the use of visual or chemical cues. However, the line of sight to the female will often be blocked by intervening stems and leaves, so visual cues of female location are probably used only at very close range, when the male is on the same stem as the female (as in periodical cicadas; see Cooley & Marshall 2001). In contrast, directional accuracy was high up to 30 cm from the female, and for most of that distance the use of visual cues is unlikely. Airborne chemical cues, while used by some Hemiptera for long-range attraction (Cökl & Virant-Doberlet 2003), do not seem to be used for guiding within-plant search, perhaps reflecting the difficulty of tracking an airborne odour plume by walking along the three-dimensional branching structure of the host plant. Finally, vibration-guided searching has been experimentally demonstrated in two other species in the same subfamily, including nymphs responding to food recruitment signals (Cocroft 2005) and males searching for stationary, signalling females (J. Gibson & R. B. Cocroft, unpublished data).

The picture that emerges from characterization of individual male search paths is that mate searching in *T. gibbera* is a challenging process with significant sources of error that can contribute to inefficiency and increased time to locate the female. Furthermore, in the presence of two males, females will duet with both individuals and mate with the first to arrive. The process, therefore, seems vulnerable to a competitive strategy that reduces the directional information available to a rival male.

How does the presence of a second mate-searching male influence the behaviour of male *T. gibbera*? In the presence of a rival, males produced a new signal type that overlapped the second section of the advertisement signal; this overlapping signal was produced during up to 82% of advertisement signals when two males were duetting with a female. Playback of a duet to individual males predictably elicited this signal. The presence of an overlapping signal inhibited female responses, with overlapped signals eliciting about one-third as many replies as nonoverlapped signals. We conclude that the overlapping signal produced by competing males functions to disrupt the duet, probably through signal masking (see below).

What are the likely costs and benefits of masking the signal of a rival male? Female response signals are important for localization,

so the lack of a response probably decreases a male's localization efficiency. Given that the female will duet with more than one male and mate with the first to arrive, any behaviour that increases the time required by a rival to reach the female has the potential to increase mating success. A male can produce a masking signal during a competitor's advertisement signal without decreasing its own production of advertisement signals, because males typically produce their bouts of advertisement signals in alternation. However, the information about the female's location provided by her response signal is available to both males, so by preventing a female from responding a male would seem to incur an information cost. Whether this cost is higher for the male whose signal was masked may depend on whether males elicit female replies at points during their searches when such information is particularly valuable.

Disruption of duetting through the production of masking signals may be common among species using vibrational communication, given that mate searching often involves duets, and that multiple individuals are often found on the same plant. Masking signals have also been demonstrated in the leafhopper *Scaphoideus titanus* (Mazzoni et al. 2009a, b). In contrast to the masking signals of *T. gibbera*, however, the masking signals of eavesdropping male *S. titanus* overlap the female's reply, preventing the duetting male from locating the female or indeed from continuing to duet (Mazzoni et al. 2009b). Possible masking signals have also been suggested for the treehopper *Ennya chrysur* (Miranda 2006), based on the observation that eavesdropping males sometimes produce a distinctive signal that overlaps with a rival male's courtship signal.

Specialized masking signals occur in some periodical cicadas (*Magicicada* spp.; Cooley & Marshall 2001), and there are close parallels with the masking signals of *T. gibbera*. First, male cicadas that are duetting with a female produce buzzes during the advertisement signals of a rival male, and the buzzes are timed to overlap with the second portion of the male advertisement signal, which is the most effective in eliciting a female wing flick. Buzzes cause a marked reduction in female responses to advertisement signals: when a buzz occurs during the terminal portion of the male's advertisement signal, female *M. septendecim* are only 25–65% as likely to produce a wing flick, compared to their responses to advertisement signals alone. However, the role of the signals in mate competition is different in *T. gibbera* and *Magicicada*. For cicadas, Cooley & Marshall (2001) suggested that the rival male does not perceive the interference signal (which ends just at the end of the rival's advertisement signal), and that when it succeeds

in suppressing female wing flicks, the rival male remains unaware of the female's presence and will leave and continue with its call-fly mate-searching behaviour. In contrast, in *T. gibbera*, both males are duetting with the female, and both produce masking signals in response to the advertisement signals of the other. In *T. gibbera*, then, masking the advertisement signal functions not to prevent a second male from perceiving the presence of a female but to prevent the rival from locating the female.

In the phaneropterine katydid *Elephantodeta nobilis*, eavesdropping males often insert several pulses near the end of a duetting male's advertisement signal; this behaviour does not decrease the female's probability of responding, but it changes the female response latency and causes at least some females to approach the satellite rather than the duetting male (Bailey et al. 2006). In another phaneropterine (*Caedicia* sp.), duetting males pre-empt the possibility of eavesdropping by producing a high-amplitude signal immediately after the female's reply (Hammond & Bailey 2003). During playback of a duet from two speakers, listening males approached the speaker playing the female signal, but this response was abolished by playback of the masking signal. In *T. gibbera*, masking of the female response also occurred during mating trials in which two males duetted with a single female; in several cases, both the duetting and the eavesdropping male produced a masking signal during the same female response signal. The function of masking the female reply signal has not yet been investigated, but it is possible that it makes the female signal more difficult to recognize and/or locate.

We have described the reduction of female responses by the presence of an overlapping signal as masking, which occurs when the threshold of audibility of one sound is increased by the presence of another sound (Gelfand 2010). However, the means by which the overlapping signal reduces female responses requires further investigation. In the trials with two *T. gibbera* males in this study, the masking signals were about half the duration of the pulsed section of the advertisement signal with which they overlapped (187 ± 56 ms versus 370 ± 33 ms). If only simultaneous masking is occurring, such that the pulses that follow the mask are perceived, then presumably the female response is dependent on perception of pulses during a short time window after the whine. Forward masking (which occurs when the increased threshold for perception of a sound is raised for a period after the offset of a masking sound) is another possibility, in which case the masking signal would also reduce the likelihood that the female perceives the pulses that follow the mask. It is also unclear why the mask is produced at the frequency of the whine, rather than that of the pulses. This might occur if lower-frequency masks are effective at masking higher-frequency signals, as occurs in humans (Gelfand 2010). Alternatively, the combination of pulses + mask might be perceived as a continuation of the whine section of the signal; a similar function was proposed for the 'buzz' in periodical cicadas. In that case, males produce a constant-frequency component followed by a 'frequency downslur', and the latter is necessary for triggering the female response (Cooley & Marshall 2001). Production of a 'buzz' during the downslur reduces the likelihood that females will respond to a signal. Because the buzz is produced at the same frequency as the first, constant-frequency section of the signal, Cooley & Marshall (2001) speculated that the female perceives only a continuous constant-frequency signal, and fails to perceive the downslur. In addition to masking, it is also possible that the combination of pulses and overlapping signal results in perceptual fusion (Bregman 1990), such that the female perceives not the pulses, but rather a distinct signal resulting from the combination.

In this study, vibrational playback of masking signals was more effective at preventing female responses than were the masking

signals of interacting males. There are several possible explanations for this difference. If there is spatial release from masking (Bregman 1990), then masking would be relatively ineffective when males were on opposite sides of the female. Furthermore, the effectiveness of masking is influenced by the relative amplitudes of mask and target (Gelfand 2010; see also Bailey & Field 2000). In this study, during playbacks the amplitudes of mask and target were relatively similar (both set equal to natural amplitudes at the source), while during natural signalling interactions, masks produced by a male further from the female would probably be relatively ineffective at masking the signal of a male closer to the female. In addition, the masking signals varied substantially in duration, as did the target pulses, and while the relative duration of mask and pulses was fixed within a single playback replicate, in natural signalling interactions the two were variable, and their relative durations may influence the effectiveness of masking. Current experiments are testing the importance of each of these aspects on the effectiveness of masking. Such experiments may also shed light on the related question of how an advertisement signal might evolve to be less easily masked, and how competing males might alter their behaviour in ways that make signal masking by a rival less effective.

In summary, in multiple insect lineages in which male–female duets occur, males produce specialized masking signals whose function is to prevent signal detection or recognition: a katydid, a group of cicadas, a leafhopper and now a treehopper (Cooley & Marshall 2001; Bailey et al. 2006; Mazzoni et al. 2009a, b; this study). Signal interference and masking are a general problem in animal communication, especially in contexts such as the choruses of frogs and acoustic insects where multiple individuals are signalling in close proximity at the same time (Gerhardt & Huber 2002). The related problems of how signallers can behave to avoid signal interference, and how receivers can extract information about individual signallers, have been studied in contexts in which multiple signallers are producing similar signals, or a signaller is producing a signal in the presence of noise (Bee & Micheyl 2008). Competitive signal interference can occur in chorusing species, for example through signal-timing interactions that can result in advantages to the individual whose signal slightly precedes that of the other (Greenfield 2002). We know of no examples in chorusing species of masking signals similar to those described here, although in principle such masking should be possible. Whether specialized masking signals have evolved in any chorusing species is unknown, but the apparent lack of such signals may reflect the cooperative nature of some choruses (Greenfield 1994) or a lower payoff to such behaviour in a context in which neighbours are producing signals for hours and the timing of female arrival is unpredictable. In any case, the evolution of masking signals is of interest from a psychoacoustic perspective, because these signals are free of the constraint of being attractive to females, and their only known function is to disrupt communication. While the study of masking has largely been devoted to understanding how receivers can detect a signal in the presence of noise, masking signals present the related but distinct problem of what noise properties can most effectively prevent signal detection.

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References

- Bailey, W. J.** 2003. Insect duets: underlying mechanisms and their evolution. *Physiological Entomology*, **28**, 157–174.
- Bailey, W. J. & Field, G.** 2000. Acoustic satellite behaviour in the Australian bushcricket *Elephantodeta nobilis* (Phaneropterinae, Tettigoniidae, Orthoptera). *Animal Behaviour*, **59**, 361–369.
- Bailey, W., MacLeay, C. & Gordon, T.** 2006. Acoustic mimicry and disruptive alternative calling tactics in an Australian bushcricket (*Caedicia*; Phaneropterinae; Tettigoniidae; Orthoptera): does mating influence male calling tactic? *Physiological Entomology*, **31**, 201–210.
- Bee, M. A. & Micheyl, C.** 2008. The cocktail party problem: what is it? How can it be solved? And why should animal behaviorists study it? *Journal of Comparative Psychology*, **122**, 235–251.
- Bregman, A. S.** 1990. *Auditory Scene Analysis*. Cambridge, Massachusetts: MIT Press.
- Brumm, H. & Slabbekoorn, H.** 2005. Acoustic communication in noise. *Advances in the Study of Behavior*, **35**, 151–209.
- Claridge, M. F.** 1985. Acoustic signals in the Homoptera: behavior, taxonomy and evolution. *Annual Review of Entomology*, **30**, 297–317.
- Cocroft, R. B.** 2005. Vibrational communication facilitates cooperative foraging in a phloem-feeding insect. *Proceedings of the Royal Society B*, **272**, 1023–1029.
- Cocroft, R. B. & Rodríguez, R. L.** 2005. The behavioral ecology of insect vibrational communication. *BioScience*, **55**, 323–334.
- Cocroft, R. B., Tieu, T. D., Hoy, R. R. & Miles, R. N.** 2000. Directionality in the mechanical response to substrate vibration in a treehopper (Hemiptera: Membracidae: *Umbonia crassicornis*). *Journal of Comparative Physiology A*, **186**, 695–705.
- Cokl, A. & Virant-Doberlet, M.** 2003. Communication with substrate-borne signals in small plant-dwelling insects. *Annual Review of Entomology*, **48**, 29–50.
- Cooley, J. R. & Marshall, D. C.** 2001. Sexual signaling in periodical cicadas, *Magicicada* spp. (Hemiptera: Cicadidae). *Behaviour*, **138**, 827–855.
- Gelfand, S. A.** 2010. *Hearing: An Introduction to Psychological and Physiological Acoustics*. 5th edn. New York: Marcel Dekker.
- Gerhardt, H. C. & Huber, F.** 2002. *Acoustic Communication in Insects and Anurans: Common Problems and Diverse Solutions*. Chicago: University of Chicago Press.
- Greenfield, M. D.** 1994. Cooperation and conflict in the evolution of signal interactions. *Annual Review of Ecology and Systematics*, **25**, 97–126.
- Greenfield, M. D.** 2002. *Signalers and Receivers: Mechanisms and Evolution of Arthropod Communication*. Oxford: Oxford University Press.
- Hall, M. E.** 2004. A review of hypotheses for the functions of avian duetting. *Behavioral Ecology and Sociobiology*, **55**, 415–430.
- Hammond, T. J. & Bailey, W. J.** 2003. Eavesdropping and defensive auditory masking in an Australian bushcricket *Caedicia* (Phaneropterinae: Tettigoniidae: Orthoptera). *Behaviour*, **140**, 79–95.
- Heller, K.-G.** 1990. Evolution and song pattern in east Mediterranean Phaneropterinae: constraints by the communication system. In: *The Tettigoniidae: Biology, Systematics and Evolution* (Ed. by W. J. Bailey & D. C. F. Rentz), pp. 130–151. Bathurst: Crawford House Press.
- Kopp, D. D. & Yonke, K. R.** 1973. The treehoppers of Missouri. Part 1: Subfamilies Centrotinae, Hoplophorioninae, and Membracinae. *Journal of the Kansas Entomological Society*, **46**, 42–64.
- Mazzoni, V., Prešern, J., Lucchi, A. & Virant-Doberlet, M.** 2009a. Reproductive strategy of the Nearctic leafhopper *Scaphoideus titanus* Ball (Hemiptera: Cicadellidae). *Bulletin of Entomological Research*, **99**, 401–441.
- Mazzoni, V., Lucchi, V., Cokl, A., Prešern, J. & Virant-Doberlet, M.** 2009b. Disruption of the reproductive behaviour of *Scaphoideus titanus* by playback of vibrational signals. *Entomologia Experimentalis et Applicata*, **133**, 174–185.
- Michelsen, A., Fink, F., Gogala, M. & Traue, D.** 1982. Plants as transmission channels for insect vibrational songs. *Behavioral Ecology and Sociobiology*, **11**, 269–281.
- Miranda, X.** 2006. Substrate-borne signal repertoire and courtship jamming by adults of *Ennya chrysur* (Hemiptera: Membracidae). *Annals of the Entomological Society of America*, **99**, 374–386.
- Rodríguez, R. L., Ramaswamy, K. & Cocroft, R. B.** 2006. Evidence that female preferences have shaped male signal evolution in a clade of specialized plant-feeding insects. *Proceedings of the Royal Society B*, **273**, 2585–2593.
- Shuster, S. M. & Wade, M. J.** 2003. *Mating Systems and Strategies*. Princeton, New Jersey: Princeton University Press.
- Strümpel, H.** 1974. Beitrag zur Kenntnis der neotropischen Membraciden Gattung *Tylopelta* Fowler, 1894. *Entomologische Mitteilungen aus dem Zoologischen Museum Hamburg*, **4**, 531–540.
- Svensson, M.** 1996. Sexual selection in moths: the role of chemical communication. *Biological Reviews*, **71**, 113–135.
- Tobias, J. A. & Seddon, N.** 2009. Signal jamming mediates sexual conflict in a duetting bird. *Current Biology*, **19**, 1–6.
- Virant-Doberlet, M. & Cokl, A.** 2004. Vibrational communication in insects. *Neotropical Entomology*, **33**, 121–134.
- Virant-Doberlet, M., Cokl, A. & Zorovic, M.** 2006. Use of substrate vibrations for orientation: from behaviour to physiology. In: *Insect Sounds and Communication: Physiology, Behaviour, Ecology and Evolution* (Ed. by S. Drosopoulos & M. F. Claridge), pp. 81–97. Boca Raton, Florida: Taylor & Francis.