

HOW WELL ARE FREQUENCY SENSITIVITIES OF GRASSHOPPER EARS TUNED TO SPECIES-SPECIFIC SONG SPECTRA?

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Summary

Grasshoppers of 20 acridid species were examined using spectral analysis, laser vibrometry and electrophysiology to determine whether the song spectra, the best frequencies of tympanal-membrane vibrations and the threshold curves of the tympanal nerves are adapted to one another. The songs of almost all species have a relatively broad-band maximum in the region between 20 and 40 kHz and a narrower peak between 5 and 15 kHz. There are clear interspecific differences in the latter, which are not correlated with the length of the body or of the elytra. At the site of attachment of the low-frequency receptors (a-cells), the tympanal membrane oscillates with maximal amplitude in the region from 5 to 10 kHz. At the attachment site of the high-frequency receptors (d-cells), there is also a maximum in this region as well as another around 15–20 kHz. The tympanal nerve is most sensitive to tones between 5 and 10 kHz, with another sensitivity maximum between 25 and 35 kHz. The species may differ from one another in the position of the low-frequency peaks of the membrane oscillation, of the nerve activity and of the song spectra. No correlation was found between the

characteristic frequency of the membrane oscillation and the area of the tympanal membrane.

Within a given species, the frequency for maximal oscillation of the membrane at the attachment site of the low-frequency receptors and the frequency for maximal sensitivity of the tympanal nerve are in most cases very close to the low-frequency peak in the song spectrum. In the high-frequency range, the situation is different: here, the position of the peak in the song spectrum is not correlated with the membrane oscillation maximum at the attachment site of the high-frequency receptors, although there is a correlation between the song spectrum and the sensitivity of the tympanal nerve. On the whole, therefore, hearing in acridid grasshoppers is quite well adjusted to the frequency spectra of the songs, partly because the tympanal membrane acts as a frequency filter in the low-frequency range.

Key words: grasshoppers, sound production, hearing, frequency tuning, laser vibrometry.

Introduction

Intra- and interspecific communication, by whatever sensory modality, is possible only if the transmitter and the receiver are sufficiently matched to one another. When auditory signals are employed, two factors enter into this matching: the amplitude modulation of the signal (i.e. its temporal pattern) and its frequency spectrum.

Whereas analysis of the temporal pattern of sound pulses and chirps is primarily achieved by a central nervous network, with little filtering by the ears, this is not the case for frequency spectra. In many cases, tympanal membranes and tympanal receptors are tuned to the main frequencies of the conspecific sound signals. Examples have been found in bats (Kössl, 1994), frogs (Capranica and Rose, 1983), cicadas (Popov, 1990), crickets (Huber *et al.* 1990; Paton *et al.* 1977; Libersat *et al.* 1994) and bushcrickets (Jatho *et al.* 1994).

The Acrididae have never been the subject of experiments to

compare the frequencies in the song with the frequency sensitivity of the ear, even though species in the acridid subfamily Gomphocerinae are distinguished by particularly highly developed auditory communication. The lack of such studies is unsurprising given that their song spectra, unlike those of the crickets for instance, are extremely broad-band (Dumortier, 1963); thus, there was no reason to expect them to have any particular significance. The main consideration, however, has been the convincing demonstration by von Helversen (1972) and von Helversen and von Helversen (1981, 1994) that the temporal pattern of the song subunits – that is, the grouping of pulses into syllables and the repetition of the latter to form chirps – plays the decisive role in intraspecific communication.

Nevertheless, it has not been ruled out that a special adaptation of the oscillatory characteristics of the tympanal membrane and of the frequency-sensitivity of the tympanal

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receptors to the song spectrum might also contribute to this species-identification process. The study described here was undertaken in order to answer this question, by testing males of 18 acridid species of the subfamily Gomphocerinae, as well as two of the subfamily Oedipodinae (including the migratory locust *Locusta migratoria*), using a combination of biophysical and electrophysiological methods.

Materials and methods

Animals

Male grasshoppers of the species *Chorthippus albomarginatus*, *Ch. biguttulus*, *Ch. brunneus*, *Ch. dorsatus*, *Ch. montanus*, *Ch. parallelus*, *Gomphocerus rufus*, *Mecostethus grossus*, *Myrmeleotettix maculatus*, *Omocestus viridulus*, *Stenobothrus lineatus* and *St. stigmaticus* were collected near Göttingen, Germany, and in the surrounding area. *Chorthippus apricarius* and *Stenobothrus nigromaculatus* were caught at Badra near the Kyffhäuser, *Omocestus haemorrhoidalis* at Bitterfeld, *Chorthippus mollis* at Erlangen, *Chrysochraon dispar* at Seewiesen and the species *Gomphocerus sibiricus* and *Stenobothrus rubicundus* at Pontresina, Switzerland. *Locusta migratoria* were obtained from a colony at the Zoological Institute in Göttingen.

The experiments were all performed at an ambient temperature of 25–28 °C in a sound-insulated laboratory.

The size of the tympanal membranes was measured by drawing their contours with the help of a drawing mirror attached to the microscope, cutting out the sketches and weighing them on an analytical balance. By comparing the mass of these cut-outs with that of a reference sheet of known size, the area of the tympana could easily be determined.

Acoustic stimulation

The acoustic stimuli were presented using a high-frequency loudspeaker (Dynaudio D-21 AF) with a flat frequency response from 2 to 40 kHz. According to the data sheet, the deviations were within a limit of ± 1 dB. The speaker was positioned at 90° to the long axis of the animal, at a distance of 33 cm.

For the measurement of auditory threshold curves, trapezoidally modulated sinusoidal tones (10 ms duration, 1 ms rise time, 2 Hz repetition rate) were generated by an acoustic stimulator (built in the Zoological Institute, Göttingen, Germany). The animals were stimulated with these signals by changing the frequency in 1 kHz steps and gradually increasing the sound intensity at each frequency. During stimulation at a 2 Hz repetition rate, no habituation of the nerve response was observed.

In experiments on tympanal-membrane oscillation, the stimulus was a frequency-modulated signal 7.8 ms in duration, produced by a digital/analog converter (DAC) integrated into a Fast Fourier Transformation (FFT) analyzer (Hewlett Packard 3567A). These so-called ‘chirps’ contained all frequencies from 1 to 40 kHz at almost the same amplitudes. With this form of stimulation, the intensity was 75 dB SPL 1 cm above the animal. Sound intensity was measured with a

1/4 inch Brüel & Kjær microphone. The animal was surrounded with sound-absorbing material in such a way that, during acoustic stimulation with ‘chirps’, flat frequency spectra were measured 1 cm above the preparation. The sound field closer to the tympanal membrane could not be mapped in detail as no probe microphone was available; therefore, we cannot completely rule out the possibility of different acoustic conditions due to anatomical differences between the species.

Song recording

The grasshoppers’ songs were recorded using a Brüel & Kjær microphone (1/2 inch, type 4133) with a flat frequency response from 2 to 40 kHz, connected to a Brüel & Kjær measurement amplifier (type 2608). The signals were stored on magnetic tape (Racal Store 7DS) and subsequently input to the FFT analyzer.

Laser measurements

To measure the velocity of acoustically induced oscillations of the tympanal membrane, a laser vibrometer (Polytec OFV 2100 with measurement head OFV 300) was used. This technique makes use of the Doppler effect: the fact that the wavelength of the light reflected from a moving object changes in proportion to the object’s velocity.

In these experiments, the intact animal was fixed with kneadable adhesive to a plate measuring 5 cm×5 cm mounted on a ball joint so that the position of the animal could be changed as desired. The plate was large enough that a small micromanipulator holding the electrode for nerve recording could be attached to it. For further details of the preparation and the principle of laser measurement, see Meyer and Elsner (1995) and Meyer and Hedwig (1995). A very detailed description of the laser technique has been published by Lewin *et al.* (1990).

Electrophysiological recording

The auditory thresholds of the individual animals were determined by recording the summed activity of all auditory receptors extracellularly from the tympanal nerve (nerve 6). A hook electrode made of steel wire (diameter 30 μ m) insulated with varnish was used for this recording; the reference electrode was placed in contact with the haemolymph in its immediate vicinity. To protect the tympanal nerve from drying out and to prevent a short circuit, it was insulated with Vaseline. During the dissection, care was taken to leave the tracheal and air-sac system as nearly intact as possible, as it affects the function of the tympanal organs.

Data processing

The signals representing membrane oscillation and the recordings of the song were input to a PC-supported FFT analyzer (Hewlett Packard 3567A) for analysis. The same system was used to generate acoustic signals, to store the measurements and to produce graphs of the spectra. By averaging over 50 (for the membrane oscillations) or 100 (for the songs) data blocks (length 7.8 ms), the signal-to-noise ratio was improved by a factor of 50 or 100, respectively.

As an example of the details observable in single spectra, a characteristic spectrum is shown in Fig. 1 for one individual of each of 14 species. The intraspecific variability is indicated in each graph by the shaded area, which represents the envelope of the superimposed spectra of several (4–6) individuals of the same sex. In order to compare the maxima more easily, the frequency spectra in Fig. 1 have been normalized to their maximal value (=0 dB). That is, negative dB values indicate the amplitude difference from the maximum.

The grasshoppers' auditory threshold was determined qualitatively by listening through earphones to a signal representing the extracellularly recorded tympanal nerve activity. The threshold curves in Fig. 1 (fourth column) represent the mean values ($N=4-6$) for each of seven species, and the shaded area shows the calculated standard deviation.

In addition, the characteristic frequencies (CF) of the tympanal vibrations, song spectra and threshold curves of the tympanal nerve were determined (Table 1); the CFs are the mean frequencies at the maximum or minimum of the curve. Most spectra have two peaks (see Fig. 1), and in these cases Table 1 gives two values, one in the low-frequency (LF) and one in the high-frequency (HF) range. It also gives the mean (calculated for up to six individuals) and the standard deviation in each case.

Results

The song spectrum and the spectra of the membrane vibrations at the two receptor attachment sites – those of the low-

frequency (a-cells) and high-frequency (d-cells) receptors – were obtained for males of 18 central European species of Gomphocerinae as well as for *Mecostethus grossus* and for the migratory locust *Locusta migratoria* (both Oedipodinae) over the frequency range 0–40 kHz (Fig. 1; Table 1). For nine of these species, the threshold curve of the tympanal nerve was also measured by recording the summed activity in the nerve (seven examples are shown in Fig. 1). Table 1 shows the characteristic frequencies (CF, also called 'best frequencies' below) at the peaks of these curves averaged over several animals (usually 4–6) and the standard deviations. The great differences in temporal pattern (amplitude modulation) of the songs were not considered in the present study; oscillograms have been published by Elsner (1974b), Meyer (1994) and Fries (1994).

Spectra of the courtship songs

Position of the frequency bands

With few exceptions (e.g. *Ch. brunneus*), the frequency spectrum of the songs is bimodal (Fig. 1, column 1; Table 1). In most cases, the two peaks are separated by a deep, narrow-band minimum. Even when the temporal pattern of hindleg movement and hence the sound pattern it generates are highly temperature-dependent (von Helversen, 1972), the shape of the song spectra, in particular the position of the maxima, does not change with temperature; this has been demonstrated by recordings from *Chorthippus biguttulus* in the range 22–45°C (Meyer, 1994).

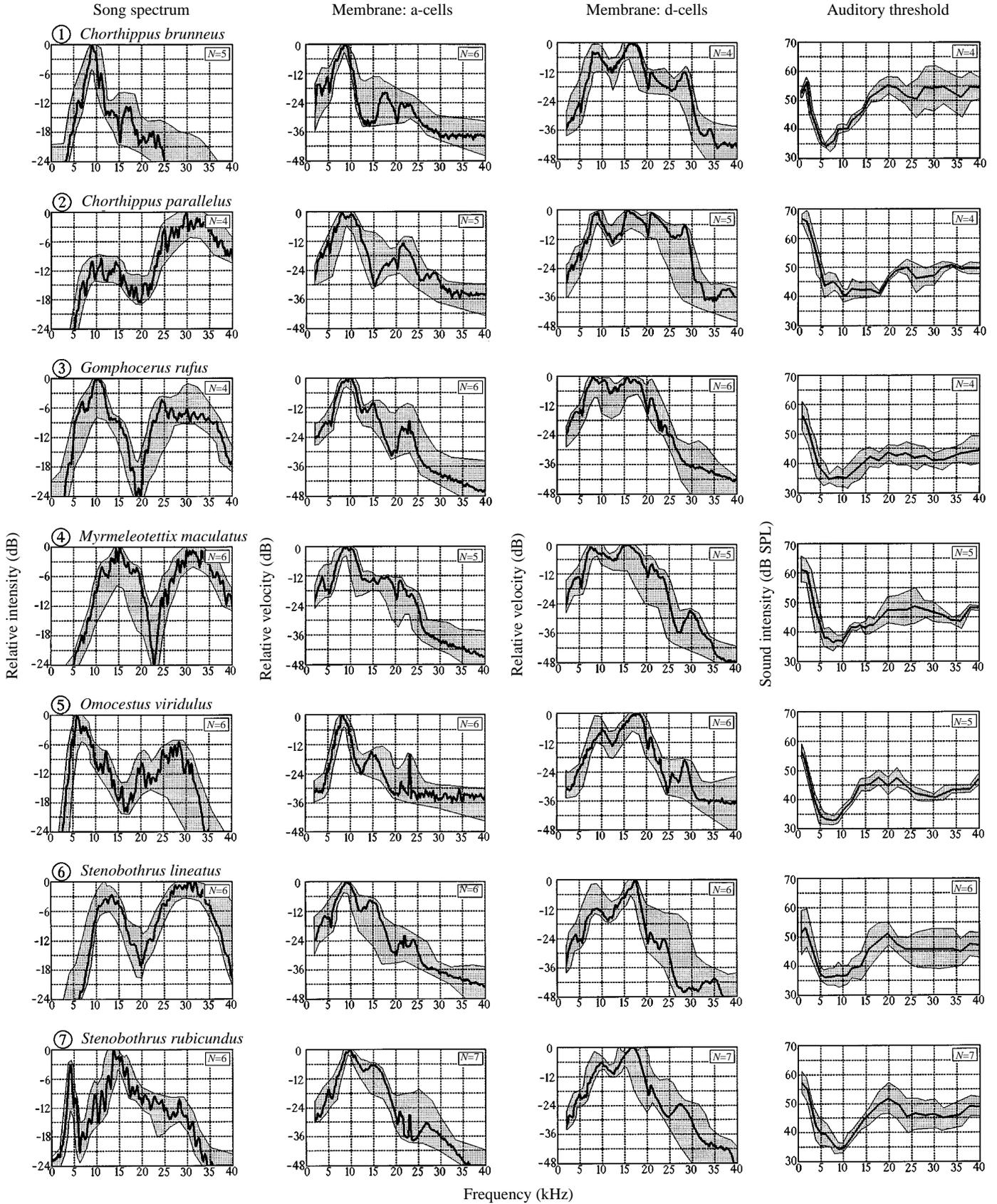
The broad-band high-frequency region present in the spectra of all 20 species except *Chorthippus brunneus* and

Table 1. The characteristic frequencies (kHz) of song spectra, of membrane vibrations at the attachment sites of the a- and d-cells and of the sensitivity maxima of the tympanal nerve in 20 species of Acrididae

Species	Song spectrum		a-cells	d-cells		Nerve 6	
	LF	HF		LF	HF	LF	HF
1 <i>Chorthippus brunneus</i>	9.72±0.57	–	8.81±0.39	8.92±0.42	16.65±0.83	7.3±0.81	25.33±0.94
2 <i>Ch. parallelus</i>	9.98±0.13	30.5±0.48	9.36±0.45	9.28±0.78	16.28±0.61	10.33±0.47	28.0±1.63
3 <i>Gomphocerus rufus</i>	8.57±1.15	30.08±1.2	8.94±0.45	8.57±0.56	16.25±1.11	8.31±0.81	30.66±0.94
4 <i>Myrmeleotettix maculatus</i>	15.1±0.11	31.31±0.44	8.74±0.49	8.26±0.53	16.76±1.26	9.05±1.25	30.8±2.5
5 <i>Omocestus viridulus</i>	6.98±0.45	28.96±0.31	8.09±0.31	8.75±0.94	15.9±1.19	7.13±0.89	29.2±0.98
6 <i>Stenobothrus lineatus</i>	12.29±0.78	30.41±1.21	8.64±0.46	8.61±0.41	16.06±1.28	8.25±1.37	30.5±1.65
7 <i>St. rubicundus</i>	4.17±0.09*	14.68±0.19	9.47±0.3	9.29±0.38	16.29±1.8	9.17±0.68	28.3±2.52
8 <i>Ch. apricarius</i>	15.55±0.44	30.4±0.45	8.59±0.14	8.57±0.13	14.72±0.27	–	–
9 <i>Ch. dorsatus</i>	7.58±0.32	28.35±1.23	7.91±0.74	7.77±0.19	15.18±0.94	–	–
10 <i>Ch. montanus</i>	7.22±0.52	29.13±2.66	7.6±0.47	8.29±0.61	16.35±0.57	–	–
11 <i>G. sibiricus</i>	7.71±0.55	30.56±1.67	8.55±0.52	8.61±0.24	15.46±0.82	–	–
12 <i>Mecostethus grossus</i>	8.51±0.32	25.96±0.96	7.53±0.27	7.84±0.39	15.06±0.66	–	–
13 <i>St. nigromaculatus</i>	8.96±0.62	34.42±0.65	9.19±0.32	8.06±0.41	14.08±0.73	–	–
14 <i>St. stigmaticus</i>	7.9±0.14	31.07±0.71	8.37±0.45	8.34±0.51	16.46±0.94	–	–
15 <i>Ch. albomarginatus</i>	8.66±1.04	31.02±0.94	8.58±0.86	9.47±0.61	15.84±1.49	–	–
16 <i>Ch. biguttulus</i>	6.57±0.49	29.8±1.43	7.77±0.78	8.05±0.45	15.2±0.75	7.1±0.71	28.0 ±1.9
17 <i>Ch. mollis</i>	10.24±0.94	30.7±2.25	10.08±0.38	9.45±0.54	16.68±0.69	9.89±1.09	31.0 ±2.2
18 <i>Chrysochraon dispar</i>	10.11±0.49	29.87±1.47	10.03±0.34	9.94±0.22	16.04±0.53	–	–
19 <i>O. haemorrhoidalis</i>	9.98±0.4	33.2±2.1	9.51±0.77	9.38±0.68	15.72±0.9	–	–
20 <i>Locusta migratoria</i>	7.84±0.57	–	7.44±0.45	7.57±0.53	16.91±1.36	–	–

LF, HF, low- and high-frequency range, respectively.

Values are means ± S.D., $N=4-6$. *Wing stridulation.



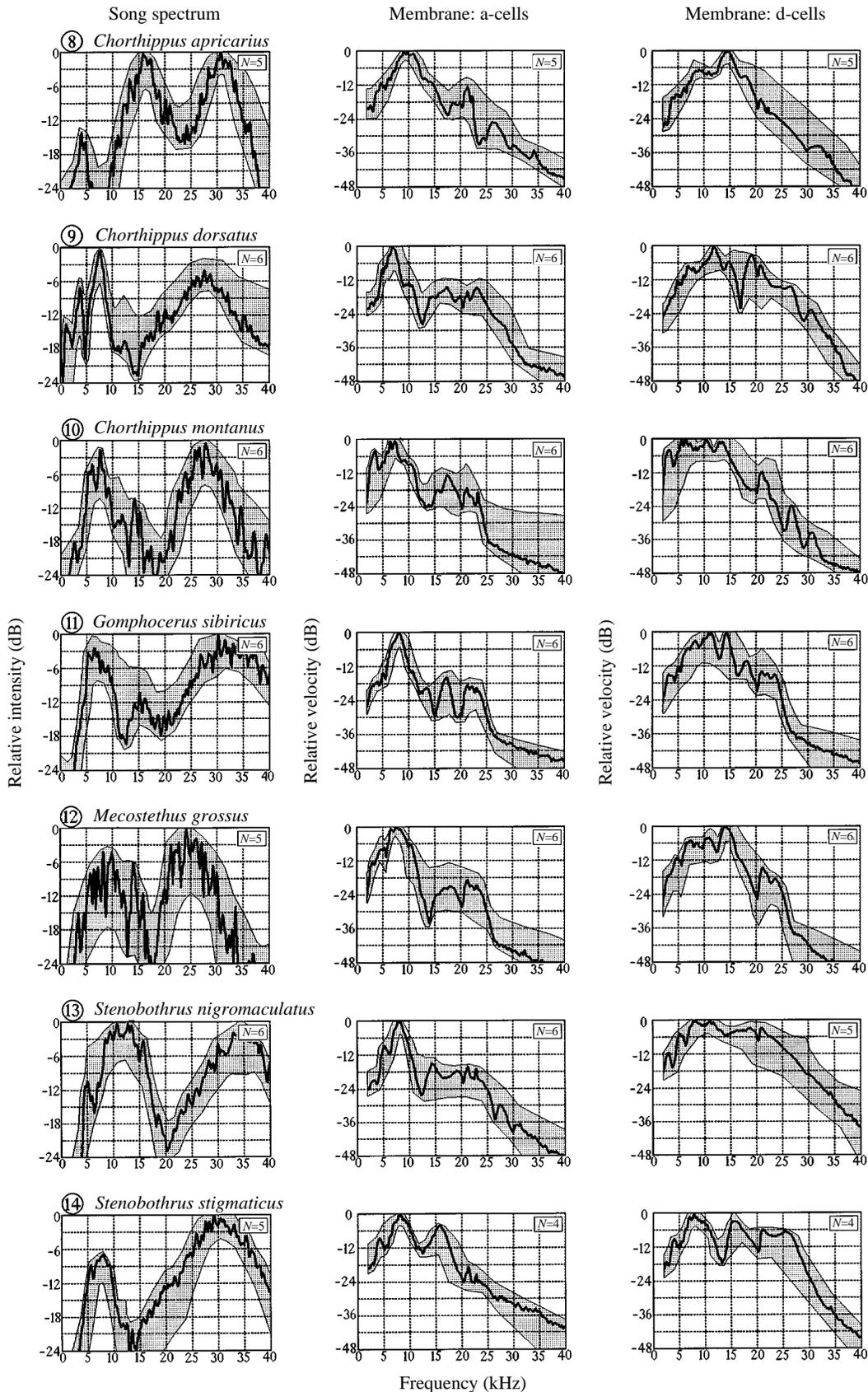


Fig. 1. Frequency spectra of the courtship songs (column 1) and the oscillations of the tympanal membrane at the attachment sites of the a-cells (column 2) and the d-cells (column 3) in 14 males of different species of the acridid subfamily Gomphocerinae. The curves are normalized by setting the maximum of each to 0 dB. Each curve was obtained from one individual; the range of variation among different individuals is indicated by the shaded band. For seven of these species, the mean (\pm s.d.) threshold curves of the tympanal nerve are also shown (column 4). The means of the characteristic frequencies of the spectra and of the threshold curves are listed in Table 1 together with the standard deviations.

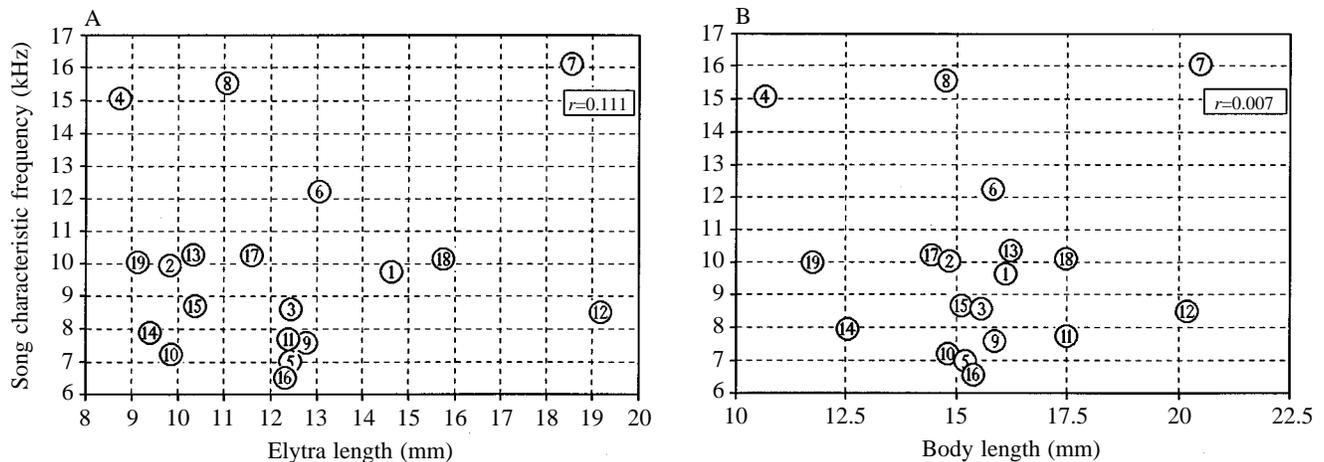


Fig. 2. Relationships between the length of the elytra (A) or that of the body (B) and the characteristic frequency in the low-frequency region of the songs of the males of 19 acridid species. The correlation coefficient r is also given. The numbers identify the species (see Table 2).

Stenobothrus rubicundus begins at approximately 15–20 kHz and extends to 40 kHz. In most species, the peak is at 30 kHz, but it can be slightly higher or lower depending on the species (Table 1).

There is considerably greater species-specific variation, in both the bandwidth and the position of the maximum, in the relatively narrow low-frequency region of the song spectrum at around 5–15 kHz. For instance, the low-frequency peak is at 7.2 kHz for *Chorthippus montanus*, at 9.7 kHz for *Chorthippus brunneus* and at 15.6 kHz for *Chorthippus apricarius*. Other differences can be seen by comparing, for example, *Stenobothrus nigromaculatus* with *Stenobothrus stigmaticus*, or *Chorthippus parallelus* and *Gomphocerus rufus* with *Myrmeleotettix maculatus*.

Some species, such as *Chorthippus albomarginatus* (von Helversen, 1986), *Myrmeleotettix maculatus* (Fries, 1994) and *Stenobothrus lineatus* (Elsner, 1974b), produce songs that can be subdivided into separate phases on the basis of marked differences in temporal structure. As a rule, the frequency maxima are slightly shifted in the different phases, but the most notable feature is that there can be major changes in the relative magnitude of the two frequency components. For example, in phase I of the song of *Stenobothrus lineatus*, the low-frequency peak is almost the only visible component. The high-frequency component appears only in phase II and becomes progressively more dominant as the intensity of the song in this phase increases.

The song of *Stenobothrus rubicundus* is unusual for the Gomphocerinae in that when the courtship song begins it stridulates in the ordinary way, striking the hindlegs against the forewings, but at the end it beats the hindwings sonorously against each other (Elsner, 1974a; Elsner and Wasser, 1995a,b,c). The spectrogram of the leg stridulation is very broad-band (5–40 kHz) and has only a single maximum, at 16 kHz. The wing stridulation, however, produces sounds with two main frequency regions, a broad-band one that more or less coincides with the leg-stridulation spectrum and a narrow-band region at 5 kHz (Fig. 1; Table 1).

Test for dependence of the spectra on the lengths of elytra and body

During stridulation, a row of teeth on the inner side of the hindleg femur is rubbed against a thickened vein, the vena radialis media, on the forewing. It is therefore conceivable that the species-specific differences, especially in the low-frequency part of the song spectra, might be related to differences in the length of the forewings or in body length. To test this, the characteristic frequency in the low-frequency region for each species was plotted against the length of the elytra (Fig. 2A) or of the body (Fig. 2B; Table 2; all values are means for six individuals). No dependence was discernible in either case: the correlation coefficient for elytra length was 0.111 and that for body length was 0.007.

It should also be mentioned in this regard that previous resonance measurements at various places on the forewings of *Chorthippus biguttulus* have shown that this grasshopper, unlike crickets (Nocke, 1971) for example, does not have a special wing field with resonance characteristics that could account for the song spectrum.

Vibration spectra of the tympanal membrane Spectra at the site of attachment of the low-frequency receptors

Although there are three groups of low-frequency receptors (a-, b- and c-cells), the membrane oscillation spectra at their different points of attachment differ only very slightly from one another (Meyer, 1994). The present measurements were therefore always made at the attachment site of the a-cells, which are the most numerous (Gray, 1960). At this point, the membrane oscillates with greatest amplitude at frequencies in the range between 5 and 10 kHz (Fig. 1, column 2; Table 1), with clear species-specific differences in the position of the maximum. These details will be considered further in a comparison of the membrane sensitivity with the song spectra (see Discussion).

Table 2. Body size, elytra length and tympanal membrane area for males of 20 species of Acrididae

Species	Body length (mm)	Elytra length (mm)	Tympanum area (mm ²)
1 <i>Chorthippus brunneus</i>	16.1±0.35	14.62±0.64	0.729±0.09
2 <i>Ch. parallelus</i>	14.83±0.23	9.8±0.32	0.488±0.04
3 <i>Gomphocerus rufus</i>	15.6±0.4	12.34±0.61	1.175±0.11
4 <i>Myrmeleotettix maculatus</i>	10.66±0.37	8.76±0.49	0.388±0.07
5 <i>Omocestus viridulus</i>	15.16±0.23	12.4±0.31	0.782±0.09
6 <i>Stenobothrus lineatus</i>	15.85±0.35	13.1±0.41	0.747±0.12
7 <i>St. rubicundus</i>	20.5±0.9	18.55±0.37	1.49±0.13
8 <i>Ch. apricarius</i>	14.75±0.44	11.05±0.15	0.937±0.12
9 <i>Ch. dorsatus</i>	15.9±0.49	12.78±0.47	0.89±0.12
10 <i>Ch. montanus</i>	14.77±0.65	9.38±0.33	0.543±0.05
11 <i>G. sibiricus</i>	17.5±0.7	12.33±0.29	0.792±0.12
12 <i>Mecostethus grossus</i>	20.2±0.41	19.13±0.28	1.366±0.22
13 <i>St. nigromaculatus</i>	16.25±0.24	10.33±0.38	0.827±0.07
14 <i>St. stigmaticus</i>	12.57±0.49	9.4±0.4	0.61±0.03
15 <i>Ch. albomarginatus</i>	15.1±0.54	10.38±0.44	0.488±0.05
16 <i>Ch. biguttulus</i>	15.35±0.63	12.29±0.75	0.57±0.02
17 <i>Ch. mollis</i>	14.44±0.64	11.58±0.41	0.529±0.05
18 <i>Chrysochraon dispar</i>	17.5±0.35	15.75±0.35	0.469±0.09
19 <i>O. haemorrhoidales</i>	11.75±0.25	9.12±0.31	0.457±0.06
20 <i>Locusta migratoria</i>	45.5±0.57	41.1±1.53	2.32±0.24

Values are means ± S.D., N=4–6.

In the higher-frequency region, the sensitivity of the membrane at this site falls rapidly, although in some species – and sometimes in certain individuals – secondary maxima may be discernible at around 18 and 22 kHz (Fig. 1, column 2).

Spectra at the site of attachment of the high-frequency receptors

In all the species tested, there was a remarkably broad-band vibration spectrum at the attachment site of the high-frequency receptors (Fig. 1, third column; Table 1). It includes all the frequencies in the spectrum found at the site of the low-frequency receptors (a-cells): in all experiments, the amplitude of membrane oscillation between 5 and 10 kHz was at least as great at the d-cell site as at the a-cell site. This becomes clear when non-normalized curves are compared (see Meyer and Elsner, 1995; Fig. 1). For example, in *Chorthippus biguttulus*, the velocity of membrane vibrations at 7.5 kHz reaches a maximum of 1.43 mm s⁻¹ at the site of both the low- and the high-frequency receptors. In addition, there is another peak at higher frequencies, between 14 and 17 kHz in most species (Table 1). At this frequency, the velocity of the membrane vibrations at the d-cell site is 2.26 mm s⁻¹, i. e. 0.83 mm s⁻¹ above that at 7.5 kHz. In the ultrasonic region, above 25 kHz, even at the d-cell site, the membrane is only slightly sensitive to airborne sound.

Like the song spectra, the vibration spectra of the tympanal membrane are entirely independent of temperature in the physiological range. Measurements from *Chorthippus biguttulus* between 20 and 41 °C have produced identical curves (Meyer, 1994).

Test for dependence of the vibration spectra on the size of the tympanum

As mentioned above, there are species-specific differences in the best frequency at the site of attachment of the low-frequency receptors. One of the factors that determine the resonant frequency of a membrane is its size. In the gomphocerines studied here, the area of the tympanal membrane varies between 0.39 mm² (*Myrmeleotettix maculatus*) and 1.49 mm² (*Stenobothrus rubicundus*) (Table 2). Because the resonant frequency of a membrane is inversely proportional to its radius, if all other parameters are constant the characteristic frequency in *M. maculatus* might be expected to be twice as high as in *St. rubicundus*.

Surprisingly, plots of vibration maxima (averaged over six animals in each case) at both the low- and the high-frequency receptor insertion sites *versus* tympanum area, shown for 20 species in Fig. 3, give not the slightest hint of such a relationship; the correlation coefficient is 0.192 for the a-cell site and only 0.004 for the d-cell site. Consider the species mentioned above as examples of extreme tympanal membrane sizes: the oscillation maximum for *St. rubicundus* is at 9.5 kHz, actually higher than the 8.7 kHz maximum of the very small membrane of *M. maculatus*. Conversely, the characteristic frequencies in *Chorthippus biguttulus* and *Ch. mollis* differ from one another by as much as 2.5–3 kHz, although the membranes are both approximately 0.6 mm² in area.

The most astonishing result is that even the oscillation spectra in *Locusta migratoria*, with a tympanum 2.3 mm² in area and thus 3–5 times the size of those of most

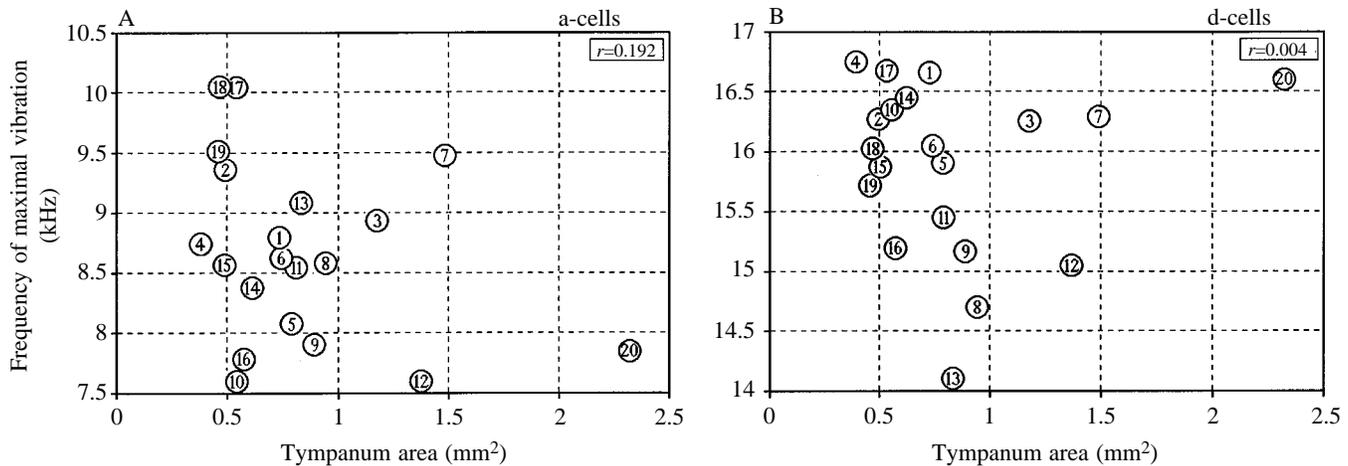


Fig. 3. Relationships between the area of the tympanal membrane and the frequency for maximal membrane oscillation at the site of attachment of the a-cells (A) or the d-cells (B) for the males of 20 acridid species. The correlation coefficient r is also given. The numbers identify the species (see Table 2).

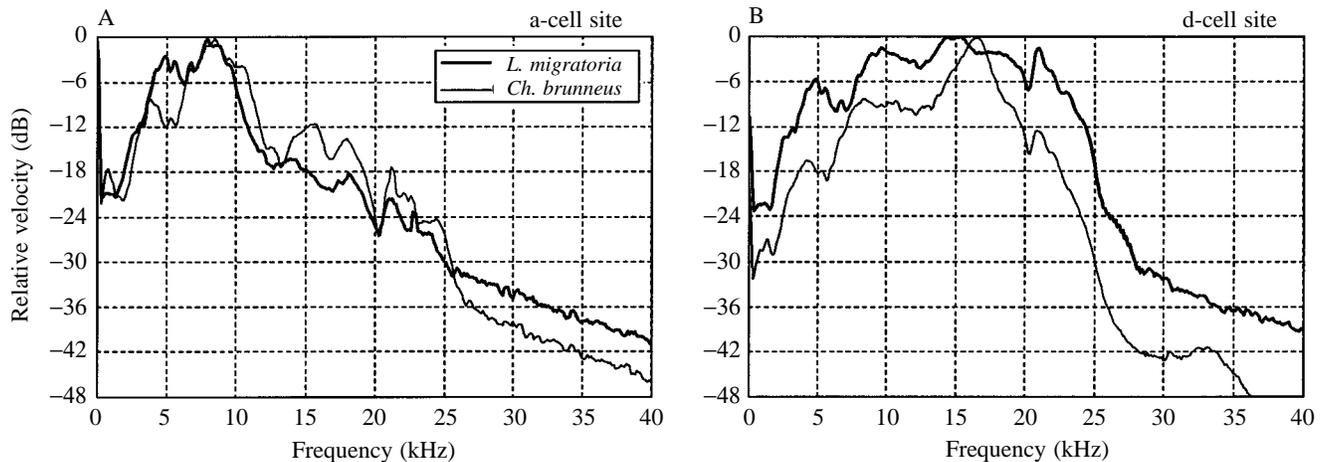


Fig. 4. Frequency spectra of tympanal membrane vibration at the site of attachment of the a-cells (A) or the d-cells (B) in *Locusta migratoria* and *Chorthippus brunneus*.

Gomphocerinae, are not outside the range of the other species. At both of the two receptor attachment sites, the CFs are practically identical with those of, for example, *Chorthippus brunneus*, which has a tympanal membrane measuring only 0.73 mm² (Table 1; Fig. 4).

Frequency sensitivity of the tympanal nerve

Shape of the auditory threshold curves

In nine of the gomphocerine species tested, the summed activity of the tympanal nerve was recorded extracellularly with hook electrodes in order to obtain threshold curves in the frequency range between 1 and 40 kHz (Fig. 1, seven shown in fourth column). The curves are fairly similar in all nine species; there are usually two regions of relatively high sensitivity, one at low frequencies and the other in the ultrasonic region (Table 1). Sensitivity is greatest between 5 and 10 kHz, where the threshold falls to between 30 and 40 dB SPL. There are some differences among the species here: for

instance, the best frequency of *Chorthippus brunneus* is 7.3 kHz, while that of *Stenobothrus rubicundus* is 9.2 kHz. Values for *Chorthippus biguttulus* (7.1 kHz) and *Ch. mollis* (9.9 kHz) differ by almost 3 kHz (Table 1).

In the ultrasonic region, between 25 and 35 kHz, the threshold is lowered to about 45 dB SPL (Fig. 1, column 4); the bandwidth of this second sensitivity maximum is considerably broader than that of the first. In most, but not all, species it is separated from the lower-frequency maximum by a region of elevated threshold (approximately 55 dB SPL) near 20 kHz.

Discussion

General remarks about the song and oscillation spectra and the spectral sensitivity of the tympanal nerve

This comparative study has revealed common features in the spectra of the song and the tympanal membrane vibration and

in the spectral sensitivity of the tympanal nerve in the 20 species examined, as well as certain species-specific differences.

Song spectra

In almost all species, the spectrum of the male's song has two peaks. There is a broad-band component in the ultrasonic region, at around 30 kHz, and usually a narrow-band region between 5 and 15 kHz. The position of the latter maximum has the greatest interspecific variability. On the basis of the present results, all that can be said about these species-specific features or about the general form of the song spectra is that neither body size nor wing length is a contributing factor.

Membrane spectra

It is also unclear why the general shapes of the vibration spectra at the sites of attachment of the high- and low-frequency receptors, and the positions of the peaks, again have many features common to all species but also species-specific peculiarities. The most surprising finding is that the position of the best frequency has nothing to do with the area of the tympanum. An equation published by Michelsen (1971) gives the resonant frequency in the ideal case of a homogeneously structured, circular membrane in a vacuum as follows:

$$f = 0.383 \frac{1}{a} \sqrt{\frac{T}{\sigma}},$$

where f is resonant frequency (Hz), a is radius (m), T is tension per unit length (N m^{-1}) and σ is mass per unit area ($\text{kg m}^{-2} = \text{N s}^2 \text{m}^{-3}$).

This equation (for details, see Morse, 1948) is undoubtedly only a rough approximation to the biological situation, in which the membrane is neither in a vacuum nor circular and homogeneous in structure. Nevertheless, it clearly states that, as the radius is enlarged, the resonant frequency should become markedly smaller, which is not at all in accordance with the present results. Similarly, as the membrane becomes thicker, which it is likely to do as the area increases, the frequency should shift to lower values – which is also not the case in the present study. The only parameter that remains as a determinant of resonant frequency is the membrane tension, which would have to increase in larger and thicker tympana in order to keep the frequency the same as that measured in smaller animals.

Tympanal nerve

In all the species investigated, the measured auditory threshold curves exhibit a particularly high sensitivity maximum in the low-frequency region, between 5 and 10 kHz, and a considerably less sharp increase in sensitivity in the ultrasonic region, between 25 and 35 kHz. In evaluating multi-unit recordings, it should be kept in mind that the amplitude of the summed action potentials depends on both the number and the synchronicity of the excited receptor cells. Given that there are 65 low-frequency receptors and only 12 high-frequency

receptors (data from Gray, 1960, for *Locusta migratoria*), the summed potential in response to stimulation at low frequencies would be expected to be larger than that to high-frequency stimulation. Moreover, it should be easier to detect the threshold when many low-frequency receptors are excited rather than when a few high-frequency ones are excited. Despite these qualifications, the relatively simple method of recording extracellularly with hook electrodes is useful, especially in the low-frequency range, for monitoring the overall activity of the auditory sense cells so that it can be compared with the spectra of the songs and the vibration spectra of the tympanal membrane.

Comparison of the song spectra, the spectra of membrane vibration and the spectral sensitivity of the tympanal nerve

The central question in the present investigation was to what extent the frequency spectra of the grasshoppers' songs, the vibration spectra of the tympanal membrane and the spectral sensitivity of the tympanal receptors are matched to one another and, hence, might contribute to auditory communication in these insects.

Song and membrane spectra

The song spectra of the various species exhibit remarkable resemblances but also certain species-specific differences, especially in the low-frequency region. The same applies to the vibration spectra at the site of attachment of the low-frequency receptors (Fig. 1). In Fig. 5, the characteristic frequencies of the song and membrane are plotted against one another, for the low- and high-frequency membrane regions.

In the low-frequency region, they are evidently well correlated (correlation coefficient 0.75; $P < 0.001$); the calculated regression line departs only slightly from the line for which peaks are equal. Disregarding *Stenobothrus lineatus*, the best frequencies of the song differ by at most 1 kHz from those of membrane vibration at the attachment site of the a-cells.

The species *Stenobothrus rubicundus*, *Myrmeleottetix maculatus* and *Chorthippus apricarius* are special cases, for in their songs the low-frequency CF is between 15 and 16 kHz, quite different from the frequency of maximal vibration at the attachment site of the low-frequency receptors (8.5–9.5 kHz). In these species, however, the oscillation maxima at the high-frequency receptor attachment site occur at 14–17 kHz, and thus are well-matched to the best frequencies of the songs.

In general then, even at the level of the tympanal membrane, there is a distinct species-specific adaptation to the best frequencies of the species' own song in the low-frequency region, up to 16 kHz.

The high-frequency components of the songs, however, are not adapted to the oscillation maxima at the attachment site of the high-frequency receptors (d-cells): the former peak at around 30 kHz (Table 1), but in this region the membrane gives only a weak response to airborne sound. The velocity and amplitude of the membrane vibration fall sharply beginning at frequencies as low as 20 kHz (Fig. 1, fourth column). This

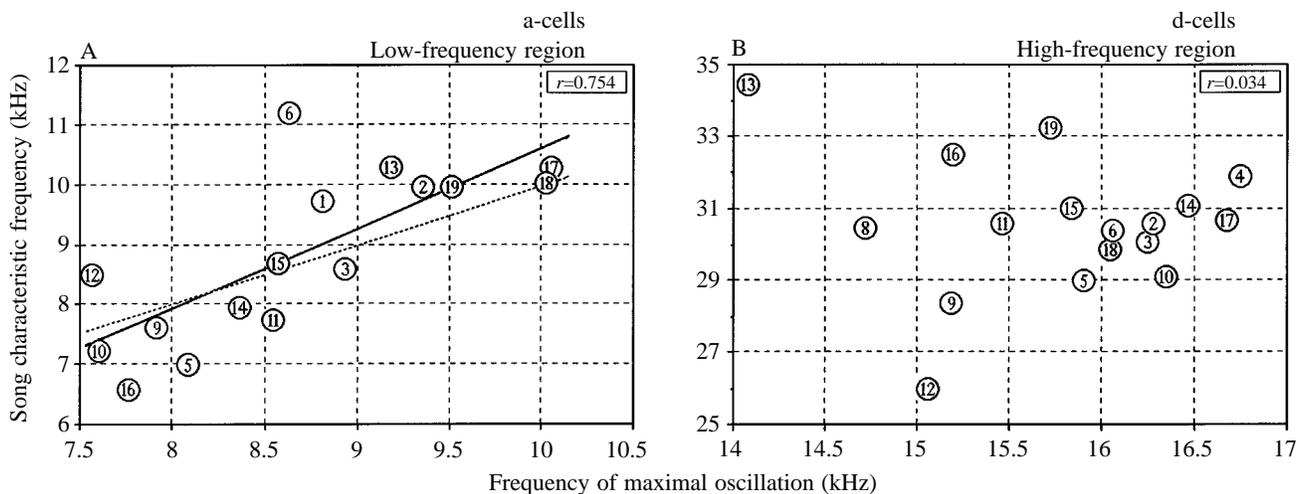


Fig. 5. Relationships between the frequency of maximal tympanal membrane oscillation at the site of the a-cells (A) or the d-cells (B) and the characteristic frequency of the male song of 17 acridid species. Continuous line, regression line ($P<0.001$); dotted line, line for which peaks are equal. The correlation coefficient r is also given. The numbers identify the species (see Table 1).

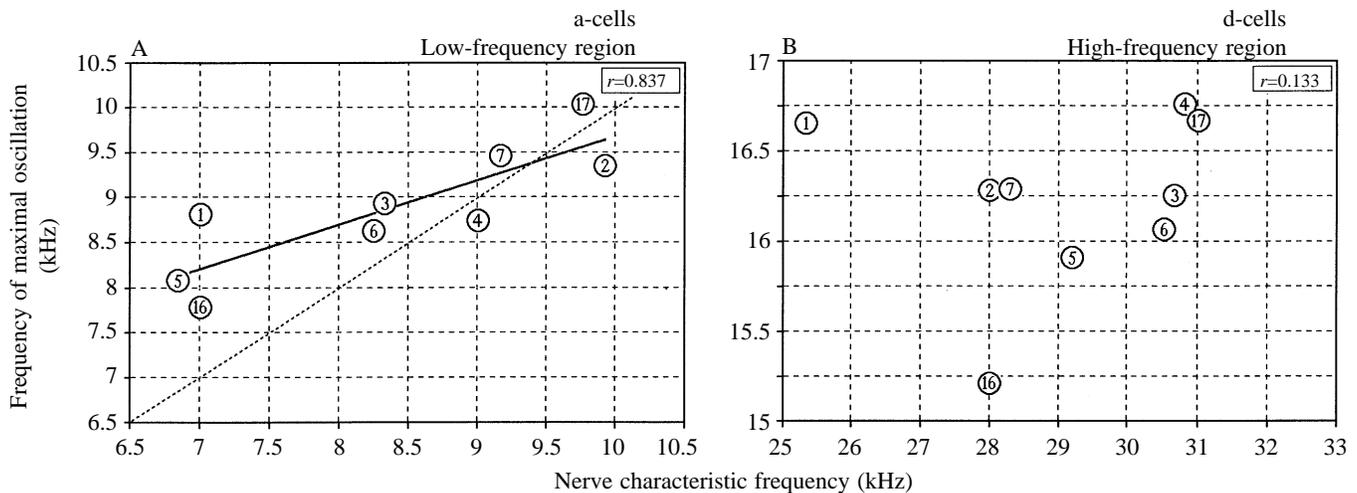


Fig. 6. Relationships between the characteristic frequencies of maximal sensitivity of the tympanal nerve in the low-frequency or high-frequency region and the frequency of maximal tympanal membrane oscillation at the site of attachment of the a-cells (A) or the d-cells (B) in the males of nine acridid species. Continuous line, regression line ($P<0.01$); dotted line, line for which peaks are equal. The correlation coefficient r is also given. The numbers identify the species (see Table 1).

mismatch is evident in the plot of Fig. 5B, which shows no correlation ($r=0.034$).

Oscillation spectra of the membrane and auditory threshold curves

The membrane oscillations are not a direct indicator of hearing and its adaptation to the song spectra. It is therefore necessary to check whether the best frequencies of the tympanal nerves are correlated with those of membrane vibration. In Fig. 6, these values are plotted for the nine species for which summed recordings from the tympanal nerve are available. Clearly, in the low-frequency region, species with higher-frequency membrane oscillation also have higher-frequency sensitivity maxima in the tympanal nerve. The correlation of these two quantities is high ($r=0.837$, $P<0.01$).

In contrast, the slight lowering of auditory threshold in the region between 25 and 35 kHz cannot be ascribed directly to tympanal-membrane oscillation, which has its best frequency near 16 kHz and a sharp cut-off above 20 kHz (Fig. 1, third column). This major discrepancy between the best frequencies may be related to the special morphology at the attachment site of the high-frequency receptors (d-cells). Their dendrites form a structure separate from the main part of Müller's organ, called the fusiform body; this is an independent unit that extends to the pyriform vesicle, a cuticular structure on the thin part of the tympanal membrane (Gray, 1960). Because of this anatomical arrangement, the fusiform body has its own resonant frequency, different from that of the rest of Müller's organ. In the migratory locust *Locusta migratoria*, the resonant frequency of the fusiform body has been found to be 13 kHz,

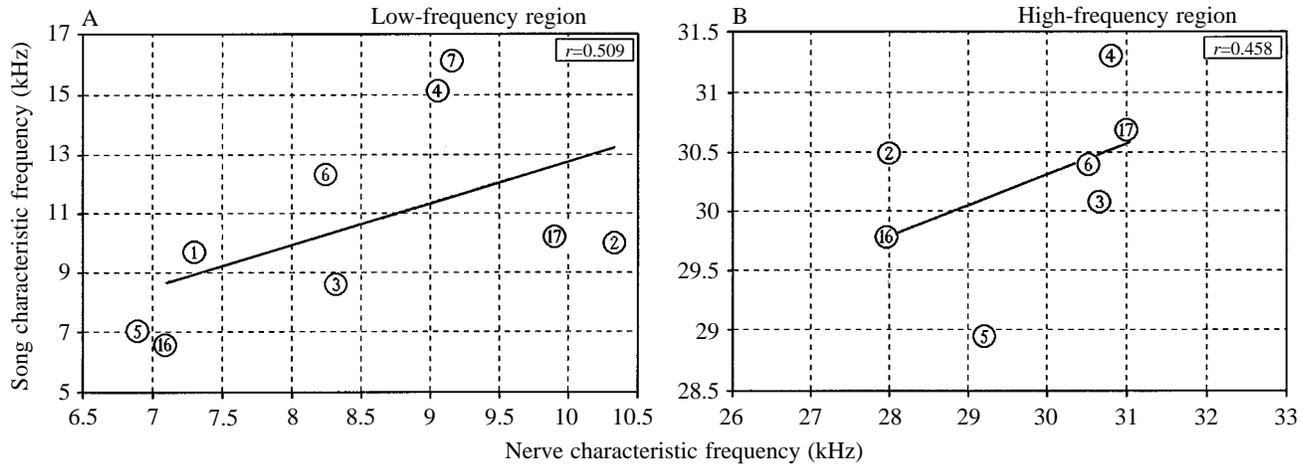


Fig. 7. (A,B) Relationships between the characteristic frequencies of maximal sensitivity of the tympanal nerve in the low-frequency or high-frequency region and the characteristic frequency of the male song in nine acridid species. Continuous line, regression line ($P < 0.05$). The correlation coefficient r is also given. The numbers identify the species (see Table 1).

although these measurements included only the region up to 20 kHz (Breckow and Sippel, 1985). Hence, it remains an open question whether the fusiform body also resonates independently in the ultrasonic region; if so, it might account for the sensitivity of the high-frequency receptors between 25 and 35 kHz.

Song spectra and auditory threshold curve

As a final comparison, the best frequencies of the song spectra and auditory threshold are plotted against one another in Fig. 7. In the low-frequency region (Fig. 7A), seven of the nine species for which summed recordings from the tympanal nerve are available show a fairly good, though not optimal, correlation ($r=0.509$, $P < 0.05$). This is not surprising, since the best frequencies of the membrane oscillation at the attachment site of the low-frequency receptors are correlated both with the frequency maximum of the song (Fig. 5) and with the sensitivity maximum of the tympanal nerve (Fig. 6). The lack of agreement between the characteristic frequencies of the song and of nerve activity in the low-frequency region for *Myrmeleottetix maculatus* and *Stenobothrus rubicundus* can be explained by the fact that, although in these species there is an excellent correlation between nerve activity and membrane oscillations (Fig. 6), the latter are not correlated with the low-frequency components of the song (Table 1).

For the high-frequency component near 30 kHz that characterizes the songs of all the gomphocerine species studied here except *Chorthippus brunneus* and *Stenobothrus rubicundus*, there is no corresponding membrane-oscillation maximum (cf. columns 1 and 3 in Fig. 1). However, when the ultrasonic spectrum of the song is compared with the threshold curve of the tympanal nerve in this region, there is a good match (Fig. 7B). In all seven species in this group, the two maxima are quite close to one another, differing by less than 0.5 kHz in four cases. That the correlation coefficient for the group is no greater than $r=0.458$ may be due to the fact that

the auditory threshold curves were sampled only in 1 kHz steps.

Concluding remarks

These experiments have shown that in the low-frequency region (5–10 kHz) adaptations of hearing to the conspecific song frequencies can be discerned even at the level of the tympanal membrane. The membrane thus functions as a peripheral frequency filter, with bandpass characteristics matched to those of the conspecific signal. In addition, because acoustic stimuli below 4 kHz induce little membrane oscillation, abiotic background noises (the wind, for instance), but especially the songs of other animals such as cicadas or birds, can be largely filtered out by the tympanal membrane.

In the low-frequency region, the membrane vibrations directly excite the low-frequency receptors; the best frequencies of membrane and receptors are very similar. In the ultrasonic region, however, this is not the case. Here, there is a considerable frequency offset. By a mechanism that is not yet understood, the high-frequency receptors respond best at frequencies twice as high as the frequencies for maximum-amplitude membrane oscillation. As a result, the receptors are well adapted to respond to the ultrasonic components of the conspecific signal.

The net results are that hearing in the gomphocerines is well adapted to conspecific sounds and that frequency is likely to make more of a contribution to song recognition than has often been thought. This finding may provide an incentive for further study of the interspecific aspects of the adaptation, e.g. by closer examination of sympatric species, as well as its intraspecific significance, by a comparative analysis of hearing in females and the songs of males (or the reverse).

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