The Journal of Experimental Biology 206, 141-152

doi:10.1242/jeb.00070

What determines the tuning of hearing organs and the frequency of calls? A comparative study in the katydid genus *Neoconocephalus* (Orthoptera, Tettigoniidae)

Johannes Schul* and Adam C. Patterson

Division of Biological Sciences, University of Missouri, 207 Tucker Hall, Columbia, MO 65211, USA

*Author for correspondence (e-mail: schulj@missouri.edu)

Accepted 10 October 2002

Summary

The calls of five syntopic species of *Neoconocephalus* varied significantly in their spectral composition. The center-frequency of the narrow-band low-frequency component varied from 7 kHz to 15 kHz among the five species. Hearing thresholds, as determined from whole nerve recordings, did not vary accordingly among the five species but were lowest in the range from 16 kHz to 18 kHz in all five species. Iso-intensity response functions were flat for stimulus intensities up to 27 dB above threshold, indicating an even distribution of the best frequencies of individual receptor cells. At higher stimulus intensities, the intensity/response functions were steeper at frequencies above 35 kHz than at lower frequencies. This suggests the presence of a second receptor cell population for such high frequencies, with 25–30 dB higher thresholds. This receptor cell population is interpreted as an adaptation for bat avoidance. The transmission properties of the *Neoconocephalus* habitat (grassland) had low-pass characteristics for pure tones. Frequencies below 10 kHz passed almost unaffected, while attenuation in excess of spherical attenuation increased at higher frequencies. Considering these transmission properties and the tuning of female hearing sensitivity, call frequencies of approximately 9–10 kHz should be most effective as communication signals in this group of insects. It is discussed that the frequency of male calls is strongly influenced by bat predation and by the transmission properties of the habitat but is not strongly influenced by the tuning of the female hearing system.

Key words: *Neoconocephalus*, acoustic communication, frequency tuning, bushcricket, hearing threshold, call spectrum, sound transmission.

Introduction

Communication systems are only functional when there is a sufficient match between the signal properties and the sensitivity of the receiver. In the case of an acoustic communication system, the hearing organs are usually tuned to the main frequency components of the calls: numerous examples have been described in insects (e.g. Huber et al., 1990a: Paton et al., 1977; Popov, 1990; Meyer and Elsner, 1996), frogs (e.g. Capranica and Rose, 1983) and bats (Kössl, 1994). This matching has probably evolved by reciprocal selection on both signalers and receivers (Endler, 1992). Nevertheless, there are a number of exceptions in which a distinct mismatch between call frequency and auditory tuning is found (e.g. Bailey and Römer, 1991; Heller et al., 1997; Huber et al., 1990b; Mason, 1991; Ryan et al., 1990). In some cases, the mismatch between receiver tuning and call frequency acts in the context of sexual selection (Anderson, 1994), by causing a female preference for a main call frequency outside of the population mean (e.g. Nocke, 1972; Ryan et al., 1990). While in these cases the mismatch is usually not very pronounced, several other cases of striking differences between receiver tuning and call frequencies exist that cannot be explained by selection in the context of the communication system alone (e.g. Heller et al., 1997; Mason, 1991). In these cases of significant mismatches, other evolutionary forces are likely to be involved.

Further causes of selection leading to a mismatch in the communication system could arise in other behavioral contexts, such as predator avoidance. Many organisms use their hearing organs to detect and avoid acoustically hunting predators (e.g. bats; Moiseff et al., 1978; Hoy, 1992). Also, an organism's communication signals can be targeted by acoustically orienting predators or parasitoids as a way to localize their prey or hosts (e.g. Cade, 1975). Thus, calls and hearing organs might be affected by selection pressures from potentially conflicting forces. Selection on the communication system can also be caused by environmental factors. Signal degradation occurs in most cases during the passage of the signal through the environment. Masking noise may originate from the environment itself (e.g. moving water) or be produced by other noisy animals. In addition to these potential selection pressures, non-selective evolutionary forces (e.g. mutation, genetic drift) might have a strong impact on the evolution of

communication systems. Furthermore, physical, morphological and physiological constraints might limit the adaptive evolution of both call production and the hearing system.

The description of mismatches between signal spectrum and tuning of the hearing organ in one or a few species usually does not allow one to determine which evolutionary forces contribute to this phenomenon. The influences of predation, environment, etc. can usually be estimated at best, and the interpretation of the signal/sensory system mismatch remains speculative (e.g. Heller et al., 1997; Bailey and Römer, 1991). A comparative approach that studies several species that are similar in some, but different in other, potentially important factors for the evolution of the communication system might allow one to single out the influence of individual evolutionary forces.

In the present study, we focus on a group of five katydid species of the genus *Neoconocephalus* (Orthoptera: Tettigoniidae) with largely sympatric and synchronic occurrences (Greenfield, 1990), which differ significantly in the spectral composition of their calls. We compare the response properties of the hearing organs (tuning and iso-intensity responses) in these five species and quantify the influence of their grassland habitat on signal propagation. Because of the similar morphologies and lifestyles and the cohabitation of the five species, the influences of factors other than communication itself (e.g. by environment or predators) on the evolution of the communication system should be similar among these five species.

Materials and methods

Animals

Males and females of five species of *Neoconocephalus* [*N. bivocatus* Walker, Whitesell and Alexander, *N. ensiger* (Harris), *N. nebrascensis* (Bruner), *N. robustus* (Scudder) and *N. retusus* (Scudder)] were collected around Columbia, MO and Lawrence, KA, USA. Males were collected as adults, whereas females were collected as nymphs and reared to adulthood. The insects were kept in outdoor enclosures until they were used in the experiments. The coloration of the fastigum allows identification of most species (Froeschner, 1954), except for *N. robustus* and *N. bivocatus*, which are identical in this feature (Walker et al., 1973). Males of these two species were differentiated using the temporal pattern of their calls, and the females were differentiated by the ratio of ovipositor length to hind femur length (Walker et al., 1973). Only specimens that could be identified unequivocally were used in the experiment. The hind femur length was used as a measurement for body size of the individuals used in the neurophysiological experiments (Table 1).

Call recordings and analysis

Male calls were recorded in an anechoic chamber at an ambient temperature of 25°C. The specimens were placed in small screen cages 15 cm in diameter. A microphone was placed 20 cm dorsal of the calling male. Calls were recorded with a $\frac{1}{4}$" free field microphone (40BF, G.R.A.S., Vedbaek, Denmark), amplified (G.R.A.S. 26 AC and 12 AA), high-pass filtered (1000 Hz, KH3202, Krohn Hite, Avon, MA, USA) and digitized using a custom-made A/D-converter system (16-bit resolution, 250 kHz sampling rate). This setup provided a flat (±1 dB) frequency response in the range from 2 kHz to 70 kHz.

Amplitude spectra were calculated with a computer program (BatSound 1.0, Pettersson, Uppsala, Sweden) by fast Fourier transformation (FFT; Hamming window, frame length 1024) and averaged over a 1 s section of each call. The spectra of the calls of all species had a narrow-band low-frequency component and broad components of lower amplitude in the ultrasound range (Fig. 1A). In the spectra, we measured the frequency with the highest amplitude and the width of the low-frequency component at –3 dB and –10 dB amplitude. $Q_{3\,dB}$ and $Q_{10\,dB}$ values were calculated as the ratio of center frequency to bandwidth at –3 dB and –10 dB, respectively.

Tympanal nerve recordings

The animals were anesthetized by brief exposure to CO_2 and fixed ventral side up on a free-standing metal holder with a wax–resin mixture. The forelegs were fixed on a wire holder, perpendicular to the body axis, in a natural position. The cuticle covering the entrance of the tympanal nerve into the prothoracic ganglion was removed, and exposed tissue was covered with saline. A silver wire, inserted into the abdomen, served as the indifferent electrode.

Experiments took place in an anechoic chamber (1.2 m×1.2 m×0.7 m) at 24–26°C. Whole nerve recordings were obtained using electrolytically sharpened tungsten hook-electrodes (diameter 50–70 μm) placed at the entrance of the tympanic nerve into the prothoracic ganglion. The recording site was covered with silicone-grease (Baysilone) in order to prevent drying of the nerve. The recorded signals were amplified using a custom-made amplifier, band-pass filtered (120–4000 Hz, Krohn Hite 3342) and digitized (12-bit A/D converter, 10 kHz sampling rate).

The stimuli were delivered *via* one loudspeaker (Technics 10TH400C) located 70 cm from the preparation, perpendicular to the body axis of the animal. The stimuli were generated using a computer and a 16-bit DA-converter system (sampling rate 250 kHz). The signals were amplified and their amplitude manipulated by a computer-controlled attenuator in steps of 3 dB. The amplitudes of the signals were calibrated at the position of the insect using a B&K 2231 sound level meter (Bruel and Kjaer, Naerum, Denmark) and a $\frac{1}{4}$" free field microphone (G.R.A.S. 40BF). Sound measurements were obtained on the preparation site with no animal present. Signal amplitudes are given in dB peak SPL (re 2×10^{-5} Pa), which is, for sine waves, 3 dB above the respective root-mean-square (rms) value. At the recording site, slight echoic influences were unavoidable, but these influences did not alter the intensity or the envelope of the signals by more than ±1 dB.

Thresholds were determined for sinusoids in the range of

4 kHz to 80 kHz (1 kHz steps from 4 kHz to 10 kHz; 2 kHz steps from 10 kHz to 20 kHz; 5 kHz steps from 20 kHz to 50 kHz; 10 kHz steps from 50 kHz to 80 kHz). Stimuli had a trapezoid-shaped envelope with a rise and fall time of 1 ms and a 10 ms plateau time. Each frequency was played back at 20 different amplitude attenuations in steps of 3 dB (total amplitude range 57 dB). The stimulus protocol included the playback of 'no stimulus' (i.e. a digital stimulus consisting only of zeros) at the same attenuation settings as the other stimuli. These stimuli provided the baseline for the threshold determination and controlled for the noise generated by the amplifiers and the computer-controlled attenuator. Absolute stimulus amplitudes for each frequency were set so that the lowest amplitude tested was well below threshold. All stimulus combinations were presented 25 times: all frequency/amplitude combinations, including the 'no stimulus', were presented once, and then the whole sequence was repeated 25 times. This procedure guaranteed almost simultaneous measurement of all stimulus combinations, thus excluding effects due to changes in recording quality. During each repeat of the stimulus sequence, each frequency was presented from low to high amplitudes, and the frequencies were sorted from low to high. A pause of 350 ms was kept between different amplitudes of the same frequency; between frequencies, a pause of 3500 ms was kept. Because the main purpose of these experiments was to determine the hearing thresholds, the non-random presentation of stimulus intensities should have no impact on the results, because the presentation at threshold level was preceded by below-threshold stimulation. Also, all frequencies were treated identically, so that possible influences of the preceding stimulation would affect all frequencies in the same way.

Analysis of neurophysiological data

The digitized recordings of 15–25 responses of each stimulus/amplitude combination were averaged. We excluded stimulation cycles from the analysis when amplitude disturbances (e.g. due to movements of the insect) occurred or when the amplitude of the recordings diminished. Averaged responses well above threshold resembled damped oscillations (see Fig. 5A), the peak-to-peak amplitude of which was measured for each stimulus combination.

In order to determine the threshold for the different frequencies, we first calculated the mean and S.E.M. for the peak-to-peak amplitudes of the recordings obtained during the 20 presentations of 'no-stimulus'. This mean, plus 2 S.E.M., was used as the threshold criterion. Then, an intensity/response function was constructed for each frequency using the measured amplitudes of the averaged responses. These functions were smoothed by calculating the gliding mean over three values. Starting at the lowest stimulus intensity, we searched the smoothed curve for the stimulus intensity in which the response amplitude increased above the threshold criterion determined from the 'no-stimulus' recording. By linear interpolation of the steepness of the intensity/response function at this point, the threshold was determined to a resolution of 1 dB.

The intensity/response functions that were constructed for each frequency were also used to compare the response magnitudes at certain levels above threshold (+9 dB, +18 dB, +27 dB and +36 dB) between different frequencies. Response magnitudes were measured as the peak-to-peak amplitude of the averaged responses. This amplitude is mainly determined by the number of cells responding and the level of synchronization (Pollack and Faulkes, 1998; Schul, 1999). To compare the level of synchronization among different stimuli, we measured the breadth of the first peak in the averaged recording (Pollack and Faulkes, 1998; see Fig. 5).

Sound transmission in the field

The attenuation of the sound frequencies from 5 kHz to 40 kHz during transmission was measured through vegetation typical for the biotope of *Neoconocephalus*. Measurements took place in Rock Bridge State Park in Columbia, MO, USA. The field site was a grassland with tall stalks (2.0–2.5 m) and a dense understory of grass blades (0.75–1.0 m). The vegetation was uniform in the range of our experiment. Males of all five species studied were heard calling at this site. Measurements were made during the calling season of *Neoconocephalus*, in early September 2001 from 17:00 h to 21:00 h, immediately before the calling activity of *Neoconocephalus* began.

Stimulus-playback was performed with the same setup and identical signals as in the neurophysiological experiment detailed above. The loudspeaker was placed at the upper edge of the understory at a height of 85 cm. We normally found calling males at similar positions in the vegetation. The sound was recorded at several distances from the stimulus (1 m, 2 m, 5 m, 10 m and 20m) with a $\frac{1}{2}$" free-field microphone (G.R.A.S. 40AG) placed at 1.5 m height in the vegetation. The signals were also recorded at a distance of 17 cm without any vegetation between loudspeaker and microphone. During all recordings, the microphone and loudspeaker were on axis to each other.

The recorded signals were amplified (G.R.A.S. 26AC and 12AA), high-pass filtered (1000 Hz, Krohn Hite 3202) and recorded with a Pioneer D-C88 DAT recorder (sampling rate 96 kHz, frequency response up to 40 kHz). We also recorded a calibration signal (94 dB SPL, 1000 Hz, Bruel & Kjaer 4231) with the same gain settings as the recordings for each frequency, to allow the comparison of absolute signal amplitudes among the recordings at different distances. The signals were later digitized (250 kHz sampling rate, 16-bit resolution) and 50 repeats of each frequency were averaged to improve the signal-to-noise ratio. The averaged recordings were then filtered with a digital band-pass filter (bandwidth 2 kHz, CoolEdit 2000, Syntrillum Software) that was centered around each stimulus frequency, to further eliminate noise from the stimuli. We then measured the rms amplitude for the 10 ms plateau of each stimulus recorded at the six distances.

For each frequency, we plotted attenuation *vs* distance and calculated the best-fit curve for attenuation using the formula: $y=a*x+b*\log(x)+c$. In this function, the term $b*\log(x)$

represents the spherical attenuation, and the term *a**x* includes atmospheric attenuation and attenuation due to reflection, absorption and diffraction by the vegetation ('excess attenuation'). The best-fit functions, which for all frequencies had r^2 values of above 0.9, were used for further analysis. Excess attenuation was calculated as the difference between the best-fit curves and the theoretical curve calculated for spherical attenuation alone (6 dB per double distance; see Fig. 7).

Results

Call spectra

The spectral composition of the calls of the five *Neoconocephalus* species tested here had a similar general structure. Highest amplitudes were present in a narrow-band low-frequency component, and the frequency components at ultrasonic frequencies were at least 20 dB softer than the low-frequency band in the averaged spectra (Fig. 1). The variability within each species was very low for the low-frequency peak but was high for the ultrasonic components. There were significant differences between the species in the position of the low-frequency peak: in *N. retusus* and *N. ensiger*, this component was centered around 15 kHz; in *N. nebrascensis* and *N. bivocatus*, this peak occurred at approximately 10 kHz; and in *N. robustus*, the low-frequency peak occurred at 7 kHz (Fig. 2, Table 1). Thus, the main frequency component of the five *Neoconocephalus* species varied in the order of more than one octave (7–15 kHz).

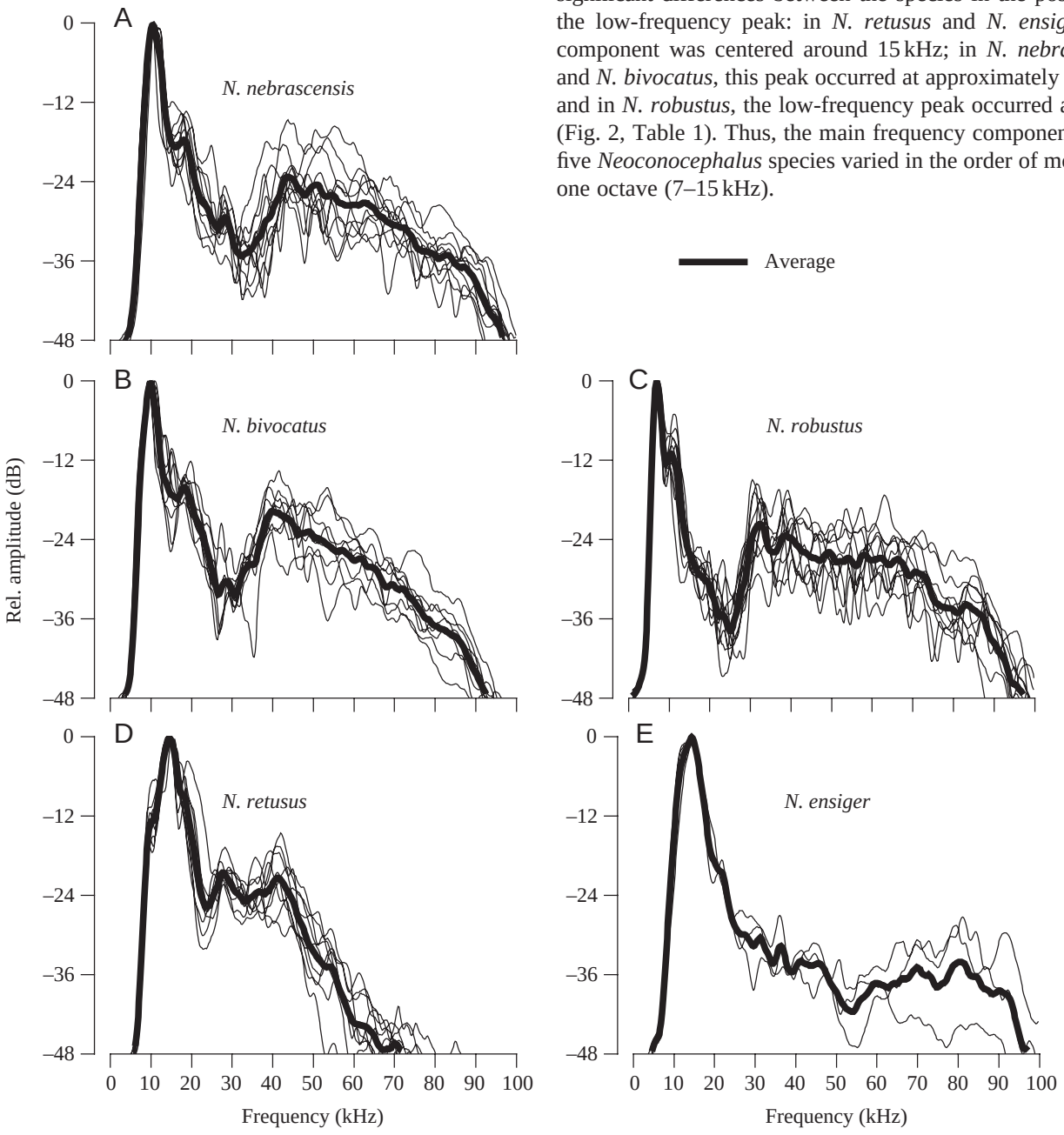

Fig. 1. Spectra of male calls of five species of *Neoconocephalus*: (A) *N. nebrascensis* (*N*=10), (B) *N. bivocatus* (*N*=8), (C) *N. robustus* (*N*=10), (D) *N. retusus* (*N*=9) and (E) *N. ensiger* (*N*=3). The thick line represents the averaged spectrum of each species, while the thin lines denote the individual spectra contributing to the averaged spectrum. Spectra are calculated as fast Fourier transform (1024 points window length) averaged over 1 s.

Table 1. *Characteristics of the five* Neoconocephalus *species studied*

	Femur length (mm)		Low frequency component of calls				Hearing sensitivity (dB peak SPL)	
	Males (*N*)	Females (*N*)	Peak frequency (kHz)	$Q_{10\,dB}$	$Q_{3\,dB}$	*N*	Males (*N*)	Females (*N*)
N. retusus	20.4±0.7 (5)	23.8±1.3 (5)	14.8±0.56	2.37±0.55	6.36±2.36	8	33.2±1.8 (9)	30.3±4.3 (10)
N. ensiger	22.1±1.5 (5)	24.5 (2)	14.5±0.22	2.25±0.45	4.46±1.71	4	31.1±2.6 (8)	29.8±4.7 (4)
N. nebrascensis	24.2±1.0 (6)	26.0±0.9 (9)	10.4±0.29	2.66±0.48	4.97±1.40	9	27.4±3.8 (10)	27.7±1.6 (8)
N. bivocatus	25.3±1.3 (6)	28.3±1.5 (5)	10.1±0.57	2.46±0.45	5.46±1.11	7	28.5±2.8 (11)	26.6±3.7 (7)
N. robustus	28.1±0.7 (10)	27.6 (3)	7.0±0.28	2.05±1.08	6.53±1.49	8	29.3±3.3 (9)	30.9±4.3 (5)

The low-frequency components of the five species have $Q_{3\,dB}$ values between 4.5 and 6 and $Q_{10\,dB}$ values of approximately 2.5 (Table 1). The low-frequency band of all species except *N. robustus* was fairly symmetrical around the peak amplitude. In *N. robustus*, a secondary peak was present in all individual call spectra (see Fig. 1C), which resulted in the asymmetrical position of the –10 dB band relative to the peak frequency.

Hearing thresholds

Hearing thresholds were determined independently for males and females of all species. Fig. 3 shows the mean threshold curves for males and females of the five *Neoconocephalus* species. We found highest sensitivity for all five species in the range from 16 kHz to 20 kHz, with absolute thresholds of approximately 30 dB SPL. The absolute sensitivity was similar in all five species, with a tendency for larger species to be more sensitive (Table 1). There were no significant differences between the threshold curves obtained in males and females in any of the five species. There was a non-significant tendency for females to be slightly more sensitive than their male counterparts, except for *N. robustus*, where females were slightly less sensitive. This again reflects differences in body size, with males of all species being smaller in our sample than the females, except for *N. robustus*, where the males were larger than the females. The shape and general tuning of males and females were similar in all five species studied here (but see Faure and Hoy, 2000b for sex-specific differences in the tuning of an auditory interneuron in *N. ensiger*). Therefore, for comparison of the tuning among the five species, we pooled the data for males and females of each species.

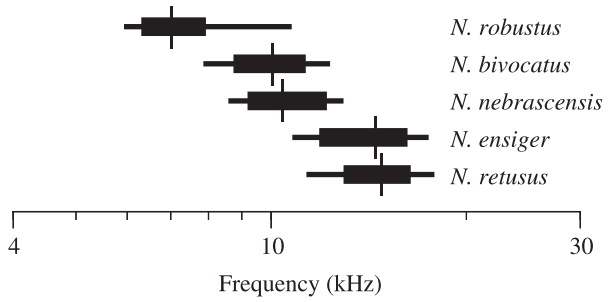

Fig. 2. Position and width of the low-frequency band of the calls of five *Neoconocephalus* species at –3 dB (thick bar) and –10 dB (thin bar): *N. nebrascensis* (*N*=10), *N. bivocatus* (*N*=8), *N. robustus* (*N*=10), *N. retusus* (*N*=9) and *N. ensiger* (*N*=3). The position of the mean peak frequency is indicated by a vertical line.

The relative spectral sensitivity of the five species is compared in Fig. 4. The tuning of the five species was similar, with highest sensitivities occurring in the range of 16–20 kHz. Threshold values remained low for frequencies down to 9 kHz. Below 9 kHz, the steepness of the roll-off of the threshold curve increased to 20–25 dB octave^{-1} in all species. For frequencies above 20 kHz, the roll-off was approximately 8–10 dB octave^{-1} in all species tested. The main difference among the hearing thresholds of the five species is the increase of thresholds from the best frequencies at 16–20 kHz down to 9 kHz. In *N. robustus* and *N. bivocatus*, the increase of threshold in this range is less than 3 dB, while in *N. ensiger*, the threshold at 9 kHz was 8 dB higher than at 18 kHz. *N. nebrascensis* and *N. retusus* were intermediate, with threshold increases of 5–6 dB between 18 kHz and 9 kHz.

Comparing the tuning of the auditory thresholds with the dominant frequencies of the calls revealed a mismatch between calls and hearing in all five species. This was most apparent in *N. robustus*, where hearing sensitivity at the dominant call frequency (7 kHz) was 9 dB lower than at the best frequency of the threshold curve (18 kHz). In *N. nebrascensis*, sensitivity at the dominant call frequency (10 kHz) was 7 dB lower than at 18 kHz. In the other three species, the mismatch was less pronounced, with reduced sensitivity at their respective call frequency of 4–5 dB (*N. retusus* and *N. ensiger*) or 3 dB (*N. bivocatus*). Given the distinct differences in dominant call frequency among the five *Neoconocephalus* species, the tuning of hearing thresholds did not seem to reflect these differences in the calls.

Above-threshold responses

The averaged responses obtained from the tympanic nerve recordings resembled damped oscillations (Fig. 5A). The response magnitudes, measured as peak-to-peak amplitudes of the averaged recordings, increased with stimulus intensity. For stimulus frequencies up to 30 kHz, this increase was linear up to 30 dB above threshold; above this intensity, response magnitude began to saturate (Fig. 5B). For higher stimulus frequencies (40–70 kHz), the increase of response magnitude was similar to that of lower frequencies up to 24 dB above threshold. At higher intensities, the steepness of the

intensity/response function for these high frequencies increased and continued to rise at the higher rate up to the highest intensities tested (Fig. 5B). This increased steepness of high frequencies at high intensities could be caused either by an increased number of neurons responding or by a higher spike synchronization (Pollack and Faulkes, 1998). To distinguish between these possibilities, we measured the breadth of the first spike in the compound action potential (Fig. 5C). The width did not vary systematically over stimulus intensity at both 12 kHz and 50 kHz nor did it differ significantly between high and low frequencies. This indicates that spike synchronization remains constant over the intensity range tested.

To compare the intensity/response functions in the complete frequency range tested, in Fig. 6 we show iso-intensity responses (relative to threshold) for all five species. For stimulus intensities of 9 dB, 18 dB and 27 dB above threshold, the response magnitudes did not vary systematically with frequency, indicating similar intensity/response functions for the frequency range between 5 kHz and 80 kHz up to 27 dB above threshold. An additional increase of stimulus intensity to 36 dB above threshold revealed a strong, non-linear increase of response amplitudes for ultrasonic frequencies between 35 kHz and 70 kHz, while below 30 kHz, the response magnitudes increase at the same, or lower, rate than at lower stimulus intensities. We found the same general pattern of above-threshold response magnitudes in all five species tested (Fig. 6).

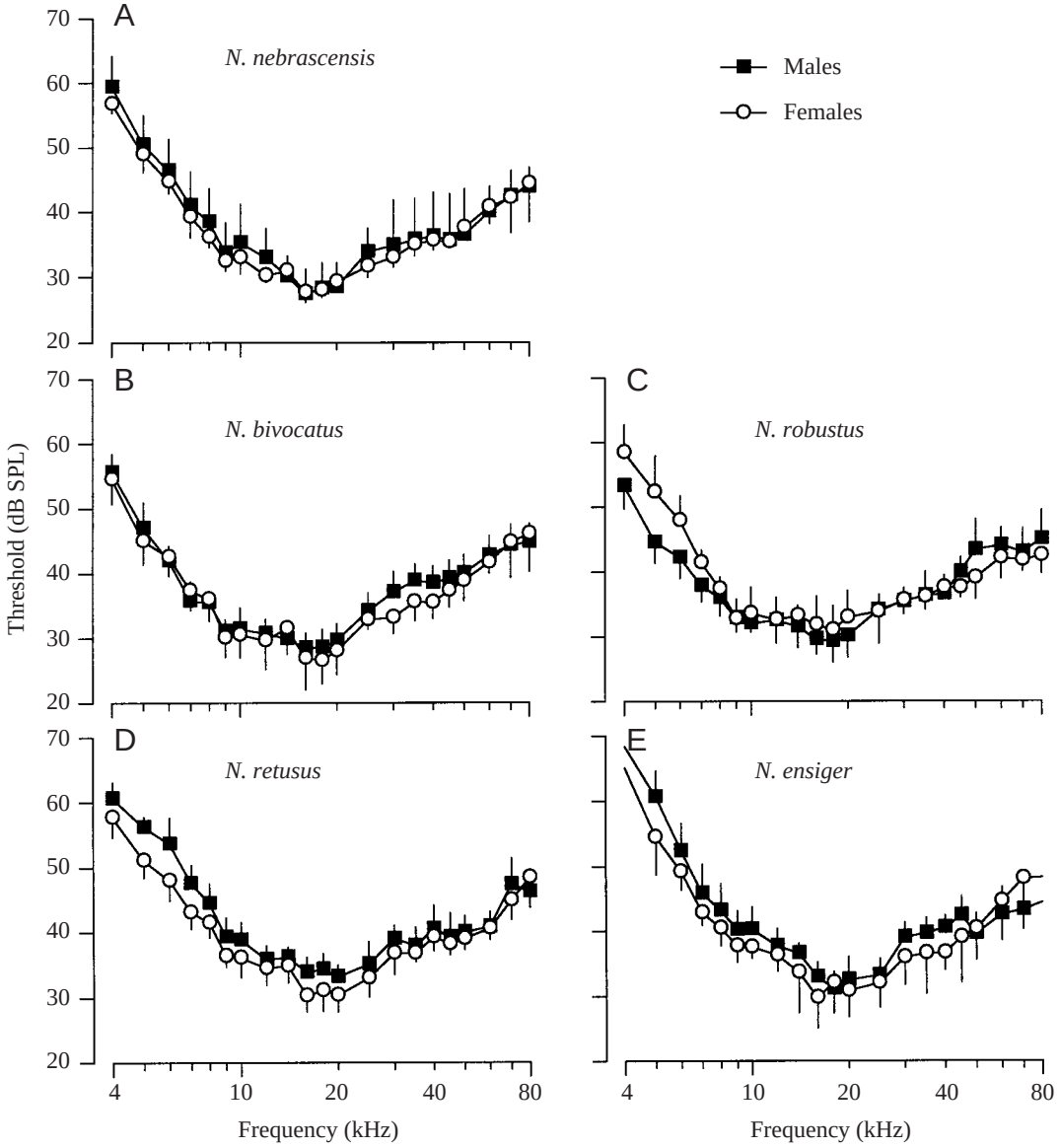

Fig. 3. Hearing threshold (mean ± S.D.) of males (filled squares) and females (open circles) of five species of *Neoconocephalus*: (A) *N. nebrascensis* males (*N*=10 in seven insects) and females (*N*=8 in five insects), (B) *N. bivocatus* males (*N*=11 in six insects) and females (*N*=8 in five insects), (C) *N. robustus* males (*N*=9 in six insects) and females (*N*=5 in three insects), (D) *N. retusus* males (*N*=9 in five insects) and females (*N*=10 in five insects) and (E) *N. ensiger* males (*N*=8 in five insects) and females (*N*=4 in two insects).

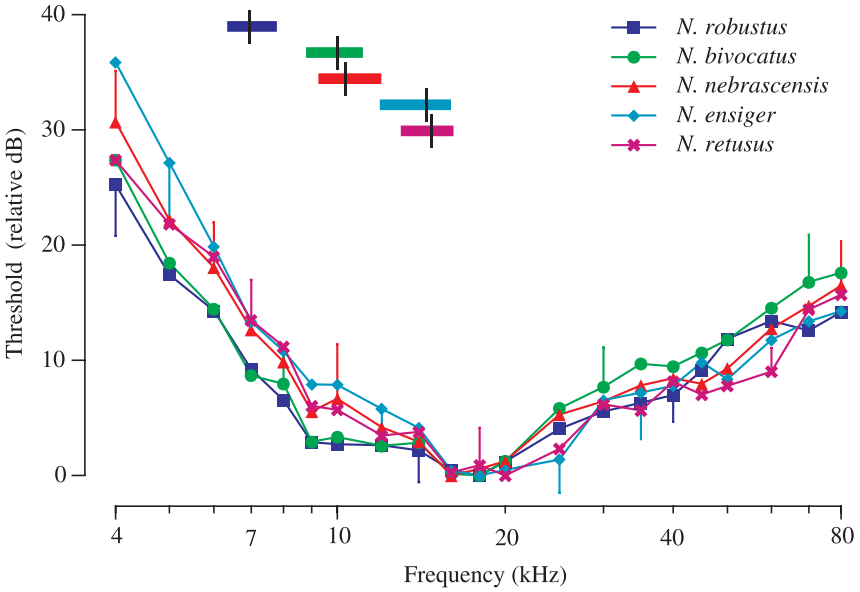

Fig. 4. Hearing thresholds (mean ± S.D.) of five species of *Neoconocephalus* relative to the highest sensitivity. Absolute sensitivity at this point ranged between 27.5 dB SPL and 31.8 dB SPL. The peak frequency (vertical lines) and the –3 dB band (horizontal lines) of the low-frequency component of the calls are indicated by vertical lines. *N. robustus*, *N*=14 in nine animals; *N. bivocatus*, *N*=19 in 11 animals; *N. nebrascensis*, *N*=18 in 12 animals; *N. ensiger*, *N*=12 in seven animals; *N. retusus*, *N*=19 in 10 animals.

Transmission in the field

We measured the attenuation during the transmission through a typical biotope of *Neoconocephalus* for sinusoidal stimuli in the range of 5 kHz to 40 kHz. As expected (Keuper and Kühne, 1983), the attenuation of high frequencies was much more severe than for lower frequencies (Fig. 7): at frequencies below 10 kHz, attenuation was only little more than spreading loss (–6 dB per double distance), while at 40 kHz, the excess attenuation (i.e. attenuation additional to the spreading loss) was more than 60 dB at 20 m distance. The excess attenuation is plotted against frequency in Fig. 8. In the range from 5 kHz to 9 kHz, excess attenuation was hardly recognizable (below 4 dB at 20 m distance) and did not increase with frequency. For frequencies above 9 kHz, excess attenuation increased with increasing frequency (Fig. 8). Thus, the grassland vegetation of the *Neoconocephalus* biotope acted like a low-pass filter by not significantly affecting frequencies below 10 kHz but increasingly dampening higher frequencies. This is in contrast to measurements in habitats with shrubs and trees, where excess attenuation increases linearly with frequency from at least 5 kHz to 40 kHz (Römer and Lewald, 1992).

'Effectiveness' of frequencies for communication

The effectiveness of a signal frequency during communication, i.e. how effectively the signal stimulates the receiver's sensory system, is determined by the

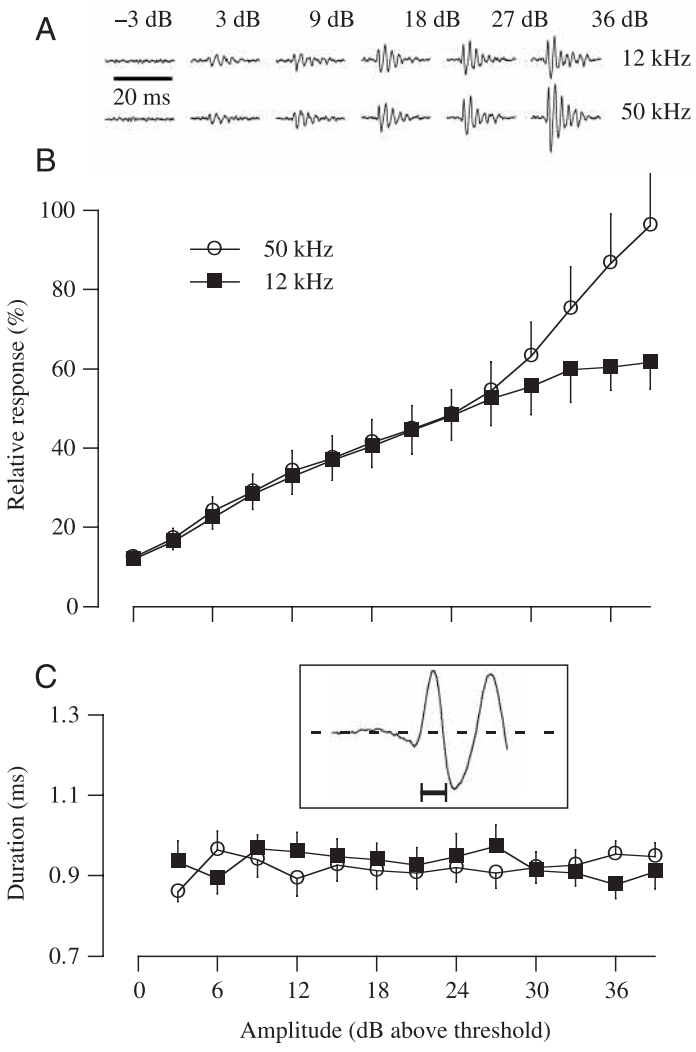

Fig. 5. Response amplitudes of the averaged tympanic nerve responses in *Neoconocephalus nebrascensis*. (A) Example of the averaged responses recorded from the tympanic nerve to 12 kHz and 50 kHz sine waves (10 ms duration; 1 ms rise/fall time) of various amplitudes. Stimulus amplitudes are given relative to the threshold at each frequency. (B) Stimulus amplitude/response function of the mean response amplitudes (mean ± S.E.M., *N*=15 in nine animals) of *N. nebrascensis* during stimulation with 12 kHz and 50 kHz. Stimulus amplitudes are given relative to the threshold at both frequencies. Response amplitudes are given as arbitrary units. (C) Breadth of the first oscillations (see inset) of the averaged responses (mean ± S.E.M., *N*=15 in nine animals) of *N. nebrascensis* during stimulation with 12 kHz and 50 kHz. Stimulus amplitudes are given relative to the threshold at both frequencies.

sensitivity of the receiver and the transmission properties of the biotope. We calculated the theoretical effectiveness of call frequencies in the range of 6–18 kHz, assuming that calls were produced with an amplitude of 110 dB SPL at 20 cm distance, which is approximately the amplitude of male calls of the five species (U. Büttner and J. Schul, unpublished observations). Using the hearing thresholds (Fig. 3) and the transmission functions through the biotope (Figs 7, 8), we calculated the amplitude at which a female would perceive the call (i.e. its amplitude above the hearing threshold) over distance. At 18 kHz (the frequency of highest hearing sensitivity), this perceived amplitude is high at short distances but declines rapidly as the distance increases (Fig. 9). The perceived amplitude of lower frequencies (e.g. 9 kHz; Fig. 9) is lower at short distances because of higher hearing thresholds. But the decline of perceived amplitude of lower frequencies (e.g. 9 kHz; Fig. 9) with increasing distance is less steep than at 18 kHz because of the lower excess attenuation. The two amplitude functions cross at a 'break-even' distance (arrow in Fig. 9): at shorter distances, the higher frequency is perceived as louder by the female; at longer distances, the lower frequency has a higher perceived amplitude.

Accordingly, we calculated the break-even distances for

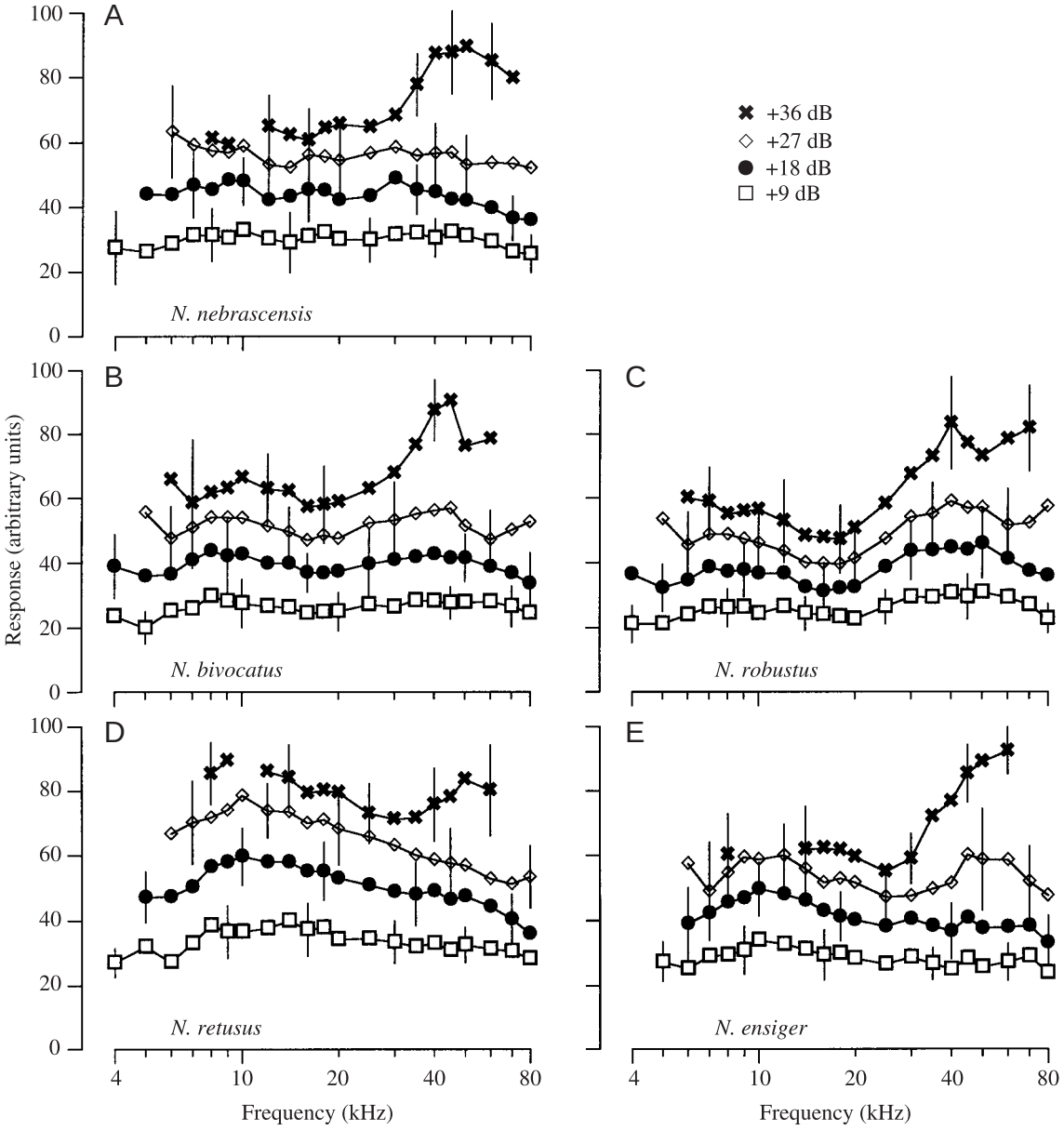

Fig. 6. Iso-intensity response function of the averaged responses recorded from the tympanic nerve in five species of *Neoconocephalus* (mean ± S.E.M.). Response amplitudes were measured at 9 dB, 18 dB, 27 dB and 36 dB above threshold of each frequency. (A) *N. nebrascensis*, *N*=15 in nine animals; (B) *N. bivocatus*, *N*=19 in 11 animals; (C) *N. robustus*, *N*=14 in nine animals; (D) *N. retusus*, *N*=19 in 10 animals; (E) *N. ensiger*, *N*=12 in seven animals.

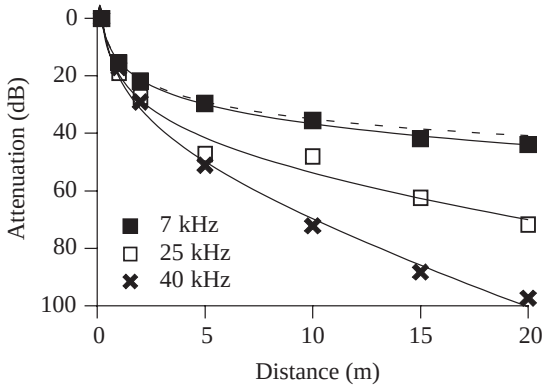

Fig. 7. Attenuation of pure-tone sound pulses (7–40 kHz) over distance, as measured in a typical biotope of the five *Neoconocephalus* species. The curve fittings (solid lines) were calculated using the formula $y=a*x+b*\log(x)+c$. The dotted line indicates the theoretical attenuation due to spherical spreading alone (6 dB per double distance).

frequencies between 7 kHz and 14 kHz, relative to an 18 kHz signal, for all five species (Fig. 10). For all five species, this distance was short in the range from 9 kHz to 14 kHz: for *N. robustus* and *N. bivocatus*, it was below 1 m; for the other three species, it ranged between 1 m and 2.3 m. Below 9 kHz, the break-even distance increased sharply in all five species. This increase is due to the increasing hearing thresholds of all species below 9 kHz (Figs 3, 4) and the fact that the transmission properties of the biotope do not change for frequencies below 9 kHz (Fig. 8). At frequencies above 9 kHz, the higher hearing thresholds compared with 18 kHz (Figs 3, 4) are offset at short distances by the higher excess attenuation at the higher frequency (Fig. 8).

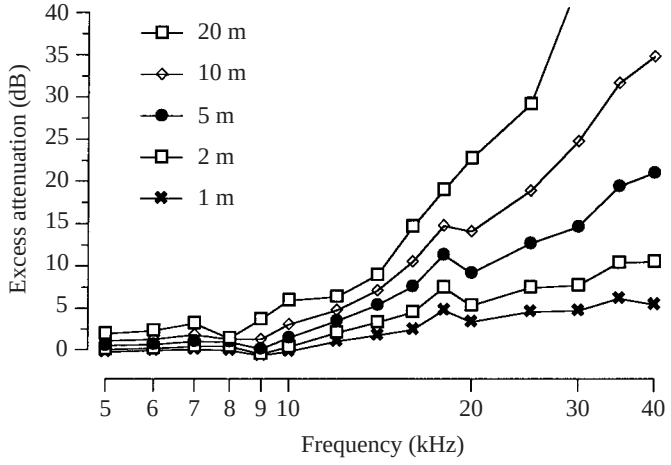

Fig. 8. Attenuation of pure-tone sound pulses (5–40 kHz) exceeding the spherical attenuation of 6 dB per double distance ('excess attenuation') at various distances. The attenuation was measured in a typical grassland habitat of the five *Neoconocephalus* species; the data shown here are taken from the best-fit curves for each frequency (see Materials and methods; Fig. 6).

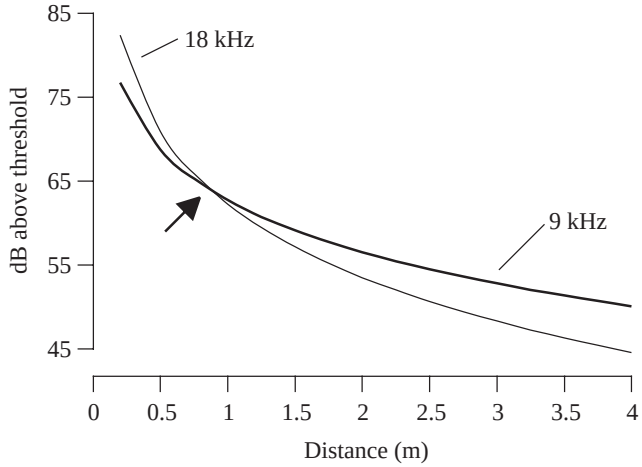

Fig. 9. Sound intensity above hearing threshold of pure-tone signals of 9 kHz and 18 kHz, as perceived by a female *Neoconocephalus nebrascensis*, over distance between sender and receiver. Data were calculated by assuming a signal amplitude of 110 dB SPL at 20 cm distance and using the measured data for the attenuation during sound transmission of pure tones (Figs 6, 7) and the hearing thresholds (Fig. 4). At approximately 1 m distance, both pure-tone signals are perceived at the same relative intensity ('break-even' distance; arrow).

Discussion

In this study, we compared hearing thresholds with the frequencies of the male calls in a group of closely related katydid species. Although the call frequencies of the five species differed considerably, the response properties of the tympanic organ, as revealed by whole nerve recordings, were similar among the species; the overall shapes of their threshold curves did not differ, and all species were most sensitive in the frequency range of 16–20 kHz. Also, there was a mismatch between the dominant frequency of the male call and the best frequency of hearing sensitivity in all five species tested.

Influences on the tuning of the hearing organ

Crickets and katydids (tettigoniids) use their auditory sensory system mainly in two behavioral contexts: acoustic communication and bat avoidance. Thus, selective pressure for high hearing sensitivity stems from two signal classes with different spectral properties: conspecific communication signals and bat echolocation calls. In most crickets, auditory sensitivity is high in two frequency ranges (Pollack and Imaizumi, 1999): the frequency of the calling songs (usually 3–9 kHz) and the frequency range of many bat echolocation calls (25–60 kHz; Fenton et al., 1998). Many katydid species have calls with broad band spectra or with several distinct frequency bands, which commonly extend from 10 kHz to 60 kHz (Heller, 1988). Hearing sensitivity in such species usually has a broad frequency range of highest sensitivity, comprising both the frequency range of their communication signals and bat echolocation calls (e.g. Kalmring et al., 1990).

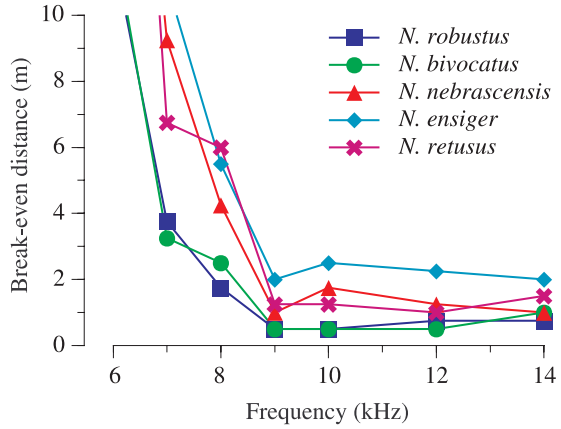

Fig. 10. Break-even distances for pure-tone signals relative to an 18 kHz signal. At the break-even distance, both signals are perceived by a female with the same intensity above threshold (see Fig. 8). For longer distances, the lower frequency is perceived louder, whereas for shorter distances, 18 kHz is perceived louder.

The communication signals of *Neoconcephalus* are more 'cricket-like' than 'katydid-like' in that the main energy component of the call is in a narrow low-frequency band and only minor ultrasound components are present in the calls (Fig. 1; Greenfield, 1990; Libersat and Hoy, 1991). The low amplitude of these ultrasound components and the high intraspecific variability suggest little, if any, importance for communication; if they were important for female phonotaxis, i.e. if they would make a call more attractive or better localizable, sexual selection theory would predict a pronounced ultrasound component in male calls (similar to most other katydid species) and also lower variability within male calls of each species for this trait (Anderson, 1994). Therefore, it is most likely that the pronounced low-frequency component of male calls is mainly, if not exclusively, used for communication between males and females.

Surprisingly, in all five species of *Neoconcephalus*, the frequency range of highest sensitivity of the hearing organ was not tuned to either communication signals or to bat echolocation calls but to an intermediate frequency range around 18 kHz (Fig. 4). This mismatched tuning could nevertheless be a by-product of the above-mentioned selective pressures in combination with the limitations caused by the biophysics of the hearing mechanism. In crickets, the high, narrow-band selectivity in the low-frequency range is due to the transmission properties of the tracheal system, which constitutes the main sound input for the hearing system (Michelsen et al., 1994). In katydids, the acoustic trachea acts as a finite exponential horn, which has high-pass rather than band-pass characteristics (Hoffmann and Jatho, 1995). The cut-off frequency of the exponential horn largely determines the low-frequency roll-off of hearing thresholds. Towards high frequencies, the gain of the exponential horn remains high, and the decrease in sensitivity towards high frequency is probably due to the mechanical properties of the receptor organ itself. This mechanism leads to a broad frequency range of high sensitivity rather than to a W-shaped threshold curve, as found in crickets. In *Neoconcephalus*, we found evidence for special adaptations to hearing bats (see below), which suggest a strong selective pressure for high sensitivity in the ultrasonic frequency range. The acoustic trachea of the katydid hearing system, with its broad frequency range of high gain, probably prevents the evolution of a sensitivity maximum of 40–50 kHz, but the sensitivity in this range can be increased by an increase of the overall sensitivity. In conclusion, we suggest that the tuning of the five *Neoconcephalus* species is the consequence of selection for high sensitivity in the frequency range of the conspecific signals (7–15 kHz) and of bat echolocation calls (30–60 kHz). Highest sensitivity around 18 kHz is probably a consequence of selection for high sensitivity in the two adjacent frequency ranges.

Adaptations to hearing bats

The intensity/response functions of all five *Neoconcephalus* species showed a peculiarity at ultrasonic frequencies between 35 kHz and 70 kHz; for stimulus intensities higher than 25–30 dB above threshold, the intensity response function was more than twice as steep than that at lower stimulus amplitudes (Fig. 5). Iso-intensity functions of call responses were flat for lower stimulus intensities (9–27 dB; Fig. 6) in the complete frequency range tested, indicating that similar numbers of receptor cells contribute to the compound action potential at each stimulus frequency. This, in turn, suggests that best frequencies of individual receptor cells are evenly distributed along the tuning curve of the whole hearing organ (Pollack and Faulkes, 1998; Schul, 1999), as was found for several katydid species (e.g. Kalmring et al., 1990; Römer, 1983; but see Stölting and Stumpner, 1998). The increase in the slope of the intensity response function at high intensities in the ultrasonic range cannot be attributed to an increased spike synchronization, because the width of the compound action potential remains constant. Rather, the increased slope is explained by an increased number of cells responding, i.e. a second receptor cell population begins responding to ultrasonic stimuli. This receptor cell population could either be a group of receptors tuned to ultrasonic frequencies, but with 25–30 dB higher thresholds, or could be cells tuned to lower frequencies with a secondary sensitivity maximum at the higher frequencies. In crickets, most receptor cells tuned to ultrasonic frequencies have such secondary sensitivity maxima at lower frequencies close to the carrier frequency of the calling song (Imaizumi and Pollack, 1999), whereas threshold curves described for katydid receptor cells do not show such secondary peaks. Stölting and Stumpner (1998) demonstrate that receptor cells of the intermediate organ may have high frequency auditory tuning with thresholds that are 25 dB higher than that of receptor cells in the crista acoustica, the major hearing organ in katydids. Thus, receptor cells of the intermediate organ could be the second receptor cell population responding to ultrasonic stimuli. Our whole nerve recordings do not allow us to decide between the two possibilities; single

cell recordings of auditory receptor cells are required to answer this question.

The presence of a second group of receptor cells responding to ultrasound with 25–30 dB higher thresholds is reminiscent of the auditory system in some moths (Roeder, 1967). The ear of the noctuid moth is comprised of only two receptor cells (A1 and A2). A1 and A2 have nearly identical tuning curves, but the A2 cell is approximately 20 dB less sensitive than the A1 cell (Roeder, 1967). Noctuid moths show graded responses to bat calls: negative phonotaxis at low echolocation call intensities, and erratic flight maneuvers at high intensities. The switch between these behaviors is probably related to the intensity range fractioning provided by the A1 and A2 receptors (reviewed in Yager, 1999).

The approximate threshold of the second receptor cell population at 40 kHz described here is in the range of 70–75 dB SPL. A bat echolocation call reaching an insect as large as *Neoconocephalus* with this amplitude would probably produce an echo that the bat would be able to hear, thus indicating an immediate danger for the insect (Schulze and Schul, 2001). Bat avoidance behaviors have been described in *N. ensiger* both during flight (Libersat and Hoy, 1991) and calling (Faure and Hoy, 2000a), and thresholds in both situations were at 70–75 dB SPL. The correlation between behavioral and neuronal thresholds suggests that the second receptor cell population determines the behavioral threshold for bat-avoidance behavior in *Neoconocephalus*. Therefore, we interpret its presence as an adaptation for predator detection.

Why do males not call at the frequency of highest female sensitivity?

The amplitude of a male call is probably the single most important factor determining its overall attractiveness. Call amplitude was found to be the most important factor for intraspecific female choice (Arak et al., 1990); with all other parameters equal, amplitude differences of as little as 1–2 dB have been reported to reliably cause female preferences for the louder signal (e.g. Römer et al., 1998). Therefore, selection should favor male call frequencies that are perceived by females as the loudest. Which call frequency is optimal for the male depends on the tuning of the female hearing system and the sound-transmission properties of the biotope. In the case of the *Neoconocephalus* species studied here, females are most sensitive for frequencies around 18 kHz (Fig. 4). However, sounds are best transmitted through grasslands at lower frequencies; excess attenuation is lowest for frequencies below 10 kHz and increases with increasing frequencies above 10 kHz. Therefore, at short distances, when the transmission through the biotope has only little effect, a call frequency of 18 kHz would be optimal. At longer distances, beyond the break-even distance (Fig. 10), call frequencies of 9–10 kHz seem ideal. The relevant distance for female choice is as long or longer than half the nearest neighbor distance of calling males, because females must choose (for the latest) when they are sitting between two calling males.

Male *Neoconocephalus* are usually spaced 3–10 m apart (J. Schul, unpublished observation). Therefore, female phonotaxis should usually take place at considerably longer distances than at the break-even distances. Thus, the optimal call frequency for males of all five *Neoconocephalus* species is 9–10 kHz. Males of two species (*N. bivocatus* and *N. nebrascensis*) call at this frequency, while two species (*N. retusus* and *N. ensiger*) call at considerably higher frequencies (approximately 15 kHz) and *N. robustus* calls at lower frequencies (approximately 7 kHz).

At this point, we can only speculate as to which factors might be responsible for these discrepancies between the call frequency and the predicted optimal frequency in three of the five species. *N. retusus* and *N. ensiger*, which both call above the predicted optimal frequency, are the smallest of the five species (Table 1). A physiological constraint such as body size could hinder the evolution of lower call frequencies in two ways. First, males might be too small to produce the lower frequency effectively; i.e. they would lose more in absolute call amplitude than they would gain in improved transmission (reviewed in Bennet-Clark, 1998). Second, the females might be too small to generate enough sound shadow to localize the lower call frequency effectively; because katydid ears function as pressure receivers (Michelsen et al., 1994), directional information is derived from intensity differences between the sound entrances of both ears. These intensity differences are caused by diffraction on the insect's body, which strongly depends on body size (Michelsen, 1994). As *N. retusus* and *N. ensiger* are the smallest of the five species, their body size might be too small to generate sufficient directional information at the optimal call frequency of 9–10 kHz. In this case, their call frequency of 15 kHz would be a trade-off between attractiveness for and localizability by the females. Such size constraints would not explain the situation in *N. robustus*, where males call at 7 kHz rather than at 10 kHz. A possible explanation here could be the need for this species to signal in a 'private channel' (Narins, 1995) to avoid masking of their signals by signals of other noisy animals.

Concluding remarks

Although the five species of *Neoconocephalus* studied here differ considerably in the spectral composition of their calls, the tuning properties of their hearing systems are very similar. The tuning of the hearing system seems to be largely determined by the influence of factors such as bat detection and the morphology of the hearing system. The call frequency is not strongly influenced by the tuning of the hearing organ due to constraints imposed by the transmission properties of the biotope: the high pass characteristics of the grassland habitat favor call frequencies of 10 kHz for all species. Thus, the mismatch between call frequencies and tuning of the hearing systems seems to be mainly a consequence of bat predation, which favors high sensitivity at ultrasonic frequencies, and the low pass transmission properties of the biotope, which favor a call frequency lower than the best frequency of the hearing organ.

We wish to thank Sarah L. Bush and Rex B. Cocroft for important and helpful discussions during the experimental phase of the project and the preparation of the manuscript. We are grateful to Ursula K. Büttner who helped collect the insects and perform the call recordings. This research was supported by a grant of the Research Council of the University of Missouri – Columbia to J.S. (URC-01-063).

References

Anderson, M. B. (1994). *Sexual Selection*. Princeton, NJ: Princeton University Press.

Arak, A., Eiriksson, T. and Radesater, T. (1990). The adaptive significance of acoustic spacing in male bushcrickets *Tettigonia viridissima*: a pertubation experiment. *Behav. Ecol. Sociobiol.* **26**, 1-8.

Bailey, W. J. and Römer, H. (1991). Sexual differences in auditory sensitivity: mismatch of hearing threshold and call frequency in a tettigoniid (Orthoptera, Tettigoniidae: Zaprochilinae). *J. Comp. Physiol. A* **169**, 349-353.

Bennet-Clark, H. C. (1998). Size and scale effects as constraints in insect sound communication. *Phil. Trans. R. Soc. Lond. B* **353**, 407-419.

Capranica, R. R. and Rose, G. (1983). Frequency and temporal processing in the auditory system of anurans. In *Neuroethology and Behavioral Physiology* (ed. F. Huber and H. Markl), pp. 136-152. Heidelberg: Springer.

Cade, W. H. (1975). Acoustically orienting parasitoids: fly phonotaxis to cricket song. *Science* **190**, 1312-1313.

Endler, J. A. (1992). Signals, signal conditions and the direction of evolution. *Am. Nat.* **139**, S125-S153.

Faure, P. A. and Hoy, R. R. (2000a). The sound of silence: cessation of singing and song pausing are ultrasound-induced startle behaviors in the katydid *Neoconocephalus ensiger* (Orthoptera; Tettigoniidae). *J. Comp. Physiol. A* **186**, 129-142.

Faure, P. A. and Hoy, R. R. (2000b). Neuroethology of the katydid T-cell I. Tuning and responses to pure tones. *J. Exp. Biol.* **203**, 3225-3242.

Fenton, M. B., Portfors, C. V., Rautenbach, I. L. and Waterman, J. M. (1998). Compromises: sound frequencies used in echolocation by aerial-feeding bats. *Can. J. Zool.* **76**, 1174-1182.

Froeschner, R. C. (1954). The grasshoppers and other Orthoptera of Iowa. *Iowa State College J. Sci.* **29**, 163-354.

Greenfield, M. D. (1990). Evolution of acoustic communication in the genus *Neoconocephalus*: Discontinuous songs, synchrony, and interspecific interactions. In *The Tettigoniidae: Biology, Systematics and Evolution* (ed W. J. Bailey and D. C. F. Rentz), pp. 71-97. Heidelberg: Springer.

Heller, K.-G. (1988). *Die Bioakustik der europäischen Laubheuschrecken*. Weikersheim, Germany: Verlag J. Margraf.

Heller, K.-G., Schul, J. and Ingrisch, S. (1997). Sex-specific differences in song frequency and tuning of the ears in some duetting bushcrickets (Orthoptera: Tettigonioidea: Phaneropteridae). *Zoology* **100**, 110-118.

Hoffmann, E. and Jatho, M. (1995). The acoustic trachea of tettigoniids as an exponential horn: theoretical calculations and bioacoustical measurements. *J. Acoust. Soc. Am.* **98**, 1845-1851.

Hoy, R. R. (1992). The evolution of hearing in insects as an adaptation of predation from bats. In *The Evolutionary Biology of Hearing* (ed. D. B. Webster, R. R. Fay and A. N. Popper), pp. 115-129. New York: Springer Verlag.

Huber, F., Moore, T. E. and Loher, W. (1990a). *Cricket Behavior and Neurobiology*. Ithaca, NY: Cornell University Press.

Huber, F., Kleindienst, H.-U., Moore, T., Schildberger, K. and Weber, T. (1990b). Acoustic communication in periodic cicadas: neuronal responses to songs of sympatric species. In *Sensory Systems and Communication in Arthropods* (ed. F. G. Gribakin, K. Wiese and A. Popov), pp. 217-228. Basel, Switzerland: Birkhäuser.

Imaizumi, K. and Pollack, G. S. (1999). Neuronal coding of sound frequency by cricket auditory receptors. *J. Neurosci.* **19**, 1508-1516.

Kalmring, K., Schröder, J., Rössler, W. and Bailey, W. J. (1990). Resolution of time and frequency patterns in the tympanal organs of Tettigoniids. II. Its basis at the single receptor level. *Zool. Jb. Physiol.* **94**, 203-215.

Keuper, A. and Kühne, R. (1983). The acoustic behaviour of the bushcricket *Tettigonia cantans.* II. Transmission of airborne-sound and vibration signals in the biotope. *Behav. Proc.* **8**, 125-145.

Kössl, M. (1994). Evidence for a mechanical filter in the cochlea of the 'constant frequency' bats, *Rhinolophous rouxi* and *Pteronotus parnellii*. *Hearing Res.* **72**, 73-80.

Libersat, F. and Hoy, R. R. (1991). Ultrasonic startle behavior in bushcrickets (Orthoptera; Tettigoniidae). *J. Comp. Physiol. A* **169**, 507-514.

Mason, A. C. (1991). Hearing in a primitive ensiferan: the auditory system of *Cyphoderris monstrosa* (Orthoptera: Haglidae). *J. Comp. Physiol. A* **168**, 351-363.

Meyer, J. and Elsner, N. (1996). How well are frequency sensitivities of grasshoppers ears tuned to the species-specific song spectra? *J. Exp. Biol.* **199**, 1631-1642.

Michelsen, A. (1994). Directional hearing in crickets and other small animals. In *Neural Basis of Behavioural Adaptations* (ed. K. Schildberger and N. Elsner), pp. 195-207. Stuttgart, New York: Fischer.

Michelsen, A., Heller, K.-G., Stumpner, A. and Rohrseitz, K. (1994). A new biophysical method to determine the gain of the acoustic trachea in bushcrickets. *J. Comp Physiol. A* **175**, 145-151.

Moiseff, A., Pollack, G. S. and Hoy, R. R. (1978). Steering response of flying crickets to sound and ultrasound: mate attraction and predator avoidance. *Proc. Natl. Acad. Sci. USA* **75**, 4052-4056.

Narins, P. (1995). Frog communication. *Sci. Am.* **273**, 62-67.

Nocke, H. (1972). Physiological aspects of sound communication in crickets (*Gryllus campestris* L.). *J. Comp. Physiol.* **80**, 141-162.

Paton, J. A., Capranica, R. R., Dragsten, P. R. and Webb, W. W. (1977). Physical basis for auditory frequency analysis in field crickets (Gryllidae). *J. Comp. Physiol. A* **119**, 221-240.

Pollack, G. S. and Faulkes, Z. (1998). Representation of behaviourally relevant sound frequencies by auditory receptors in the cricket *Teleogryllus oceanicus*. *J. Exp. Biol.* **201**, 155-163.

Pollack, G. S. and Imaizumi, K. (1999). Neural analysis of sound frequency in insects. *BioEssays* **21**, 295-303.

Popov, A. V. (1990). Co-evolution of sound production and hearing in insects. In *Sensory Systems and Communication in Arthropods* (ed. F. G. Gribakin, K. Wiese and A. V. Popov), pp. 301-304. Basel, Switzerland: Birkhäuser.

Roeder, K. D. (1967). *Nerve Cells and Insect Behavior*. Cambridge, MA: Harvard University Press.

Römer, H. (1983). Tonotopic organization of the auditory neuropile in the bushcricket *Tettigonia viridissima*. *Nature* **306**, 60-62.

Römer, H. and Lewald, J. (1992). High-frequency sound transmission in natural habitats: implications for the evolution of insect acoustic communication. *Behav. Ecol. Sociobiol.* **29**, 437-444.

Römer, H., Spickermann, M. and Bailey, W. (1998). Sensory basis for sound intensity discrimination in the bushcricket *Requena verticalis* (Tettigoniidae, Orthoptera). *J. Comp. Physiol. A* **182**, 595-607.

Ryan, M. J., Fox, J. H., Wilczynski, W. and Rand, A. S. (1990). Sexual selection by sensory exploitation in the frog *Physalaemus pustulosus. Nature* **343**, 66-67.

Schul, J. (1999). Neuronal basis for spectral song discrimination in the bushcricket *Tettigonia cantans. J. Comp. Physiol. A* **184**, 457-461.

Schulze, W. and Schul, J. (2001). Ultrasound avoidance behaviour in the bushcricket *Tettigonia viridissima* (Orthoptera, Tettigoniidae). *J. Exp. Biol.* **204**, 733-740.

Stölting, H. and Stumpner, A. (1998). Tonotopical organization of auditory receptors of the bushcricket *Pholidoptera griseoaptera* (De Geer 1773) (Tettigoniidae, Decticinae). *Cell Tissue Res.* **294**, 377-386.

Walker, T. J., Whitesell, J. J. and Alexander, R. D. (1973). The robust conehead: two widespread sibling species (Orthoptera: Tettigoniidae: *Neoconocephalus 'robustus'*). *Ohio J. Sci.* **73**, 321-330.

Yager, D. D. (1999). Structure, development, and evolution of insect auditory systems. *Microsc. Res. Tech.* **47**, 380-400.