

Steering responses of flying crickets to sound and ultrasound: Mate attraction and predator avoidance*

(phonotaxis/acoustic communication/neuroethology/frequency discrimination/insect hearing)

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ABSTRACT We investigated the steering responses of tethered, flying adult female crickets (*Teleogryllus oceanicus*) to acoustic stimulation. Crickets responded directionally to directional sound stimulation by bending their abdomens and hind legs to one side. We interpret this response as an attempt to turn. When stimulated with a model of conspecific calling song with a carrier frequency of from 3 to 9 kHz, crickets turned toward the sound source. We believe that this indicates a positive phonotactic response of flying females to calling, conspecific males. When offered a choice between conspecific song and the song of another species, females turned exclusively toward conspecific song, demonstrating that the response is species specific. The direction of the response is dependent on the carrier frequency of the song, and it demonstrates frequency discrimination. Females turned toward calling song when it was played at carrier frequencies from 3 to 9 kHz, but they turned away from the same song pattern played at carrier frequencies from 30 to 70 kHz. This negative phonotaxis to ultrasonic stimuli suggests that crickets, like some other nocturnal flying insects, can evade bats by acoustic detection.

An important problem in neuroethology concerns the mechanisms by which animals detect and interpret signals produced by other animals. This question is of general significance because all animals must discriminate between potential mates, rivals, and predators. In many animals, males produce acoustic signals in order to attract conspecific females for mating. A model system for the study of acoustically mediated mate attraction is found in crickets. Male crickets emit an acoustic signal (calling song) that attracts conspecific females. The signal consists of a series of sound pulses of relatively pure carrier frequency (generally between 3 and 6 kHz), arranged in a genetically determined temporal pattern (1, 2). The calling songs of species that overlap each other in space and time differ from one another in carrier frequency, temporal pattern, or both (3). Females use these species-specific differences to detect conspecific calling song and respond by walking or flying to its source (4-12).

Most crickets are nocturnally active; some species fly at night (13). Like other night-flying insects, they are vulnerable to predation by bats. Bats locate their prey by emitting ultrasonic signals and monitoring their echoes (14). Some insects can detect these ultrasonic signals and use this information to avoid predation. Moths of several families (Noctuidae, Arctiidae, Geometridae) for example, fly away from low-intensity ultrasound (such as might be produced by a distant bat) and perform other evasive maneuvers (e.g., power dives) in response to higher intensities of ultrasound (15, 16). Green lacewings (Chrysopidae) cease flying and drop to the ground when they detect bat cries (17, 18). These behaviors effectively reduce the likelihood of being captured.

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In this paper we describe the role of carrier frequency in the phonotactic behavior of crickets. We describe the behavior of tethered, flying crickets stimulated with their conspecific calling song pattern played at various carrier frequencies. At frequencies similar to those produced by calling males, crickets performed steering movements toward the sound source; at higher frequencies, such as those produced by hunting bats, they steered away from the sound source.

METHODS

Adult virgin *Teleogryllus oceanicus* females were selected from a laboratory culture within one month of the imaginal molt. Each cricket was tethered by the pronotum to a holder and suspended in a wind stream (wind speed 1-3 m/sec). This procedure induced most crickets to fly. Room temperature was maintained between 24°C and 26°C. Each animal's behavior was monitored and taped for later analysis with a Sony AV 3400 video system. A photocell mounted on the video monitor screen indicated abdominal position. As the image of the cricket's abdomen moved across the screen the amount of light falling on the photocell, and consequently its dc voltage, varied. Thus, the amplitude of the photocell output reflected the instantaneous position of the abdomen.

The auditory stimulus was a train of electronically synthesized sound pulses (rise and fall times, 5 msec; duration, 30 msec), with the temporal pattern of *T. oceanicus* calling song recorded at $24.5 \pm 1^\circ$ (1). The carrier frequency of the sound pulses was produced by a Hewlett-Packard 200 CD wide-range oscillator. The train of pulses was attenuated (Hewlett-Packard HP 350D), amplified (Crown D150), and delivered through either a piezoelectric tweeter for frequencies up to 40 kHz, or an ultrasonic transducer (19) for higher frequencies (up to 100 kHz).

The speakers were placed level with the cricket, 90° to the right and left with respect to the longitudinal body axis, at a distance of 0.5 m. Peak sound pressure levels were measured at the position occupied by the cricket (Bruel and Kjaer 2209 sound level meter, Bruel and Kjaer 4135 1/4-inch microphone). All sound pressure levels are expressed in decibels relative to 0.0002 dynes/cm² (20 μPa) (dB SPL).

We determined thresholds as follows. For each animal, we presented the stimulus at each frequency (always beginning at 3 kHz and progressing systematically to 100 kHz) at sub-threshold intensity and increased intensity in 5-dB steps until

Abbreviation: dB SPL, decibels sound pressure level relative to 0.0002 dynes/cm² (20 μPa).

* We dedicate this paper to Dr. Kenneth Roeder, who pioneered studies in the physiological basis of insect behavior, and whose work on the physiological basis of the evasion response of moths to bats is a classic in what is now called neuroethology. We are indebted to the many contributions of this pioneer neurobiologist, whose work obviously influences our own paper.

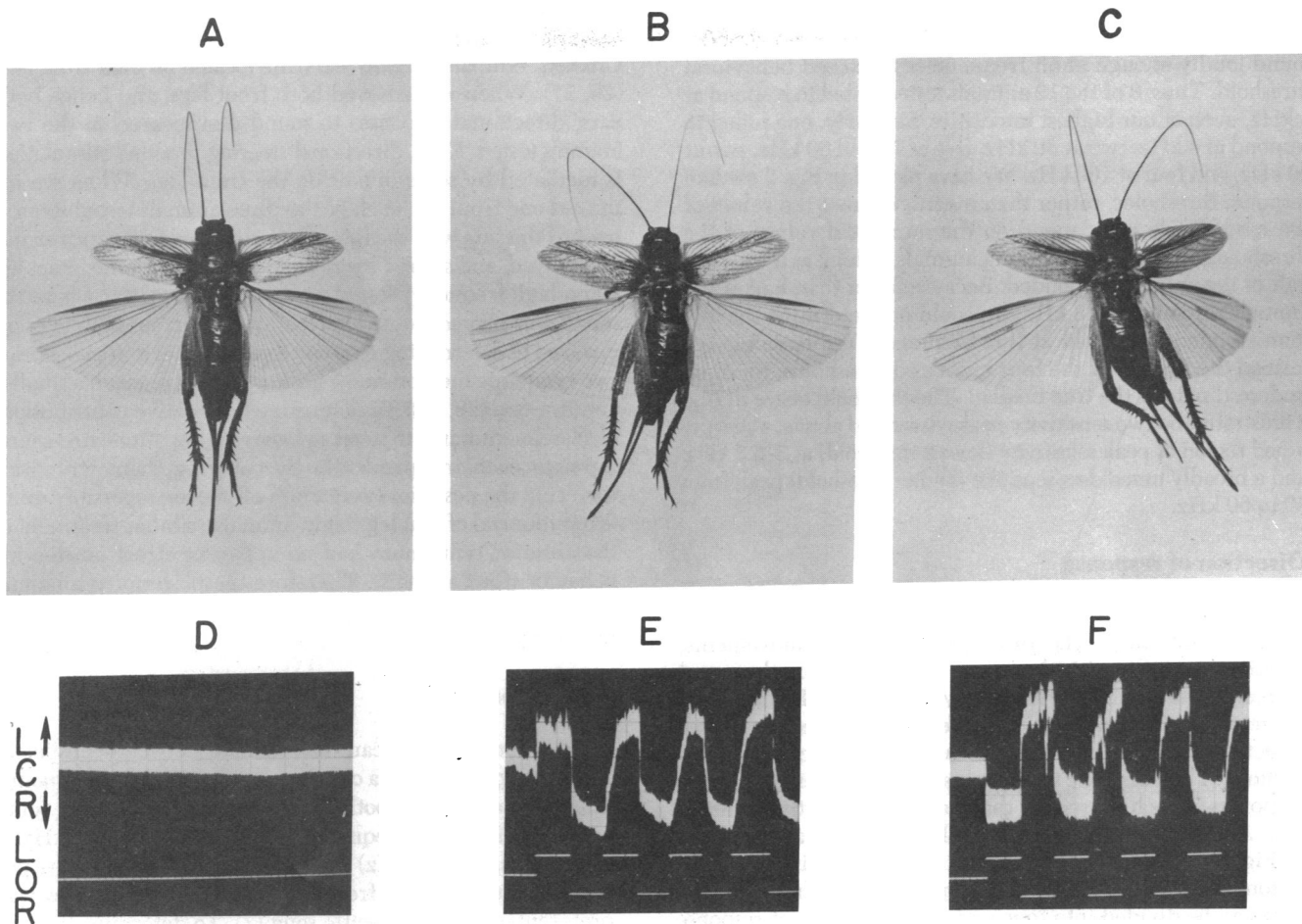


FIG. 1. Turning responses to low- and high-frequency sounds. A–C are photographs of tethered flying crickets, taken with a high-speed flash. No sound was played in A and D; in B, calling song at 5 kHz was played from the cricket's left; in C, calling song at 40 kHz was played from the cricket's left. In D–F, the upper traces indicate abdominal position (L, left; C, center; R, right), and lower traces indicate whether song was played from the left speaker (L) or the right speaker (R), or whether both speakers were turned off (0). E, 5 kHz; F, 40 kHz. Time bar below E: 50 sec.

a clear orientation response (judged by directly viewing the animal's abdomen and legs on the video monitor) occurred.[†]

RESULTS

Steering behavior

In the absence of acoustic stimulation, most crickets assumed a straight and symmetrical flight posture, with the abdomen and metathoracic legs extended directly backwards (Fig. 1A). Not all animals flew in the wind stream; those that did not fly, or did not fly straight in the absence of acoustic stimulation, were not used for experimentation. In response to a supra-threshold sound stimulus the cricket bent her abdomen and hindlegs to one side (Fig. 1B and C) and maintained this position for the duration of stimulation (Fig. 1E and F). Similar movements occur during turning in the yaw plane in flying locusts (20–22) and moths (16). Presumably these movements increase aerodynamic drag on one side and thus facilitate turning toward that side (22). Thus we interpret movements of the abdomen and hindlegs to the right (or left) as attempts to turn toward the right (or left).

Responses such as those illustrated in Fig. 1 occurred con-

sistently in more than 80% of the crickets that flew (i.e., flapped their wings) in the windstream. The remaining crickets either failed to respond at all or had unstable response thresholds, and were not used for data collection. Occasionally, a cricket failed to flap its wings but did assume a characteristic flight posture in which the fore- and midlegs were held close to the body, the abdomen was elevated, and the hindlegs were extended behind (23). When sound was presented to such crickets some did not respond at all, some began to fly and perform steering movements, and some performed steering movements even though they failed to actually beat their wings. Data included in the following analyses were collected only from crickets that were actually flying.

Frequency sensitivity

The behavioral thresholds of 12 animals were measured over the range from 3 kHz to 100 kHz. Each animal exhibited two sensitivity peaks. In the range between 3 and 20 kHz each animal had a lowest threshold between 4.5 and 5.5 kHz; above 20 kHz each animal had a lowest threshold between 35 and 80 kHz. With the exception of a single animal, sensitivity was greatest (threshold was lowest) in the lower of these two frequency ranges.

These results are summarized in Fig. 2, which depicts the median threshold for all 12 animals at the various frequencies tested. The sensitivity of the cricket's ear varies sharply as a

[†] The findings reported here are not an artifact of the ascending order of frequencies of successive stimuli; similar results were obtained in preliminary experiments in which we alternated between low- and high-frequency stimuli.

function of sound frequency (24, 25). Because of the relative insensitivity of the ear at some frequencies, we could not play sound loudly enough at all frequencies to exceed behavioral threshold. Thus, 8 of the 12 animals tested failed to respond at 3 kHz, even at our highest intensities. Similarly, one failed to respond at 4 kHz, two at 50 kHz, five at 70 and 80 kHz, two at 90 kHz, and four at 100 kHz. We have plotted in Fig. 2 median response thresholds, rather than means, because the values of the medians do not depend on the numerical values of the thresholds of the nonresponding animals, as long as more than half of the animals responded. Because fewer than half of the animals responded at 3 kHz, we could not determine the median response threshold at this frequency. We have plotted instead the highest of the four measured thresholds; this is an underestimate of the true median. The threshold curve in Fig. 2 illustrates the two sensitivity peaks described above; a sharply tuned region of peak sensitivity (lowest threshold) at 5–5.5 kHz, and a broadly tuned, less-sensitive (higher threshold) peak from 40 to 60 kHz.

Direction of response

We have plotted in Fig. 3 the direction of the response at threshold intensity. The direction of turning movements, whether toward (positive) or away (negative) from the sound source, depends on carrier frequency of the sound. In some cases the response was bimodal, that is, the cricket steered first in one direction and then the other. Such cases were scored as ambiguous. The bars, in Fig. 3 represent the percentages of all responses at each frequency that were either positive or negative. Ambiguous responses, although not plotted, are reflected in Fig. 3 by the failure of the positive and negative segments of some bars to total 100%. The frequency range from 3 to 100 kHz can be divided into four regions on the basis of response sign. From 3 kHz to 9 kHz, responses were predominantly positive; from 10 to 25 kHz, response signs changed systematically from positive to negative; from 30 to 70 kHz, responses were mostly negative; above 70 kHz, positive responses were again evident.

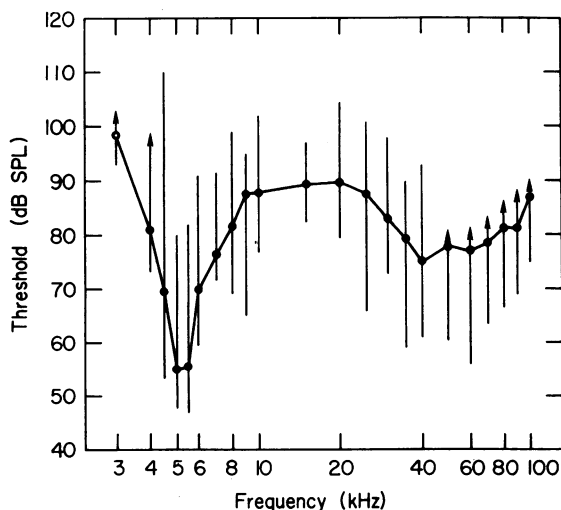


FIG. 2. Frequency sensitivity of steering behavior. Filled circles indicate median threshold intensities for 12 animals. The open circle at 3 kHz is an underestimate of the median threshold (see text). Vertical bars indicate ranges. Arrows indicate that some animals did not respond at (i.e., had thresholds greater than) the maximum available intensity. The position of the arrowhead indicates this maximum intensity.

Sensory modality

Crickets' tympanal organs (ears) are located on their front legs (26, 27). When we removed both front legs, and hence both ears, directional responses to sound disappeared in the two animals tested. Thus, directional steering to sound stimulation is mediated by sense organs on the front legs. When we removed one front leg, each of the three animals tested steered toward the side with the intact leg when low frequency sound was played, and steered away from the side with the intact leg when high frequency sound was played, no matter where the sound was played from. Therefore, inputs from both legs are involved in determining response direction. Each front leg bears two tympanic membranes; a large posterior one and a smaller anterior one (26, 27). To determine the relative contributions of these membranes to steering behavior, we attenuated sound input to each independently by covering them with wax. Covering the posterior tympanum of one leg reversibly mimicked removal of that leg (eight animals); similar treatment of the anterior tympanum had no apparent effect on steering behavior (four animals). Therefore the posterior tympanum provides the major input for steering responses to both low and high frequency sounds.

Species specificity

T. oceanicus females can discriminate between conspecific calling song and that of a closely related species, *T. commodus*. The two songs differ both in the temporal pattern of sound pulses and in carrier frequency (*T. oceanicus*, 4.5–5.4 kHz; *T. commodus*, 3.5–4.4 kHz) (7, 28). When the two songs are presented simultaneously from separate speakers females walk preferentially to conspecific song (7). To determine whether flying *T. oceanicus* females could also distinguish between the songs, we offered both songs simultaneously from two separate speakers; the *T. oceanicus* song at 4.5 kHz and the *T. commodus* song at 3.8 kHz. Both songs were played at the same intensity. This intensity was chosen so that each song, presented along, elicited a positive steering response. When both songs were presented simultaneously, each of the five crickets tested steered exclusively toward *T. oceanicus* song (Fig. 4).

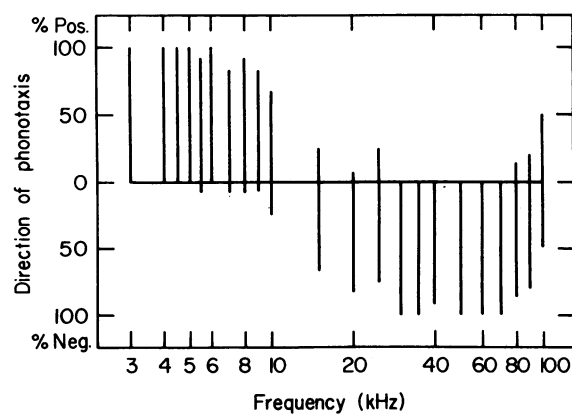


FIG. 3. Direction of phonotaxis at various frequencies. For each frequency all responses were classified as positive, negative, or ambiguous (see text). Vertical bars indicate the numbers of positive and negative responses, expressed as percentages of the number of total responses at that frequency. There were 4 responses at 3 kHz, 11 at 4 kHz, 12 at 5–40 kHz, 10 at 50 kHz, 8 at 60 kHz, 7 at 70 and 80 kHz, 10 at 90 kHz, and 8 at 100 kHz.

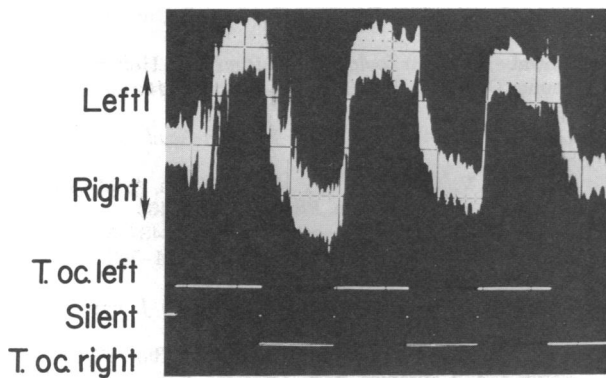


FIG. 4. Species-specific phonotaxis. The upper trace indicates abdominal position. *T. oceanicus* calling song was played from one speaker and *T. commodus* song was played simultaneously from the opposite speaker. The lower trace indicates which of the two speakers played *T. oceanicus* song. Time bar: 10 sec.

DISCUSSION

In the experiments described here we demonstrate that tethered, flying *T. oceanicus* perform well-defined steering movements in response to acoustic stimulation. These steering responses bear close parallels to the phonotactic behavior of freely moving crickets. The frequency sensitivity of phonotaxis, in all crickets studied to date, is closely matched to the carrier frequency of the species' calling song (10–12). This is also true for the steering behavior reported here; the peak in sensitivity at 5–5.5 kHz corresponds to the carrier frequency of *T. oceanicus* calling song (4.5–5.4 kHz). Phonotaxis in all species studied is species specific; females are more likely to locomote toward a source of conspecific song than toward a source of heterospecific song (5–12). Such species specificity is also evident in steering behavior; *T. oceanicus* females presented with both *T. oceanicus* and *T. commodus* calling songs steered exclusively toward the *T. oceanicus* song. Because of these parallels we feel that steering behavior in tethered, flying crickets accurately reflects phonotactic tendencies. It is reasonable to suppose that phonotaxis in flying *T. oceanicus* may supplement phonotaxis performed by these crickets while walking. Field experiments have shown that flying crickets perform species-specific phonotaxis under natural conditions. Ulagaraj and Walker (8) simultaneously broadcast the calling songs of *Scapteriscus acletus* and *S. vicinus* from separate outdoor speakers. They found that flying crickets were preferentially attracted to the speaker playing their conspecific calling song.

The occurrence of steering movements away from high frequency sound suggests that crickets, like other insects, possess a bat evasion system. This finding confirms an earlier report by Popov *et al.* (29), who found that tethered flying *Gryllus bimaculatus* swing the arm to which they are tethered away from a speaker playing high frequency sound. We found that negative phonotaxis was most sensitive in the frequency range from 40 to 60 kHz. This range corresponds closely with the frequency sensitivity of the auditory receptors of moths (30) and green lacewings (31). A similar sensitivity peak in the ultrasonic range was reported for crickets in the only study we know of to include this frequency range (32). The close correspondence between the ultrasonic frequency sensitivities of these insects (which represent three orders) is not surprising, in view of the fact that this range encompasses the dominant sound frequencies of bat cries (14). The thresholds for behavioral responses to ultrasound that we observed are substantially higher than those of moths [40 dB SPL (16)] but are comparable

to those observed in green lacewings [70 dB SPL (17)].

Walking crickets determine the direction of a sound source by integrating information from the two ears (33). Our experiments with crickets having only one front leg confirm this finding. Bailey and Thomson (34) found that of the two tympana on each front leg, the posterior one provides the major input to the ear. We confirm this result and extend it into the ultrasonic range. Biophysical studies (35, 36) also implicate the posterior (rather than anterior) tympanum as being of major importance; however, frequencies greater than 30 kHz were not investigated.

The difference in direction of steering to identically patterned low- and high-frequency sound represents a clear-cut behavioral demonstration of frequency discrimination in insects. Because both the low- and high-frequency stimuli were presented at behaviorally equivalent intensities, i.e., at threshold, the difference in steering direction must be due to true frequency discrimination, rather than to apparent intensity differences at different frequencies. Earlier studies by Popov and Shuvalov (12) suggested that crickets perform frequency discrimination. They found that freely walking adult females do positive phonotaxis to calling song at frequencies of 4–4.5 kHz, but that tethered flying females perform escape movements to 100-msec tones of higher frequencies. However, because the crickets were walking in one case and flying in the other, and because the stimulus was calling song in one case and long tones in the other, an unambiguous interpretation of these results is difficult. Physiological studies have demonstrated frequency discrimination in insect nervous systems (37–39); our results show clearly that insects can use this capability in a behavioral context.

Previous studies of phonotaxis concentrated on the behavior of freely locomoting crickets. Such studies have permitted the observation of behavior under conditions that approximate the natural environment. Unfortunately, these same conditions hamper detailed analyses of the neural mechanisms underlying the behavior. The steering movements described in this paper represent a simple, well-defined behavior that apparently reflects phonotactic tendencies, but which should nevertheless permit us to study the physiological basis of the behavior in detail. Insect flight has been successfully studied in restrained, dissected animals (40, 41). The behavior reported in this paper, steering movements of the abdomen and hind legs, has been studied in locusts at the level of individual, identified neurons (21, 42). It may thus be possible for us to study the neuronal organization of phonotactic behavior in detail. Such an approach would complement other work on insect audition, which has concentrated on the processing of auditory information (2). Furthermore, it should be possible to record from the auditory pathways of tethered, flying crickets while simultaneously monitoring either phonotactic steering movements or their neural correlates. This will allow us to correlate the activity of auditory neurons with the animal's behavior.

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