The Journal of Experimental Biology 205, 1285–1292 (2002)
Printed in Great Britain © The Company of Biologists Limited 2002
JEB3790

Song discrimination by male cicadas *Cicada barbara lusitanica* (Homoptera, Cicadidae)

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Accepted 18 February 2002

Summary

***Cicada barbara lusitanica* males presented a stereotyped singing response behaviour when exposed to a playback of the conspecific song. Males preferred (as measured by the time taken to sing) the conspecific signal to heterospecific songs that differed markedly in temporal pattern. Manipulation of the gross temporal pattern of *C. barbara* calling song significantly reduced stimulus attractiveness. Indeed, *C. barbara* males stopped responding to stimuli in which the temporal pattern approached the characteristic *C. orni* song, a sympatric and closely related species. If present in females, the preference for stimuli with pauses not exceeding 30 ms could reflect the evolution of a behavioural pre-copulatory isolating mechanism based on song analysis. Males discriminated frequencies within 3–15 kHz, clearly preferring 6 and 9 kHz; both these frequencies matched the main spectral peaks of the song. The preference for specific frequencies was not associated with maximum neuronal excitation as estimated by auditory nerve recordings, which suggests that this frequency-dependent behaviour is not based on the strength of the auditory system's response to different frequencies. Rather, it is likely to reflect fine frequency resolution in the central nervous system.**

Key words: cicada, *Cicada barbara lusitanica*, song discrimination, temporal pattern, frequency discrimination.

Introduction

In acoustically active insects (e.g. crickets, grasshoppers, mole crickets, cicadas), males often advertise by means of a conspicuous song. This song may encode information about the identity of the species (e.g. Popov et al., 1974), which can be used by other individuals to discriminate conspecifics from heterospecific sympatric species.

The ability to discriminate the conspecific signal from heterospecific songs has been studied in different insect groups (e.g. crickets: Popov and Shuvalov, 1977; Weber et al., 1981; grasshoppers: von Helversen and von Helversen, 1983; Stumpner and von Helversen, 1994). In order to assess the relative importance of particular song parameters in song discrimination, most studies have used the phonotactic preference of reproductively receptive females or any other stereotyped response to a modified or a natural song playback. Both the time pattern (e.g. Pollack and Hoy, 1979; Hennig and Weber, 1999) and the frequency spectrum (e.g. Popov et al., 1975; Oldfield, 1980) of the signal have been shown to be involved in species discrimination. In cicadas, however, few studies have addressed this question, and the relative importance of the song parameters in species recognition is still poorly known. Some evidence indicates that the frequency spectrum of the signal carries information about the species-identity of a calling male (Doolan and Young, 1989; Daws et al., 1997), whereas the temporal pattern might reveal the quality of the sender at a short distance (Doolan and Young, 1989).

Cicadas have traditionally been difficult to work with in the laboratory, and female flight phonotaxis has been difficult to observe in some species (Daws et al., 1997). On the other hand, male cicadas have been described to respond by calling when stimulated with the conspecific song (Villet, 1992; Fonseca, 1994). *Cicada barbara* males are such an example and, alternatively, the response of males towards natural and modified songs can be used to determine the relative importance of song parameters in song discrimination. This method has proved reliable for some orthopteran species in which either males or females respond to a song stimulus by singing a response song (Skovmand and Pedersen, 1983). Similarly, Simmons et al. (1971) induced cicada males to sing by presenting sound stimuli and used this behaviour to investigate song production and hearing in periodical cicadas.

Hence, we used the stereotyped singing response behaviour of males to analyse song discrimination in *C. barbara*. We investigated the following questions: (i) can *C. barbara* males discriminate the conspecific calling signal from the songs of other sympatric cicadas? If so, (ii) which parameters of the song are involved in song discrimination? We demonstrate that, in contrast to what is presently known for cicadas (Doolan and Young, 1989; Daws et al., 1997), the temporal pattern can influence long-range communication. Moreover, the mechanism underlying the frequency-related behaviour in *C. barbara* males differs from what has been described for another cicada species (Daws et al., 1997).

Materials and methods

Animals

Adult males of the species *Cicada barbara lusitanica* Boulard, were caught on the south-west coast of Portugal and transported on a feeding plant to the experimental site. The cicadas were kept indoors at room temperature (20–25 °C) on an *Olea europaea* tree branch.

Experimental arrangement

All behavioural experiments were conducted outdoors in a cylindrical cage, 100 cm in diameter and 60 cm high, supported by four legs (60 cm) and covered with green insect net (Fig. 1). The structure of the arena was made of iron rods 0.8 cm thick. During the experiments, stimuli were individually presented by a loudspeaker (Dynaudio D28/2) positioned 60 cm away from the centre of the arena. A laptop (Toshiba 230 CX) equipped with a Yamaha OPL3-SAx soundboard generated the stimuli, delivered at a D/A rate of 44 kHz to an amplifier (Phoenix Gold QX 4040) through a step attenuator, allowing an accurate control of the signal amplitude. The amplitude of the sound stimulus at the stick was calibrated with a Radio Shack (catalogue no. 33-2050) sound-level meter.

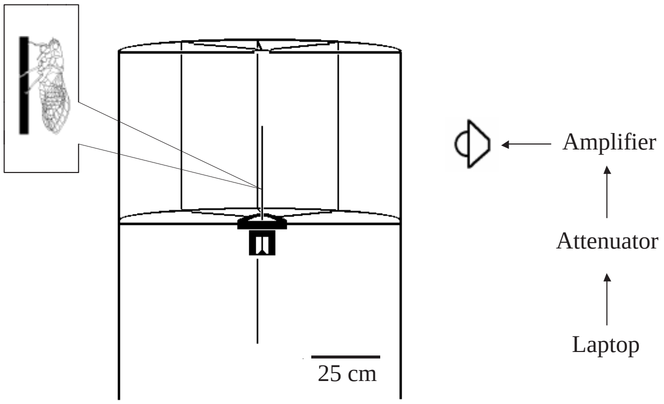

Fig. 1. Experimental arrangement. Only one half (180 °) of the cage is represented. At the centre of the arena base, a small hole (5 cm in diameter) allowed a 60 cm long and 1 cm thick wood stick to project from a platform situated 15 cm below the base of the cage. The stimuli were presented by a loudspeaker positioned 60 cm away from the centre of the arena and 90 cm from the ground.

Experimental design

Each male was tested individually under one or two of the five different behavioural experiments (see Stimulus design). For each experiment, the test procedure was as follows: individual males were placed on the base of a wooden stick 60 cm long placed at the centre of the arena, and they immediately walked upwards. When an animal entered the arena, a sound stimulus was presented by the loudspeaker and singing activity by a male was taken as a positive response. The time elapsed from the onset of the playback was recorded and the stimulus was turned off. A failure to respond during 3 min of consecutive playback was taken as a negative response. In either case, the experiment continued with the presentation of a different stimulus after moving the cicada back to the base of the stick.

Each behavioural experiment consisted of a predefined set of test stimuli (T) presented in random order. To control for motivational changes, a positive control (PC, the conspecific song at 90 dB sound pressure level (SPL) was always required before and after two consecutive test stimuli. An animal with high motivation would always respond to this positive control; failure to respond to this stimulus would mean that the results from the two previous stimuli were discarded before resuming the series. Thus, the sequence of stimuli was always PC-T-T-PC-T-T-PC and so on, until all the stimuli to be tested were presented. Among the test stimuli there were always blank stimuli in which no sound was presented (NC); these represented a control for possible responses in the absence of sound stimuli and the number of these stimuli equalled the number of intensities tested (e.g. if the stimuli were tested at two different intensities, two NC would be randomly positioned among the T). The positive and negative control responses are not presented in the Results section. They were used to evaluate the motivation of each animal during a test series and not for statistical comparisons with the test stimuli. Animals that did not respond to the PC within 40 s or that responded to the NC within 120 s were not used for analysis.

Stimulus design

Five behavioural experiments were designed to investigate specific questions related to song discrimination by *C. barbara* males.

Experiment 1

Are *C. barbara* males able to discriminate the conspecific song from the calling songs of other sympatric cicada species? Males were presented with four test stimuli, consisting of the calling songs of *C. barbara*, *Tibicina quadrisignata* (Hagen), *Tettigetta argentata* (Olivier) and *Cicada orni* L. (Fig. 2). To avoid pseudoreplication problems (Kroodsma, 1989; McGregor et al., 1992), recordings from four different animals of each species were used. The calling songs were presented at 70 and 90 dB SPL.

Experiment 2

Is song discrimination affected by manipulation of the gross temporal pattern? The gross temporal pattern of the songs of *C. barbara* and *C. orni* (sympatric and closely related species) were modified while retaining the characteristic frequency spectrum. The continuous trill of *C. barbara* was modified into a train of sound pulses by inserting 120 ms pauses every 64 ms. Pauses of 40–120 ms are found in natural *C. orni* calling songs.

C. orni song was modified by removing the pauses between echemes, resulting in a continuous trill signal. Natural conspecific songs were also used in this experiment to compare the attractiveness of the modified songs. All stimuli were presented at 70 and 90 dB SPL.

Experiments 3 and 4

Which parameters of the temporal pattern are used by *C. barbara* males to discriminate their song from the song of the sister species *C. orni*? These experiments were designed to investigate the effect of varying the sound pulse duration, pause duration and the duty cycle (i.e. pulse duration/pulse period). In experiment 3, the pause was kept constant at 30 ms while the pulse duration was varied between 30 and 240 ms. In a second variant of experiment 3, the pause was set to 15 ms while the pulse duration varied between 15 and 180 ms. By contrast, in experiment 4 the pulse duration was set at 60 ms, whilst the pause varied between 7.5 and 120 ms. This range was selected to include pauses that were shorter (7.5 and 15 ms), similar (30 and 60 ms) and larger (120 ms) than the average pause found in most *C. orni* songs. The test stimuli were presented at 80 dB SPL in both experiments.

Experiment 5

Can differences in frequency spectrum be used to improve song discrimination between two trilled songs? *C. barbara* males were presented with six pure-frequency tones (3, 4, 6, 9, 12 and 15 kHz) and the conspecific calling song. This experiment also allowed us to investigate frequency discrimination. All test stimuli were presented at 80 dB SPL.

Auditory nerve recordings

Seven cicada males, wings and legs removed, were waxed to a holder by their pro- and mesonotum with the ventral side facing upwards. The auditory nerves were exposed by ventral dissection and the preparation was kept moist with insect Ringer. Each auditory nerve was recorded with a single Vaseline-insulated tungsten hook. Computer-generated sound stimuli (100 kHz conversion rate, 12 bit resolution) were delivered at the ipsilateral side as pure-tone sound pulses (25 ms duration, 5 ms ramps, frequency range 0.5–25 kHz) by a Dynaudio D28/2 loudspeaker. The sound stimulus amplitude was conditioned by two serially connected computer controlled attenuators. One allowed compensation for the frequency response of the loudspeaker and the other delivered the signal in 5 dB steps ranging from 30 to 90 dB SPL (±0.5 dB). For each frequency and intensity, five stimuli were presented. A microphone B&K 4135 calibrated with a piston phone (B&K 4220) and placed at the position later occupied by the cicada (22 cm from the loudspeaker) allowed for adjustments in stimulus intensity and echo control. The echoes were minimised by lining the Faraday cage and the apparatus close to the preparation with sound-absorbing material (illsonic).

Stimulus and nerve activity were stored in a DAT tape recorder (TEAC RD-120TE). Off-line analysis of digitised nerve recordings (10 kHz, Data translation DT 2821-F-8 di) was performed with purpose-designed software. For each male, the auditory nerve suprathreshold response was estimated as the average peak amplitude of the summed excitation of the auditory receptors for the five repetitions of each frequency and intensity.

Statistics

All behavioural experiments were conducted in a within-subjects (repeated-measures) design, i.e. each animal was tested for all the experimental conditions of a particular test series (see Ferguson, 1981; Schweigert, 1994 for details of this experimental design). When data met the assumptions for inferential statistics, a one-way or two-way within-subjects analysis of variance (ANOVA) was performed. Data that could not meet these assumptions were log-transformed before use in subsequent analysis. *A posteriori* comparisons of means were performed using the Tukey HSD test; in this case, only the *P*-level for the comparison is presented. In experiments with one repeated-measures factor with only two levels, a related *t*-test was used for statistical comparisons.

The average time taken by individuals to sing following sound stimulation, i.e. the response latency, is expressed as the mean±95 % confidence interval.

Results

Experiment 1: can C. barbara *males discriminate the conspecific song from the calling songs of other sympatric cicada species?*

Individual males were presented with the calling songs of four cicada species, *Cicada barbara*, *Tibicina quadrisignata*, *Tettigetta argentata* and *Cicada orni* (Fig. 2).

Males were clearly able to discriminate the conspecific song (CS) from the calling signals of *T. argentata* and *C. orni*, as measured by the time taken to respond to these stimuli (Fig. 2A). Indeed, the CS elicited singing activity significantly faster than did both heterospecific songs ($P<0.001$, both comparisons). The calling song of *T. quadrisignata*, very similar in temporal pattern to that of *C. barbara*, proved as effective as the CS in promoting singing behaviour at both 70 and 90 dB SPL ($P>0.05$, both comparisons).

Thus, *C. barbara* males presented a consistent stereotyped behavioural response towards the CS that was statistically different from the behavioural responses towards the songs of two other sympatric cicada species, *Cicada orni* and *T. argentata*. This experiment also indicated that pulsed songs (*T. argentata*, *C. orni*) were less attractive than trilled songs (*C. barbara*, *T. quadrisignata*), suggesting a role of the temporal pattern in song discrimination.

Experiment 2: is song discrimination affected by manipulation of the gross temporal pattern?

Four stimuli were used: two calling songs (*C. barbara* and *C. orni*) and the same songs but with the gross temporal pattern manipulated (*C. barbara* with pauses and *C. orni* without pauses).

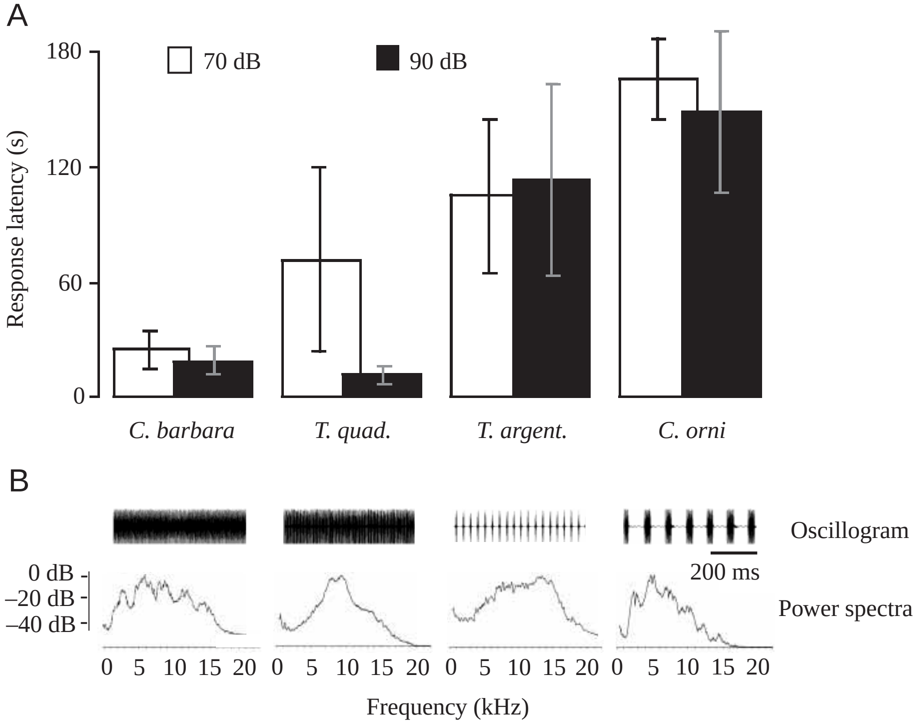

Fig. 2. (A) Time taken by *Cicada barbara* males to respond to the songs of four sympatric cicadas presented at 70 and 90 dB. Values are means ± 95 % confidence intervals (N=8). (B) Oscillograms and power spectra of the songs of *Cicada barbara*, *Tibicina quadrisignata*, *Tettigetta argentata* and *Cicada orni*.

The modified *C. barbara* song (with pauses) was significantly less attractive than the natural calling song (P<0.001). Moreover, the modified *C. orni* song (without pauses) proved as attractive as the *C. barbara* song (P>0.05, Fig. 3). Hence, manipulation of the gross temporal characteristics of *C. barbara* and *C. orni* songs significantly affected the attractiveness of both stimuli. Note that the frequency spectrum was not manipulated in either modified song.

Experiments 3 and 4: which parameters of the temporal pattern are used by C. barbara *males to discriminate their song from that of the sister species* C. orni*?*

Manipulation of the gross temporal pattern had a strong effect on song discrimination. Hence, in the following experiments three parameters of the temporal pattern (pulse duration, pause duration and duty cycle) were manipulated and signal discrimination investigated.

In Experiment 3, a constant pause duration (30 ms) and variable pulse duration (30–240 ms) was used (Fig. 4A). No significant differences were observed between stimuli ($F_{4,28}$=2.5, P>0.05). A stimulus in which a 30 ms pause alternated with a 30 ms sound pulse was as effective in stimulating singing activity as a stimulus with 30 ms pauses alternating with 240 ms pulses, although the duty cycle (DC) had increased from 50 % to 89 %. Hence, pulse duration and duty cycle do not seem to influence signal discrimination significantly. Similar results were obtained when using constant 15 ms pauses ($F_{4,24}$=0.7, P>0.05).

In Experiment 4, constant pulse duration (60 ms) and variable pause duration (7.5–120 ms) was used (Fig. 4B). Significant differences were found between stimuli as the pause duration was varied ($F_{4,16}$=44.9, P<0.001). The efficiency of a stimulus with a 60 ms sound pulse duration decreased significantly as the pause duration in the song

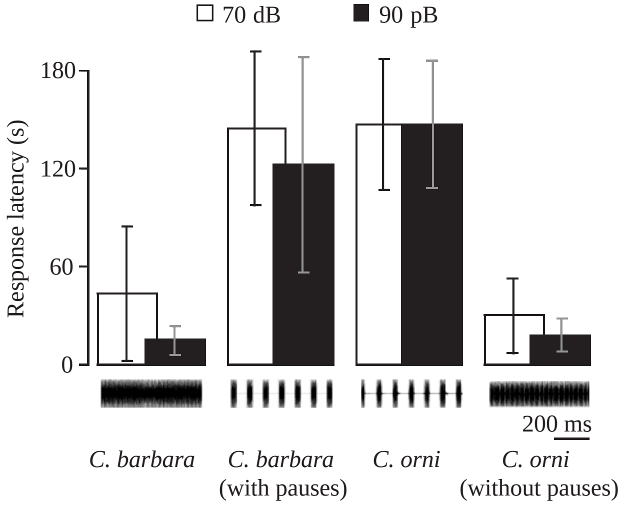

Fig. 3. Effects of manipulation of the gross temporal pattern on song discrimination. Time taken by *Cicada barbara* males to respond to unmanipulated and manipulated songs of *C. barbara* and *C. orni*. Oscillograms are presented below the histogram. When pauses were inserted into *C. barbara* song the stimulus became unattractive and males could not discriminate it from the song of *C. orni*. Removing the pauses within *C. orni* song resulted in a very attractive stimulus, indicating that males are not able to discriminate between these species using only the differences in the frequency spectra. Values are means ± 95 % confidence intervals (N=5).

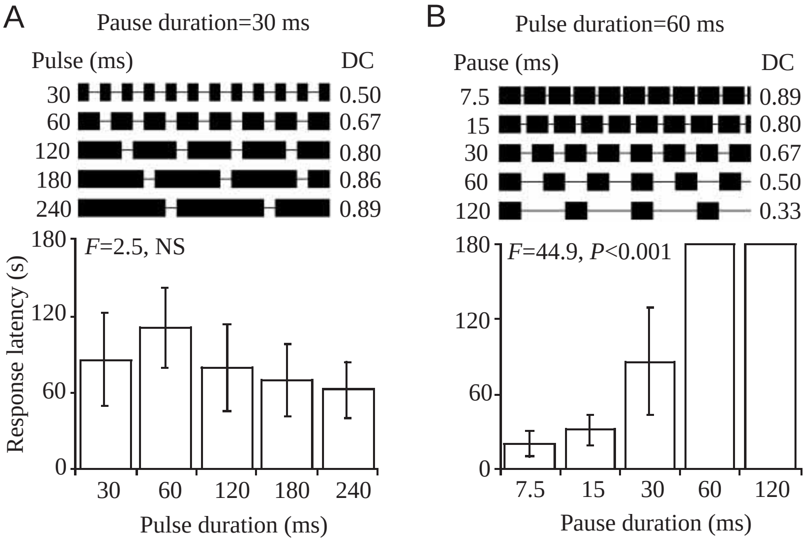

Fig. 4. The effect of varying pulse and pause duration on signal discrimination. The pulses for all stimuli were obtained from *C. barbara* calling song. (A) The pause duration was kept constant at 30 ms while the pulse duration was varied from 30 to 240 ms. All stimuli were equally effective (N=8). (B) The pause duration was varied between 7.5 and 120 ms while the pulse duration was fixed at 60 ms. Stimulus efficiency (measured as response latency to stimulus) decreased significantly with increasing pause duration. Values are means ± 95 % confidence intervals (N=5). DC, duty cycle (pulse duration/pulse period); NS, not significant.

approached the range found in *C. orni* songs (40–120 ms). Experiment 3 demonstrated that both the duty cycle and the pulse duration were not correlated with the response latency, suggesting that duty cycle variation is unlikely to be responsible for the effects observed in Experiment 4. Rather, *C. barbara* males respond preferentially to continuous songs or to signals with short periodic pauses (⩽15 ms).

The previous experiments demonstrate that *C. barbara* males discriminate continuous trills from pulsed songs (if pulses ≥30 ms). Using this temporal cue alone, however, is not sufficient to discriminate the conspecific song from the songs of some sympatric species (e.g. *Tibicina quadrisignata*, Fig. 2B). Hence, we further investigated if differences in the frequency spectrum might be used as a complementary cue for species discrimination.

Experiment 5: can differences in frequency spectrum be used to improve song discrimination between two trilled songs?

Both *C. barbara* and *T. quadrisignata* songs are continuous trills, but the former has a main frequency spectrum peak around 6 kHz whereas the latter is centred around 9 kHz (Fig. 2B). Hence, spectrum differences might be used to help discriminate between these signals. We investigated if (i) significant differences in response could be observed between different frequencies and (ii) if certain frequencies alone could be sufficient to elicit singing behaviour as well as the conspecific song (Fig. 5).

Significant differences in the response latency were observed between frequencies ($F_{6,78}$=24.2, P<0.001), with *C. barbara* males discriminating tones differing by 1–2 kHz (e.g. 3 *versus* 4 kHz, P<0.01; 4 *versus* 6 kHz, P<0.01). The most effective tones were 6 and 9 kHz, but males could not significantly discriminate between these stimuli (P>0.05). On the other hand, only the 6 kHz tone induced singing behaviour as rapidly as the conspecific song (P>0.05).

Thus, although *C. barbara* males responded better to some frequencies than to others, the small differences in the response latency between the 6 and 9 kHz tones indicate that these are unlikely to enhance song discrimination significantly between *C. barbara* and *T. quadrisignata* songs.

Auditory nerve recordings

C. barbara males were more stimulated to sing by some frequencies than others. Importantly, the previous experiment also demonstrated that cicadas were able to discriminate frequencies differing by only 1 kHz (e.g. 3 *versus* 4 kHz). To investigate if this discrimination could be attributed to differences in overall excitation of the auditory system, frequency-dependent excitation at the auditory nerve (AN) was measured in seven males (Fig. 6). The frequencies 3 kH and 4 kHz induced the largest but similar levels of AN excitation at the three intensities tested. This demonstrated that the frequency discrimination observed in the behavioural

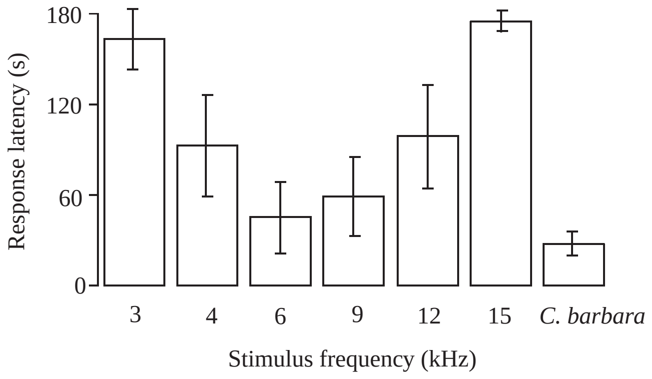

Fig. 5. Frequency discrimination by *Cicada barbara* males. Each male was stimulated with six sinusoidal tones at 80 dB SPL and the time taken by males to respond was measured. The conspecific song at the same sound intensity was presented for comparison. The 6 and 9 kHz tones were the most attractive. Singing behaviour was frequently not induced with 3 and 15 kHz tones within 180 s. Values are means ± 95 % confidence intervals (N=14).

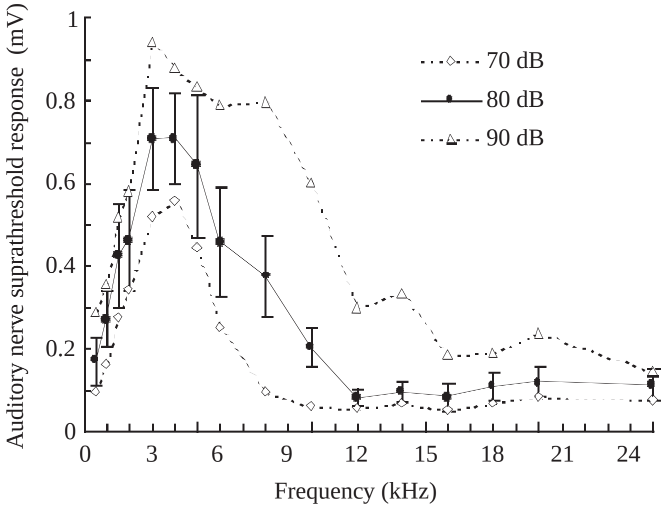

Fig. 6. Frequency-dependent auditory nerve suprathreshold response, estimated by the summed excitation of the auditory receptors. Values are means (N=7). Error bars represent 95 % confidence intervals. Variability at 70 and 90 dB SPL was similar to that shown at 80 dB SPL.

experiments between 3 and 4 kHz (Fig. 5) cannot be explained by differences in overall auditory afferent excitation. Moreover, although 6 kHz was the most effective tone in behavioural experiments (Fig. 5), the AN excitation induced by this frequency was lower than the excitation at 3 and 4 kHz, both at 70 and 80 dB SPL. Above 8 kHz, AN excitation decreased even further.

Discussion

Behavioural experiments: the role of time and frequency domains in song discrimination

Cicada barbara is found sympatrically and synchronously distributed with other cicada species, including the closely related *Cicada orni*. These cicadas have specific mating signals (Fonseca, 1994), and a pre-copulatory isolating mechanism based on song analysis is believed to have evolved to maintain species integrity. Indeed, some behavioural evidence indicates that acoustic analysis is important for the isolation of closely related insect species (Perdeck, 1957; von Helversen and von Helversen, 1994; Hennig and Weber, 1999). For cicadas, however, only two studies have addressed the role of the different song parameters in species recognition (Doolan and Young, 1989; Daws et al., 1997), namely the temporal pattern and the frequency spectrum of the signal.

We have demonstrated that specific manipulation of the gross temporal pattern of the song significantly affected song discrimination. Indeed, experiments in which pauses were introduced into the conspecific song revealed that *C. barbara* males stopped responding when these pauses exceeded 30 ms (see Fig. 4B), a pause approaching what can be found in the song of the closely related species *C. orni*. Hence, the acoustic system of *C. barbara* clearly uses the gross temporal song structure for long-range communication, a feature common to other insects (molecrickets: Ulagaraj and Walker, 1975; crickets: Pollack and Hoy, 1979; grasshoppers: von Helversen and von Helversen, 1994). Still, and unlike the present findings, Doolan and Young (1989) reported that long-range song recognition by tethered flying *Cystosoma saundersii* females was not affected by gross manipulation of the song structure. A near ninefold increase in the pause separating individual pulses had no significant effect on female steering preferences. However, in the cicada *Cystosoma saundersii* the frequency spectrum of the song could be used as a sufficient cue for species recognition since, to our knowledge, there are no other sympatric insect species using the same frequency channel (800 Hz). Thus, evolutionary pressure to develop a long-range recognition system sensitive to the temporal properties of the signal might not have occurred.

On the other hand, for *C. barbara* the temporal pattern alone was not sufficient for correct discrimination between all heterospecific sympatric cicadas, since some also have continuous songs (e.g. *T. quadrisignata*). We initially hypothesised that differences in the frequency spectrum could enhance discrimination in such cases. Indeed, the frequency content of a song was demonstrated to be important for species recognition in various insect groups (Popov et al., 1975; Ulagaraj and Walker, 1975; Bailey et al., 1990). However, although the main spectral peaks of *C. barbara* and *T. quadrisignata* differ by approximately 3 kHz, these differences were not observed to enhance song discrimination significantly.

Thus, the song recognition system present in *C. barbara* males is able to discriminate songs that differ significantly in the gross temporal pattern. This clearly reflected the ability to discriminate stimuli on the basis of the pause duration (Fig. 4B). A high-pass filter, possibly based on its temporal summation properties, may be responsible for the discrimination ability described here (Weber and Thorson, 1989; Stumpner et al., 1991; von Helversen and von Helversen, 1994). If present in females, such a recognition system would allow for efficient behavioural discrimination between *C. barbara* and *C. orni* males.

Frequency-dependent behaviour: preferential attraction for particular frequencies due to the tuned auditory system or active choice based on fine frequency analysis?

C. barbara males demonstrated a behavioural preference for specific frequencies (Fig. 5). This behaviour is thought to be possible in cicadas through at least two mechanisms: (i) a tuned peripheral auditory system that allows only some frequencies to excite the central nervous system (CNS) (Daws et al., 1997) and (ii) active choice based on fine frequency analysis in the CNS (Fonseca et al., 2000).

Daws et al. (1997) have shown that female frequency-selective phonotaxis in the bladder cicada *Cystosoma saundersii* was associated with the strength of the auditory system's response to an acoustic signal. At a defined sound intensity, frequencies that evoked a stronger auditory excitation were more attractive than frequencies causing a weaker excitation. Hence, the attractiveness of a particular sound frequency in *C. saundersii* reflected the tuning curve of the auditory system, as obtained by auditory nerve recordings. A similar mechanism has been suggested for other insect species (Ulagaraj and Walker, 1975; Huber, 1983; Bailey et al., 1990; Pollack and El-Feghaly, 1993).

In contrast, our results indicate that there is no correlation between the behavioural response and auditory excitation in *C. barbara* males. Indeed, the 3 and 4 kHz tones induced similar peripheral auditory responses but a significantly different behaviour, as measured by the response latency (Figs 5 and 6). In the same way, there was a sevenfold difference in AN excitation between 4 and 12 kHz at 80 dB, but both tones were similarly effective in the behavioural experiments. Thus, the frequency-dependent behaviour observed in *C. barbara* males is not a consequence of differential excitation of the tuned peripheral auditory system (see also 3 *versus* 6 kHz, Figs 5 and 6). Rather, this behaviour seems to involve fine frequency analysis in the central nervous system (CNS), which has been physiologically demonstrated in another cicada species (*Tettigetta josei*; Fonseca et al., 2000). Indeed, frequencies that induced similar responses at the auditory nerve were discriminated in behavioural experiments. Moreover, the most attractive frequency was 6 kHz. This frequency corresponds to the main spectral peak of the calling song but induces a weaker peripheral excitation than other frequencies (e.g. 3 and 4 kHz). If the frequency information was instead processed through an intensity-dependent system, as is believed to occur in *C. saundersii* (Daws et al., 1997), *C. barbara* males would present a preference for frequencies with a sound intensity well below the highest energy peak of the song (3 kHz, –12 dB). This would result in a 'behavioural mismatch' that would be an important constraint for long-range communication. Interestingly, males of *Magicicada cassini* presented a behavioural response to pure-frequency tones (Simmons et al., 1971) that is not correlated with the auditory tuning curve, as measured by the summed response of the receptors (Huber et al., 1990). Indeed, despite having the majority of the receptor cells more excited at 2 kHz than at 6 kHz, in behavioural experiments the males responded significantly better to the tone corresponding to the spectral peak of the calling song (5–6 kHz). This suggests that, as in *C. barbara* males, in *M. cassini* the frequency-related behaviour involves a mechanism different from that described for *Cystosoma saundersii* but similar to *Cicada barbara*.

Several insect species have a fine frequency resolution in the peripheral auditory system, with ears sensitive to a broad range of sound frequencies and with different receptor cells encoding different frequencies (Schildberger et al., 1989). For instance, crickets have a tonotopic organisation of the auditory organ with different fine-tuned receptor cells (Oldfield et al., 1986), allowing a fine frequency discrimination at the periphery. However, the resolution allowed by the insect ear is frequently reduced within the CNS. In crickets, the frequency resolution at the periphery is pooled in the CNS through two parallel channels, one centred at 5 kHz and used for acoustic communication (Schildberger, 1994) and the other a broad-band channel important for the detection of echolocation calls of bats (Nolen and Hoy, 1984). Hence, in cricket species only a categorical perception of frequencies seems to take place in the brain (Wyttenbach et al., 1996).

Cicadas are also likely to have a high frequency resolution at the periphery, with up to 2000 receptor cells in each ear. However, recently it was demonstrated that a good frequency resolution is maintained in the CNS (Fonseca et al., 2000). At least eight ascending interneurons in *Tettigetta josei* are tuned to different frequencies with Q_{10dB} values similar to most lower vertebrates (where Q is a measure of frequency tuning and can be derived from a neuron's tuning curve; Q_{10dB} corresponds to the quotient between the frequency at which a neuron shows the lowest threshold and the bandwidth at 10 dB above that threshold), providing that cicada with a capacity for fine frequency resolution in the brain that is remarkably high for insects. Here, we showed that individual *C. barbara* males can discriminate frequencies that differ by 1–2 kHz (see 3–6 kHz, Fig. 5). Thus, this behavioural data similarly indicates that cicadas have fine representation of frequencies in the CNS.

We thank Tim Guilford, Rafael Marquez and Manuel Carmo Gomes for earlier discussions on the experimental design. M.A.R. was funded by PRODEP, Medida III.

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