

Effects of acoustic environment on male calling activity and timing in Neotropical forest katydids

Laurel B. Symes^{1,2} · Rachel A. Page¹ · Hannah M. ter Hofstede²

Received: 10 March 2016 / Revised: 18 May 2016 / Accepted: 30 May 2016
© Springer-Verlag Berlin Heidelberg 2016

Abstract

Many characteristics of signals can convey information, but the exact timing of the signal often matters as well. The timing of signals is shaped by selective pressures including mate preferences, predation, and competition. In many insect communities, male calling to attract females is persistent and pervasive, and signal timing interactions among individuals are relatively common. In Neotropical forests, many katydid species are represented in the acoustic environment, but calls are usually short (<40 ms) and infrequent (<10 s of sound per individual per night), characteristics that have likely evolved in response to intense predation by insectivorous bats. We test two alternative hypotheses about signal timing in environments where signaling is rare and costly, either that timing is absent due to the unpredictable nature of the signals or that the rarity of signals places a premium on signal timing and attention to the acoustic environment. We tested these hypotheses by broadcasting conspecific calls, heterospecific calls, and silence to eight species of katydids and measuring calling activity and call timing in each playback treatment. All species changed the amount or timing of calling (or both) as a result of the playbacks, but species responded differently to playbacks,

with some calling more or less during specific treatments and some showing differences in the timing of calls relative to playbacks. Although short latency signal timing was not observed, this study shows that Neotropical forest katydids are responsive to their acoustic environment despite an exceptionally low rate of signaling.

Significance statement

In many species, males produce signals to attract females, and studies show that the timing of these signals relative to other stimuli can play an important role in mate attraction and predator avoidance. Most of these studies have investigated species that are prolific signalers, due to the ease of collecting data. Here, we extend these theories and test them in Neotropical forest katydids, which produce very short and sporadic acoustic signals. We find that these insects do not display the fast competitive interactions seen in more prolific signalers, but still adjust both the timing and amount of calling in response to what they hear. These findings reveal that insects that signal rarely are still attending to the signaling of others and that their behavior can be strongly affected by the signals of other insects, including insects of other species.

Communicated by D. Gwynne

Electronic supplementary material The online version of this article (doi:10.1007/s00265-016-2157-4) contains supplementary material, which is available to authorized users.

✉ Laurel B. Symes
laurel.symes@dartmouth.edu

¹ Smithsonian Tropical Research Institute, Apartado 0843-03092, Balboa, Ancón, República de Panamá

² Dartmouth College, Department of Biological Sciences, 78 College St., Hanover, NH 03755, USA

Keywords Copiphorinae · Orthoptera · Phaneropterinae · Pseudophyllinae

Introduction

Although the structure of communication signals is important for conveying information from the sender to a receiver, the decision to signal and the exact timing of the signal are also critical in behavioral interactions (Colavita 1974; Shaw and Galliard 1987; Greenfield 1994; Dyson et al. 1994; Greenfield 2005; Brumm 2006; Höbel and Gerhardt 2007). Signal timing

can be shaped by many selective pressures including characteristics of the transmission medium, sources of interference, receiver biases, mate preferences, predation, and competition (Klump and Gerhardt 1992; Grafe 1996; Gerhardt and Huber 2002; Brumm 2006; Höbel 2010; Siegert et al. 2011). For example, signal timing underlies mating duets, which occur in taxa from birds and primates to insects such as katydids, fireflies, and tree hoppers, where the timing of the signal is an important component of mate recognition (Lloyd 1984; Heller and von Helversen 1986; Geissmann 2002; Bailey 2003; Mennill et al. 2006; Rodriguez and Cocroft 2006; Henry and Wells 2010). Signal timing can also arise because calling while others are calling reduces individual conspicuousness to predators, for example, by making it difficult for predators to focus attention on a single individual (Ryan et al. 1981; Alem et al. 2011). The importance of signal timing also pervades male-male competition, with many species engaging in signal jamming and other competitive interactions (Greenfield 1994; Snedden and Greenfield 1998; Höbel 2010). Males will compete to produce the first signal, or to overlap another male's signal so that his is the last sound that a female hears (Greenfield et al. 1997; Snedden and Greenfield 1998; Hartbauer et al. 2005).

In many environments, insect choruses can be loud, pervasive, and repetitive (Greenfield 1994; Walker and Moore 2013; Symes et al. 2015), but in Neotropical forests, the situation can be quite different. Species diversity is extremely high, meaning that signal space is shared by many different species, including at least 75 species of katydids, as well as other acoustically communicating species (Symes et al. personal observation, Nickle et al. 1992). The Neotropics are predictably diverse, but the signaling activity and signal structure of Neotropical forest katydids is often profoundly different from katydid species in other habitats. Many Neotropical forest katydid species produce calls that are short (often <40 ms), repeated infrequently, and have most of the energy at ultrasonic frequencies that travel relatively short distances in rainforest vegetation (Gwynne and Bailey 1988; Romer and Lewald 1992; Romer and Romer 1993; Montealegre-Z and Morris 1999; Montealegre-Z et al. 2006; Montealegre-Z 2009; Heller and Hemp 2014; Sarria-S et al. 2014). Several Neotropical bat species are known to use the calls of katydids to locate them as prey (Belwood and Morris 1987; Romer et al. 2010; Falk et al. 2015), and it is believed that the low calling rate and other features that make Neotropical katydid calls difficult to track have arisen as a result of predation by bats (Rantz 1975; Belwood and Morris 1987; Morris et al. 1994; Lang et al. 2006; Falk et al. 2015) and potentially other acoustically orienting predators such as parasitoid flies (Lehmann 2003).

The exceptionally low duty cycle of many Neotropical forest katydids raises the question: when signals are short, infrequent, and costly, what are the consequences for the timing of

signaling behavior? Due to the ease of data collection, the majority of studies on signal timing have investigated species that are prolific signalers, resulting in a non-representative sample of species in the literature on this topic. We test two alternative hypotheses about how communication functions in environments where signaling is rare and costly. The first hypothesis is that signal timing is no longer central to competition or mate attraction, with the prediction that Neotropical katydids will show no evidence of timing their calls with conspecific or heterospecific calls. The second hypothesis is that signal timing is critical and organisms attend carefully to the behavior of neighbors, with the prediction that Neotropical katydids will time their calls with conspecific calls, heterospecific calls, or both. We tested the predictions of these hypotheses by measuring (1) the amount of signaling and (2) the timing of signals by Neotropical katydids in different acoustic conditions.

Methods

Study animals

Katydidids were captured at night from lights around buildings on Barro Colorado Island, Panama, in January and February 2014. Individuals were identified to species using the key by Nickle et al. (1992) and Orthoptera Species File Online (Eades et al. 2016). Katydidids were housed in individual mesh cages with ad libitum water and food (cat food and apple) until testing. To maximize our ability to detect shared and divergent patterns of response, we used eight species with a nested taxonomic structure. We used three subfamilies of Tettigoniidae (Conocephalinae, Phaneropterinae, and Pseudophyllinae), five genera within the Phaneropterinae (*Arota*, *Chloroscirtus*, *Lamprophyllum*, *Phylloptera*, and *Viadana*), and two species within the genus *Arota*. (Fig. 1, Supplemental Fig. 1): (Phaneropterinae: *Arota festae* ($n = 5$), *Arota panamae* ($n = 4$), *Chloroscirtus discocercus* ($n = 8$), *Lamprophyllum micans* ($n = 13$), *Phylloptera dimidiata* ($n = 6$), *Viadana zetterstedti* ($n = 12$); Pseudophyllinae: *Cocconotus wheeleri* ($n = 7$); Conocephalinae: *Copiphora brevirostris* ($n = 5$)). Katydidids were released at the completion of the experiment.

Sound stimuli

Male katydidids were exposed to playbacks of conspecific calls, heterospecific calls, and silence. To obtain calls of male katydidids for use in these playback experiments, isolated males were placed in cylindrical metal mesh cages (72 × 150 mm, D × H) that were surrounded by acoustic foam to reduce sound reflections. Calls were recorded using a condenser microphone (CM16, Avisoft Bioacoustics, Berlin Germany) placed

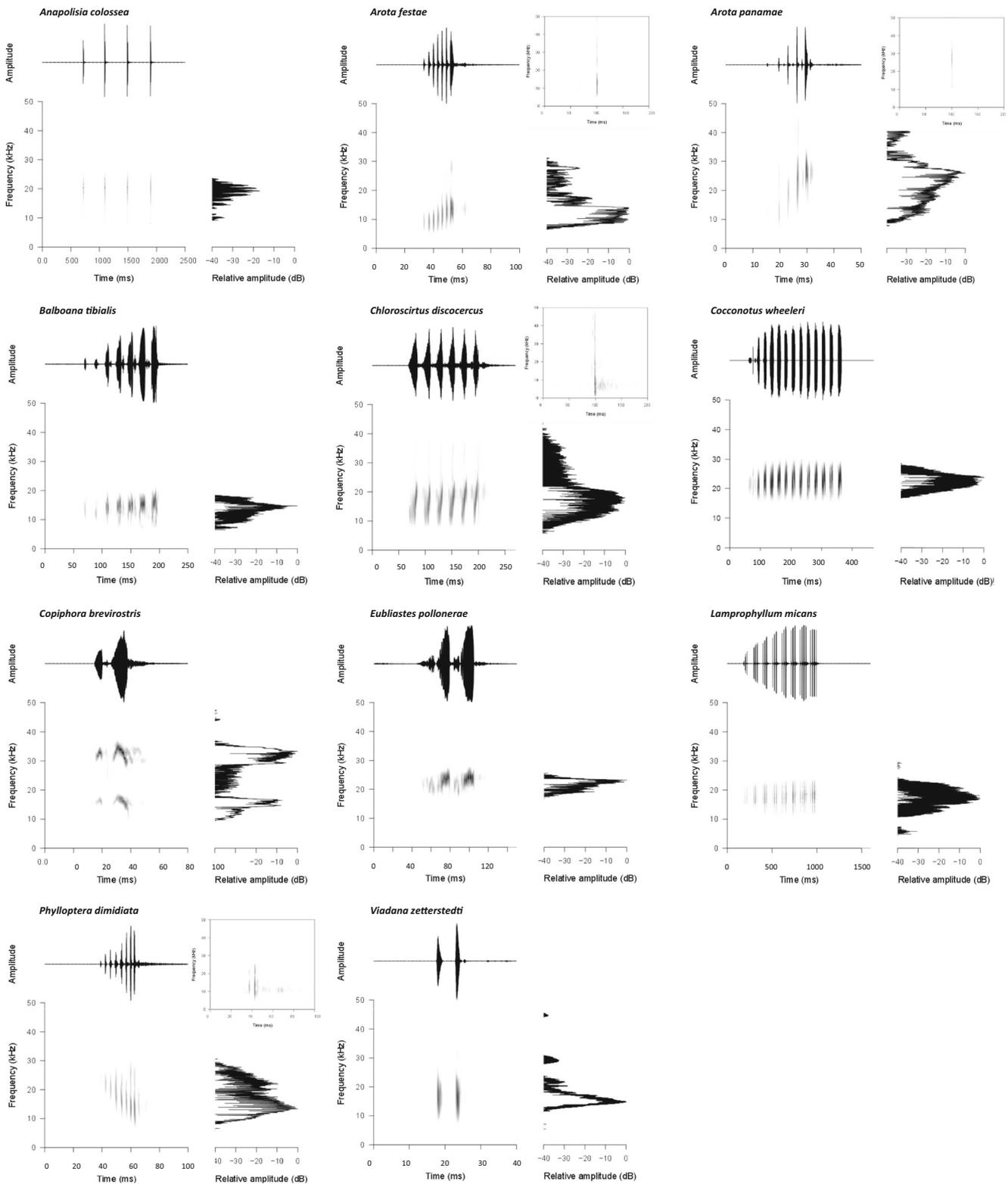


Fig. 1 Example recordings of the male call from 11 Neotropical katydid species (eight focal species and three species used as heterospecific playbacks). The oscillogram (*top left panel*), spectrogram (*bottom left*

panel), and power spectrum (*bottom right panel*) are given for each species. For species that produced ticks, a spectrogram of the tick is shown in a box (*upper right panel*)

30 cm from the cage, an A/D converter (UltraSoundGate 416H, Avisoft), and a laptop running Avisoft Recorder

software with a sampling rate of 250 k-samples/s. We applied a custom frequency response filter that was the inverse of the

microphone frequency response using SASLab analysis software (Avisoft Bioacoustics). This filter corrected for changes to the relative amplitudes of frequencies in the recording introduced by the frequency response of the microphone. This filter reduced or amplified different frequencies to generate audio files with accurate power spectra (realistic amplitudes at each frequency). We used these filtered recordings for measurements of spectral parameters of the calls. Likewise, we applied a filter to the calls based on the frequency response of the individual speaker before broadcasting calls to katydids during experiments. The silence playback was a .wav file containing no sound.

To determine whether the similarity of conspecific and heterospecific signals predicted response to heterospecific signals, we measured acoustic characteristics for male calls (3–9 individuals/species, 2–20 calls/individual) using SASLab analysis software (Avisoft Bioacoustics). Calls consisted of multiple short sound pulses (Fig. 1) and we counted the number of pulses for each call. We measured call duration (the time from the start of the first pulse to the end of the last pulse in the call) and four spectral parameters from power spectra for each call (FFT length 512, Hamming window): (1) peak frequency (frequency with the most energy), (2) lowest frequency – 20 dB below the peak, (3) highest frequency – 20 dB below the peak, and (4) bandwidth (highest frequency minus lowest frequency). For each katydid species, the mean value for each call parameter was calculated by first averaging the value across calls for each individual, and then averaging across the means for each individual to calculate the mean value for the species.

Katydid calling activity during three acoustic conditions

Male katydids were placed in the same cages as described above and an ultrasonic speaker (Vifa model 60108, Avisoft) was placed 30 cm from the center of the cage. The speaker was connected to a laptop running Avisoft Recorder software via an amplifier (UltraSoundGate Player 216H, model 70118, Avisoft). Sound levels for sound stimuli were calibrated prior to experiments by recording sound stimuli using a 1/4" microphone with a flat frequency response (type 4939, ± 2 dB from 0.004–100 kHz; Brüel and Kjær, Nærum, Denmark) and adjusting amplitudes relative to a calibration tone (type 4231, Brüel and Kjær). Due to the extremely short durations of some katydid calls, sound levels were calculated as peak equivalent SPL (peSPL: the r.m.s. level re. 20 μ Pa of a sinusoid with the same peak-to-peak-amplitude as the pulse; (Burkard 2006)). During each treatment period, the calls produced by the test katydid were recorded using the same microphone, A/D converter, and software described above for recording the katydid calls used as stimulus files.

We recorded calling activity of male katydids during exposure to the stimuli described above (conspecific calls, heterospecific calls, and silence). For every treatment, a block

consisted of the sound stimulus (a single call or silence) broadcast once per minute for 27 min. Throughout the night, each katydid experienced two blocks of silence, three to four blocks of conspecific calls, and four blocks of heterospecific calls broadcast in random order and each separated by 15 min of silence. To mimic an environment with multiple individuals at different distances, conspecific and heterospecific calls were broadcast at 60, 65, and 70 dB peSPL at the center of the cage. These amplitudes are well above the neural thresholds for sound in Neotropical katydids studied so far (ter Hofstede et al. 2010). For the conspecific treatment, we broadcast one call per minute, cycling through three different call amplitudes for 27 min to represent individuals at different distances. Three individuals of the focal species each contributed a call that was used to generate one of the three amplitude treatments. This order of the three amplitudes was repeated throughout each 27-min conspecific block. For the heterospecific treatment, we broadcast one call of *Anapolisia colossea* at 60 dB peSPL, one call of *Balboana tibialis* at 65 dB peSPL, and one call of *C. wheeleri* (or *Eubliastes pollonerae* when the test species was *C. wheeleri*) at 70 dB peSPL. This order of the three call types and amplitudes was also repeated throughout each 27-min heterospecific block. Observers were not blinded to insect identity, but call events were detected using automated triggering thresholds and call timing was analyzed using uniform R code, minimizing the potential for experimenter bias.

Statistical analysis

We conducted analyses to quantify the amount of time spent calling per night and to test whether the amount and timing of calling changed during playbacks. All statistical analyses were run using R (R Core Team 2015).

To estimate the time spent calling per night, we multiplied the number of calls produced per individual by the mean call duration of the species. The number of calls per individual was measured for 1 h of silence as well as for an 8-h night that included the playbacks and periods of silence. We tested whether call characteristics were correlated with the number of calls produced or the time spent calling using Spearman rank correlations, because the mean values across species were not normally distributed.

To test whether calling activity differed between treatments, we counted the number of calls produced by each katydid during each treatment (conspecific calls, heterospecific calls, and silence). Analyses were conducted using generalized linear models (R package: lme4 using the glmer function with a binomial distribution as the link function). For each species, we tested whether treatment affected the amount of calling activity by constructing a model with treatment as a fixed effect and individual as a random effect and comparing this model against a model that included only

the random effect of individual identity. Playback treatments contained multiple amplitudes of conspecific calls and multiple species and amplitudes of heterospecific calls. Therefore, we also tested within treatment whether specific playbacks had different effects on calling by comparing a model that contained only a random effect of individual identity against a model that also included a fixed effect of stimulus identity.

To quantify call timing behavior, we assessed two aspects of call timing: endogenous call period and call timing during stimulus playback. To determine whether these species had an endogenous rhythm to their calls, we measured the call period (the time from the start of one call to the start of the next) during silent periods when conspecific and heterospecific calls were not played. To determine whether playback treatments affected call timing, we divided the 1-min time periods between acoustic stimuli into time bins of 10 s, with the response variable being the presence or absence of calls during these time bins. We ran generalized linear mixed models using the `glmer` function in the `lme4` package of R using a binomial distribution as the link function. We tested multiple models with katydid individual as a random effect plus one or more of four fixed effects: species of focal katydid, acoustic condition (silence, conspecific call, heterospecific call), order (first, second, or third stimulus in playback series), and time bin (10 s bins of time since call produced). The fixed effect time bin allowed us to assess if katydids were timing their calls relative to the acoustic stimulus. To assess the contribution of each fixed effect and particular interaction terms to explaining the variance in katydid calling, we used likelihood ratio tests to compare models that did or did not contain each fixed effect and interaction term. Specific comparisons are provided in the results.

Results

Male katydids recorded in this study produced calls that varied widely in temporal and spectral features depending on species (Fig. 1, Table 1, Supplemental Fig. 1). As reported in previous studies (Belwood and Morris 1987, Belwood 1988), the amount of calling song produced by Neotropical katydids was extremely low both during our experimental treatments and during the remainder of the night that followed the completion of playbacks (Fig. 2). For the eight focal species, the average time spent calling in an 8-h night was 6.3 s (standard error 4.2 s; species means ranged from 1.5 to 35.5 s), with an average of 80.2 calls per night (standard error 23.2 calls; range 4.4–190 calls) (Fig. 2). During an hour when no calls were played back and only ambient sound was heard, the time spent calling ranged from 0.06 to 3.30 s across the eight species, with a mean of 0.57 s of sound per hour and a standard error of 0.39 s (Supplemental Fig. 2). The average number of calls

produced by the eight species during this hour ranged from 0.1 to 17.8 calls per hour (standard error of 2.2 calls; average of 6.3 calls, Supplemental Fig. 2). The duration of the call was positively correlated with the time spent calling ($\rho = 0.93$, $p = 0.002$), but there was no other correlation between the call characteristics and the time spent calling or number of calls produced (Supplemental Table 1).

Depending on the katydid species, we found evidence to support both hypotheses about signal timing in species that rarely signal. Two species (*A. panamae* and *C. brevisrostris*) did not change the amount of calling with social context, producing an equivalent amount of calling song in each of the three sound treatments (silence, conspecific calls, and heterospecific calls; Fig. 3a, see Supplemental Table 2 for statistical results). In the remaining six species, social context influenced the amount of signaling in a variety of ways. Two species (*A. festae* and *C. discocercus*) produced more calls during conspecific call playbacks than during the other two sound treatments (Fig. 3b). Four species (*C. wheeleri*, *L. micans*, *P. dimidiata*, and *V. zetterstedti*) produced the most calls during heterospecific call stimuli (Fig. 3c). For two species (*P. dimidiata* and *V. zetterstedti*), the amount of calling during heterospecific calls was only significantly different from the silent treatment, and in two species (*C. wheeleri* and *L. micans*) males produced more calls during the heterospecific call treatment than during the conspecific call treatment.

A detailed look at which stimuli lead to increased calling shows that some species had different responses to different playbacks within a treatment. Although there was variation in the responses of katydids to the specific playbacks of each treatment, we found statistical differences in only two cases. *C. discocercus* increased calling specifically in response to playbacks of the heterospecific *A. colosseae* (Fig. 4a). The short ticks produced by *C. discocercus* are very similar to the individual pulses of sound produced by *A. colosseae*, in that they are both short duration, broadband signals (Fig. 1). After conspecific playbacks, *C. discocercus* called more, so it may be responding the same way to conspecific calls and similar heterospecific calls, representing a potential case of signal confusion, at least among males. Alternatively, *C. discocercus* may simply be responding to the onset of loud broadband sound. *A. festae* was much more likely to call after high amplitude conspecific playbacks (Fig. 4b), suggesting that the distance of a signaling competitor will change the behavior of males in this species.

When comparing models that included an order term that accounted for the position of the stimulus in the playback series, the order term generally failed to improve the fit of the model, meaning that insects did not acclimate to the playbacks. The exception to this was *A. festae*, where individuals became less responsive to the playbacks through the course of the trial ($\chi^2 = 14.2$, $p = 0.0002$).

Table 1 Mean acoustic characteristics for the calls of males of 11 Neotropical katydid species (mean \pm s.e.m.). See “Methods” for definitions of acoustic characteristics

	Duration of call (ms)	No. of pulses	Low freq (kHz)	High freq (kHz)	Bandwidth (kHz)	Peak freq (kHz)	Number of katydids
<i>Anapolistia colosseae</i>	1964.5 \pm 405.4	5.6 \pm 0.8	13.1 \pm 2.4	25.4 \pm 0.9	12.3 \pm 3.1	20.1 \pm 0.5	9
<i>Arota festae</i>	23.4 \pm 1.8	8.3 \pm 0.4	8.1 \pm 0.1	16.8 \pm 0.5	8.7 \pm 0.5	10.7 \pm 0.3	4
<i>Arota panamae</i>	15.4 \pm 0.8	5.0 \pm 0.2	15.4 \pm 0.7	31.7 \pm 0.9	16.4 \pm 0.9	24.2 \pm 0.5	9
<i>Balboana tibialis</i>	140.2 \pm 18.1	7.2 \pm 0.7	10.4 \pm 0.2	15.6 \pm 0.4	5.2 \pm 0.3	13.7 \pm 0.6	3
<i>Chloroscirtus discocercus</i>	140.7 \pm 6.7	6.4 \pm 0.2	13.5 \pm 1.1	21.9 \pm 0.7	8.4 \pm 0.8	18.6 \pm 0.9	5
<i>Cocconotus wheeleri</i>	247.3 \pm 32.7	11.4 \pm 1.4	20.7 \pm 0.4	27.4 \pm 0.6	6.7 \pm 0.5	24.8 \pm 0.4	6
<i>Copiphora breviostris</i>	36.0 \pm 3.0	3.0 \pm 0.2	27.8 \pm 0.3	34.4 \pm 0.4	6.7 \pm 0.5	32.8 \pm 0.4	9
<i>Eubliastes pollonerae</i>	57.7 \pm 3.1	4.0 \pm 0	21.1 \pm 1.3	25.5 \pm 1.5	4.3 \pm 1.5	24.2 \pm 1.3	5
<i>Lamprophyllum micans</i>	875.7 \pm 19.1	60.7 \pm 6.1	14.0 \pm 0.3	22.6 \pm 0.4	8.6 \pm 0.4	18.3 \pm 0.5	5
<i>Phylloptera dimidiata</i>	22.0 \pm 1.0	7.6 \pm 0.4	11.3 \pm 0.3	23.8 \pm 0.4	12.5 \pm 0.4	16.5 \pm 0.7	7
<i>Viadana zetterstedti</i>	8.6 \pm 0.2	2.0 \pm 0.01	14.8 \pm 0.2	18.9 \pm 0.3	4.1 \pm 0.2	16.1 \pm 0.2	9

The Neotropical forest katydids that we recorded showed little evidence of a precise endogenous calling rhythm, although it was relatively common for playbacks to affect signal timing. When katydids were experiencing ambient sound without conspecific or heterospecific playbacks, there was often some regularity in the timing between calls. Across species, the typical intervals between calls ranged from 2 s to more than 2 min. These intervals were not precise, however, with calls often occurring within a window of 10 or 15 s, rather than at an exact interval after the proceeding call (Supplemental Fig. 3, Column 3). The approximate nature of the time between calls would make it essentially impossible for another individual to anticipate and supersede the call of the signaler.

Although precise repetition rates and fast-scale signal timing were rare or absent, four broad patterns related to signal timing occurred: no effect of playbacks on the timing of signals, timing in response to both conspecifics and heterospecifics, to conspecifics only, and to heterospecifics only (Fig. 5, see Supplemental Table 3 for statistical results). *Chloroscirtus discocercus* and *P. dimidiata* showed no evidence of signal timing, even though both species called more during conspecific or heterospecific playbacks than during silence. In *A. festae*, calling was significantly more likely immediately after conspecific calls (although still not fast enough for call overlapping), while *A. panamae* displayed a non-significant trend toward delayed calling after conspecific playbacks. In *V. zetterstedti*, calling was significantly more likely immediately after heterospecifics, with a non-significant trend toward fast reaction to conspecifics as well. *C. breviostris* displayed a non-significant trend in call timing, with lower calling activity immediate after heterospecific calls. Calling in *C. wheeleri* was significantly delayed by both conspecific and heterospecific calls. This is interesting because *C. wheeleri* still called more in the presence of heterospecifics than in silence, suggesting an immediate inhibition of calling, but higher overall calling activity. *L. micans* also showed a non-significant trend that is consistent with inhibition of calling, but called so rarely that there was no statistical support for the pattern. Both *C. wheeleri* and *L. micans* produced calls with much longer duration than other focal species, suggesting that longer calls may be associated with a strong inhibitory response to con- and heterospecific calls.

Discussion

Calling activity is remarkably rare in these species of Neotropical forest katydids, but signaling interactions remain pervasive and diverse. In terms of amount of calling, we found that species fell into three broad categories: calling more, less, or the same in the different acoustic treatments. In addition, some species showed evidence of timing their calls relative to

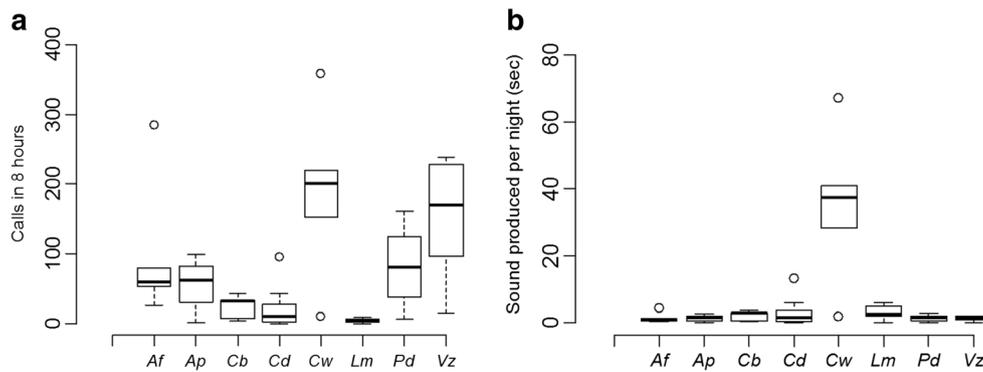


Fig. 2 Amount of calling per night by eight species of Neotropical katydids (8 h of recording, including all calls produced during playbacks). **a** Number of calls produced per night. **b** Total time spent calling per night (s number of calls times call duration). *Af* *Arota festae* ($N=5$), *Ap* *Arota panama* ($N=4$), *Cb* *Copiphora brevirostris* ($N=5$), *Cw*

Cocconotus wheeleri ($N=6$), *Cd* *Chloroscirtus discocercus* ($N=7$), *Lm* *Lamprophyllum micans* ($N=10$), *Pd* *Phylloptera dimidiata* ($N=6$), *Vz* *Viadana zetterstedti* ($N=10$). Horizontal lines are medians, boxes are quartiles, and whiskers are ± 1.5 IQR

the acoustic stimuli, showing inhibition or stimulation of calling activity. The fast-scale signal timing of species from other environments, however, is notably absent in these tropical katydid species.

The total time each katydid spent calling per night was extremely low, providing additional quantitative support for previous reports of low calling activity (Belwood and Morris 1987; Romer et al. 2010). Individuals of most species produced less than 10 s of sound per night (Fig. 2). No katydid produced more than 6 min of sound over an 8-h nocturnal time period. The low sound production is a function of low call rate, but also short call duration (Fig. 2). For example, many individuals called throughout the night, but produced calls that

were less than 50 ms in duration (Table 1). These numbers stand in contrast to the calling activity of Orthopterans from a tropical evergreen forest in India, where calls tend to be longer with lower dominant frequencies (Diwakar and Balakrishnan 2007a).

Although calling was infrequent, katydids attend to the signaling of others. One of the most striking patterns was that the acoustic environment affected overall calling activity even when it did not generate signal timing interactions (Fig. 3). Only two of the eight species (*C. brevirostris* and *A. panama*) had comparable calling activity across all three acoustic environments (playback of conspecifics, heterospecifics, and silence). This indicates that many species are changing behavior

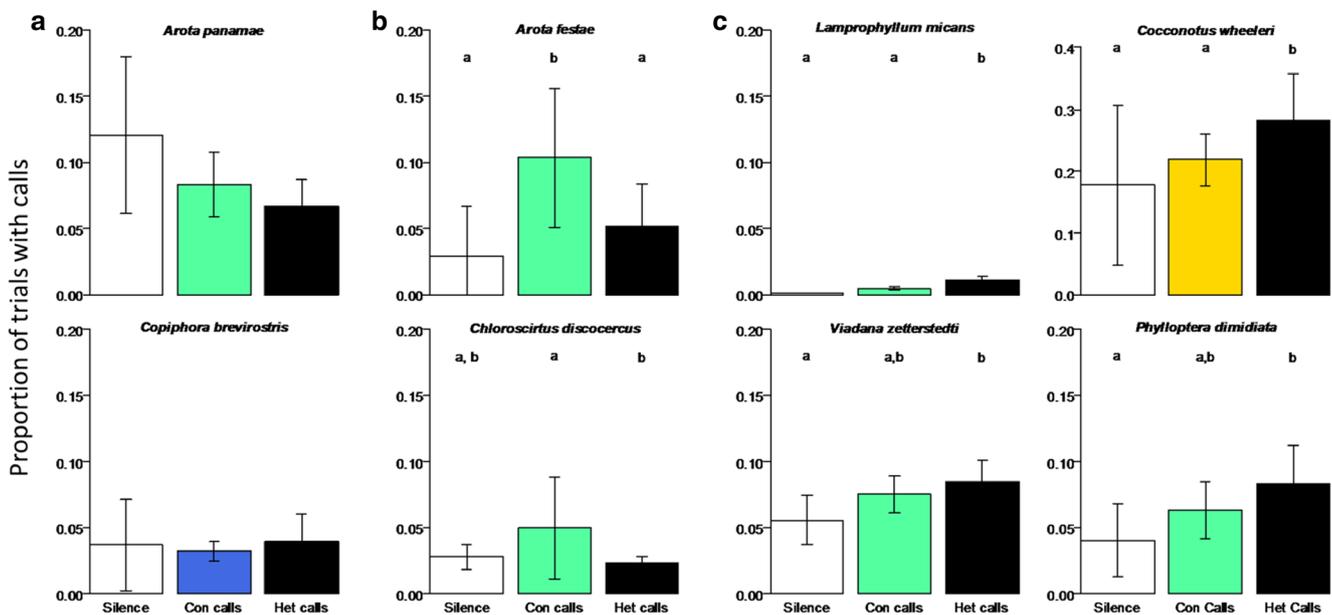


Fig. 3 Calling activity of eight species of katydids during three playback conditions (silence (white bars), conspecific calls (colored bars), and heterospecific calls (black bars)). **a** Calling activity did not differ significantly across treatments for two species. **b** Two species called most during the conspecific playback treatment. **c** Four species called

the most during heterospecific playbacks. *Conspecific bar color* shows family affiliation: Conocephalinae (blue), Phaneropterinae (green), or Pseudophyllinae (yellow). Within a species, *different letters* denote treatments that are significantly different (see Supplementary Table 1 for p values)

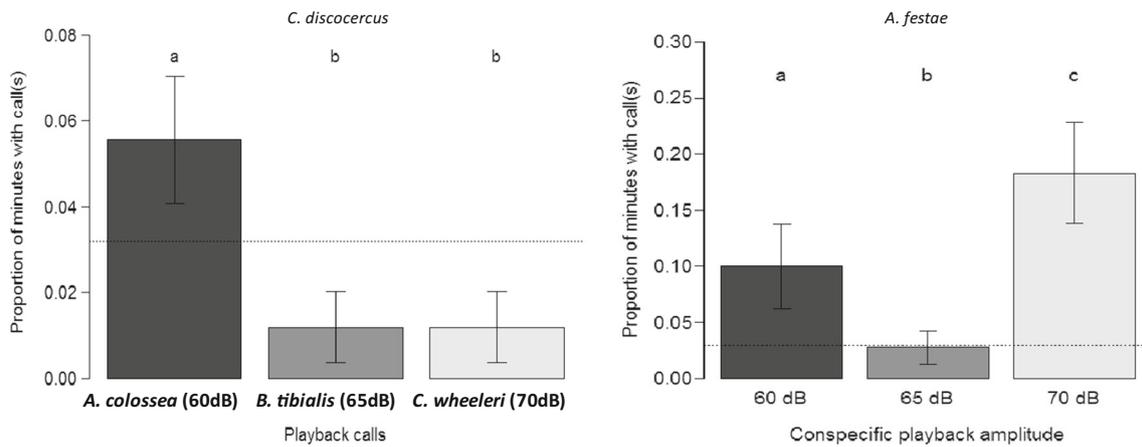


Fig. 4 Two species showed different responses to the stimuli within playback treatments. **a** *C. discocercus* called more when hearing *A. colosseae* than when hearing two other species of heterospecifics. **b** *A. festae* called more during the highest amplitude playback treatment of

conspecific song. Horizontal dotted lines show calling activity in silence. Within panels, different letters denote treatments with significantly different calling activity (see Supplementary Table 1 for *p* values)

in response to katydid sound (Greenfield et al. 1997; Snedden and Greenfield 1998; Hartbauer et al. 2005). In two species (*L. micans* and *C. wheeleri*), the presence of heterospecific calls elevated calling activity relative to both conspecific and silent playbacks. Two additional species (*P. dimidiata* and *V. zetterstedti*) also showed a pattern in which calling was

highest in the heterospecific playback treatment. In these species, calling was significantly higher in the heterospecific playback than in silence, but was not significantly elevated over the conspecific treatment. The ability of heterospecific calls to elevate calling rate was particularly notable in *L. micans*, which had a low overall call rate and almost never

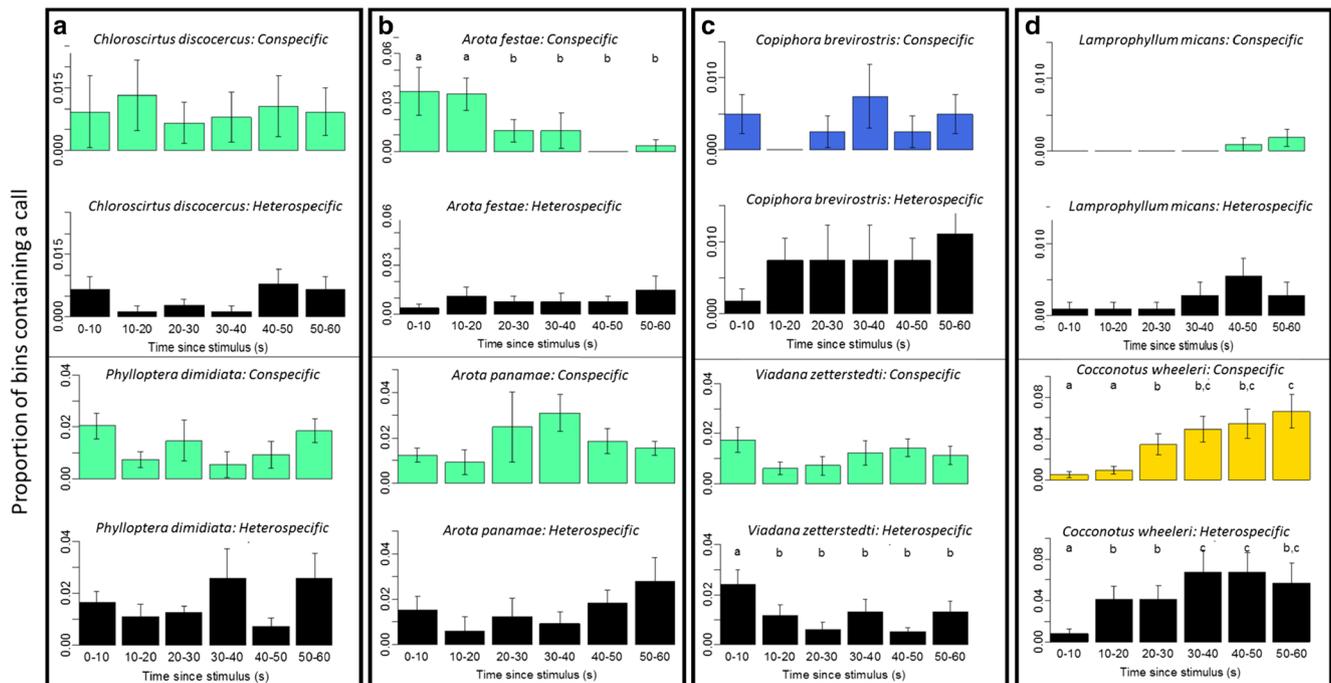


Fig. 5 Call timing of eight species of katydid. Some species showed little evidence that playbacks affected male signal timing (**a**), whereas males of other species tended to respond to conspecific playbacks (**b**), heterospecific playbacks (**c**), and conspecific and heterospecific playbacks (**d**). For each species, colored bars show call timing relative to the conspecific playback stimulus. Black bars show call timing relative

to the heterospecific playback stimulus. Conspecific bar color shows family affiliation: Conocephalinae (blue), Phaneropterinae (green), or Pseudophyllinae (yellow). Within panels, different letters denote time bins with significantly different calling activity (see Supplementary Table 2 for *p* values)

called while hearing conspecifics or silence. Unlike many tropical species that produce short calls (<50 ms), *L. micans* produces calls that are nearly a second long.

It is notable that calling is highest in the heterospecific playback condition. If insects monitored only ambient sound level to gauge competition or predation risk, conspecific and heterospecific playbacks should have comparable responses, especially for those species that produce relatively long calls. Elevated calling in response to conspecifics could evolve because the presence of calling indicates the absence of predation or because it represents an environment where intense competition favors increased risk-taking (Greenfield 1983). However, calling that is elevated by the presence of heterospecific calls might mean that the presence of calling reflects the absence of shared predators (Alem et al. 2011). Calling may also be elevated during heterospecific playbacks if species occupy different microhabitats (Diwakar and Balakrishnan 2007b) and playbacks included species that are seldom heard by the focal species. Under this hypothesis, playbacks of unfamiliar heterospecific calls could act as foreign stimuli that elicit defensive calling activity. However, this hypothesis predicts that the activity of focal species would be increasing in response only to playbacks of certain heterospecifics, rather than increasing as a general response to heterospecific playbacks. Although multiple factors may explain the pervasive effect of heterospecifics, the fact that heterospecific signals do influence signaling behavior creates the potential for a broad and intricate web of intra- and inter-specific acoustic interactions (Tobias et al. 2014).

Two species (*C. discocercus* and *A. festae*) had elevated calling activity during conspecific playbacks. Both species produce both calls and ticks, short percussive sounds that have been described for the males of at least one other phaneropterine species, *Caedicia* sp., from Australia (Bailey et al. 2006). In the case of *Caedicia* sp., these ticks are produced by males to disrupt the mating opportunities of conspecific neighboring males (Bailey et al. 2006). The presence of these acoustic features suggests that male-male interactions may be particularly important in these species. While *C. discocercus* only showed an overall elevation in calling rate in response to conspecific playbacks, in *A. festae*, the probability of calling was elevated for approximately 20 s after playback. *Arota festae* was also sensitive to playback amplitude with the loudest conspecific playback (representing the closest male) eliciting the greatest response (Fig. 5a). *C. discocercus* had relatively low calling activity during heterospecific playbacks, although the calling activity varied depending on the identity of the heterospecific (Fig. 5b). For example, calling activity was high after hearing the call of a particular heterospecific, *A. colossea*.

The katydids examined in this study did not display precise call intervals, but did adjust timing in response to what they heard. When calling in the absence of playbacks, several

species had a somewhat regular interval between calls, ranging from a few seconds to several minutes (Fig. S2). However, these call periods were approximate, with calls tending to arrive within a window of time, rather than at a rhythmic interval. The lack of a precise endogenous rhythm likely limits the predictability of the next call and the ability of males to anticipate and supersede the calls of other males. The acoustic environment did affect the signal timing of these eight katydid species, showing four broad clusters of response types: timing affected by both conspecifics and heterospecifics, by conspecifics only, by heterospecifics only, and call timing not affected by playbacks (Fig. 5). It is notable that these clusters are not united by taxonomy or similarity in call parameters. Species from different subfamilies (e.g., *C. wheeleri* and *L. micans*) show mechanistically similar responses while similar congeners show very different responses (e.g., *A. festae* and *A. panamae*). The fact that taxonomically diverse species show similar patterns of response suggests that there may be a few signaling strategies that successfully balance mate attraction, predation, and intraspecific competition. One strategy that is conspicuously absent in any of these species is short latency signal timing. The rarity of this type of signal timing interaction is striking compared to their prevalence in other environments. Short latency signal timing has been reported in tropical species that call from protected locations (Greenfield and Roizen 1993). The fact that signal timing has been observed in tropical species means that tropical conditions are not antithetical to signal timing, but rather that characteristics of the forest environment, likely predation by acoustically localizing bats and associated low calling activity, may make signal timing an ineffective strategy for many tropical forest katydid species (Belwood and Morris 1987; Kalka and Kalko 2006; Falk et al. 2015).

Although Neotropical forest katydid species are sensitive to the acoustic environment, the rarity of calls raises the question of how males and females find each other in these forests. Although male calls are short, most males do continue to produce sound sporadically throughout the night, potentially providing enough acoustic information for a motivated female. In Phaneropterines, duetting between males and females could facilitate localization once the signal is detected (Heller et al. 2015). Tremulation (body vibrations that propagate through the substrate) has been documented in several species of tropical katydids and forms an important component of mate localization in these species (Morris 1980; Morris et al. 1994). Although vibratory cues can alert predators that are on the same substrate, they provide protection against aerial predators such as bats that hone in on the male call (Römer et al. 2010). It may be that the airborne acoustic signal serves as a beacon that brings the female to the vicinity, while vibratory cues are used to complete the localization process. If particular species occur preferentially at specific heights or on particular plants, these preferences could further enhance encounter

rates. If the mate searching process is time consuming and dangerous, it may help to explain the large spermatophore gifts that males offer females (spermatophores are often 0.3–1 g) (Morris 1980; Del Castillo and Gwynne 2007). One possibility is that the energy conserved through low acoustic signal rate is used in tremulation and/or invested in the nuptial gift, maintaining high male investment, but shifting the modality (Gwynne 2001). A deeper understanding of the ecology, evolution, and spatial distribution of these insects will be important for understanding the interspecific differences in signaling.

Neotropical forest katydid species produce relatively little sound, but still alter calling activity and timing in response to the acoustic environment. In several cases, focal species respond differently to playbacks of heterospecifics than to playbacks of conspecifics or silence. The effect of the heterospecific community on signaling behavior suggests that the heterospecific community likely has substantial impacts on communication patterns, possibly as a result of signal interference, signal confusion, or shared predation risk (Phelps et al. 2007; Coleman 2008; Magrath et al. 2015). The findings of this study reveals that even animals with low levels of signaling can have extensive intra- and interspecific interactions around signaling.

Acknowledgments Funding and logistical support was provided by the Smithsonian Tropical Research Institute to LBS and RAP and by Dartmouth College to HMtH. Full permits for the project were obtained through the Smithsonian Tropical Research Institute. Thank you to L. Höger for assistance with data analysis. Thank you to the staff at Barro Colorado Island for logistical and administrative support.

References

- Alem S, Koselj K, Siemers BM, Greenfield MD (2011) Bat predation and the evolution of leks in acoustic moths. *Behav Ecol Sociobiol* 65: 2105–2116
- Bailey WJ (2003) Insect duets: underlying mechanisms and their evolution. *Physiol Entomol* 28:157–174
- 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? *Physiol Entomol* 31:201–210
- Belwood JJ (1988) The influence of bat predation on calling behavior in neotropical forest katydids (Insecta: Orthoptera: Tettigoniidae). PhD Thesis, University of Florida
- Belwood JJ, Morris GK (1987) Bat predation and its influence on calling behavior in neotropical katydids. *Science* 238:64–67
- Brumm H (2006) Signalling through acoustic windows: nightingales avoid interspecific competition by short-term adjustment of song timing. *J Comp Physiol A* 192:1279–1285
- Burkard R (2006) Calibration of acoustic transients. *Brain Res* 1091:27–31
- Colavita FB (1974) Human sensory dominance. *Percept Psychophys* 16: 409–412
- Coleman SW (2008) Mourning dove (*Zenaida macroura*) wing-whistles may contain threat-related information for con- and hetero-specifics. *Naturwissenschaften* 95:981–986
- Del Castillo R, Gwynne D (2007) Increase in song frequency decreases spermatophore size: correlative evidence of a macroevolutionary trade-off in katydids (Orthoptera: Tettigoniidae). *J Evol Biol* 20: 1028–1036
- Diwakar S, Balakrishnan R (2007a) The assemblage of acoustically communicating crickets of a tropical evergreen forest in southern India: call diversity and diel calling patterns. *Bioacoustics* 16:113–135
- Diwakar S, Balakrishnan R (2007b) Vertical stratification in an acoustically communicating ensiferan assemblage of a tropical evergreen forest in southern India. *J Trop Ecol* 23:479–486
- Dyson ML, Henzi SP, Passmore NI (1994) The effect of changes in the relative timing of signals during female phonotaxis in the reed frog, *Hyperolius marmoratus*. *Anim Behav* 48:679–685
- Eades D, Otte D, Cigliano M, Braun H (2016) Orthoptera species file online. Version 2.0/4.0 [WWW document]. URL <http://Orthoptera.SpeciesFile.org>
- Falk JJ, ter Hofstede HM, Jones PL, Dixon MM, Faure PA, Kalko EK, Page RA (2015) Sensory-based niche partitioning in a multiple predator–multiple prey community. *Proc R Soc Lond B Biol Sci* 282:20150520
- Geissmann T (2002) Duet-splitting and the evolution of gibbon songs. *Biol Rev Camb Philos Soc* 77:57–76
- Gerhardt HC, Huber F (2002) Acoustic communication in insects and anurans: common problems and diverse solutions. University of Chicago Press, Chicago
- Grafe TU (1996) The function of call alternation in the African reed frog (*Hyperolius marmoratus*): precise call timing prevents auditory masking. *Behav Ecol Sociobiol* 38:149–158
- Greenfield MD (1983) Unsynchronized chorusing in the coneheaded katydid *Neoconocephalus affinis* (Beauvois). *Anim Behav* 31:102–112
- Greenfield MD (1994) Cooperation and conflict in the evolution of signal interactions. *Annu Rev Ecol Syst*:97–126
- Greenfield MD (2005) Mechanisms and evolution of communal sexual displays in arthropods and anurans. *Adv Study Behav* 35:1–62
- Greenfield MD, Roizen I (1993) Katydid synchronous chorusing is an evolutionarily stable outcome of female choice. *Nature* 364:618–620
- Greenfield MD, Tourtellot MK, Snedden WA (1997) Precedence effects and the evolution of chorusing. *Proceedings of the Royal Society of London. Series B: Biological Sciences* 264:1355–1361
- Gwynne DT (2001) Katydid and bush-crickets: reproductive behavior and evolution of the Tettigoniidae. Cornell University Press, New York
- Gwynne DT, Bailey WJ (1988) Mating system, mate choice and ultrasonic calling in a zaprochiline katydid (Orthoptera: Tettigoniidae). *Behaviour* 105:202–223
- Hartbauer M, Kratzer S, Steiner K, Römer H (2005) Mechanisms for synchrony and alternation in song interactions of the bushcricket *Mecopoda elongata* (Tettigoniidae: Orthoptera). *J Comp Physiol A* 191:175–188
- Heller K, Hemp C (2014) Fiddler on the tree—A bush-cricket species with unusual stridulatory organs and song.
- Heller K, von Helversen D (1986) Acoustic communication in phaneropterid bushcrickets: species-specific delay of female stridulatory response and matching male sensory time window. *Behav Ecol Sociobiol* 18:189–198
- Heller K, Hemp C, Ingrisch S, Liu C (2015) Acoustic communication in Phaneropterinae (Tettigoniidea)—a global review with some new data. *J Orthop Res* 24:7–18
- Henry CS, Wells MM (2010) Acoustic niche partitioning in two cryptic sibling species of *Chrysoperla* green lacewings that must duet

- before mating. *Anim Behav* 80:991–1003. doi:[10.1016/j.anbehav.2010.08.021](https://doi.org/10.1016/j.anbehav.2010.08.021)
- Höbel G (2010) Interaction between signal timing and signal feature preferences: causes and implications for sexual selection. *Anim Behav* 79:1257–1266
- Höbel G, Gerhardt HC (2007) Sources of selection on signal timing in a tree frog. *Ethology* 113:973–982
- Kalka M, Kalko EK (2006) Gleaning bats as underestimated predators of herbivorous insects: diet of *Micronycteris microtis* (Phyllostomidae) in Panama. *J Trop Ecol* 22:1–10
- Klump GM, Gerhardt HC (1992) Mechanisms and function of call-timing in male-male interactions in frogs. In: Playback and studies of animal communication. Springer, New York, pp. 153–174
- Lang AB, Kalko EK, Römer H, Bockholdt C, Dechmann DK (2006) Activity levels of bats and katydids in relation to the lunar cycle. *Oecologia* 146:659–666
- Lehmann GU (2003) Review of biogeography, host range and evolution of acoustic hunting in Orniini (Insecta, Diptera, Tachinidae), parasitoids of night-calling bushcrickets and crickets (Insecta, Orthoptera, Ensifera). *Zoologischer Anzeiger-A Journal of Comparative Zoology* 242:107–120
- Lloyd JE (1984) Evolution of a firefly flash code. *Fla Entomol*:228–239
- Magrath RD, Haff TM, Fallow PM, Radford AN (2015) Eavesdropping on heterospecific alarm calls: from mechanisms to consequences. *Biol Rev* 90:560–586
- Mennill DJ, Burt JM, Fristrup KM, Vehrencamp SL (2006) Accuracy of an acoustic location system for monitoring the position of duetting songbirds in tropical forest. *J Acoust Soc Am* 119:2832–2839. doi:[10.1121/1.2184988](https://doi.org/10.1121/1.2184988)
- Montealegre-Z F (2009) Scale effects and constraints for sound production in katydids (Orthoptera: Tettigoniidae): correlated evolution between morphology and signal parameters. *J Evol Biol* 22:355–366
- Montealegre-Z F, Morris GK (1999) Songs and systematics of some Tettigoniidae from Colombia and Ecuador I. Pseudophyllinae (Orthoptera). *Journal of Orthoptera Research*:163–236
- Montealegre-Z F, Morris GK, Mason AC (2006) Generation of extreme ultrasonics in rainforest katydids. *J Exp Biol* 209:4923–4937. doi:[10.1242/jeb.02608](https://doi.org/10.1242/jeb.02608)
- Morris GK (1980) Calling display and mating behaviour of *Copiphora rhinoceros* Pictet (Orthoptera: Tettigoniidae). *Anim Behav* 28:42–IN1
- Morris G, Mason A, Wall P, Belwood J (1994) High ultrasonic and tremulation signals in neotropical katydids (Orthoptera: Tettigoniidae). *J Zool* 233:129–163
- Nickle DA, Quintero D, Aiello A (1992) Katydids of Panama (Orthoptera: Tettigoniidae) insect of Panama and Mesoamerica. Oxford Science Publications:142–184
- Phelps SM, Rand AS, Ryan MJ (2007) The mixed-species chorus as public information: túngara frogs eavesdrop on a heterospecific. *Behav Ecol* 18:108–114
- R Core Team (2015) R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. ISBN 3–900051–07–0, URL <http://www.R-project.org/>
- Rentz D (1975) Two new katydids of the genus *Melanonotus* from Costa Rica with comments on their life history strategies (Tettigoniidae: Pseudophyllinae). *Entomol News* 86:129–140
- Rodriguez RL, Cocroft RB (2006) Divergence in female duetting signals in the *Enchenopa binotata* species complex of treehoppers (Hemiptera: Membracidae). *Ethology* 112:1231–1238
- Romer H (1993) Environmental and biological constraints for the evolution of long-range signalling and hearing in acoustic insects philosophical transactions of the Royal Society B. *Biological Sciences* 340:179–185
- Romer H, Lewald J (1992) High-frequency sound transmission in natural habitats: implications for the evolution of insect acoustic communication. *Behav Ecol Sociobiol* 29:437–444
- Romer H, Lang A, Hartbauer M (2010) The signaller's dilemma: a cost-benefit analysis of public and private communication. *PLoS One* 5: e13325. doi:[10.1371/journal.pone.0013325](https://doi.org/10.1371/journal.pone.0013325)
- Ryan MJ, Tuttle MD, Taft LK (1981) The costs and benefits of frog chorusing behavior. *Behav Ecol Sociobiol* 8:273–278
- Sarria-S F, Morris G, Windmill J (2014) Shrinking wings for ultrasonic pitch production: hyperintense ultra-short-wavelength calls in a new genus of neotropical katydids. Tettigoniidae, Orthoptera
- Shaw KC, Galliard P (1987) Acoustic and mating behavior of a Mexican katydid, *Pterophylla beltrani* (Orthoptera: Tettigoniidae). *Fla Entomol*:354–368
- Siegert ME, Romer H, Hashim R, Hartbauer M (2011) Neuronal correlates of a preference for leading signals in the synchronizing bushcricket *Mecopoda elongata* (Orthoptera, Tettigoniidae). *J Exp Biol* 214:3924–3934. doi:[10.1242/jeb.057901](https://doi.org/10.1242/jeb.057901)
- Snedden W, Greenfield MD (1998) Females prefer leading males: relative call timing and sexual selection in katydid choruses. *Anim Behav* 56:1091–1098
- Symes L, Ayres M, Cowdery C, Costello R (2015) Signal diversification in *Oecanthus* tree crickets is shaped by energetic, morphometric, and acoustic trade-offs. *Evolution* 69:1518–1527
- ter Hofstede HM, Kalko EKV, Fullard JH (2010) Auditory-based defence against gleaning bats in neotropical katydids (Orthoptera: Tettigoniidae). *J Comp Physiol A* 196:349–358
- Tobias JA, Planque R, Cram DL, Seddon N (2014) Species interactions and the structure of complex communication networks. *Proc Natl Acad Sci U S A* 111:1020–1025. doi:[10.1073/pnas.1314337111](https://doi.org/10.1073/pnas.1314337111)
- Walker TJ, Moore TE (2013) Singing insects of North America. University of Florida. <http://entnemdept.ifas.ufl.edu/walker/Buzz/crickets.htm>