# Calling Songs, Duets, and Auditory Tuning in Two Cryptic Katydids (Tettigoniidae: Phaneropterinae: *Amblycorypha*)

T. G. FORREST,[1] DAWN R. LAJOIE, AND DAWN CUSICK

Department of Biology, University of North Carolina at Asheville, Asheville, NC 28804

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**ABSTRACT** In most katydids, females listen to and locate a stationary, singing male for mating. Pair formation differs in phaneropterine katydids where pairs form duets and the male typically finds the female after hearing her acoustic reply to his song. We recorded the duetting behavior of two cryptic species of phaneropterines, *Amblycorypha rotundifolia* (Scudder) and *Amblycorypha alexanderi* Walker (Tettigoniidae: Phaneropterinae), from populations in their zone of sympatry. The songs of the two species differed in their temporal properties, and the duets differed in the timing of the female's replies with respect to the male's song. We also measured the hearing sensitivity and auditory tuning in these species by recording extracellular neural responses to sound stimuli varying in frequency and intensity. Individuals of both species were most sensitive to frequencies near 13 kHz, which corresponds to the frequencies of the males' calling songs and to the peak frequency in the females' tick responses. Both species also responded to pulses with ultrasonic carrier frequencies. For higher amplitude stimuli, neural responses had shorter latencies and more action potentials. Latency functions differed for low-frequency and high-frequency stimuli. These data form the basis for understanding how auditory processing and sexual selection might be involved in the recent divergence of these two cryptic species.

**KEY WORDS** communication, acoustic behavior, phonoresponse

Acoustic communication by animals is a window through which biologists can view the processes of evolution. For insects, acoustic signals are usually associated with reproduction and therefore are important determinants of evolutionary success. Perhaps the best known insect signals are the species-specific calls of ensiferan Orthoptera (crickets and katydids) that are generated using a file and scraper mechanism located on the forewings (Bennet-Clark 1989, Gwynne 2001, Gerhardt and Huber 2002). Usually, the males produce long-range acoustic signals (calling songs), which function in attracting mates (Alexander 1967, Searcy and Andersson 1986). Song parameters enable the females to recognize and discriminate among conspecifics, promoting pair formation. Males often use their acoustic signals in territorial displays, adjusting the temporal rhythms of their songs to synchronize or alternate with their calling neighbors (Alexander 1975, Greenfield and Shaw 1983). Because of their function in mate choice and during competitive interactions among males, the acoustic signals of crickets and katydids should be under strong sexual selection (Gerhardt and Huber 2002, Greenfield, 2002).

Pair formation among katydids usually involves a silent female using a calling song to home in on a stationary mate's location. One katydid subfamily, the Phaneropterinae, exhibits a more complex acoustic signaling system (Spooner 1964, 1968). Females of most phaneropterines produce an acoustic "tick" in response to the male's calling song. The pair forms a duet (Bailey 2003), exchanging acoustic signals during phonotaxis. Males typically locate the female by tracking her responses during the duet (Shaw et al. 1990, Dobler et al. 1994, Zimmermann et al. 1988). However, pair formation can be highly variable with either or both sexes moving toward the other during the acoustic exchanges (Heller 1990, Spooner 1995).

The timing of the signals in the duet can be extremely precise and requires that each individual detect and localize the signals of their partner, often while moving through a noisy environment. Tympanal organs located on the forelegs are responsible for sound reception in ensiferan Orthoptera. Hearing can be sharply tuned to the species' calling song, particularly among the phaneropterine katydids, to enhance reception of the signal (Dobler et al. 1994). In addition to detecting the sounds of conspecific males and females during social interaction, hearing can function in detecting sounds produced by predators (Pollack and Hoy 1989, Weber and Thorson 1989, Hoy 1992). The neural networks that relay and process auditory information in crickets and katydids are composed of a relatively small number of auditory receptors and interneurons. Yet, across animal taxa, striking similar-

[1] Corresponding author, e-mail: tforrest@unca.edu.

ities exist in the neural responses for coding intensity, frequency and time of acoustic signals (Gerhardt and Huber 2002). Given the simplicity of the orthopteran nervous system and their stereotypic responses to acoustic stimuli, crickets and katydids serve as excellent models to study the neural substrate of auditory processing.

The calling songs of *Amblycorypha*, a North American genus of phaneropterines, are extremely diverse, ranging from simple sound units of *Amblycorypha carinata* Rehn & Hebard to the multicomponent songs of the *uhleri* complex, which are the most complex songs in the Ensifera (Walker and Dew 1972, Walker 2004). Given the complex acoustic interactions characteristic of *Amblycorypha* calling songs and their duets, studies of communication within this group may provide information about the roles of signal detection and sexual selection in adaptive speciation (Dieckmann et al. 2004). A recent revision of the *Amblycorypha rotundifolia* (Scudder) complex distinguished three eastern species: *Amblycorypha alexanderi* Walker, *Amblycorypha bartrami* Walker, and *Amblycorypha. rotundifolia* (Walker et al. 2003). At present, these three cryptic species can only be identified on the basis of differences in the male's calling songs. The morphological similarities among species in the *rotundifolia* complex suggest a recent divergence, and a comparative approach that examines communication within and between species of *Amblycorypha* should offer important insights into the evolution of signal complexity and speciation.

Our work investigates communication and hearing in *A. alexanderi* and *A. rotundifolia* from populations in North Carolina, where their geographic distributions overlap. To examine auditory processing, we measured their neural responses to sound stimuli and determined their spectral sensitivities, response latencies, and intensity response functions. We describe the relationship between the neurophysiological tuning and the acoustic signals produced by duetting males and females. Our data represent the first neural recordings for this genus and we compare our findings with those from other tettigoniids.

## Materials and Methods

**Animal Collection, Care, and Housing.** *A. rotundifolia* and *A. alexanderi* were collected from herbaceous plants along roadsides in Buncombe, Henderson, and McDowell counties, North Carolina. Adult males were located after sunset by homing in on their calling song and netting them. Females emit a brief, quiet sound that is difficult to hear in the field. Therefore, females and silent juveniles were found by scanning the vegetation near calling males with a headlight. In the laboratory, the captured individuals were housed separately in 10- by 10- by 9-cm plastic cages and were provided water, lettuce, apple, and dry cat or dog food ad libitum. Animals were kept at 22–25°C with natural photoperiod.

**Song Recording and Analysis.** We recorded male calling songs and female tick responses with a Tascam DA-P1 digital audio tape recorder (48-kHz sampling frequency) and Sennheiser ME 66 shotgun microphones (frequency response 50–20,000 Hz ± 2.5 dB) powered with Sennheiser K6 power modules. To reduce reverberations, katydids were housed in 10- by 10- by 9-cm screened cages above Sonex sound-attenuating foam. When recording interactions between pairs, the song of each individual was recorded onto a separate channel of the recorder. The distance between pairs varied from 1 to 3 m. The shotgun microphones were kept 15 cm from each individual's cage and directed at the cage to improve the acoustic isolation between channels. Temperatures during the recordings ranged from 22 to 24°C.

For analysis, recorded signals were transferred directly to a computer where we made temporal and spectral measurements using CoolEdit96 or Adobe Audition software. Unless stated otherwise, all measurements were made to the nearest 1 ms with a frequency resolution of 57 Hz. Delays in the female tick replies were always measured from the onset of the previous syllable of the male. Because measured variation in the delays was large (range 100–600 ms; see below), we did not take into account travel time of signals between the cages (34 cm/ms; ≈3–9 ms). For the calling songs of the males, we defined a syllable as the sound produced by a single complete wing cycle and measured the syllable period from a point on one syllable, usually the beginning of the closing stroke, to the corresponding point on the next syllable.

**Physiological Preparation.** Immediately before dissection, katydids were cold anesthetized for 5–8 min. Using low-melting temperature wax, katydids were then affixed, ventral side up, to a custom-made insect holder. The prothoracic legs were restrained in a seminatural position, by waxing each tarsus to the holder. Care was taken to ensure the integrity of the tympana. The ventral cervical membrane was removed to expose the connectives between the subesophageal and prothoracic ganglion. During the dissection, we minimized trauma to internal acoustic trachea and auditory spiracles and we kept the preparation moist with standard insect saline. One of the cervical connectives was then hooked with an electrolytically sharpened, tungsten electrode, and petroleum jelly was applied to the nerve–electrode coupling to prevent desiccation and to isolate the nerve from a tungsten reference electrode placed in the insect's abdomen.

**Stimulus Generation and Presentation.** Electrophysiological recordings were made inside a 1.3- by 0.85- by 0.75-m sound-attenuating chamber lined with Sonex foam. The temperature in the chamber averaged 24°C (range 23–25°C). For individual tuning curves, pure-tone pulses (10-ms duration including 1-ms raised-cosine ramps) were broadcast at varying frequencies between 1.5 and 57.8 kHz. Stimuli were generated with custom software and were broadcast using a TDT 16-bit DA3-2 converter at a sampling period of 5.76 $\mu$s (≈174 kHz). To prevent aliasing, the output of the DA was filtered at 69 kHz using a Kemo VBF eight Dual Variable Filter. A Harmon/Kardon Amplifier (HK620) drove the speakers (catalog no.

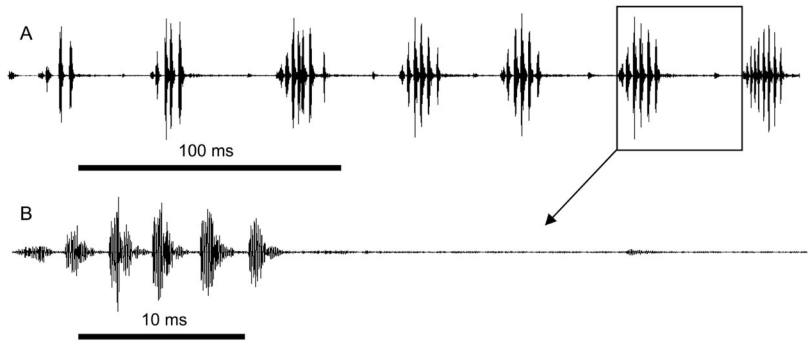


**Fig. 1.** Oscillograms of portions of an *A. rotundifolia* male calling song (at 22.8°C). (A) Seven-syllable phrase. (B) Single syllable from phrase in A indicated by the rectangle. Note that the syllable is composed of six high-amplitude pulses presumably produced during the closing stroke of the tegmina followed by a single low-amplitude pulse that represents the opening stroke.

40-1310B, Realistic, Super Tweeter and catalog no. 40-1221, Optimus, Bullet Horn Tweeter), which were located 30 cm from the preparation at 90°L relative to the katydid's longitudinal axis.

Sound pressure levels (SPLs) of the stimuli were controlled using a TDT PA-4 programmable attenuator and were calibrated using Larson Davis 2520 ¼-in. microphone (PRM910B preamp and 2200C power supply) with protective grid located at the position of the insect's ipsilateral tympanum. The calibration of the microphone was checked using a Larson Davis acoustic calibrator (Cal 200). Sound pressure levels are expressed in decibels relative to 20 $\mu$Pa.

Neural responses were amplified using an A-M Systems model 1700 differential AC amplifier (high pass 300 Hz, low pass 5 kHz) and were recorded using a TDT 16-bit AD2 sampled at 10 kHz. Custom software controlled the SPL, frequency of the stimulus presentation, and the recording of the amplified neural response. Each stimulus (frequency by SPL) was presented five times at a rate of 1/s. Recorded data were stored on the computer and analyzed off-line to determine neural threshold, first spike latency to the nearest 0.1 ms, and spike counts for each stimulus presentation.

**Physiological Analyses.** Auditory threshold was defined as the minimum SPL required to elicit correlated neural responses in at least three of five stimulus presentations. Tuning curves were based on the average of all individual's thresholds as a function of stimulus frequency. Best frequency (BF) for each sex was defined as the frequency with the lowest average threshold.

For each individual, the response latency (milliseconds) and number of spikes was averaged over the five stimulus presentations at the same sensation level (SL in decibels above threshold) and frequency. These means were then averaged across individuals. Because measurements of latencies and spike counts were performed off-line, data were excluded when traces were excessively noisy or when the average was based on fewer than two individuals.

## Results

**Male Songs.** Our song descriptions are based on our laboratory recordings and are similar to descriptions for *A. rotundifolia* and *A. alexanderi* given by Walker et al. (2003). The spectra of the males' songs are broadband, with most of the energy between 9 and 15

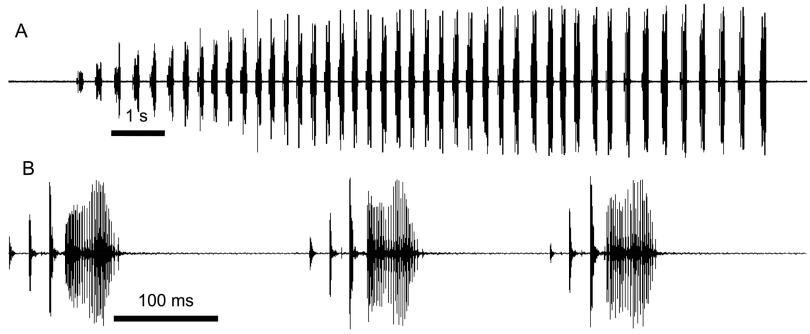


**Fig. 2.** Oscillograms of portions of an *A. alexanderi* male calling song (at 22.9°C). (A) A 13-s series consisting of 42 syllables. (B) Three syllables showing the complex changes in amplitude during each wing-movement cycle; each syllable is composed of three initial impulses followed by a longer terminal "buzz."

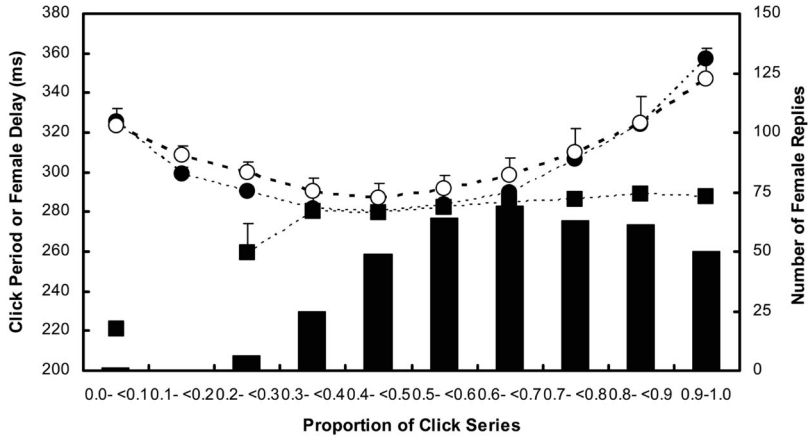


**Fig. 3.** Temporal changes in calling song and female response delays during the click series of male *A. alexanderi*. Circles are the average ± SE syllable periods during solo singing (open; $n = 5$) and duetting (closed; $n = 4$). Bars represent the frequency distribution of when female responses occurred during the click series. The squares are the average ± SE of the response delays represented in the frequency distribution.

kHz (see below). Calling songs of the two species have similar spectra but differ greatly in their temporal properties.

Male *A. rotundifolia* produce variable duration (≈140 to >7,000 ms) phrases consisting of a series of syllables (also see Walker et al. 2003). Figure 1A shows a phrase with seven syllables. The closing stroke of each syllable is composed of one to nine pulses, each 1–3-ms in duration (Fig. 1B). Syllables at the beginning of a phrase tend to have fewer pulses (one to three) than those at the end of the phrase. Syllable periods ranged from 37 to 46 ms for recording temperatures between 22 and 24°C, corresponding to rates of 27–22/s. These rates are the fastest in the species complex and are perceived by the human ear as a rattle. Normally the males' songs begin with short phrases that increase in duration (and amplitude) culminating in a long duration phrase (of several seconds often with >200 syllables) that may then be followed by several shorter duration phrases. Phrases are separated by silent intervals that are also highly variable in duration (≈140–6,000 ms).

**Table 1. Peak frequencies of the acoustic replies of duetting *Amblycorypha* females ($n = 4$ *A. rotundifolia* females responding to three different males and three *A. alexanderi* females duetting with four different males)**

| Male no. | Female no. | Responses ($n$) | Responses (% between phrases) | Peak frequency[a] ± SD (kHz) |
|---|---|---|---|---|
| *A. rotundifolia* | | | | |
| 07 | 01 | 39 | 85 | 12.3 ± 1.5 |
| 01 | 02 | 92 | 74 | 11.2 ± 0.5 |
| 07 | 02 | 159 | 89 | |
| 02 | 03 | 46 | 89 | 12.3 ± 1.2 |
| 07 | 04 | 37 | 81 | 11.2 ± 1.4 |
| *A. alexanderi* | | | | |
| 03 | 03 | 110 | | 13.0 ± 0.7 |
| 02 | 03 | 133 | | |
| 01 | 05 | 74 | | 13.3 ± 0.1 |
| 06 | 06 | 81 | | 12.0 ± 0.9 |

*A. rotundifolia* females responded most often during the interphrase intervals of the songs.

[a] Average of 10 responses from each female.

Songs of *A. alexanderi* males consist of a series of ≈20–60 syllables (Fig. 2A) that differ significantly from those in the rattling song of *A. rotundifolia.* The syllable rate of *A. alexanderi*, ≈3.5/s at 24°C is much slower than *A. rotundifolia* and is the slowest in the complex (Walker et al. 2003). Each syllable is easily resolved by the human ear and is heard as a click. Whereas the syllables of *A. rotundifolia* are composed of similar pulses, the sounds produced by the closing stroke of the tegmina of *A. alexanderi* are temporally complex, composed of one to four pulses followed by a terminal buzz that contains 10 or more rapid impulses (Fig. 2B). The complexity of the syllable suggests that changes in the rates of wing closure occur during each wing cycle. During the series the click rates also change (Fig. 3, open circles). Because the series vary in duration and in the number of clicks, we divided each series into 10 equal intervals and averaged the click periods during each of the 10 intervals. At 24°C, the syllables (clicks) at the beginning of the series are produced at ≈3/s (period 325 ms). The rates gradually increase and remain relatively constant at ≈3.5/s (period 290 ms) in the middle third of the series. Then, rates decrease during the last half of the series (Fig. 3, open circles).

**Female Replies.** Like most phaneropterine katydids, females of the two species replied to males' songs with ticks. Often, several ticks were produced in close succession (2–5 ticks with periods of a few milliseconds). When multiple ticks were separated by <10 ms we used only the time to the first tick answer for analysis.

We recorded 373 tick answers from four *A. rotundifolia* females that answered three different males. Most (81%) of the female answers occurred during the silent intervals between phrases (Table 1; Fig. 4A).

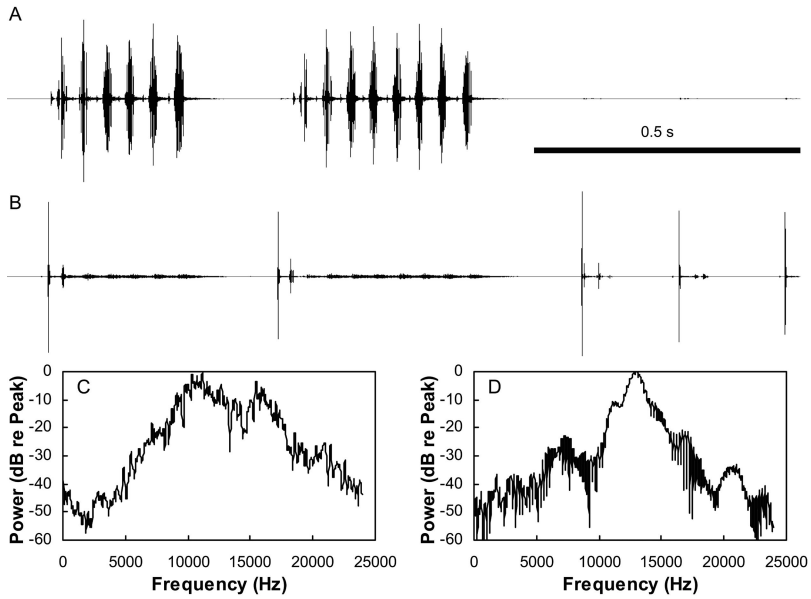


**Fig. 4.** Oscillograms (A and B) and spectra (C and D) of *A. rotundifolia* duetting pair recorded simultaneously (at 22.8°C). (A) Last two phrases of male calling song consisting of six and eight syllables, respectively. (B) Female tick responses. Note that the tick preceding the first male's phrase is the female's response to the previous phrase of the male (not shown). Male signal can be seen in female channel. (C and D) Power spectra of single syllable of male and single tick response of female, respectively. Both signals are broadband (10–15 kHz), but the female's tick has a well-defined peak around 13 kHz.

For those answers that occurred during the males' phrase (19%), there seemed to be no relationship in the timing of the answers with respect to the syllables in the song. Approximately 30% of the replies occurred during the acoustic portion of the syllable, and 70% occurred during the silent portion of the syllable, almost exactly the ratio of sound/silence in that part of *A. rotundifolia*'s calling song. Using only the females' first responses that occurred between phrases ($n = 162$), the response delays were variable, ranging

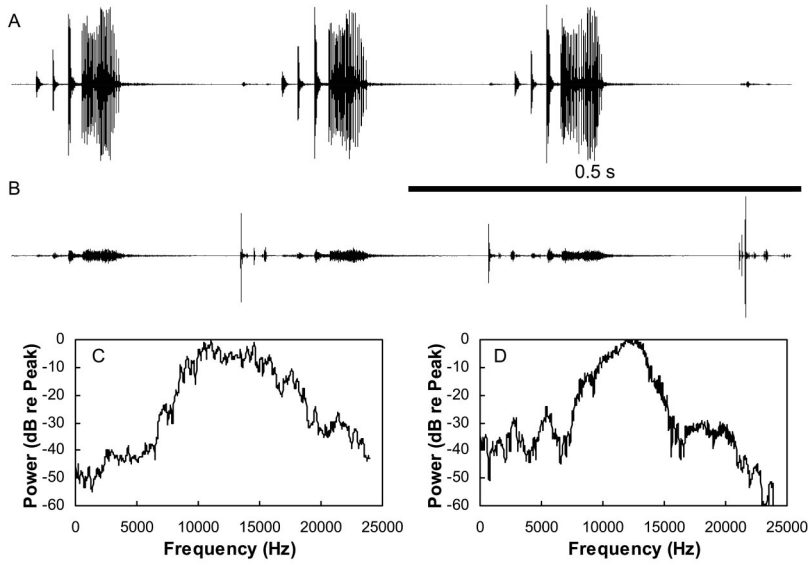


**Fig. 5.** Oscillograms (A and B) and spectra (C and D) of *A. alexanderi* duetting pair recorded simultaneously (at 22.9°C). (A) Three syllables of male calling song. (B) Female tick responses emitted between syllables of male. Note: Male's syllables are evident in female channel. (C and D) Power spectra of single syllable of male and single tick response of female *A. alexanderi*, respectively. Females' ticks have a well-defined peak around 13 kHz compared with the broadband (10–15 kHz) signal of the male.

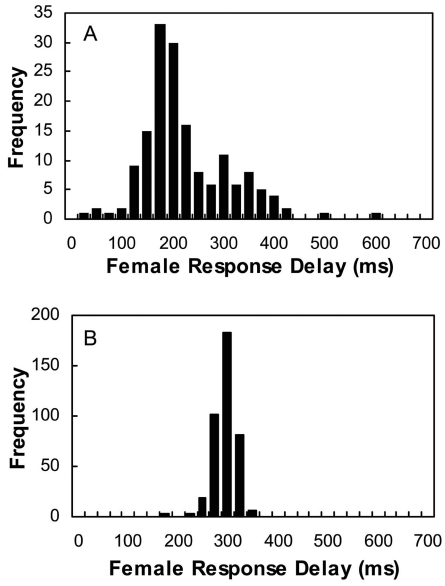


**Fig. 6.** Frequency distributions of female response delays. (A) *A. rotundifolia* ($n$ = 162, average = 220 ms, range 12–817 ms, and SD = 106 ms). (B) *A. alexanderi* ($n$ = 398, average = 284 ms, range 161–345 ms, and SD = 24 ms).

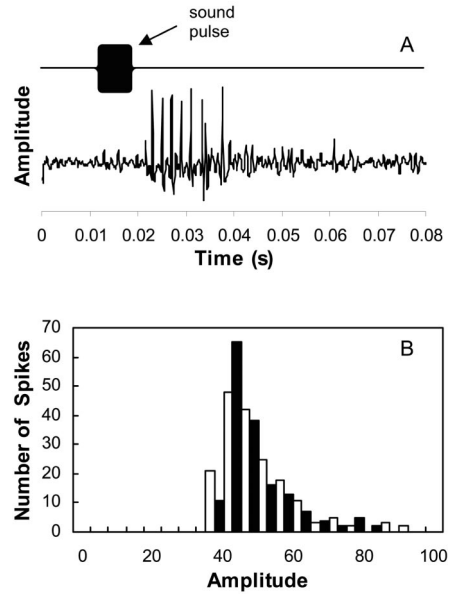


**Fig. 7.** (A) Neural response of *A. rotundifolia* male to a single, 10-ms pulse with a 10-kHz carrier at 55-dB SPL. The recording is extracellular using a hook electrode around the neck connective. (B) Distribution of spike amplitudes (arbitrary units) from the neural responses of an *A. rotundifolia* male to stimuli near the calling song (solid bars; 12 and 13 kHz, $n$ = 163, and average = 48.9 U) and in the ultrasound (open bars; 34 and 43 kHz, $n$ = 180, and average = 45.8 U).

from 12 to >800 ms and averaging ≈220 ms (Fig. 6A). Females often produced a series of ticks after the final phrase of the male's song. The period between these ticks were very precise with most periods between 200 and 250 ms (Fig. 4).

The duets between *A. alexanderi* pairs were more precise in their timing than those of *A. rotundifolia* (Fig. 5). We recorded 398 replies from three females answering four different males. For each duet, we measured the periods of the male songs and the delays of female replies during 10 click series (normalizing for the different durations and number of clicks in the series). Just as we found with solo singing males, duetting males showed the same change in syllable periods during each series of clicks (Fig. 3, compare open and closed circles). Approximately 80% of the female responses occurred during the last half of the click series (Fig. 3, bars). Those that occurred early in the series tended to have shorter delays relative to the preceding syllable of the male. Replies in the last half of the males' series showed a precise, constant delay of ≈280 ms (at 23–24°C; Fig. 3, squares). The timing of the female replies was such that answers given during the middle portion of the series were produced during the pulsed part of the subsequent click, whereas those given later in the series, when the males' periods increased, were produced in the silence between the syllables. The response delays of *A. alexanderi* varied much less than those of *A. rotundifolia* (coefficient of variation [CV] = 8 versus 48%, respectively; compare Fig. 6A and B). On average, the response delays of *A. alexanderi* females were 284 ms ($n$ = 398; range 161–345 ms; SD = 24 ms).

Spectral analysis of the female answers showed that their ticks also were broadband but with a measurable peak (often 6–12 dB above the rest of the spectrum; Figs. 4D and 5D for *A. rotundifolia* and *A. alexanderi*, respectively). The average peak frequency for ten answers of each female was between 11.2 and 13.3 kHz (Table 1).

**Neural Responses.** Fig. 7A shows a typical extracellular, neural response recorded from a neck connective electrode. The distributions of spike amplitudes were unimodal (Fig. 7B), indicating that a single auditory interneuron was consistently responding to the sound stimuli. Moreover, the distributions of the neural response to frequencies around the calling song and to those in the ultrasound were the same, indicating that the same neuron was responding across the frequency range we used (Fig. 7B).

The BFs measured for *A. rotundifolia* were 13 and 12 kHz for males ($n$ = 5) and females ($n$ = 5), respectively (Fig. 8). We also measured the auditory tuning for 15 *A. alexanderi* males, and their BF was also 13 kHz. Thus, the BF corresponded to the frequency range of the male's song and to the peak frequency of the female's tick answer. Thresholds for frequencies near the calling song were ≈30 dB SPL (Fig. 8). The tuning curves of all three groups were similar, and we found no sex difference in tuning between male and female *A. rotundifolia* (Fig. 8). The neuron also responded to ultrasonic pulses, as indicated by thresholds of 55–59 dB for stimuli between 43 and 58 kHz.

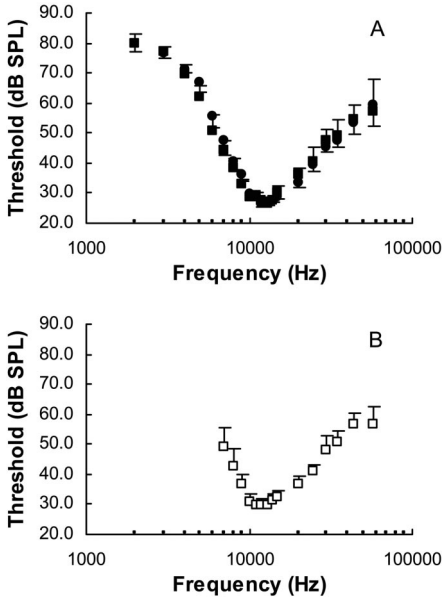


**Fig. 8.** Audiograms for (A) *A. rotundifolia* (closed squares, $n = 5$ males; closed circles, $n = 5$ females) and (B) *A. alexanderi* (open squares, $n = 15$ males). Points represent the average ± SD lowest sound pressure level eliciting a correlated neural response in three of five responses at each test frequency.

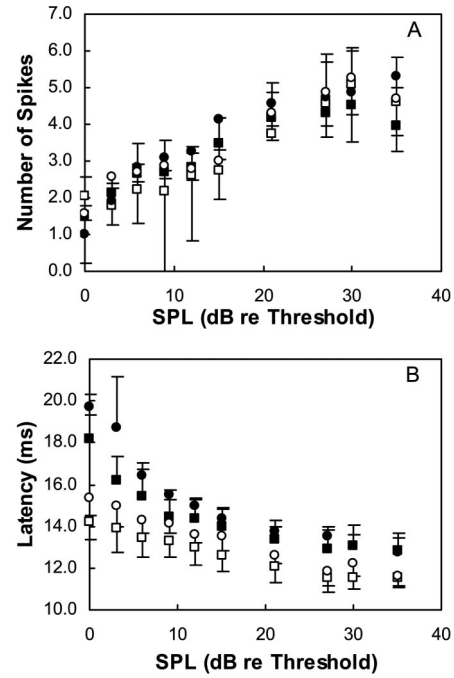


**Fig. 9.** Intensity response functions for *A. rotundifolia* males (circles, $n = 5$) and females (squares, $n = 5$). Points are the averaged ± SD spike counts (A) and latency (B) of neural responses to single 10-ms pulses at 13 kHz (closed symbols) and 35 kHz (open symbols) as a function of SPL normalized to each individual's threshold.

As the intensity of the sound stimulus increased, the average number of spikes increased, and the average latency of the neural response decreased (data only for *A. rotundifolia*). The neural response had a small dynamic range (≈25 dB) at both ultrasonic (35 kHz) and calling song frequencies (13 kHz). The average number of spikes increased from ≈1 spike/stimulus presentation near threshold to maximum of five to six spikes/stimulus presentation for intensities >20 dB above threshold (Fig. 9A). For sound pulses at 13 kHz, the latency of the neural response decreased from 18 to 20 ms at threshold to a minimum near 14 ms at intensities >30 dB above threshold (Fig. 9B). The cell responded more quickly to ultrasonic pulses at 35 kHz and had latencies of 14–16 ms at threshold that decreased to 12 ms for stimuli >30 dB above threshold (Fig. 9B).

## Discussion

**Male Songs.** Galliart and Shaw (1991, 1992, 1996) worked extensively on acoustic communication and the reproductive biology of *Amblycorypha parvipennis* Stäl, the western species in the *rotundifolia* group. Like most of the species in the complex, *A. parvipennis* males emit phrases consisting of a series of syllables. In the field, males chorus with neighbors such that their phrases alternate but with some portions of the phrases overlapping in time. Within the overlapping regions of the phrases, the syllables are synchronized (Fulton 1928, Shaw et al. 1990). The songs of *A. alexanderi* are similar to, but slower than those of *A. parvipennis*, and we have observed neighboring male *A. alexanderi* synchronizing their clicks. Like *A. parvipennis*, *A. alexanderi* clicks are complex and suggest that wing movement cycles (i.e., syllables) involve more than simple opening and closing strokes. Complex wing movement cycles seem to be common in phaneropterines (see Heller 1990 for a comparative study of wing movement, syllables, and sound production by Barbistini katydids), including species in the *Amblycorypha uhleri* complex (Walker 2004, Walker and Dew 1972).

The calling song of male *A. rotundifolia* also consists of phrases composed of a series of syllables, with phrases and silent interphrase intervals that are highly variable in duration. The syllables, composed of one to nine simple impulses, are produced at rates of ≈26/s (25°C). They are the fastest rates in the group, ≈4 times the rate of *A. parvipennis* and ≈8 times the rate of *A. alexanderi* (Walker et al. 2003).

**Female Replies and Duetting.** Both species form duets. Female *A. rotundifolia* differ in the timing of their replies compared with those of the other species in the *rotundifolia* complex. Females of the other species in the complex, including *A. alexanderi*, typically respond between the syllables within the male's series (Shaw et al. 1990, Walker et al. 2003). However, the syllable rates of *A. rotundifolia* males are apparently too fast for females to time their answers between syllables. Females usually produced tick answers dur-

ing the silent intervals between phrases. Most (81%) of the female answers occurred during these interphrase intervals. When the answers occurred during the rattle portion of the song (19%), they had no consistent temporal relationship with the syllables of the male song. The reply latencies of *A. rotundifolia* females are shorter (mean 220 ms versus 284 ms) and more variable (CV = 48 versus 8%) than those of *A. alexanderi* (Fig. 6), suggesting differences in female response strategies between the two species. The precision with which *A. alexanderi* duets are timed indicates that these temporal relationships are important in recognition and that male responses may require that female replies occur during a specific time window (Heller and von Helversen 1986, Robinson et al. 1986, von Helversen et al. 2001).

Spectra of the songs of *A. rotundifolia* and its sympatric congener, *A. alexanderi*, are identical; therefore, other song parameters (e.g., the temporal patterning of the males' songs and differences in timing of the duet) probably provide information for mate choice that maintains reproductive isolation between the two species. Within a species, females may make more fine-tuned discrimination among males using temporal properties that predict male size (Galliart and Shaw 1992, 1996; Tuckerman et al. 1993).

Phaneropterine katydids exhibit at least four different pair-forming strategies that involve one or both of the sexes moving toward the sounds emitted by the other sex (Spooner 1995). Spooner (1995) described pair formation in *Amblycorypha* "fast clicker" where males and females showed positive phonotaxis to the sounds produced by the opposite sex. *A. parvipennis* females also moved toward signaling males and preferentially responded by phonotaxis and phonoresponse to males whose songs were louder than, longer than, or leading an alternative song (Galliart and Shaw 1996).

**Neurophysiology.** Our neurophysiological results indicate that the auditory system of male and female *A. rotundifolia* and *A. alexanderi* males are tuned to the frequencies of their duetting signals (Fig. 8). Auditory filtering and tuning of hearing organs to the spectra of their signals is a general trend found in many other Orthoptera (Stumpner 1997, 1999; Mason et al. 1998; Faure and Hoy 2000; Tauber and Pener 2000) and most likely optimizes the reception of conspecific signals in a field of background noise (Dobler et al. 1994). It is interesting that the songs of the two sexes have similar frequency content, considering that the signals are generated by different, independently evolved structures on the forewings (Nickle and Carlysle 1975). Differences in the female stridulatory files can be used to separate *A. bartrami* from *A. alexanderi* and *A. rotundifolia*, but apparently they do not distinguish *A. alexanderi* from *A. rotundifolia* (Nickle and Carlysle 1975). Unfortunately, no spectral analyses of *A. bartrami* female ticks have been done for a comparison.

The match that we found between *Amblycorypha* signal spectra and auditory tuning is probably the result of coevolution of the auditory and signaling systems. However, in some katydids the auditory system does not seem to be tuned to their signals. A comparative study of tuning in *Neoconocephalus* showed similar auditory sensitivity in five species with widely different calling song spectra. The tuning, which was best near 16 kHz, seems to be a compromise between efficient detection of mating signals (7–15 kHz) and selection for hearing high frequency signals of echolocating bats (Schul and Patterson 2003). One Australian katydid, *Sciarasaga quadrata* Rentz, also has a tuning mismatch that results in a 20-dB reduction of sensitivity to the male's call (Romer and Bailey 1998). Romer and Bailey (1998) found that *S. quadrata* could close their auditory spiracle and by doing so, individuals improved the auditory tuning to conspecific call, filtered the songs of other congeneric species, and improved the signal-to-noise ratio in the field.

Acute sensitivity is a notable characteristic of *A. rotundifolia* and *A. alexanderi*, as evidenced by the lowest threshold (≈30 dB at 13 kHz) in their audiogram (Fig. 8). Given the pair formation system in these species, in which males home in on the female's low intensity acoustic response, acute hearing may be indicative of the difficulty males face detecting and localizing the short, quiet responses of females. In addition, male katydids frequently aggregate and chorus with one another in the field (Greenfield and Shaw 1983). Thus, males must hear the songs of competitors as well as the answers of females, whereas females must hear the songs to select a mate. *A. rotundifolia* males participate in bout synchrony, a type of chorusing in which nearby males sing in unison (Greenfield and Shaw 1983). *A. alexanderi* males also chorus but synchronize syllables with nearby neighbors. If there is a specific window of time after a male's signal where female answers are detected, synchrony may allow a male to eavesdrop on duets by synchronizing his "clock" with that of a duetting competitor (see Bailey and Field 2000 and Hammond and Bailey 2003 for discussion of acoustic satellite behavior, eavesdropping, and countermeasures in Australian phaneropterines).

Because we recorded extracellularly, the interneuron we detected was not identified. Frequency distributions of the amplitudes of the neural response indicate that we recorded the response of a single auditory interneuron and that this neuron responds to both calling song frequencies and ultrasound (Figs. 7 and 8).

It seems likely that the cell we were detecting is TN1, an auditory interneuron with large axons ascending and descending from the prothoracic ganglion (for review, see Faure and Hoy 2000). Because of its size, the response of TN1 is very easy to detect in extracellular recording. The cell we recorded is broadly tuned, responding to both ultrasonic and low-frequency stimuli. Tuning for TN1 has been studied in a number of katydids and is usually broad with responses similar across species (Faure and Hoy 2000). However, in all studies to date, TN1 was always tuned to higher frequencies (BF 16–30 kHz) than the cell we studied in *Amblycorypha.* When recording from the ipsilateral connective, we did not find a difference

between the sexes in the tuning of this cell. Sex differences in auditory tuning, although not typical, have been reported in T-cells of tettigoniids (*Kawanaphila nartee* Rentz: Bailey and Romer 1991; *Neoconocephalus ensiger* (Harris): Faure and Hoy 2000). Faure and Hoy (2000) found that the BFs and best thresholds of T-cells in *N. ensiger* were significantly lower in females than males and suggested that the differences in tuning might be related to size dimorphism in that species.

The spike latency functions for our recordings were influenced by stimulus frequency. At the same sensation level (decibels above threshold), response latencies were shorter in response to ultrasonic pulses compared with those to frequencies near 13 kHz. These results match those found by Faure and Hoy (2000) for TN1.

Given that the neuron we recorded from was tuned to frequencies near the acoustic signals of *Amblycorypha*, it seems likely that this neuron is involved in processing information related to pair formation. However, the neuron was also sensitive to ultrasound. Hearing serves not only to identify conspecifics but also to detect potential predators. Our results indicate that *Amblycorypha* might detect the biosonar used by hunting bats (Fig. 8, threshold of 60 dB at 40 kHz). The latencies of the neural responses to ultrasound pulses were shorter than those to calling song frequencies (Fig. 9B), a feature often associated with predator detection and avoidance (Hoy 1992, Forrest et al. 1995, Stumpner 1999). The increase in the relative strength of the neural response to ultrasound seems consistent with the importance of predator detection to the animal and the latency and intensity response functions were comparable to those measured from other species that exhibit acoustic startle responses to bat ultrasound.

Flying nocturnal insects face significant threat of predation from bats (Nolen and Hoy 1986, Schul et al. 2000), and the detection of ultrasound can reduce predation risk (Mason et al. 1998). It is interesting that members of the *rotundifolia* group of *Amblycorypha* are flightless and can only make short hops of <2 m. It is therefore unlikely that they are exposed to aerial hunting bats. There are several untested hypotheses that might explain ultrasound sensitivity in *A. rotundifolia* and *A. alexanderi*. 1) Gleaning bats might hunt *Amblycorypha*, especially males that call from exposed locations on the tops of shrubs. 2) The acoustic signals are broadband. Although our equipment did not allow us to analyze frequencies above 24 kHz, recordings of *A. parvipennis* showed that some portions of the signals extending beyond 40 kHz (Shaw et al. 1990). Also the signals of female *A. rotundifolia* and *A. alexanderi* have a peak in their spectrum near 12 kHz and may contain "harmonics" (24 and 36 kHz). Because different frequency bands attenuate at different rates, detecting relative levels in the harmonics of female signals might allow males to estimate distance to signaling females and improve their ability to find females during phonotaxis. 3) There are three species groups of *Amblycorypha*, and species in the *oblongifolia* and *uhleri* groups can fly. Depending on the ancestry and the time of divergence, ultrasonic sensitivity might have evolved in the context of predator avoidance before divergence of the *rotundifolia* complex. 4) The ultrasound sensitivity might be important in detecting other predators that produce incidental ultrasonic sounds while foraging. Clearly future studies will be needed to determine whether detection of ultrasound by *A. rotundifolia* and *A. alexanderi* is coupled to an acoustic startle response.

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