ORIGINAL PAPER

Spatial orientation in the bushcricket *Leptophyes punctatissima* (Phaneropterinae; Orthoptera): II. Phonotaxis to elevated sound sources on a walking compensator

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Abstract The ability of the bushcricket *Leptophyes punctatissima* to orient to elevated sound sources was investigated. Males were placed on a walking compensator and oriented in response to a synthetic female reply, which was broadcast via one of five loudspeakers placed at elevations of 0°, 30°, 60°, 75° and 90°. Forward and backward movements were compensated, so that males remained at the same distance and elevation to the sound source. With increasing loudspeaker elevation, the males meandered more, and the ratio of the ideal path length to the actual path length decreased. The same was true for the correlation between stimulus angle and turn angle, and there were more turns to the wrong side with increasing loudspeaker elevation. Most males performed phonotaxis with a high acuity up to an elevation of 60°. Individuals varied strongly in their performance especially at a source elevation of 75°, where some were still very accurate in their approach, whereas the acuity of others decreased rapidly. We also describe a behaviour where males tilt their body axis to more anterior and sideward positions, both during walking and while calling on the spot. This behaviour is interpreted as a kind of directional scanning in order to actively induce changes in binaural cues.

Keywords Bushcricket · Sound localization · Azimuth · Elevation · Phonotaxis

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Abbreviations
IID   Interaural intensity difference
SPL   Sound pressure level

Introduction

Many grasshoppers, crickets and bushcrickets have evolved elaborate forms of acoustic communication in the context of pair formation, which often involves one sex performing acoustically mediated attraction to the other sex (phonotaxis) over considerable distances (review in Gerhardt and Huber 2002). With the exception of some field crickets and grasshoppers that communicate close to the ground and have to localize only in the azimuth, the majority of the orthopteran insects orients in a complex, three-dimensional habitat, where localization of a sound source involves the perception of source azimuth and elevation. It is surprising, though, that spatial orientation so far has been almost completely ignored in both behavioural and neurophysiological studies. Two exceptions concern either a predator/prey context in flying crickets (May et al. 1988; Wyttenbach and Hoy 1997) or a parasitoid fly homing in on its cricket host (Müller and Robert 2001). Thus, almost nothing was known about spatial localization performance in the context of intraspecific communication in insects.

For the duetting bushcricket *Leptophyes punctatissima* we have shown recently that males orient almost perfectly in an artificial three-dimensional grid system to horizontal, elevated and depressed sound sources broadcasting a female reply (Rheinlaender et al. 2006b). This remarkable orientation was achieved, although the insects have no specialized external ear

structures such as pinnae in mammals, where the particular shape of the external pinna provides important information for the vertical orientation of a sound source (Blauert 1983; Butler and Belendiuk 1977; Fuzessery 1996; Wotton and Simmons 2000).

In the present paper, we further investigated spatial hearing in the same Phaneropterine bushricket *L. punctatissima* using a walking compensator. The advantage of such an approach is the possibility to study the behaviour of the male at the same distance and elevation angle for many repeated trials, which contrasts to a phonotactic path in a freely moving insect. This allows to correlate the degree of stimulus elevation with quantitative measures of the orienting movement, which are correlated to the accuracy of phonotaxis.

Material and methods

Animals

Nymphs of *L. punctatissima* (Tettigoniidae; Phaneropterinae) were collected from wild stock locations in Nordkirchen (Nordrhein-Westfalen, Germany) and groups of females were kept physically and acoustically isolated from groups of males in separate wooden cages (size 90 × 50 × 40 cm). Food (*Urtica dioica, Heracleum spondylium*, *Cirsium vulgare*, *Syringa vulgaris*) and water were supplied ad libitum. Males aged between one and six weeks after the final moult were used for the experiments.

Experimental arrangement and phonotactic trials

All behavioural experiments were conducted in a darkroom (160 × 220 × 200 cm), which provided a light intensity just sufficient for the video recordings. The walls of the chamber were covered with sound absorbing material, so that reflections from the walls at the position of the males were attenuated by more than 40 dB relative to the female reply. A total of thirty males performed phonotaxis on a walking compensator (width 50 cm, length 80 cm; described in detail by Hardt 1988; Rheinlaender et al. 2006a; see Fig. 1). In front of the walking belt, and connected along its longitudinal axis, five identical loudspeakers (piezo tweeters; Motorola, bullet-type KSN1005) were positioned along a quarter circle at a distance of 60 cm from the centre of the walking belt (= release point of males). The elevation of the loudspeakers was 0°, 30°, 60°, 75° and 90°; the 90° elevation was exactly above the insect. Any forward or backward movement of the animal was compensated by the operator by turning the walking belt mechanically in the opposite direction. This maintained the walking insect at a constant distance (and thus constant intensity) to the loudspeaker. Thus, in contrast to the Kramer treadmill (Kramer 1976; Weber et al. 1981; Schmitz et al. 1982), only forward or backward movements relative to the loudspeaker are compensated, and no lateral movements are compensated (for further details see Rheinlaender et al. 2006a). Although the walking belt was operated manually, the relative low speed of a male allowed to rather precisely control its position by the operator, so that the "jitter" in the anterior/posterior direction (distance) was 2.5 cm on average and did not differ between elevations. Also, in terms of SPL, the effect was less than 0.5 dB and negligible.

Pair formation in *Leptophyes* is achieved by duetting, where the male song elicits an acoustic reply in the female, to which the male then responds by phonotaxis with very high probability. However, there is a strong demand for an accurate time delay of the female reply, which has to occur in a short time window after the male song (Hartley and Robinson 1976; Robinson 1980; Robinson et al. 1986; Heller and von Helversen 1986; Zimmermann et al. 1989; Bailey 2003). We mimicked this duetting situation in an experimental set-up as shown in Fig. 1. A singing male was placed in the centre of the walking belt, and its song was recorded with a bat detector (QMC Instruments Ltd.), which then triggered a synthetic female reply (a short click of 1 ms duration; frequency 40 kHz) from a sound generator (Burchard, akustischer Stimulator II). An electronic delay circuit produced a net time delay of 35 ms for the female reply, which is centred within the temporal window for eliciting phonotaxis. The response was broadcast via one of the five loudspeakers in front of the male. The sound pressure level (SPL) of the signal at the position of the male was 85–89 dB SPL (re 20 μPa; peak reading), calibrated with a sound level metre (Bruel & Kjaer, type 2209) and a 1/4 in. microphone (type 4135). These variations of 4 dB in the actual SPL at the position of a male resulted from lateral deviations in the males' path from a straight line on the walking belt. The SPL was at least 15 dB above the behavioural threshold for eliciting phonotaxis (see below).

The phonotactic approach of the male was monitored from above with a video system (Sony DCR-PC6E PAL) in the super nightshot modus and was observed on a screen (Sony Mini DV GV-D900E PAL). This feedback allowed to compensate the forward and backward movements of the male with the walking compensator. In addition, the complete walking
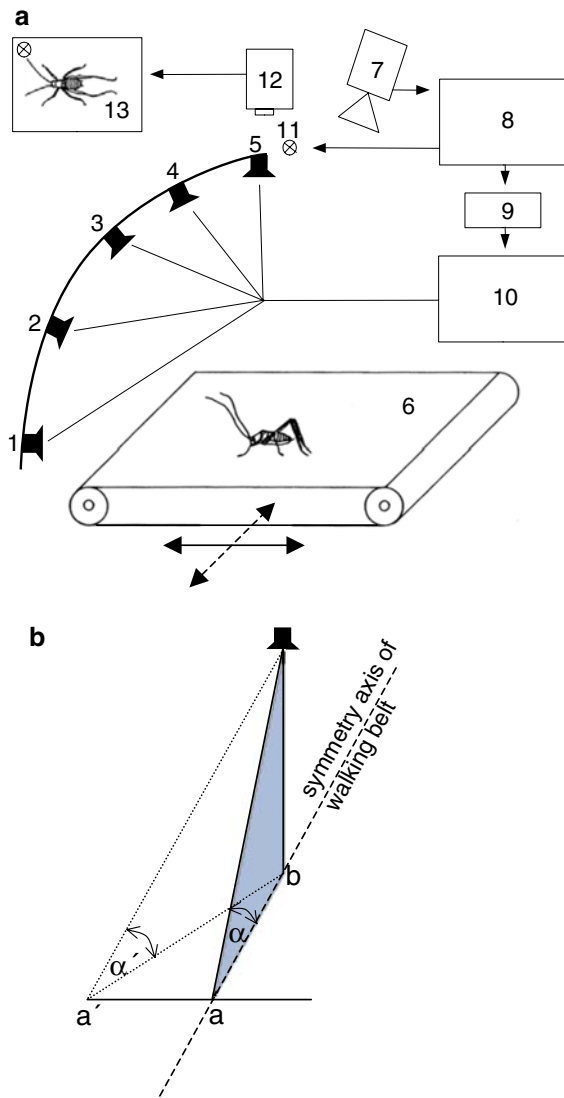
Fig. 1 a Experimental arrangement to study the vertical orientation of male *L. punctatissima* on a walking belt. The numbers refer to: *1–5* loudspeakers at elevations of 0°, 30°, 60°, 75° and 90°; *6* walking compensator; *7* microphone to record the males' call; *8* oscilloscope to display male call and synthetic female reply; *9* delay circuit; *10* electronic device to generate the female reply; *11* LED signal; *12* video camera; *13* video screen. **b** schematic diagram demonstrating the deviation of the actual elevation angle when the insect deviates from the symmetry axis on the walking belt. If the male is at position *a'*, the distance *ba'* increases compared to the distance *ba*, and the actual elevation angle α decreases. For further explanations see text

belt could be moved laterally on separate rails to keep the insect within the centre of the video frame. It should be noted that the mechanical operation of the walking belt by the experimenter apparently did not influence the orientation behaviour of the males, when compared to males orienting in a three-dimensional grid system (Rheinlaender et al. 2006b) or in the habitat (Robinson and Rheinlaender, unpublished observations). Each

male chirp also triggered a LED signal in the video frame for later analysis of the stimulus-related turn angles.

All trials were conducted at ambient temperatures between 20° and 27°. A trial started by placing one spontaneously chirping male on a starting platform (wire mesh; size 2.2 × 2.2 cm; height 3 cm) in the centre of the walking compensator. Twenty-one males were tested for all loudspeaker elevations (except 90°); each test included at least 50 cycles of male calls, female reply and subsequent phonotactic responses. After each series, for the next minute the male received no female reply and its behaviour was recorded. All males changed their walking direction or stopped walking when their calls were unanswered, demonstrating that their previous path was phonotactic. Next, the insect was placed on the starting platform again and it received a synthetic female reply from a different loudspeaker elevation. The order of the tested elevations was varied randomly.

These tests lasted for about 15 min and resulted in a total walking distance of about 12 m (see e.g. Fig. 2). In order to avoid any possible influence of the previous path taken, the tests at 90° elevation were performed with another group of nine different males. A stimulation via the loudspeaker at elevation 90° (exactly from above) regularly resulted in a disoriented, circling movement pattern (see Results). In order to decide whether such behaviour was orientation behaviour at all, at the termination of a trial at 90° elevation, the female reply was broadcast at 0° elevation for 1 min. If the male then readily oriented towards the signal, the previous path at 90° elevation was considered as phonotaxis. Occasionally, a male reached the lateral border of the walking compensator only in tests at an elevation of 90°. It was then put back to the starting point and the trial was repeated to eliminate any bias of the rim.

The behavioural thresholds of males were measured according to a method described by Zimmermann et al. (1989). A singing male was placed onto the starting platform and after each chirp received a synthetic female reply broadcast from the loudspeaker in 0° elevation at varying SPL. The area around the platform was divided into four segments, and the lowest SPL at which the male left the platform in the appropriate segment towards the sound source in three out of five trials was assessed as behavioural threshold.

Within 24 h after a phonotactic experiment, a male was placed on the walking belt for 5 min and its behaviour was recorded *without* acoustic feedback. Males either remained at the release site or moved around in circles. In no case a systematic movement to the
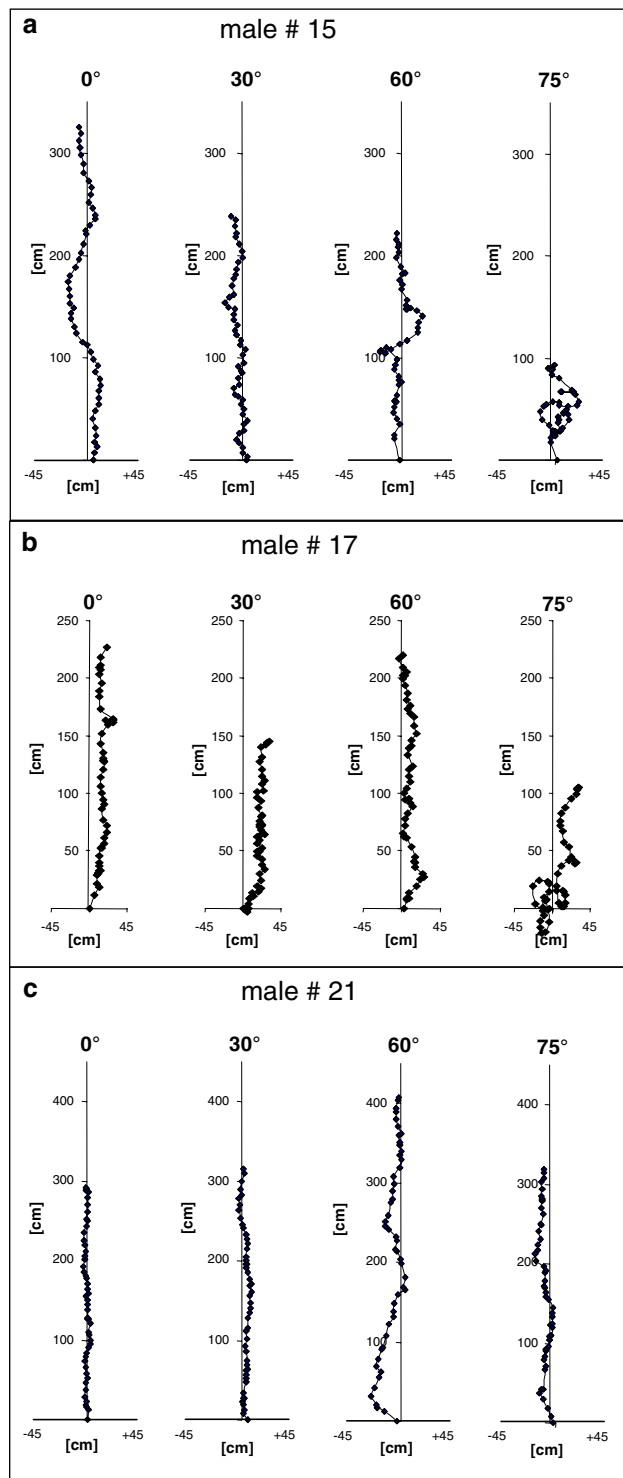
Fig. 2 Reconstructed phonotactic paths of three males on the walking belt at different loudspeaker elevations. Each *dot* refers to the position of the male when it produced his call. Note that while males in **a** and **b** are almost completely disoriented at loudspeaker elevations of 75°, the male shown in **c** performs very well at this elevation. Note also the consistent right-handed disorientation of the male shown in **b**, as evident in the walking path shifted to the right of the straight line to the loudspeaker

loudspeaker arrangement or into any other direction was observed. Thus any bias in the set-up, which may have caused movement into a certain direction, can be excluded.

Data analysis

For each male, 50 phonotactic responses were analysed per elevation. The stimulus and turn angles were evaluated in single frame-by-frame analysis with a video recorder (Sony Hi 8 EV-C2000E PAL) and monitor (Panasonic TC-15M1RC). The longitudinal axis of the male was measured at the time of the stimulus broadcast, which was identified from the LED signal in the video, and immediately after the orientation movement had ended, by monitoring the position of the pronotum and abdomen relative to the coordinate grid on the walking belt. The coordinates were also used to reconstruct the complete phonotactic path for a given elevation.

If a male was stimulated at an elevated loudspeaker position and deviated from the longitudinal axis of the loudspeaker-to-release point, this caused an inevitable decrease in the actual angle of the loudspeaker position, as illustrated in Fig. 1b. Although this was true for all loudspeaker elevations (except for the azimuth), the deviation increased with increasing elevation angle. Thus, for each male position preceding an orientation movement this deviation was calculated. The real stimulus angles ranged from 23.5° to 30° (mean ± SD, 29.7 ± 0.4) at 30° elevation, from 43.6° to 60° at 60° elevation (58.8 ± 0.9), from 50.4° to 75° at 75° elevation (71.9 ± 2.8) and from 53.1° to 90° at 90° elevation (80.8 ± 3.3). Thus, the actual stimulus angles of 30°, 60°, 75° and 90° describe a range of elevation angles, rather than a precisely defined one. As the mean values indicate, however, these ranges are rather small.

Similarly, several reasons contributed to some inaccuracy with which stimulus angles and turn angles could be determined. Though the position of the animal at the time of the stimulus was clearly defined by the LED signal in the video recording, the time for acquiring the coordinates after the orientation movement was less well defined and difficult to standardize because the turning speed varied between individuals. Furthermore, the orientation of the animal with respect to the loudspeaker fluctuated with each movement of the legs, even when the animal walked in a straight line. Finally, the parabolic surface of the monitor used for evaluating the coordinates caused some distortion, so that the overall calculated accuracy for determining stimulus and turn angles was 4.2° and 5.3°, respectively.

Statistical analysis of the data was performed using Friedman repeated measures ANOVA, followed by a post hoc Tukey-test.

Results

Phonotactic paths

To reconstruct the phonotactic paths of males their coordinates were plotted at the time when they produced a chirp. Figure 2 shows examples of three males tested at elevations from 0° to 75°, which are rather representative for all males tested. The total distance covered within 50 signal/response cycles decreased for male # 15 with increasing elevation from more than 3 m at an elevation of 0° to less than 1 m at 75° elevation (Fig. 2a). The male clearly oriented to the sound source up to an elevation of 60°, although walking backwards for a short time at this elevation. At 75°, however, the acuity of the orientation movements decreased strongly. The male circled, walked back and forth and, thus, the distance covered from the starting point was small.

Eleven out of 21 males followed this general pattern of phonotaxis with increasing elevation angles. The walking path of three males exhibited a handedness, as they consistently walked phonotactically with some deviation from the midline (see example of male # 17 in Fig. 2b). This was true for all elevation angles. In the remaining males, the phonotactic path was more or less accurate up to stimulus angles of 75°. Male # 21 was the most extreme example (Fig. 2c), and it walked almost in a straight line towards the sound source at an elevation of 0°. The maximal lateral deviation from the midline was only 3.8 cm, which resulted in the high ratio of 0.98 between the ideal and actual path. This accuracy remained almost the same with increasing elevation of the stimulus. Even at an elevation angle of 75°, the ratio between the ideal and the actual path length was 0.96, a value other individuals did not achieve even with horizontal loudspeaker positions. In contrast to male # 15, male # 21 never walked backwards, and the maximum lateral deviation from the straight direction to the sound source at 75° was 13.9 cm.

Summarizing the approaches of all males, the mean path length covered in one phonotactic approach (50 stimulus-reaction cycles) was 313 cm, and this did not differ significantly for loudspeaker elevations of up to 75°. Hence, testing all four elevations, the insects walked over a total distance of more than 12 m.

Figure 3 summarizes the results on the ratio between the ideal and the actual path obtained in all males. This ratio is a quantitative measure for the orientation acuity; males walking in a straight line towards the loudspeaker would achieve a ratio of 1.0. This ratio did not differ at elevations of 0° and 30°, but was significantly smaller at 60° compared with 0° and at 75° compared with 30°. Thus, the males´ meandering increased with increasing loudspeaker elevation. The standard deviation indicated in Fig. 3 demonstrates rather small interindividual differences at elevations of up to 60°, whereas individuals performed rather differently at 75°, where individual values ranged from 0.28 to 0.96 (compare also with Fig. 2).

As soon as *L. punctatissima* males perceive a simulated female reply, they perform a turn, the magnitude of which depends on the magnitude of the stimulus angle. The correlation between stimulus and turn angle is shown for male # 15 for different loudspeaker elevations in Fig. 4. When the loudspeaker was in the horizontal plane, almost all values were restricted to stimulus angles within ±40° of the longitudinal body axis. Thus, the male positioned itself with its body axis more or less aligned to the loudspeaker position. Stimulus-related turns occur in the first and third quadrant (stimulus/turn angles positive) in the majority of cases; turns to the incorrect side (values in the second or fourth quadrant) are rare and are restricted to the more frontal region. As a consequence, the correlation between stimulus and turn angles is rather high ($r = 0.85$). At elevations of 30° and 60°, some larger stimulus angles occurred, but there is still a high correlation between stimulus and turn angle, and incorrect turns occur with one exception only at small stimulus angles. At an elevation of 75°, however, stimulus angles up to 170° occur, and turns to the incorrect side
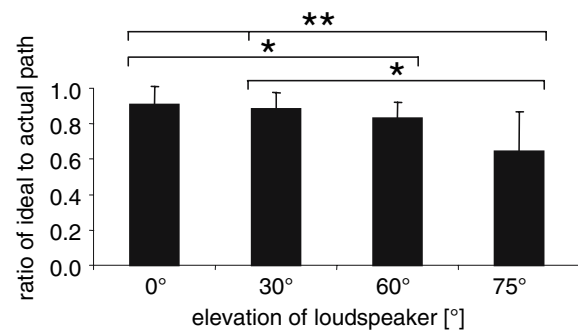

Fig. 3 Summary of the analysis of phonotactic paths of 21 males expressed as the ratio of the ideal path to the path actually taken by males. A ratio of 1.0 would represent a path in a straight line into the direction of the loudspeaker. *Asterisks* refer to the statistical significance of $P < 0.05$ and $P < 0.01$, respectively. Friedman repeated measures ANOVA, followed by a pairwise multiple comparison (Tukey-test)

Fig. 4 Relationship between stimulus angle and the subsequent turn angle analysed for one male at loudspeaker elevations up to 75° (same male # 15 as shown in Fig. 2a). Note the high correlation from $r = 0.85$ up to $r = 0.75$ at an elevation of 60°, with only few incorrect turns centred at small stimulus angles in the frontal zone of the male

Elevation 0°

$r=0,85$
$p<0,001$

Elevation 30°

$r=0,8$
$p<0,001$

Elevation 60°

$r=0,75$
$p<0,001$

Elevation 75°
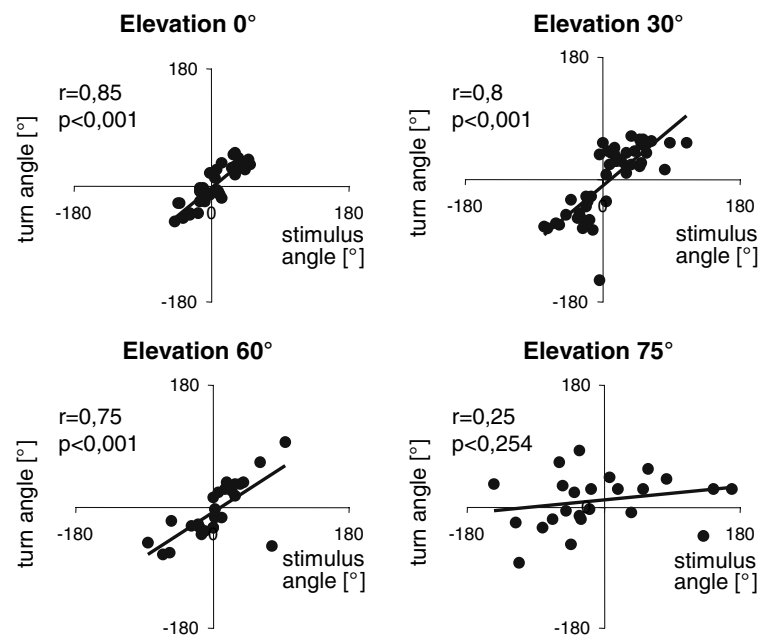
$r=0,25$
$p<0,254$

are almost as frequent as correct turns. Consequently, the correlation between stimulus angle and turn angle is low ($r = 0.25$), and the animal has obvious difficulties to orient (compare with the phonotactic path of the same male # 15 in Fig. 2a).

Figure 5 summarizes the data on stimulus-related turn angles for all tested males and elevations up to 75°. The mean correlation values for all elevations are above 0, indicating a positive correlation between stimulus and turn angle. There was no significant difference between values at elevations of 0° and 30°, whereas at 60° the correlation coefficients were significantly smaller compared with 30°. The mean value of 0.61 indicates a decreasing but still sufficient acuity. Up to an elevation of 60° the interindividual variation in the correlation coefficient was rather small (see standard deviation in Fig. 5), but with a loudspeaker elevation of 75° individual correlation coefficients varied strongly between 0.095 and 0.759. Therefore, there was no significant difference compared to 60°, although the mean correlation coefficient was smaller at 75° ($r = 0.48$).

Orientation to sound sources elevated by 90°

Nine males were also tested for their orientation behaviour to sound sources elevated by 90°. Figure 6 shows representative examples of phonotactic paths of two males under this stimulus situation where, ideally, no binaural cues should be available for the orienting insect.

Note, however, that because of the fixed assembly of the loudspeakers with the walking compensator, the actual elevation angle of 90° decreases with increasing
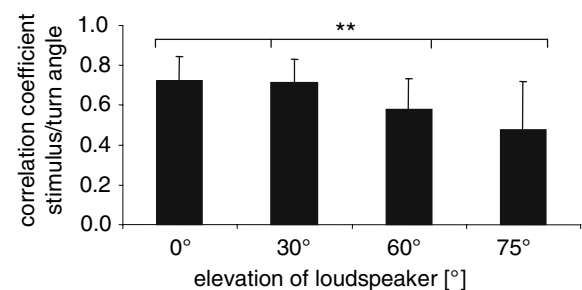
Fig. 5 Summary of the analysis of phonotactic paths of 21 males with respect to the accuracy of stimulus-related turn angles. *Asterisks* refer to a statistical significance of $P < 0.01$. Friedman repeated measures ANOVA, followed by a pairwise multiple comparison (Tukey-test)

lateral deviation of the males (see Materials and methods), so that the mean actual stimulus angle was about 80°, depending on the position of the animal. All males circled strongly under the sound source and deviated much more from the axis of loudspeaker orientation compared to smaller elevation angles. There are more missing turns at the 90° elevation (30 ± 10.4) compared to an elevation of 75° (7.7 ± 7.5).

A peculiar behaviour associated with spatial orientation can be observed in *L. punctatissima* when males tilt their head and thorax in a forward direction by up to about 45° relative to the horizontal plane, as shown in a reconstruction of video frames in Fig. 7. This movement is often associated with a shift of the longitudinal body axis by up to 30° to either side and also with bending the dorso-ventral axis to left and right. Such behaviour occurs both while the male is stationary and still singing or during walking. It has never
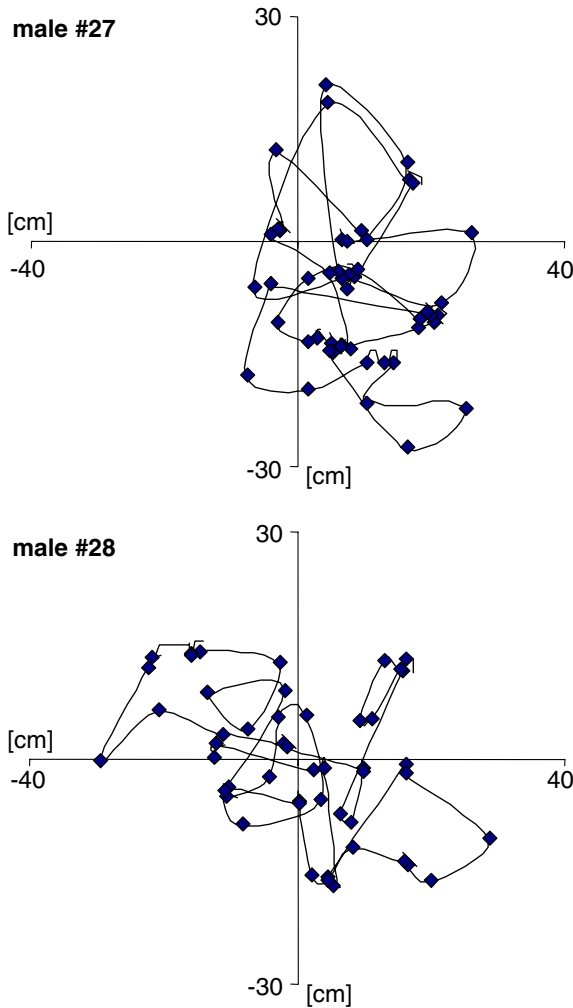
male #27

male #28

Fig. 6 Orientation behaviour of two males on the walking belt when the female reply was broadcast from above the insect at an elevation of 90°. Note the strong disorientation of both males, evident in the circling orientation behaviour
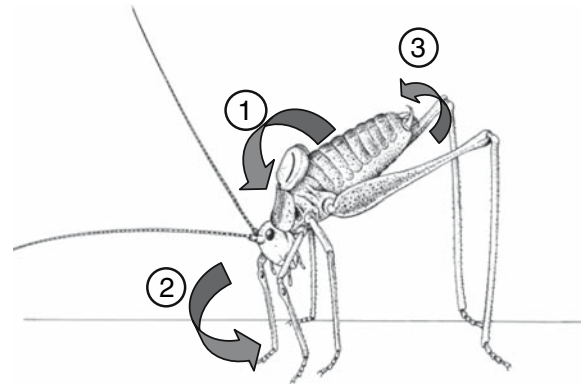

Fig. 7 Schematic reconstruction of the body posture of males of *L. punctatissima* while they exhibit the tilting behaviour. The numbered arrows indicate (*1*) the torque movement, (*2*) turning on the spot with a certain yaw angle and (*3*) a roll of the body axis to either side

been observed in males not motivated for mate finding, as indicated by their singing activity.

The frequency of tilting behaviour differed strongly between individuals. Though one male exhibited no tilting at any loudspeaker position, other males showed tilting repeatedly two to seven times in succession with a different orientation of the body in each case before continuing phonotaxis. The behaviour occurred significantly more often at 75° and 90° (mean frequency/trial 5.3 ± 5.6 and 6.9 ± 4.7, respectively) compared to 30° elevation (2.3 ± 3.6; $P < 0.02$).

Discussion

In a previous study (Rheinlaender et al. 2006b), we have investigated the ability of the duetting bushcricket *L. punctatissima* to orient to one of three loudspeakers, positioned at different levels in an artificial grid system, where the male could reach each point in space with almost equal probability. The main result was that all males tested reached the elevated, horizontal and depressed loudspeaker positions with only little deviation from the shortest possible path. This remarkable spatial orientation is achieved in spite of the fact that the insects have no specialized external ear structures as in mammals, or some owls, where the detection of the elevation of a sound source depends either on pinnae or on ear asymmetries (review in Knudsen and Konishi 1979; Heffner and Heffner 1992; Brown 1994).

Sound source elevation and accuracy of phonotaxis

The aim of the present study was to further explore the spatial orientation of *L. punctatissima* on a walking compensator. It appears counter-intuitive to study spatial orientation in an experimental paradigm where any movement of the insect away from a certain location is compensated. However, the advantage of the current approach is the fact that the orienting male always remains at the same location relative to the loudspeakers, and by repeating the loop of male call, virtual female response and orientation movement many times, we obtained quantitative measures on a number of variables of the orientation behaviour for each male and loudspeaker elevation. This would be impossible for an insect with any freedom of movement while orienting and approaching a sound source in a three-dimensional environment, whether artificial, as in our previous study, or in a natural habitat.

The hypothesis underlying our approach was that with increased elevation of a sound source the binaural

cues available for the orienting insect would decrease up to the point when the sound source is exactly above the insect (the same would, of course, be true for a depressed sound source, which was not tested here). One could expect either a more or less linear decrease of the accuracy of phonotaxis with increasing source elevation (and thus decreasing binaural cues) or a phonotaxis that remains almost unaffected until the binaural cues decrease to a threshold level below which phonotaxis is impossible.

Our results would support both interpretations: on the one hand, there appears to be a critical source elevation, where males are unable to receive reliable information concerning the location of the acoustic target, most evident in the circling behaviour on the walking platform (see e.g. Fig. 2a, b). For the majority of males, this occurred at a source elevation of 75°, but some individuals still performed extremely well at 75° (Fig. 2c). Thus, if one assumes a minimum binaural difference for successful orientation, some individuals do achieve this at higher elevation angles either due to better peripheral cues or more reliable central nervous processing. The example shown in Fig. 2c would suggest that males that perform better at high elevations, probably resulting from greater binaural symmetry or steeper excitation gradients, also perform more precise in the azimuth. However, whereas some males confirmed this prediction, other males showed a high acuity at 75° but meandered considerably at 0°.

The observed interindividual variation was the starting point for a current neuroethological approach where the individual accuracy of phonotaxis will be correlated with the individual, rather than the population mean, coding of spatial information in directionally sensitive auditory interneurons. Such a correlation of individual behavioural performance with certain measures of neuronal processing will be particularly interesting in those individuals who exhibited some handedness in their walking path (Fig. 2b), because it is tempting to speculate, that this is causally related to some asymmetry in the neuronal representation of the stimulus in direction-sensitive auditory interneurons. For example, Hardt (1988) reported results on seven individuals of the bushcricket *Tettigonia cantans* that exhibited some degree of handedness in their phonotactic behaviour, which was correlated with an asymmetry in the discharge of the pair of T-fibres (but see Bailey and Yang (2002) who found no difference in phonotaxis acuity between symmetrical female *Requena verticalis* and those with an experimentally induced asymmetry in hearing threshold).

However, most data favour the interpretation that with increasing elevation angle there is a gradual decrease in binaural information and a corresponding decrease in the accuracy of phonotaxis. For example, the ratio of the ideal to the actual path on the walking belt decreased from 0° to 75° elevation, with significant differences between 30° and 60° and also between 60° and 75° (Fig. 3). Similarly, the correlation coefficient of stimulus versus turn angles also decreased from 0° to 75°, more or less gradually (Figs. 4, 5). For both quantitative measures of the accuracy of phonotaxis, the variability was highest at 75° elevation, again indicating that individual differences may account for only little deviation from a straight path in one individual, in contrast to disoriented circling behaviour in the other (Fig. 2a, c). Another hint for the gradually decreasing acuity of phonotaxis is the higher frequency of false turns with increasing elevation. Though such turns to the wrong side only occurred at stimulus angles of ±10° of frontal stimulation, their incidence was also higher at larger stimulus angles with increasing elevation of the sound source (see Fig. 4; 60° elevation). Furthermore, stimulus angles became larger with increasing elevation, demonstrating that males meandered more.

Tilting behaviour: acoustic scanning or acoustic beaming?

A very peculiar behaviour could be observed in a number of males that we interpret as a mechanism of acoustic scanning under conditions of weak or missing localization cues. The most obvious part of the behaviour happens when the male tilts its head and thorax downwards (thus tilting behaviour, see Fig. 7), which is comparable to the torque movement in the steering of a flying insect. In addition, and often at the same time, the male performs a certain yaw-angle and rolls its body axis to one or the other side. Sometimes, the males showed the tilting behaviour repeatedly in succession, in each case with a different orientation of the longitudinal body axis. The strongest evidence for the interpretation as acoustic scanning is the fact that the frequency of tilting occurred significantly more often at 75°, when most males started to circle, and at 90°, where no binaural differences are available. We also found a higher incidence of tilting at the beginning of a trial, when the male was placed at the starting platform. Therefore, we hypothesize that by altering the orientation of the body in all three axes during tilting leads to self-induced changes of binaural differences. This is due to the fact that the main acoustic input to the ears in the forelegs is not via the two tympanal membranes, but via the acoustic spiracle and leg trachea, guiding in particular high-frequency sound to the inner side of the ear (Michelsen et al. 1994; Michelsen

1998). The described changes in body posture will lead to changes in the diffraction of the sound at the position of the spiracular opening(s) and around the body and thus to the changes in binaural cues. If, for example, the source would be elevated by 45° and at an azimuth of 60°, and the insect then performs a tilting of the thorax by 45°, the next female reply would occur at an elevation angle of 90°, with no more binaural cues available. By comparing successive binaural cues and corresponding discharge differences within the central nervous system, the insect could obtain at least a rough estimate of whether the source is above, below, left or right. In this respect, the tilting behaviour in *L. punctatissima* could be similar to that of vertebrates that move their heads to localize a sound source [see e.g. Gerhardt and Rheinlaender (1982) for frogs].

We cannot exclude, however, another possible explanation for the tilting behaviour. Because the broadcast sound amplitude is directional and highest in the dorsal direction, the function of the tilting behaviour may also be to direct the acoustic output into a particular direction, rather than obtaining directional cues about the vertical orientation of the female. Although the two explanations are not mutually exclusive, and we cannot distinguish between both on the basis of our observations, we nevertheless favour the acoustic scanning hypothesis. In our experimental paradigm, the male was in duetting contact with the female from the very first own call and perceived a reply at least 15 dB above his threshold for phonotaxis, regardless into which direction he was broadcasting his song. Beaming the acoustic output randomly into various directions would be adaptive in situations, when the male has not yet made acoustic contact with a female. Otherwise, after the acoustic contact has been lost, beaming into a particular direction from where the female reply had been detected before would be adaptive as well, but both situations never occurred in our experiments. Given the higher incidence of tilting in situations of much reduced directional cues at 75° and 90°, we would thus favour the acoustic scanning hypothesis.

Considering the short duration of only one millisecond of the female reply to which the male must orient and the small number of receptor cells that are involved in the processing of directional information, the acuity of orientation of *L. punctatissima* is remarkable. Neurophysiological studies by Hardt (1988) showed that receptor fibres stimulated with such a short female signal respond with no more than one action potential/stimulus. Thus, in contrast to other communication systems in Orthoptera, there is neither a dynamic in the intensity response function of a single receptor, nor is there a graded information available to

changing stimulus angles. Thus, the only remaining information is the number of fibres activated, because these fibres differ in their absolute sensitivity and/or tuning (Hardt 1988). In the last paper of this series we will, therefore, describe the peripheral and central nervous mechanisms underlying the remarkable spatial orientation in *L. punctatissima*.

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