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Recognition of sex in the acoustic communication of the grasshopper *Chorthippus biguttulus* (Orthoptera, Acrididae)

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Abstract Many gomphocerine grasshoppers communicate acoustically: a male's calling song is answered by a female which is approached phonotactically by the male. Signals and recognition mechanisms were investigated in *Chorthippus biguttulus* with regard to the cues which allow sex discrimination. (1) The stridulatory files on the hindfemur of both sexes are homologous in that they are derived from the same row of bristles, but convergent with respect to the "pegs". In males the pegs are derived from the bristles, and in females from the wall of the bristle's cup. (2) Male and female songs are generated by similar, probably homologous motor programs, but differ in the duration, intensity, "gappyness" of syllables, risetime of pulses, and the frequency spectra. The hindleg co-ordination during stridulation and the resulting temporal song patterns are less variable in males than in females. (3) For both sexes, recognition of a mate's signal depends on species-specific syllable structure. For males it is essential that the female syllables consist of distinct short pulses, whereas females reject "gappy" syllables. Males strongly prefer "ramped" pulses, females respond to syllables irrespective of steeply or slowly rising ramps. Males react only to the low-frequency component, whereas females prefer spectra containing both, low *and* high frequency components.

Key words Acoustic communication · Pattern recognition · Sex discrimination · Sexual selection *Chorthippus biguttulus* (Acrididae)

Introduction

Insects using acoustic communication for mate finding should be well equipped in two respects: first, individuals have to discriminate conspecific signals from the numerous songs of other species in the same biotope. Second, they have to localize and approach the conspecific partner. The simplest way to achieve these tasks is by way of unidirectional communication systems, common in crickets and many tettigoniids, where the male sings and the female recognizes, localizes and approaches the calling partner (reviews: Ewing 1989; Bailey 1991). Bidirectional communication systems are more complex; both partners stridulate and respond to each other as do some bushcricket families (review: Bailey 1991) and the gomphocerines among the acridids (Jacobs 1953; von Helversen and von Helversen 1983). In such systems, the operational sex ratio largely determines whether the male or the female, or possibly both sexes, take the risk of phonotactic approach (Heller and von Helversen 1991; Heller 1992).

In bidirectional communication systems, one would expect that evolution has enabled both sexes not only to identify conspecific signals but also to discriminate between signals of the same and the opposite sex. This is obvious for communication systems in which male and female signals are completely different, as is the case in most bushcrickets (Spooner 1968; Nickle 1976; Heller and von Helversen 1986; Dobler et al. 1994). In grasshoppers, however, males and females have a basically similar song pattern (von Helversen and von Helversen 1987; Stumpner and von Helversen 1994), raising the question of which cues of the signals guarantee species recognition and which allow discrimination of the partners' sex.

In this study we focus on those differences between male and female song that serve to discriminate between the sexes. First, we describe differences in the stridulatory apparatus and stridulation pattern of males and females. Second, we report the results of playback ex-

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Dedicated to Prof. Dr. Wolfgang Wickler on the occasion of his 65th birthday

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periments that were designed to determine the acoustic properties of male and female songs used by members of both sexes to identify appropriate mating partners. Our main goal was to learn how the different subsystems operate together in order that both recognizers can detect the species specific song and discriminate between the sexes.

The grasshopper *Chorthippus biguttulus* is a suitable experimental animal for addressing such questions, since there are two distinct and sex-specific behaviours, which can be easily released by artificial acoustical stimuli. Female stridulation occurs exclusively in response to conspecific male song (von Helversen 1972), and the male turning reaction and subsequent approach of the female occurs only in response to female song (von Helversen and von Helversen 1983).

Materials and methods

Experimental animals

All animals were collected in the field at various places in southern Germany. Females were taken as subadults and kept separated from males to keep them virgin. They were fed mainly on the grass *Dactylis glomerata*.

Morphology of the stridulation file

Air-dried hindlegs of males and females were examined by scanning electronmicroscopy (Novascan).

Sound recording and analysis

Songs of intact freely moving males and females were recorded at 30 ± 1 °C. For spectral analysis, songs were recorded using a 1/4" B&K microphone and amplifier and stored on a Racal tape recorder (at a speed of 30" per s). Spectra were analysed by a Medav spectrum analyzer. For analysis of the temporal pattern, recordings were made with a 1/2"-microphone and tape speed 7 1/2" per s (upper frequency limit: 40 kHz). Simultaneously movements of both hindlegs were recorded with an optoelectronic device (von Helversen and Elsner 1977). Leg movement recordings were digitized and analyzed using signal analysis software (Turbolab by Stemmer Software). To measure the coordination of hindlegs, the upper reversal points of each leg were marked and the time between the onsets of corresponding downstrokes of the two legs determined in milliseconds.

For measuring intervals and sound units, songs were digitized (sampling rate 100 kHz) and the maximal value of every ten datapoints was determined. When this function was differentiated, the result was the train of impacts making up the song at a 10 kHz sampling rate. This train of sound impacts was analyzed with custom-designed software (J. Schul) that detected ‘sound units’ and ‘pauses’ using the following algorithm: when the impact rate increased to 1.5 kHz or higher for at least 2 ms, this was defined as the beginning of a sound pulse; when the impact rate dropped below 1.5 kHz for longer than 2 ms, the end of the pulse was registered. This “tolerable” gap of 2 ms was adopted on the basis of the results of earlier behavioural experiments with females (von Helversen 1972).

In a second run, the distribution of syllables was evaluated. For that purpose, all sound units framed by syllable pauses were detected, i.e. by pauses > 8 ms as measured in corresponding one-leg stridulation patterns.

Tests of response behaviour

Experiments with females (except those on spectral components) were performed in a computer-controlled setup that presented song stimuli and registered responses automatically (von Helversen and von Helversen 1983). The females were placed in a sound attenuated chamber at 30 ± 1 °C (35 °C in Fig. 11) and their response probability to the different types of computer-generated song models was calculated in percent of the number of presented stimuli of this type. Tests on spectral components were run by hand. An artificial model song normally consisted of one or three phrases (phrase duration 3 s, inter-phrase interval 10 s for the latter stimuli). These models were presented in 30- to 60-s intervals, which proved to be long enough to prevent an influence of the preceding stimulus. The order of the presentation of the different model types was pseudo-random.

The behavioural experiments with males had to be run by hand because male turns could not be recorded automatically and sound had to be presented from a defined direction. All experiments were conducted in a reflection attenuating room, where the males moved freely on a cloth-covered table. The temperature was 30 ± 2 °C. The song models to be tested were stored on a tape recorder (Racal 4DS, 20 models of each test pattern) in an order that mixed effective and ineffective patterns. Attractiveness of a song model was measured as the percent turning probability to that model. [For more details of the experimental procedure see von Helversen and Rheinlaender (1988)].

In total, more than 300 animals were tested. Complete reaction curves were obtained from 111 animals (53 males and 58 females), and are presented in this paper.

Nearly all males (>95%) showed immediate phonotactic reactions to artificial sound stimuli presented with a latency of 1–3 s after the end of a spontaneous song of the male, but not all males were motivated enough to respond reliably for 1–3 h (which was necessary for the estimation of turning probabilities for a whole set of stimuli). The timing of stimulus presentation in males, of course, was determined by the male’s song frequency, as all test stimuli were presented as a response to the male’s own song. When responding to an effective female pattern the duetting could be continued over 10–25 trials before the male stopped singing. The male normally resumed singing within 1 min.

Females were more variable with respect to their motivation to respond to artificial male songs. Their responsiveness, for instance, varied with the cycle of egg laying (von Helversen 1972). Thus, to select a responsive female, a singing male was brought into the cage (size ca. 50 × 50 × 50 cm) in which the virgin females were housed, and those females that responded reliably to the male’s song were chosen for the computer-aided test procedure.

Song models

All song models were generated by multiplying a computed envelope function with continuous noise (2–40 kHz) and – depending on the experiment – subsequent filtering by using a Bruel & Kjaer Terz/Oktave-filter and/or a Kemo band pass of 48 dB per octave (for precise stimulus conditions see figure legends). Intensities (in dB SPL) refer to a continuous sound of the same spectral distribution as the signal and of that amplitude which was maximal in the signal.

Results

Song of male and female

Morphology of the stridulatory apparatus

Males and females stridulate in the same way by rubbing a file at the inner side of the hindfemora against a protruding vein of the forewings. The early detailed de-

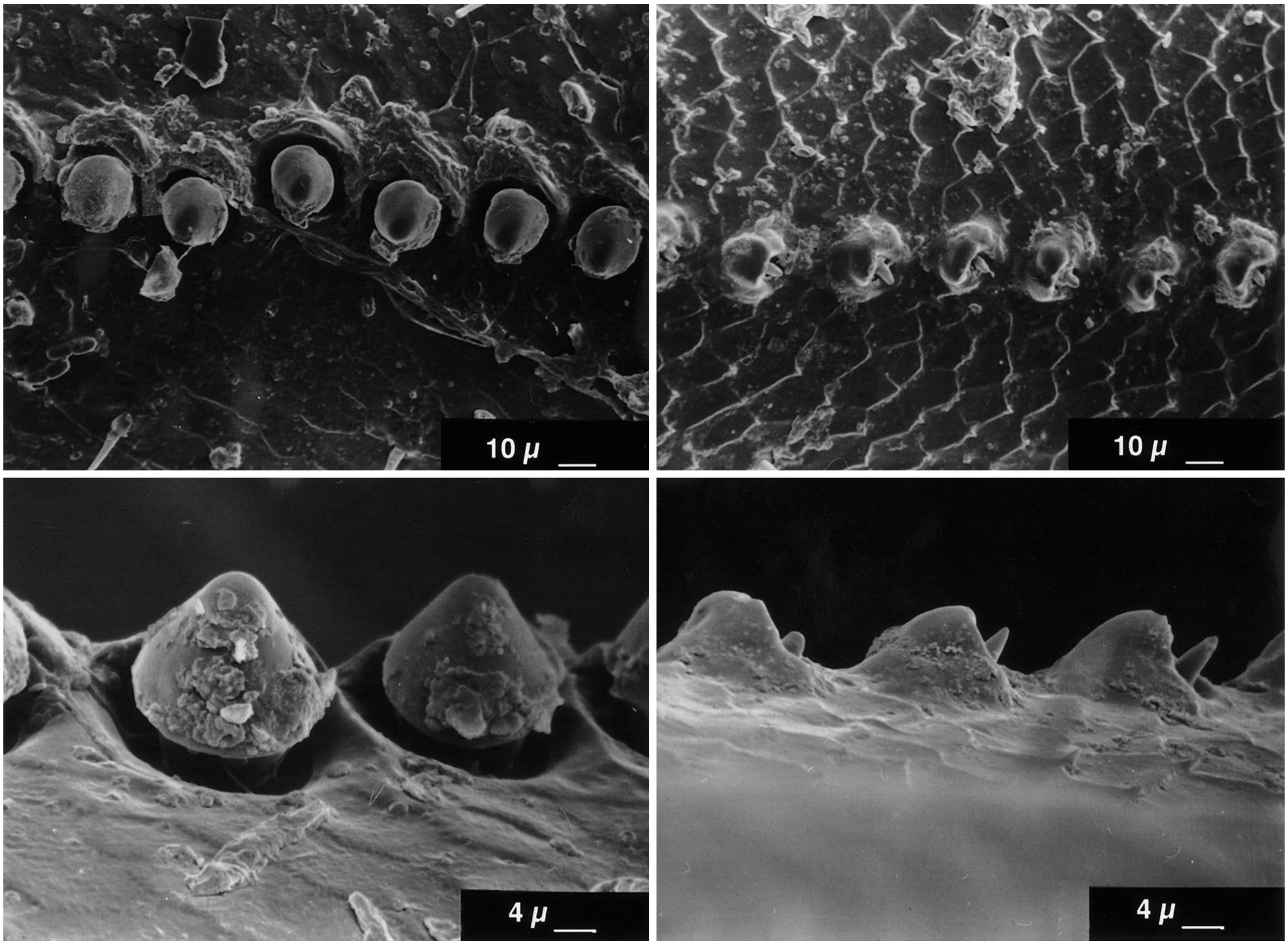

Fig. 1 Parts of male and female stridulatory files on the inner side of the hindfemora in *Ch. biguttulus*. *Upper row*: top view of male (*left*) and female file (*right*); *below*: lateral view at a greater scale, distal ends of the files point to the right (with courtesy of C. Gack)

scription of Petrunkevitch and Gaita (1901) could be confirmed by scanning electron microscopy (Fig. 1): the male's file consists of a dense row of cone shaped pegs [about 100; Jacobs (1953)], which are obviously derived from bristles. Interestingly, the female's file originates from the same, homologous row of bristles. However, a single "tooth" of her file is not a transformed bristle, but is derived from the protruding proximal edge of the sclerotized wall surrounding the cup in which the bristle inserts.

Movement pattern and sound

The male calling song of middle European *Chorthippus biguttulus* consists of several (mostly three) phrases, each with a duration of normally 2–3 s. A female that is ready to mate responds with a somewhat shorter and less intense stridulation (Jacobs 1953; Fig. 2a). The song pattern of the male, described in detail by Elsner (1974), consists of "syllables", separated by syllable pauses when the leg stops for about 10–15 ms. The syllables are typically generated by three down-up-movements, each giving rise to one short sound pulse (Fig. 2e). The pulsed pattern of the syllables is clearly visible when one of the stridulating legs is made soundless by cutting one elytron (Fig. 2d, e). In an intact animal, the gaps at the reversal points of movement are camouflaged by the stridulation of the other hindleg, as the two legs are moved slightly out of phase (upper two lines in Fig. 2d, e), thereby giving rise to continuous syllable blocks (Figs. 2b, c, 4d, f). The song pattern of males shows astonishingly little variation, when the pattern of one leg is considered (von Helversen 1972; von Helversen and von Helversen 1975).

However, the sound pattern to be recognized by male and female is the superposed pattern generated by both stridulating legs. The phase difference between the two patterns (Fig. 3) and the noisy upstroke of the delayed leg during the syllable pause largely influence the distribution of sound units and intervals produced (Fig. 4). On the one hand, if the coordination is not precisely phase-shifted throughout the syllable, then the amplitude might drop for a moment, dividing one long syllable into two or more shorter units. On the other hand, if the impact rate

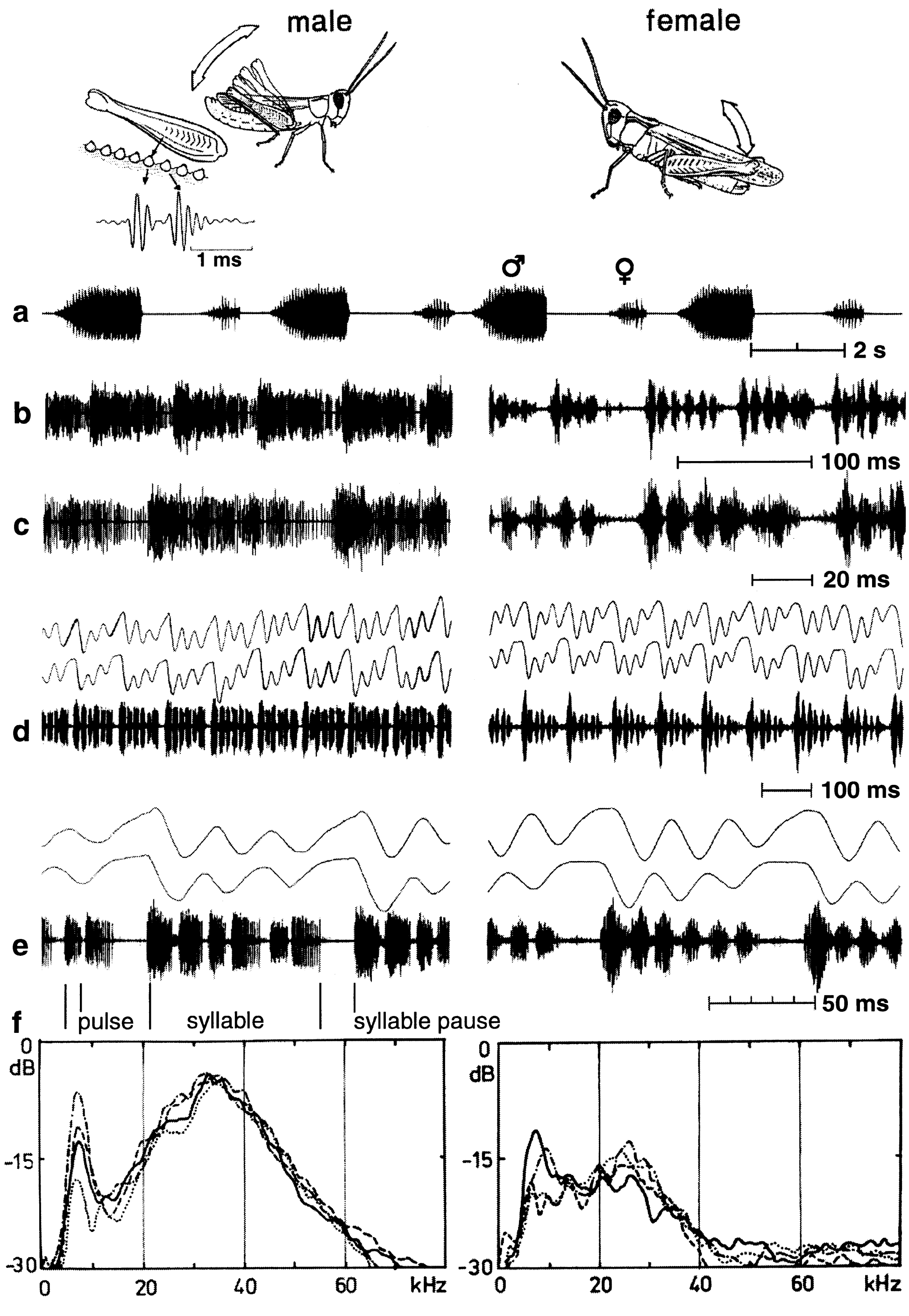

Fig. 2a–f Song pattern of male and female *Chorthippus biguttulus*: **a** response song of male and female; **b, c** parts of song phrases of intact animals stridulating with both hindlegs. Note the compact syllables of males (*left*) and the gappy and irregular syllables of females (*right*); **d** movements of hindlegs during stridulation and sound pattern of one leg, while – for sake of clarity – the other stridulating leg was made soundless by cutting the corresponding forewing; **e** same as in **d** at a larger scale to demonstrate the rectangularly modulated pulses of males and the ramp-shaped pulses of females; **f** frequency spectra of the songs of four males and four females

produced by the delayed leg is too high when the leading leg stops for the syllable pause, syllables will be enlarged at the cost of syllable pauses, or two or more syllables may fuse to form a very long sound unit. In Fig. 4 we show the distributions of intervals and sound units for song patterns produced with both hindlegs. The analysis was based on impact rates. Rates below 1.5 kHz were classified as intervals (see Materials and methods). In males, the distribution of intervals is clearly bi-modal (Fig. 4b), with one peak reflecting the syllable pauses (peak around 15 ms) and one peak reflecting the gaps between the pulses, which were not completely (or not at all) camouflaged by the superposition. In single leg stridulation, the frequency of these gaps would have been about five times the number of syllable pauses.

Thus, the analysis revealed that, compared to the stridulation pattern of a single leg, the percentage of short gaps was drastically reduced by the phase-shifted superposition of the two stridulation patterns. As a consequence, the distribution of sound units is rather broad, with four- and six-pulsed syllables around 55 and 80 ms, respectively, and the rest of shorter sound units,

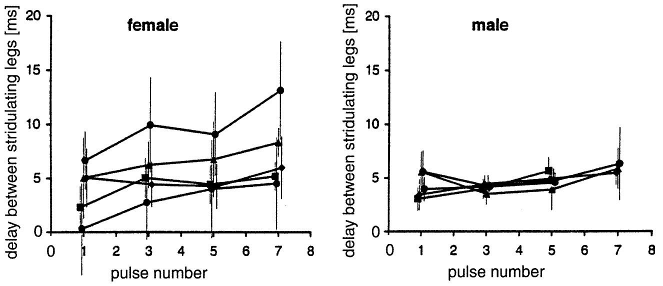

Fig. 3 Coordination of stridulating hindlegs in male (*right*) and female song pattern (*left*). Mean time delays and standard deviations between downstrokes for the uneven pulses in a syllable are given for five males and five females, respectively. Songs analyzed were recorded at 30 °C

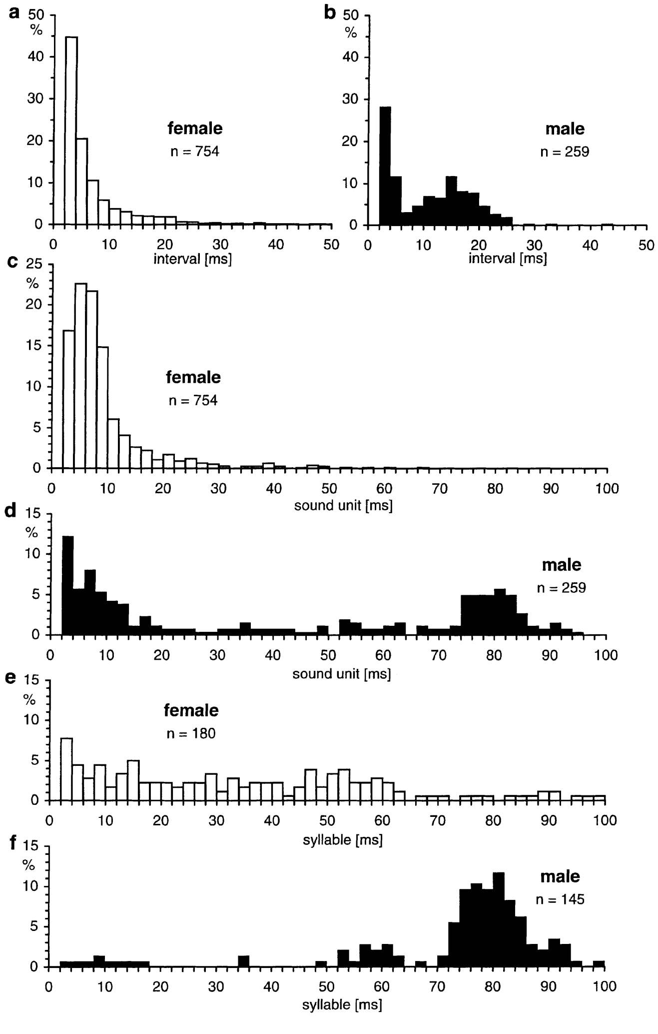

Fig. 4a–f Mean distributions of intervals and sound units and syllables in intact songs of males (*black*) and females (*white*): **a, b** intervals were defined by an impact rate lower than 1.5 kHz for at least 2 ms. In males, note the two peaks which refer to the pulse and syllable pauses, respectively; **c, d** distribution of sound units which were ≥2 ms and separated by intervals >2 ms; **e, f** distribution of syllables revealed by applying the criterion that the syllables had to be framed by syllable pauses (>8 ms). Means of seven males (one long phrase each) and five females (one to four phrases each). *N* in the histograms gives the number of detected units. Note that these numbers decrease when units fuse to syllables

which – corresponding to the gaps – originated from insufficient superposition of the two stridulation patterns (Fig. 4d). The syllable structure of the songs was clearly reflected (Fig. 4f), when the long syllable pauses (intervals > 8 ms according to Fig. 4b) were taken as a criterion to define syllables. The durations between the end of such syllable pauses and the beginning of the next are shown in Fig. 4f.

The female pattern of leg movements is very similar to the male pattern, consisting mostly of four- and six-pulsed syllables (Fig. 2d, e). The phrases are shorter, usually below 1.5 s [for quantitative data see von Helversen and von Helversen (1975)] and about 6–10 dB less intense (cf. Fig. 2a). Sex-specific differences in the sound signals may arise from the dimorphic structure of the stridulatory apparatus and from differences in the underlying neuronal pattern generators. Firstly, the single pulses are not rectangularly modulated as in the male song, but are more or less rhombic in shape (Fig. 2e) and often differ remarkably in intensity from pulse to pulse. Secondly, the coordination of the two stridulating legs is much more variable than in males (Fig. 3, left). Not only is the range of delays between the two patterns considerably larger, but also the intra-individual variation is higher compared to males. Therefore, the stridulation pattern of intact females is much more irregular than that of males, with many short pulses in the range of about 8–10 ms (Fig. 4c). Correspondingly, the relative proportion of gaps is much higher, so that the syllable pauses are not visible as a separate peak (Fig. 4a).

Nevertheless, in the female song pattern, pulses are also grouped into syllables. This is revealed when the syllables are defined by the syllable pauses. However, in females, the distribution is very broad, indicating that gaps exceeding 8 ms were frequently present in the syllables (Fig. 4e).

The frequency spectra of male and female signals also differ (Fig. 2f). In addition to a characteristic peak at about 7 kHz, the male signal contains a broad band of ultrasound frequencies with a maximum around 35 kHz. Female sound spectra are much more variable, but the relative amplitudes of the high-frequency components are usually below the low frequency components.

Selectivity of males and females to synthetic model songs

In the experiments described below, the behaviourally effective range of different song parameters was determined for males and females and compared. To do so it was necessary to hold constant all other signal parameters in an effective range, which, of course, is not necessarily identical for male and female. Therefore, all constant parameters had values close to the average of the natural male and female signal, respectively. In particular, in all males tested, the characteristic syllable structure, which had been shown to be essential for a female to recognize the conspecific male (von Helversen

1972), was present, with syllable durations between 70 and 80 ms and syllable pauses between 12 and 15 ms.

Gaps in syllables

As already observed in previous experiments (von Helversen 1972), female response probability depends strongly on whether syllables are continuous or interrupted by short gaps. Females tolerate such gaps up to 2 ms (at ≥ 30 °C and ≥ 64 dB), which seems to represent the detection threshold. The presence of longer gaps causes a pattern to become totally ineffective at this temperature and sound intensity (Fig. 5a). In contrast, males turn only towards a pattern that contains gaps within the syllables and stop turning when the gaps between pulses drop below the value of 2 ms (which is also the threshold for female acceptance of a gappy male syllable, Fig. 5a). In other words, the gap preferences of each sex not only result in attraction to the songs of the opposite sex but also in rejection of songs of the same sex.

Rise time of pulses

Rectangularly modulated pulses released only rare turns in males (Fig. 5a). However, the effectiveness of a stimulus was greatly increased when the pulses had slowly rising ramps (Fig. 5b). Females responded very moderate as long as the ramped pulses of a syllable had no gaps inbetween, but with gaps of 1 ms or longer the effectiveness of the ramped signals was completely lost (Fig. 5b).

Because gaps become larger with increasing rise time, we varied rise time at different gap widths (2, 3 and 5 ms) to separate the influence on effectiveness caused by the ramp from that caused by the gap width between pulses. While gaps within a wide range of up to 5 ms (von Helversen 1993) did not seriously affect the attractiveness of a female response song, slowly rising ramps drastically raised the turning probability of males (Fig. 6a).

In females, the effect of ramps on response probability can only be studied by introducing ramps at the beginning and/or end of syllables (not pulses, see Fig. 5b). Correspondingly, rise time influences the perceived syllable pause, which is known to significantly determine the effectiveness of a model song (von Helversen 1972). To demonstrate a possible influence of rise time, independent of pause duration, we varied the syllable pause for three different types of syllables: rectangularly modulated, and with 8 ms ramps either at the beginning or at the end of a syllable. All three curves reached about the same response level, which indicates that ramps did not seriously change attractiveness (Fig. 6b). The main difference between the response curves was a shift of the maxima towards smaller pause durations for ramped pulses, indicating that the perceived syllable pause had been enlarged by subthreshold parts of the ramps. The curves also show that ramps at the beginning have a greater effect on the shift than ramps at the end of a syllable.

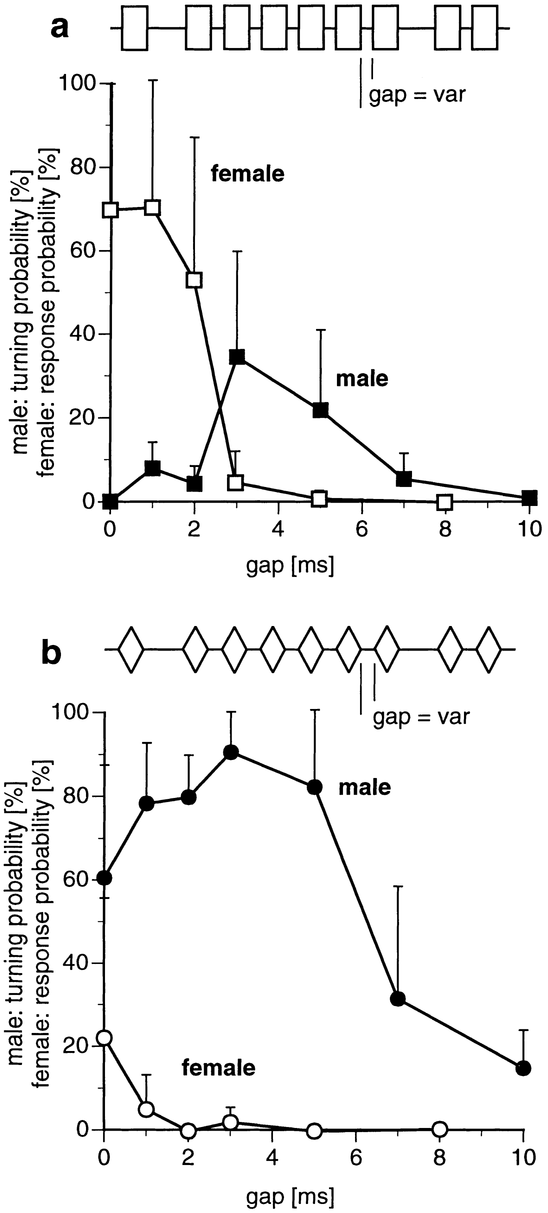

Fig. 5a, b Effect of gaps inserted in the syllables of an artificial *Ch. biguttulus* song pattern. The mean reaction curves of five males and six females are shown. The syllable duration was between 70 and 78 ms depending on the number of pulses, the syllable pause was 12 ms. The pulses were kept constant at 8 ms, rectangularly modulated in (**a**) and of rhombic shape (4 ms risetime) in (**b**). Males: 58 dB (SPL) and 4–8 kHz carrier spectrum, females: 70 dB (SPL) and 2–40 kHz carrier spectrum

Pulse and syllable duration

Since the male's turning reaction requires gappy syllables, sound events shorter than the syllables must be

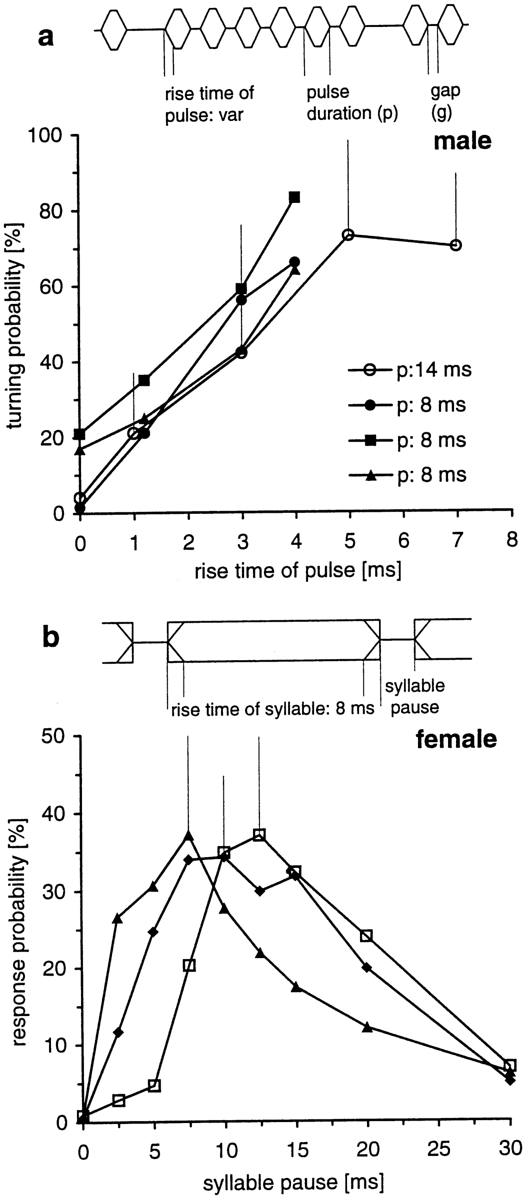

Fig. 6a, b Effect of rise time of pulses and syllables: **a** turning probability of males as a function of rise time of pulses. All curves (means of six males each, n = 91–273 per data point) show the same trend, irrespective of pulse duration (8 or 14 ms) and of gap-width between pulses (2, 3 or 5 ms). Intensity was 55–58 dB, frequency spectrum 4–8 kHz, syllable duration between 70 and 78 ms depending on gap-width and number of pulses, syllable pause 15 ms. *Bars* indicate typical standard deviation; **b** response probability of females (means and standard deviations of six animals, n = 305 per data point) as a function of syllable interval for three different shapes of syllables: rectangularly modulated (*open symbols*) or with 8 ms ramp at the beginning (*black squares*) or at the end of the syllables (*black triangles*). Note that all curves reach the same response level, indicating that ramped onsets of syllables do not seriously influence effectiveness. Syllable duration was 80 ms, intensity 70 dB (SPL)

detected. To evaluate the range of such short pulses, we offered stimuli consisting of rhombic pulses and varied their duration by enlarging rise and fall times. Necessarily then, the steepness of ramps was varied simultaneously. As long rise times do not reduce effectiveness (von Helversen 1993), the drastic decline of turning probability in Fig. 7a reflects the poor effectiveness of pulses longer than 15 ms. The suggestion that increased pulse duration drastically reduces the turning probability is corroborated by additional experiments, in which pulse duration was varied by inserting a plateau between rising and declining ramps of 3 ms each (closed symbols in Fig. 7a). The peak of both reaction curves is at the same pulse duration of 10 ms.

Interestingly, the ranges of effective sound units do not overlap for the two sexes. To release a female's response, the syllable duration has to be at least 30 ms (Fig. 7b). As the pauses between syllables critically determine the female's response, we varied syllable duration at four different values of pause durations. Syllables were accepted over a broad range of durations (Fig. 7b). The effectiveness of syllable duration was dependent on the duration of the syllable pause: short syllables were more attractive when there were short pauses and vice versa.

Sound intensity

Because the overall intensity of female songs is lower than that of male songs, we compared the intensity characteristic of male and female response behaviour (Fig. 8). Females showed a maximum response at sound pressure levels of about 60–75 dB SPL (Fig. 8b). Interestingly, the turning response of males was inhibited at such high sound pressure levels. Males reacted best at intensities over 40–60 dB SPL (Fig. 8a).

Frequency spectrum

In the experiments described so far, the spectra of the stimuli approximated those of the corresponding male and female signals. Next, we tested sexual differences for preferences of different spectral compositions.

For that purpose, in a first experiment, a frequency band (1/3 octave) was shifted along the hearing range at a constant level of 55 dB. Males responded best when the midfrequency of the 1/3 octave was 6.3 kHz (Fig. 9, black curve); at frequencies above 10 kHz no turns could be elicited either at higher or lower intensities.

Females were tested with spectra of 5 kHz bandwidth at an intensity of 66 dB; they responded to any frequency band, but at a very low rate. Only between 10 and 15 kHz did the response increase up to about 50% (Fig. 9). Considering the broad spectrum of male song, one might expect that the female would be stimulated adequately when both the low and high frequency components are present simultaneously. This is most likely the case when the females are offered a carrier frequency spectrum between 10 and 15 kHz, because in this range both low and high frequency receptor elements are activated, albeit not at their best frequencies. In fact, the response was best in this range, but was

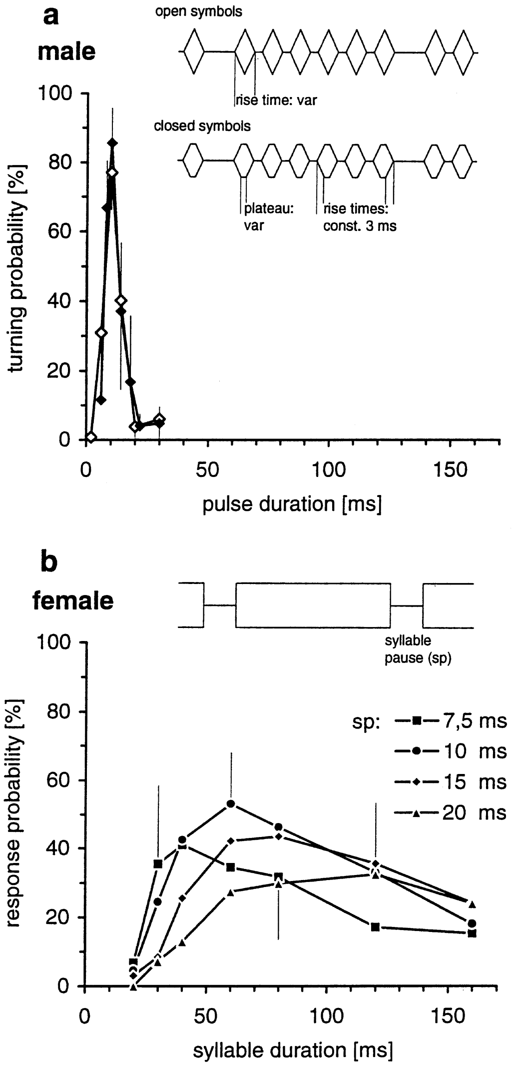

Fig. 7a, b Effect of pulse and syllable duration: **a** *open symbols*: duration of pulses was varied by increasing rise and decline of pulses (means and standard deviations of four males, n = 73–261 per data point, intensity 53 dB, spectrum 1/3 octave around 6.3 kHz); *closed symbols*: duration of pulses was varied by increasing the plateau between constant ramps of 3 ms (means and standard deviations of five males, n = 196–364 per data point, intensity 58 dB, spectrum 4–8 kHz, 30–32 °C); **b** response probability of females as a function of syllable duration. The curves give means and standard deviations of nine females (n = 427 per data point) tested at various values of syllable pauses (see *inset*), intensity 64 dB, spectrum 2–40 kHz, 35 °C

much poorer when only high- or low-frequency receptors were activated.

To test whether the combination of low and high components is indeed critical, we presented low- and high-frequency components simultaneously via two speakers positioned close to each other. The relative amplitude of the high frequency component was adjusted to be about 15 dB greater than that of the low-frequency component according to the tympanal threshold curve (A. Stumpner, pers. comm.). Presented alone, each component elicited only rare responses in females, but when presented simultaneously, the response probability increased significantly (Fig. 10b).

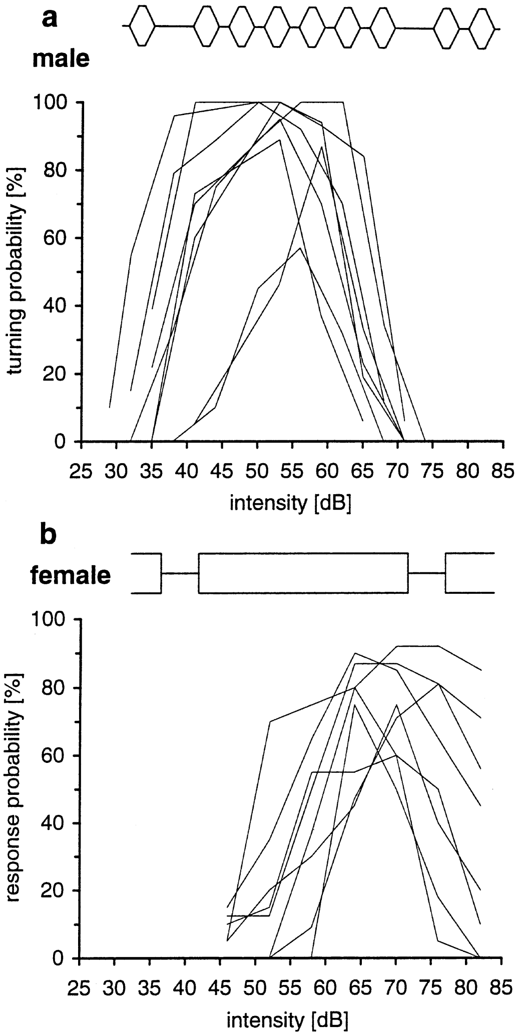

Fig. 8a, b Effect of sound intensity: **a** threshold curves of nine males (n = 91–369 per reaction curve). Stimulus: spectrum 4–8 kHz, syllable duration 72 ms composed of 10-ms pulses with 3 ms rise and decay time, syllable pause 12 ms; **b** threshold curves of eight females (n = 112–182 per reaction curve). Stimulus: syllables 80 ms, syllable pause 15 ms, spectrum 2–40 kHz

In males, turning probability was high only to the low frequency component; no reaction was observed to high frequencies (Fig. 10a), corroborating the results of Fig. 9. Interestingly, when both components were presented simultaneously, the high frequency component did not affect turning probability at intensities up to 60 dB, but drastically reduced the male's response at and above 70 dB. This result might indicate that a strong high-frequency input inhibits the turning reaction.

Is the syllable pattern as important for males as for females?

So far, the characteristic species specific syllable/pause pattern, known to be important for recognition of male song by the females (von Helversen 1972), was present in all stimuli. Is this pattern of syllables and pauses also

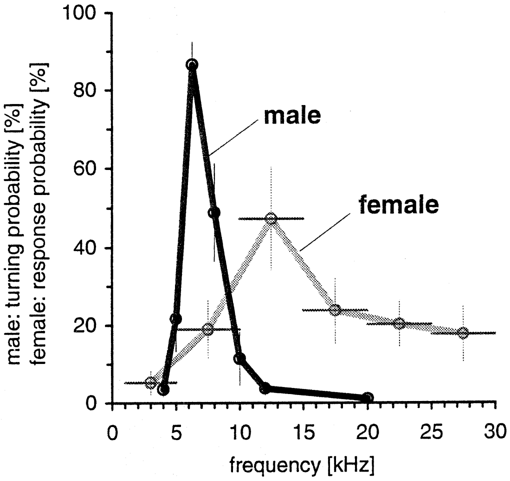

Fig. 9 Effect of carrier frequency spectrum. *Horizontal bars* indicate width of spectral bands, 1/3 octave in males and 5 kHz in females, band pass characteristic 48 dB per octave. *Black curve*: mean turning probability and standard deviations of six males tested at 55 dB (n = 102–401 per data point, syllable 72 ms, composed of six sawtooth shaped pulses with ramps of 10 ms, syllable pause 12 ms). *Hatched curve*: mean response probability and standard deviations of five females tested at 66 dB (n = 129 per data point, syllables 80 ms, pause 15 ms)

necessary for males to react, or is a series of short ramped pulses sufficient?

To test the importance of the syllable pattern, in a series of sawtooth shaped pulses (rise time 10 ms with gaps of 2 ms between the pulses) we introduced a syllable pause after every sixth pulse and varied the duration. As in females, males showed a steep increase in response probability in the range between 5 and 10 ms pause duration (Fig. 11a, b). The decline of the response curve, on the other hand, was much less prominent and much more variable in males than in females; at a pause of 30 ms the response probability of males was at most only slightly reduced, whereas it was well below 50% of the individual peak response in females (Fig. 11a).

In females, the decline of such response curves depends on intensity and is less steep at lower intensities (D. von Helversen, unpubl. obs). However, intensity dependence does not explain the variability of the decline in males; there was no difference between stimuli presented at 56 (solid lines) or 64 dB (dotted lines). More likely, the high variance in turning probability reflects the high interindividual variability between males. Thus, in addition to the short and ramped pulses, for males, the syllable pattern also strongly affects the turning probability, although the range of effective values is broader than for females.

Discussion

Homology of male and female stridulation mechanism

Male and female stridulation mechanisms are homologous in so far as both sexes rub a file on the inner side of the hind femur against a protruding vein of the elytron. In both sexes the file is derived from the same row of bristles, but interestingly must have evolved independently. In the male, the bristles are transformed to short cones and act as stridulatory pegs. In the female the bristles remain inconspicuous, whereas the proximal edges of the cups, in which the bristles insert, are sclerotized and act as stridulatory pegs. This was documented by Petrunkevitsch and Gaita (1901); however, these authors did not point out the different origin of the

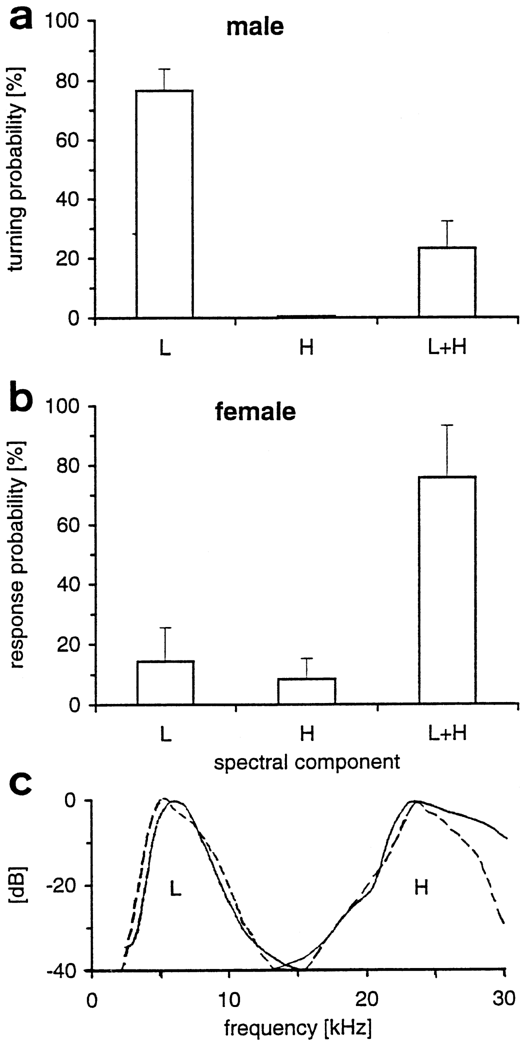

Fig. 10a–c Effect of low (L)- and high (H)-frequency components presented alone and simultaneously: **a** mean turning probability and standard deviation of six males (n = 301, 285, 375 for L, H, $L+H$). The low component was given at 58 ± 4 dB, the high component was tested at intensities between 58 and 75 dB. When both components were presented simultaneously, the high component was increased to 75 ± 3 dB, to demonstrate the reducing effect of the high component; **b** mean response probability and standard deviation of five females (n = 182, 144, 157 for L, H, $L+H$). The low and high frequency components were presented at 45 ± 3 dB and 63 ± 3 dB, respectively. Frequency spectra used for males (*solid line*): L = 5–7 kHz, filter characteristic 48 dB kHz per octave, H = 31.2 kHz (midfrequency of octave), and females (*broken line*): L = 4–8 kHz, filter characteristic 48 dB kHz per octave, H = 25 kHz (midfrequency, 1/3 octave)

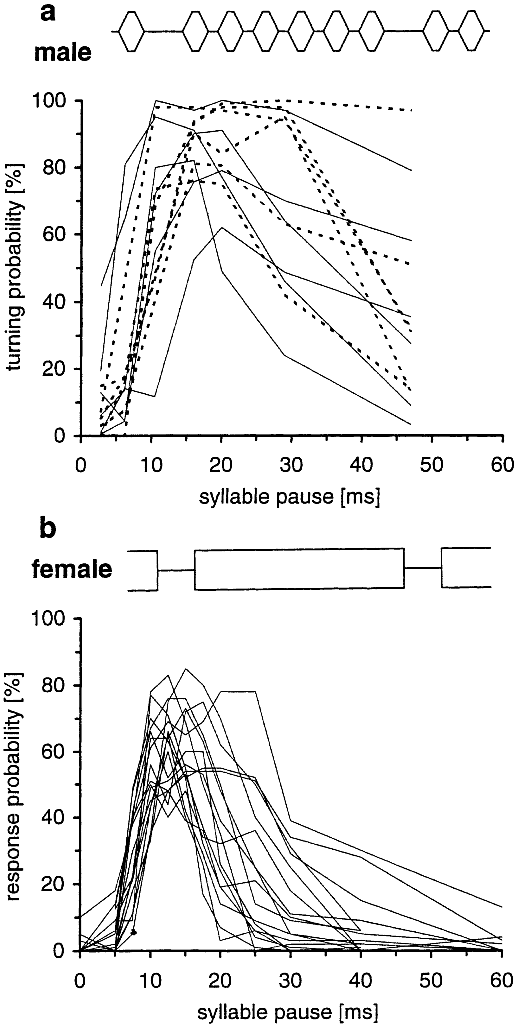

Fig. 11a, b Effect of syllable pause: **a** turning probability of 12 males (*n* = 14–81 per data point), stimulus: syllable 72 ms composed of 10-ms sawtooth-shaped pulses with 2-ms gaps, spectrum 6.3 kHz Terz at 56 (solid curves) and 64 dB SPL (dotted curves), 30–33 °C; **b** response probability of 17 females (*n* = 20–133 per data point), 35 °C, stimulus: syllable 80 ms, spectrum 2–40 kHz, 64 dB SPL

stridulatory pegs, which suggests an independent evolution of male and female stridulation. Probably in the females the file evolved later in evolution when the genetic system responsible for the male file was already sex specifically suppressed in the female.

The co-ordination of the hindlegs during stridulation is very similar in both sexes and certainly homologous. Males, however, are more precise and show a smaller inter- and intraindividual variability than females. Differences in the force with which the file is rubbed against the vein during the short down-up-strokes may explain the differences in intensity and shape of male and female pulses. Differently structured pegs may also contribute to these differences in pulse shape.

Comparison of signals and corresponding recognition mechanisms

In Table 1 male and female song characters and the corresponding requirements of the recognizing filter mechanism are listed. Thus, although the movement pattern is similar in males and females, in some respects their sound patterns show no overlap, and these are best suited for distinguishing between the sexes. For recognition of female signals, the short ramped pulses (<20 ms, Fig. 7a) are critical, whereas recognition of male signals depends on the presence of longer (>30 ms, Fig. 7b) units, the syllables, which at such duration and intensity can be produced only by the phase-shifted superposition of the movement of the two stridulating legs.

The different spectral composition of male and female signals is also involved in sex-discrimination. Thus, the differently tuned receptor elements of the grasshopper auditory system (Michelsen 1971; Römer 1976) are indeed behaviourally relevant.

The steepness of ramps is a relevant acoustic property for males but not females. While male turning probability was drastically increased by slowly rising ramps, female response probability was unaffected by ramped syllable onsets. On the other hand, accentuated onsets of syllables enhance female response probability (von Helversen and von Helversen 1983).

Sound pressure level, which of course is distance dependent, is of limited value for sex recognition and is therefore not listed above. The higher response threshold of the females might be caused by the higher threshold of high-frequency receptor elements (10–15 dB compared to a sensitive low-frequency receptor element at its best frequency; A. Stumpner, pers. comm.). The stimulation of both receptor types increased the response probability compared to stimuli with either the

Table 1 Requirements of filter mechanisms and corresponding characters of male and female song

Requirements of female filter mechanism	**Characters of male song**
(1) compact, continuous syllables without gaps and longer than 30 ms	gaps at reversal points of leg-movements camouflaged by phase difference between both hindlegs
(2) marked onsets of syllables improve efficiency (v. Helversen and v. Helversen 1983)	the first pulse of a syllable normally has a pronounced amplitude
(3) low and high frequency component necessary	broad frequency spectrum with two peaks at 7 and around 35 kHz
Requirements of male filter mechanism	**Characters of female song**
(1) gappy syllables with pulses shorter than 15 ms	distinct sound pulses
(2) slowly rising ramps of pulses	rhombic shape of pulses
(3) only low frequency component effective (high frequencies even inhibiting)	spectrum with reduced high frequency component

low or the high component. In males, which react only to the low frequency component, the behavioural threshold is about that of the electrophysiologically determined threshold of the tympanal nerve (A. Stumpner, pers. comm.). The abrupt decline of male responsiveness at sound pressure levels above 60–65 dB can be explained by the thereby increased 'absolute steepness' of ramps which become too steep to be accepted as female pulses (von Helversen 1993).

Directional hearing

Since in *Chorthippus biguttulus* it is the males which locate and approach females, one might expect female signals to be selected for localizability. In this context, the distinct short pulses in the female song may improve localization acuity for the following reasons. Firstly, the partitioning of the syllables into pulses allows a sixfold number of right/left comparisons, and this may considerably improve directional analysis compared to one based on a few syllables only. Secondly, as the latency difference (in the order of a few milliseconds) in excitation of both sides is used for lateralization (Mörchen et al. 1978, von Helversen and Rheinlaender 1988), short pulses with sufficiently long intervals in between seem to be particularly suited to avoid wrong correlations between spikes not originating from the same sound pulse.

It has been also suggested that the ramped shape of pulses could improve lateralization acuity by inducing an additional time difference between the neuronal excitation of the two sides (Adam 1977; von Helversen 1993; Krahe and Ronacher 1993).

At first sight it may be surprising that localization in *Chorthippus biguttulus* is based on the lower frequency range, as in such small animals the tympanal characteristic is rather poor at frequencies of 5–10 kHz, but much more pronounced at higher frequencies (Miller 1977; Michelsen and Rohrseitz 1995). However, in behavioural tests, the lateralization ability of *Ch. biguttulus* turned out to be astonishingly accurate, even at the low frequency peak of 7 kHz in the song spectra. A song model consisting of a 1/3-octave noise band centered at 6.3 kHz presented only 5° from the median could be lateralized correctly with about 85% probability (D. von Helversen unpubl. data).

There are two advantages of relying on the low frequency component for directional hearing. Firstly, at frequencies around 7 kHz, the hearing range will be considerably larger than that for frequencies above 20 kHz because of the smaller attenuation of low frequencies (Römer 1993) and because of the greater sensitivity of the low-frequency receptors [10–15 dB more sensitive compared to the high-frequency receptors; Römer (1976) for *Locusta*; and A. Stumpner for *Ch. biguttulus* (pers. comm.)]. Secondly, low frequencies are less susceptible to reflections and echoes in the biotope than high frequencies and hence provide more reliable directional information.

Neurophysiological correlates to behaviour

Neither the tympanal receptor elements nor the ascending metathoracic interneurones differ in males and females with respect to frequency sensitivity, threshold and temporal properties (B. Ronacher and A. Stumpner, pers. comm.). Thus, the differences in male and female recognition systems must be found at the level of the brain, where the decisive steps of recognition seem to occur (Ronacher et al. 1986; Bauer and von Helversen 1987). So far, little is known about the neuronal basis of these filter mechanisms within the brain. However, the properties of various ascending auditory neurons have been investigated thoroughly (Römer and Marquardt 1984; Römer and Seikowski 1985; Ronacher and Römer 1985; Stumpner and Ronacher 1991, Stumpner et al. 1991), and some of these neurons are likely to play a role in the recognition process.

When attempting to correlate electrophysiological data with behavioural tuning curves the main problem is the different temperatures at which the two kinds of studies were conducted. The temperature during electrophysiological recordings was normally below 25 °C, whereas in behavioral experiments the animals were tested at temperatures between 30 and 35 °C, the range at which they react reliably and frequently. Such high temperatures are especially necessary to observe the turning reaction of males, which could not be elicited at temperatures below 26 °C. Because neuronal thresholds, latencies and spike intervals are strongly dependent on temperature (Wolf 1986), correlation of behavioural and neuronal tuning curves is difficult. However, the general characteristics of neurons and whether they may be candidates for detection of male or female song, respectively, should be maintained over a wide range of temperature. Thus, neurons which are differentially activated by male and female song are more likely to be involved in sex discrimination than units responding similarly to male and female song patterns. The latter seem to be the better candidates to contribute to recognition of species specific song characters.

The AN3 neuron, in many examples, responds phasically to sound pulses even after very short gaps (>2 ms); thus, gappy syllables release considerably more spikes than continuous syllables (Stumpner et al. 1991). Also, the intensity tuning curves of many AN3 neurons correlate with those of the male turning reaction; in both, the reaction is limited to low intensities.

The AN4 neuron, described as a "gap detecting neuron", may also respond differently to stimulation by male and female song (Ronacher and Stumpner 1988). This neuron is only active when the sound units are longer than 15–20 ms, which is the time interval Stumpner and Ronacher (1991) found to be necessary to overcome the initial inhibition, which is reset with every new sound pulse. Thus, this neuron was expected to show the opposite behaviour of AN3-types; excitation when stimulated with song of intact males, and inhibition when stimulated with female song. However, Budinger (1995) and Lang (1996) showed that ramped pulses abolish the initial inhibition of AN4, causing it to spike in response to stimuli resembling female song.

In contrast to the task of identification of sex, neurons responding similiarly when stimulated with male and female song are more likely to contribute to recognition of species specific song characters. The AN12, which reliably responds to onsets of sound (provided that the preceding interval is sufficiently long), is suggested to be involved in recognition of syllable pattern (Stumpner et al. 1991). Stimulation with male and female song pattern would result in rather similar discharges, because this neuron is too slow to code the gappy structure of female song pattern.

Besides temporal discharge properties, the intensity tuning curves also give some hints which neurons are more likely to be involved in sex discrimination. Some neurons, e.g. AN1, AN3, AN11 (Stumpner and Ronacher 1991) are severely inhibited when stimulated with broadband noise at intensities above 60 dB. Such neurons seem to be less suited for processing male song at higher intensities, but would encode the less intense female song pattern. Interestingly, the neurons which, on the basis of their discharge patterns, are expected to process species specific characters (i.e. AN12, AN6 and AN4) respond to broad-band noise over the whole intensity range (Stumpner and Ronacher 1991).

Selective forces acting on sender and receiver mechanisms

The theory of sexual selection predicts that the variance of reproductive success is larger in males than in females (Bateman 1948; Trivers 1972) and, accordingly, sexual competition among males is stronger than among females. *Chorthippus biguttulus* males are able to mate several times at short intervals, but females mate only one to four times during their life time (Kriegbaum and von Helversen 1992; von Helversen and von Helversen 1994). Therefore the "operational sex ratio" is shifted far towards the males and, in the field, many males compete for few females that are ready to mate and which can choose among males, in accordance with Bateman's principles (Bateman 1948; Arnold and Duvall 1994).

Female choice in this species uses acoustical criteria (Perdeck 1957; Kriegbaum 1989; Kriegbaum and von Helversen 1992; von Helversen and von Helversen 1994). As males are selected by choosy females, the selection pressures on males and females will be asymmetric, and it may be predicted that females are more selective with respect to the acoustic signals they respond to, and males produce the more precise signals (Schmitt et al. 1994; von Helversen and von Helversen 1994).

Indeed, in *Chorthippus biguttulus*, male song is very stereotyped and precise. The accuracy of leg coordination, especially the phase shift between legs, is much higher in males than in females (Fig. 3) and the resulting syllable and syllable-pause pattern is better defined in males (Fig. 4). This is true of characters important for species recognition which are, therefore, "static" criteria (Gerhardt 1991). "Dynamic" properties (i.e. phrase duration, intensity, number of phrases per song) are more variable between males (Ryan and Keddy Hector 1992); for *Ch. biguttulus* see von Helversen and von Helversen (1994).

On the other hand, females are more selective with respect to their acoustic responses than males (for instance, evaluation of syllable pause duration, Fig. 11). The songs of all related species are rejected by confining the response to syllable durations of 30–200 ms and syllable pauses of 7–40 ms (von Helversen and von Helversen 1994). Because of the steep decline in female response probability when gaps are introduced into syllables (Fig. 5), males with an inaccurate leg coordination or with one hindleg missing, have a lower probability of eliciting a female response, and thus a drastic reduction in mating success (Kriegbaum and von Helversen 1992).

In contrast, males of *Chorthippus biguttulus*, are much less selective and will even court heterospecific females. Correspondingly, parameters that signal species specifity are not so precisely evaluated (see for instance Fig. 11).

Sex recognition

For females the pressure to discriminate between male and female songs is not so severe, since females normally sing only in response to males. For males, on the other hand, the recognition of sex by acoustic criteria is very important because, in the field, a given male is surrounded by many conspecifics singing in the vicinity; phonotactic responses to the songs of other males would waste time and energy and increase predation risk.

Therefore males evaluate nearly all the subtle differences between male and female song (see Table 1). They do not approach songs with ultrasound components (Fig. 10), steep ramps (Fig. 5), high intensities and are very sensitive to pulse duration (Fig. 7).

The hypothesis, that in general, males produce more precise signals than females but are less selective in the range of signal parameters to which they respond, is valid for species specific characters, but not for criteria used in sex recognition, in the context of which they are highly discriminatory.

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