Hearing asymmetry and auditory acuity in the Australian bushcricket *Requena verticalis* (Listroscelidinae; Tettigoniidae; Orthoptera)

Winston J. Bailey* and Suanne Yang[†]

Department of Zoology, University of Western Australia, Nedlands, Western Australia 6009, Australia

*Author for correspondence (e-mail: wbailey@cyllene.uwa.edu.au)

[†]Present address: Department of Biology, Washington State University, Vancouver Campus, 14204 NE Salmon Creek Avenue, Vancouver, WA 98686–9600, USA

Summary

Fluctuating asymmetry in bilaterally placed sensory structures of insects may be considered maladaptive; natural selection should favour a symmetrical system. Among bushcrickets or katydids, threshold hearing sensitivity is, in part, dependent on the size of the auditory spiracle in the prothorax. We tested the degree of natural asymmetry in the hearing system of the ensiferan orthopteran *Requena verticalis* by measuring the size of the auditory spiracle of females. Naturally occurring asymmetry approached 8%, which translates to a hearing bias at a threshold of only 0.8 dB. Auditory asymmetry of females was experimentally exaggerated by packing cotton wool into either the right or left auditory spiracle. We made neurophysiological recordings from the ascending auditory T-fibre in the neck connective and found that the left–right bias created by this operation approximated 5 dB; this is greater than that found in nature. For these experiments, sound was delivered to the operated side of the insect from a speaker placed at either 90° or 30° to the long-body axis. To test the influence of this induced auditory bias, free-moving females were allowed to orient towards a speaker emitting male calls at near-natural call intensities of 51 and 82 dB (SPL), 80 cm from the speaker on a flat arena. There was no variation in angle or vector between experimental and control insects, and there was no difference in acuity between intensity. We discuss the relevance of threshold measurements of bushcricket hearing systems in regard to sound localisation and, from these experiments question any role of asymmetry.

Key words: Fluctuating asymmetry, sound localisation, auditory spiracle, neurophysiology, bushcricket, *Requena verticalis*, Tettigoniidae, Orthoptera.

Introduction

Where animals depend on a comparison between left and right auditory inputs to locate their prey or mates, the reasonable expectation is that ears should remain symmetrical. However, this is not always the case, and in some animals auditory asymmetry has been shown to be adaptive, improving localisation for specialised tasks. For example, the vertical asymmetrical placement of the external auditory meatus in some species of nocturnal owls increases the perception of the elevation of certain prey-generated sounds (Payne, 1971; Knudsen et al., 1984), and asymmetry in nasal orientation of sperm whales is also considered adaptive in echolocation (Cranford, 1992). Among insects, auditory directional asymmetry in the tuning of tympana of the water bug *Corixa punctata* appears to accommodate left–right differences in the frequency response of associated air bubbles that cover the tympana (Prager and Larsen, 1981; Prager and Streng, 1982).

Earlier work, mainly with the Orthoptera, imposed asymmetry on the insect as a means of demonstrating the requirement of binaural input for acoustic orientation – the insect needs two ears to locate its prospective mate. Hence, the early studies of Regen (1924) showed quite simply that female *Gryllus campestris* with one tympanum removed still found the chirping male "but with much more difficulty". Since then numerous authors have tested the ability of various Orthoptera to orient to a sound source with unilaterally modified ears. Experimental modifications have ranged from the insect having the tympanum of one ear ablated (Murphey and Zaretsky, 1972; Zaretsky, 1972), to having one ear covered with wax or petroleum jelly (Bailey and Thomson, 1977; Bailey and Stephen, 1984), having the secondary entry of sound in bushcrickets, the auditory spiracle, variously blocked or partially blocked (Bailey and Stephen, 1984; Schmitz et al., 1988) and, perhaps the ultimate experiment, removing the foreleg altogether (Huber et al., 1984; Schmitz et al., 1988). The question was invariably one of functionality: how does the central nervous system interpret often-extreme asymmetry at the periphery? Regen's experiments, and those that followed, merely wished to show that directionality involved bilateral sensory input and that, by varying the degree of auditory asymmetry, the insect's ability to locate a sound source would change.

Measuring left–right differences in auditory input by the activity of peripheral (e.g. Hill and Oldfield, 1981) and central (e.g. Rheinlaender and Römer, 1980; Römer and Krusch, 2000) auditory neurones (reviewed in Rheinlaender and Römer, 1990) shows that the hearing system is directional. In all cases, the regions of maximum sensitivity of each ear form an ellipsoid field, with the most sensitive region normal to the long axis of the insect and the field overlapping with that of the opposite side along the midline. Moreover, left–right differences are enhanced by inhibitory activity of the central neurones (Rheinlaender and Römer, 1990). With these two threshold-determined sound fields, theory suggests that the insect should react to comparisons between each side by turning towards the more stimulated side. Predictably, any perturbation of this binaural balance should lead to a directional bias in the track of the orienting female.

Fluctuating asymmetries (FA) in nature are random changes in a trait expressed by right-minus-left differences that, in otherwise bilaterally symmetrical animals, are normally distributed about a mean of zero (Palmer and Strobeck, 1986). Where there is a link between FA and non-random mating preferences, one prediction is that the offspring of successful females indirectly benefit from the females mating with males of superior genetic quality; the so-called 'good genes' hypothesis (e.g. Liggett et al., 1993; Møller, 1991). One explanation for variation in FA within a population is the impact of developmental history, with the assumption that individuals taking longer to develop, or perhaps with less access to suitable nutrients, should show higher levels of FA than well-fed individuals (e.g. Møller and Swaddle, 1997; Simmons et al., 1999a). Within this concept females should assess male FAs directly, where FA is associated with sexual signalling (Møller, 1991, 1992), or indirectly, by evaluating signals that are correlated with FA (Simmons and Ritchie, 1996).

Faure and Hoy (2000) give a comprehensive and valuable review of auditory symmetry in insects and vertebrates. They examined the physiology of a katydid ascending auditory neurone with recognised importance in the lateralisation of sound; the ascending T-fibre. But again, these authors failed to find any manifestation of FA within the central nervous system that could be reliably linked with sexually selected traits; as they point out, such a system can only read what the peripheral system supplies. Intuitively therefore, crickets and bushcrickets should be ideal models to test hypotheses concerning sensory asymmetry; they are bilaterally symmetrical and, as indicated above, data from extensive neurophysiological and behavioural studies show how sound localisation is highly dependent on balanced bilateral hearing (Rheinlaender and Römer, 1990).

Hearing in the ensiferan Orthoptera is dominated by two auditory inputs: first, the external tympana beneath the knees of the forelegs and, second, the associated acoustic tracheae, with their often voluminous auditory bullae and open spiracle (Bailey, 1990). In bushcrickets, the auditory trachea opens through the auditory spiracle and appears the most significant feature of the insect's hearing system that influences the ear's overall sensitivity (Seymour et al., 1978). Furthermore, the size of the auditory spiracle is positively correlated with hearing sensitivity (Nocke, 1975; Bennet-Clark, 1984; Bailey, 1993), and, just as importantly, spiracle dimensions vary with body size; larger insects are more sensitive to sound than smaller insects (Bailey, 1998), and ear size and, hence, hearing ability appears to influence mating outcomes. For example, in the tettigoniid *Kawanaphilla nartee*, females with larger ears can hear the brief sounds of their males better and, as a result, achieve higher mating success (Gwynne and Bailey, 1999). Given this degree of between-individual variation in ear size among species of tettigoniids, there is at least an opportunity for small differences in ear size to influence a female's ability to track a sound source.

In this paper we take the assumptions of Faure and Hoy (2000) a step further. We first examined the degree of left–right symmetry in the auditory spiracle of female *Requena verticalis*, and we then related the degree of naturally occurring asymmetry to predicted hearing loss derived from an earlier study of this species (Bailey, 1998). Based on this evidence, we then reduced the sensitivity of one ear to a level that may be considered two standard deviations outside the natural range of variation. With this asymmetry, we measured both hearing loss, as expressed by the ascending T-fibre, and tested the unrestrained animal's ability to orient to conspecific calls on an open arena.

Materials and methods
The insect

Requena verticalis (Walker) is a common and widely distributed species of tettigoniid around metropolitan Perth (Western Australia). For these experiments, we collected immature females from the watered gardens of the campus of the University of Western Australia. Insects were fed on a diet of cat food and pollen, with water provided daily. During trials females were housed in separate containers for the duration of the experiment. The sample of females used for morphometrics was increased by collections of adult females from the field.

We found that during the phonotaxis trials, females became unresponsive to the stimulus and attempted to oviposit into the mesh surface of the arena. To overcome this problem, virgin females were allowed to lay parthenogenic eggs in sand, after which it was found that they were again responsive to the male call and could be used in the trials.

Morphology

Freshly killed females were measured in the following manner. The lengths of the pronotum and femur were measured with dial calipers to 0.01 mm as described in Bailey (1998). The aperture of the prothoracic auditory spiracle (Fig. 1) of both right and left sides was measured using the digital scanning process of Optimus™, which provided an area based on a defined perimeter. This technique was calibrated by more direct measurement using an eyepiece graticule, where
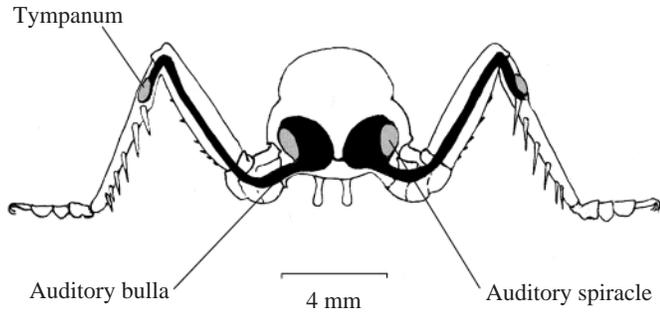
Fig. 1. A schematic view of the auditory tracheal anatomy of *Requena verticalis* viewed from anterior from the bisected animal. The open auditory spiracle is shown as grey against the black auditory bullae. (Drawn, with permission, from Bailey and Kamien, 2001).

we estimated area by measuring three of the diameters of the roughly ellipsoidal spiracle opening. We also tested for repeatability by repeating measurements on randomly chosen females.

Behaviour – Phonotaxis and asymmetry

All phonotaxis trials were carried out in a large anechoic room (4m × 5m) on an arena made of mesh that was elevated 20 cm above a carpeted floor. Two concentric circles of radii, 30 cm and 60 cm, and two perpendicular lines quartering these circles were marked on the surface of the arena. Speakers (leaf tweeters EAS-10TH400A) were mounted above the surface of arena one at each end of the perpendicular lines. The male calls, previously recorded using a Bruel and Kjaer 4133 microphone *via* a B and K 2209 sound level meter as a pre-amplifier, were stored digitally to Signal™ A-D hardware with a sampling rate of 150 000 samples s. Details of the calls of this species can be found in Schatral and Bailey (1991). There are two frequency peaks within the typical broad-frequency-band call, one at 16 kHz and the other at 28 kHz.

For every trial, speaker side was selected at random. Females were released through a hole cut in the centre of the arena from their holding vial, which was suspended by its top beneath the mesh arena. In this way insects only received directional information as they climbed to the surface. Experiments were separated into speaker intensities of 82 dB and, in a second series of trials, at 51 dB. This second series of trials resulted from a post-hoc hypothesis, based on results from the 82 dB trial, that effects of asymmetry would be more pronounced at lower rather than higher intensities.

A signal at 82 dB is equivalent to a male some 40 cm away (Bailey et al., 1993), and so females emerging from the centre of the arena some 60 cm from the speaker would experience high intensity sound. However, the power output of the speakers was conical rather than spherical, and so we compensated for the fall-off in intensity as the insect moved away from the speaker axis by using higher than natural intensities at the centre of the arena. For the second series of trials, we compared pre- and post-operational phonotaxis paths

and the time to reach two key points on the arena (see below). As part of the second series of trials with low intensity, we controlled for intensity differences on movement rates by running unblocked females at low and high intensity.

Speaker levels were calibrated using the B and K 2209 sound level meter to produce equal intensity calls at the centre of the arena of 82 dB (first trial, 2001) and 51 dB (second trial, 2002) (sound pressure level, SPL rel 20 µPa). All intensity measurements and speaker calibrations were carried out using 200 ms bursts of broad-frequency-band sounds covering the same frequencies as in the call, which were created using Signal™ at equivalent peak-to-peak levels to the male song. This obviated problems of signal measurements common for rapidly modulating signals, such as the natural insect call (see Prestwitch et al., 1989).

Females were acclimatized to the anechoic room (reverse light and temperature cycle between 20–25°C) for 24 h and remained within the room for the duration of the experiment. Trials were run under dim-red lighting suspended at a midpoint between the speakers; this reduced the impact of visual cues (see Weber et al., 1987). A female was selected at random and placed in the centre of the arena. The top of the container was removed at the same time as the male call was played. Females were allowed 5 min to respond to the sound stimulus. If no obvious phonotactic movement was discerned during this period, the trial was aborted and the female was put to one side as non-responsive. The path of responsive females was manually traced onto paper, and the times at which the female crossed the 30 cm and 60 cm circles were recorded. We have found that this rather more direct method of recording movement patterns was within 5% error of retracing movement paths from video recordings from a low-light camera mounted above the insect.

24 h prior to each experiment females were lightly anaesthetized with CO_2. We created asymmetry by inserting lightly compressed cotton wool into either of the randomly selected right or left spiracles. Once the female had completed a trial with a block, the cotton wool was removed and she was run again within minutes of the removal. Removing the cotton was a simple exercise compared with blocking. The cotton wool was retained within a vial for later weighing, thus allowing us to monitor any variation in block mass.

Neurophysiology

Female *R. verticalis*, with one randomly chosen spiracle blocked with cotton wool, as described above, were mounted inverted on a light wire frame with wax, with legs held bent and at 90° to the long body axis. The ventral nerve cord at the neck was exposed and the ipsilateral nerve elevated over a fine silver-wire hook electrode. An indifferent electrode was inserted into the abdomen. The open neck and electrode were then covered with petroleum jelly.

Speakers (see above) were mounted at a distance of 40 cm and at 90° and 30° to the female's longitudinal body axis. The stimulus consisted of a 200 ms pure tone at either 16, 22 or 28 kHz, representing the two major peaks of the male call, 16

and 28 kHz, and the mode frequency (22 kHz) of the broad-band call. The signal was exponentially tapered at the beginning and end at 5% of its total length. Calls were presented with a 1 s interval and frequency order was randomised.

Threshold was determined visually on an oscilloscope where spikes of the T-fibre could readily be detected. Sound intensity was adjusted in steps of 1 dB by passive Hewlett-Packard attenuators in line with the signal amplifier. In this way, we estimated threshold as the sound level at which nerve spikes could be counted for 50% of the presentations (for a comparable procedure, see Faure and Hoy, 2000). We repeated threshold measurements for all three frequencies and as soon as threshold had been determined for each female the cotton block was removed by fine forceps and threshold re-determined.

Because of the nature of our experimental set up, it was more convenient to measure thresholds at 90° with one set of females and then again for a second set at 30°. We selected these two angles as polar plot images of similar tettigoniids (Hill and Oldfield, 1981), with the presumption that sensitivity would be at a maximum at 90° ipsilateral and at a minimum at 90° contalateral, with a crossing-point to a null close to 30°. Sensitivity is lower towards the mid-line and, because our aim was to detect left–right differences in threshold caused by the effects of the cotton block, we restricted our measurements to these two angles.

Results

Auditory asymmetry

While spiracle area is positively correlated with body size, expressed as pronotum length (r^2=0.52, F=29.97, P<0.001, N=30) (Bailey, 1998), there was no relationship between left–right symmetry and body size (Fig. 2). Small females do not show higher levels of asymmetry than larger males and, interestingly, the range of FA experienced in hearing morphology was remarkably close to that for hind tibia length for *R. verticalis* examined by Simmons et al. (1999a).

Because our hypothesis related to relative differences either side of the insect, we calculated percent area differences as frequency within our population of *R. verticalis*. In other words, to what extent are females asymmetrical in regard to spiracle area, and what is the largest detectable left–right difference? Differences ranged from 0 to 8%, which translates to maximum area differences right–left of 0.03 mm^2. In order to relate this to expected right–left difference in threshold as dB loss (or gain) per unit of area, we used the regression of threshold on spiracle area (y=–24.97x+51.55; r^2=4.38) provided for *R. verticalis* by Bailey (1998). In this case right–left differences of 0.03 mm^2 would approximate 0.8 dB differences.

Neurophysiology

Before we attempted to test phonotaxis with insects whose ears were blocked with cotton, we needed to know if such a manipulation affected hearing thresholds and by how much.
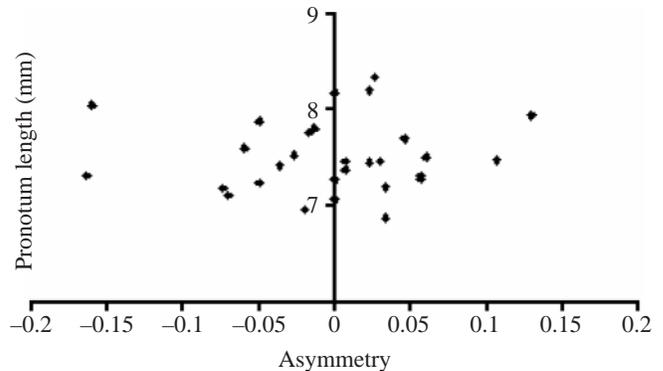

Fig. 2. Association between left–right symmetry of spiracle area and female body size expressed as pronotum length. (r^2=0.004) (N=29).

For the three different frequency treatments, which, as indicated, represented both the lower and upper peak frequencies and also the modal frequency of the call, we presented sounds at 90° to the female's body axis. The difference between threshold intensity when blocked and unblocked ranged from 5 to 6 dB. For all three treatments, the mean threshold when blocked was significantly higher than the mean threshold when unblocked (two sample t-test for means). At 16 kHz, the mean threshold was 44.3 dB when blocked and 39.0 dB when unblocked (N=11, t=9.305, P<0.001). At 22 kHz, the mean threshold was 50.7 dB when blocked and 44.7 dB when unblocked (N=19, t=11.662, P<0.001). At 28 kHz, the mean threshold was 51.4 dB when blocked and 45.3 dB when unblocked (N=11, t=11.490, P<0.001). Packing cotton wool into one of the spiracles created an asymmetry far exceeding naturally occurring asymmetry (see above). The ranges of threshold measured by the T-fiber were within 2 dB of single-neuron unit measures made by Römer et al. (1998) for *R. verticalis*.

Measurements at 30° showed a lower but wider range of 1–5 dB between blocked and unblocked spiracles and, as with the 90°-presentation angle, all blocked recordings gave higher thresholds than the unblocked condition. At 16 kHz the mean threshold was 42 dB blocked compared with 40 dB unblocked – a significant difference (N=7; t=8.23, P<0.001). Both blocked conditions for 22 kHz and 28 kHz were similarly significantly different (22 kHz, mean = 45 dB blocked, 41.9 dB unblocked; N=7, t=7.78, P<0.001; 28 kHz, mean = 48.3 dB blocked, 45.3 dB unblocked; N=7, t=13.75, P<0.001). Faure and Hoy (2000) found a consistent increase in threshold for *Neoconocephalus ensiger* when measured along the insect's midline compared with at 90°. Contrary to this expectation, we found no significant difference between blocked and unblocked threshold values for the 30°- and 90°-presentation angles.

Behaviour – phonotaxis

Regardless of threshold left–right differences in auditory symmetry created by our experiment there was no significant effect of blocking on the female's ability to find the target. The

first set of experiments used a sound intensity of 82 dB (SPL). The mean time for females to reach the 30 cm circle with a spiracle blocked was 46.2±35.23 s (mean ± S.D.) compared to without block 55.63±41.45 s (paired *t*-test, *t*=0.549, d.f.=11). Similarly, the mean time taken to reach the speaker did not significantly differ with or without the cotton block (*t*=0.562, d.f.=11). The mean time to reach the speaker when blocked was 78.57±56.66 s and 93.82±66.82 s without the block. The second series of trials, with a different set of females, and run the following season, used a call intensity of 51 dB (SPL). As with the 82 dB trial there was no effect on the female's ability to find the target (Fig. 2C), and no difference in the time taken to reach the 30 cm circle (*t*=0.73; d.f.=17) or to exit the arena (*t*=0.64; d.f.=17), between blocked and unblocked females. Furthermore, there was no difference in the time taken to exit the arena between high (first trial) and low intensity trials (*t*=0.87; d.f.=20).

For the high intensity trial, the length of the path females took to reach the speaker did not significantly differ when the spiracle was blocked or unblocked. To reach the circle at 30 cm, females travelled a mean distance of 33.24±4.30 cm (mean ± S.D.) with the block and 34.21±6.31 cm without the block (*t*=0.536; d.f.=11). To reach the speaker, females travelled a mean distance of 70.42±15.14 cm with the block and 75.0±23.87 cm without the block (*t*=0.534). We measured the rate of movement with the expectation that, although angle to the speaker may not be affecting the time taken, the decision process, correcting for right–left bias, may influence the rate (scalar measures of distance and time to the vector of rate) at which females walked to the speaker. The average rate of movement to the 30 cm mark was 1.0±0.877 cm s^{-1} (mean ± S.D.) with the block and 1.1±1.058 cm s^{-1} without the block. The difference between these two values is not significant (*t*=0.1847, d.f.=11). Similarly, the total rate of movement to the speaker was not significantly different for each treatment (block: 1.2±0.77 cm s^{-1}; without the block: 1.2±0.851 cm s^{-1}; *t*=0.15305, d.f.=11).

The paths of females in the unblocked condition, as a control, are shown in Fig. 3 (left) with the call at 82 dB measured from the centre of the arena. The paths of females with the spiracle blocked are shown for the first series of trials at 82 dB (Fig. 3, centre) and for the second series of trials at 51 dB (Fig. 3, right). The orientation of the female was measured using the angle between the line drawn from the centre through the female's position and a straight line to the speaker. The intersection of female paths along the circumference of the two circles on the arena did not significantly differ with or without the block for trials at 82 dB. The mean angle at 30 cm when blocked was –11.2°, and –4.8° without the block. At 60 cm, the mean angles for blocked and unblocked were –1.4 and +1.3°, respectively. There was no difference in the angles of blocked and unblocked conditions (Mann–Whitney test: 30 cm (*Z*=0.316) and 60 cm (*Z*=0.865)).

Spiralling behaviour appears to be a characteristic of severe auditory bias (e.g. Bailey and Stephen 1984; Schmitz et al., 1988). For the 82 dB trial, a total of six blocked females made at least one loop and, of these, five had trials where no spiralling occurred. For the 51 dB trial, six females spiralled. In the first trial, two females spiralled in a direction opposite to the side with the blocked auditory input. In addition, a total of five control females made at least one spiral and three females spiralled in the same direction in trials with and without the block. In the second trial, five females spiralled towards the unblocked side and one spiral occurred to the blocked side, a trend in line with the prediction. In the control unblocked condition, 12 insects approached the speaker from the left side and nine from the right side, the remainder moved directly to the speaker. Although sound fields would have been far from uniform across the arena, we conclude there was no speaker bias, and, moreover, speakers were chosen at random with respect to side.

We expected that females would favour the side with the highest intensity, and, in moving to the most stimulated side before or during their approach to the speaker, would make

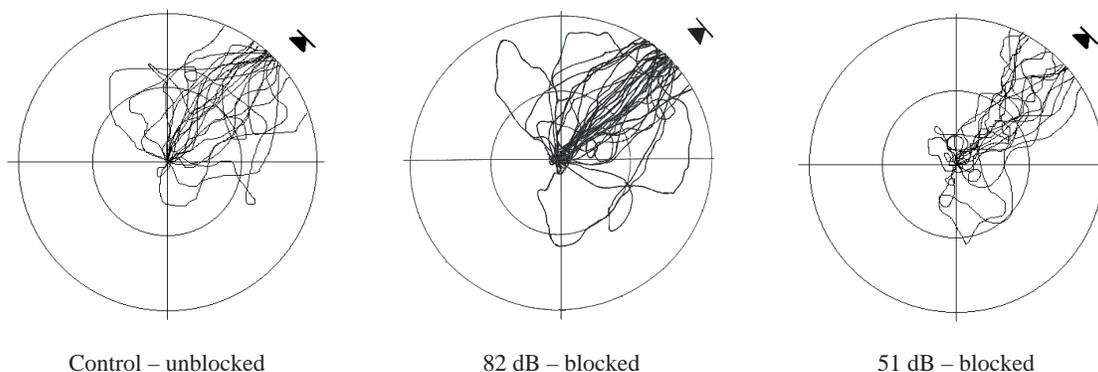| Control – unblocked | 82 dB – blocked | 51 dB – blocked |

Fig. 3. Phonotaxis by female *Requena verticalis* to speakers placed left and right of the arena. Traces of movement patterns to each side have been overlaid to the right side with the speaker position shown on right of the arena. Tracing of paths of all females (paired and unpaired cases) as (left) a control unblocked condition, with the call at 82 dB measured at the centre of the arena, (centre) with spiracle blocked with cotton at 82 dB and (right) with spiracle blocked at 51 dB. The concentric circles are distances 30 cm and 60 cm from the centre, at which point the females were timed in their progress towards the speaker (see text).

their final approach from this unblocked side. In other words, there should be a degree of bias, even though females were able to locate the target. We treated this analysis in two ways, first by using paired data only (N=12), four animals met this expectation while 3 made straight for the speaker showing no bias. The second was to include all data including repeated trials on the same female and those not included in paired tests (N=52). We found, with this larger sample, that 29 females approached the speaker from the side without the block, 14 approached from the same side as the block and 9 went straight towards the speaker (χ^2=5.23; P=0.022). However, in the second trial at 51 dB, we were unable to detect any bias in the direction of approach (Fig. 3, right).

Discussion

We have shown that the natural bilateral asymmetry of one of the controlling traits of sound input to *R. verticalis*, the size of the auditory spiracle, is so small that it has a negligible effect on a female's ability to find a sound source. Furthermore, that when the approximately 1 dB maximum left–right difference is experimentally increased to 5 dB by unilaterally blocking the spiracle with cotton wool, females show no diminution in their ability to find the target. The aim of these experiments was to demonstrate that sensory asymmetry, perhaps as a result of genetic or nutritional larval stress, could have a consequence on adult reproductive fitness; asymmetrical females would have less likelihood of finding a mate than symmetrical animals. We were unable to produce evidence for this.

The only effect on phonotaxis of unilateral blocking was a bias in the approach of orienting females responding to an 82 dB signal, and that was only detected when we combined all experimental data. If females turn to the most stimulated side then they should turn to the unblocked side in preference to the blocked side – the expectation was that females would spiral in much the same way as crickets (Schmitz et al., 1988) and bushcrickets (Bailey and Stephen, 1984) with completely blocked ears. However, the slight influence of an approximately 5 dB difference appeared to be corrected once females approached the speaker.

With the unexpected and negative finding at high intensities, we ran a second series of trials at lower sound intensity. The prediction again was that, while females may successfully orient towards the speaker at high sound intensities, because binaural differences are undoubtedly non-linear in terms of intensity (Römer et al., 1998), the effect of asymmetry should be more marked at low rather than high intensity. This was not the case, and there was no difference between the performance of females at 51 dB compared with 82 dB (SPL).

Insects and frogs appear capable of detecting extremely small differences in intensity between two or more calls arriving from more than one direction (Bailey, 1985; Latimer and Sipple, 1987; Bailey and Yeoh, 1988; Forrest and Green, 1991; Römer et al., 1998). While some of the evidence comes from the behaviour of the whole animal, much is derived from measurements of threshold, and many workers have questioned

the relevance of threshold to an orienting animal (e.g. Gerhardt, 1987). However, threshold does at least provide a reliable comparative measure of animal hearing variance as shown by earlier experiments with *R. verticalis*. Römer et al. (1998) demonstrated, at both behavioural and physiological levels, the capacity of female *R. verticalis* to detect small differences in sound intensity levels from two sources. Females would turn to the louder male call within a two-speaker trial where intensity differences of the two sources of sound were as close as 2 dB. In this case, left–right sensitivity was also reflected in physiological recordings of auditory fibers in the prothoracic ganglion. For both insects and frogs, perception of intensity differences as small as 2–3 dB improves at lower, rather than higher, call intensities. Stout and McGhee (1988) for example, found that at low-sound-pressure levels, 70% of female *Acheta domestica* chose a more intense call, differing by 5 dB, while only 12% did so at high intensities. Gerhardt (1987) similarly found that 100% of *Hyla cinerea* could detect 3 dB intensity differences at low intensities while only 50% did so at high intensities.

At a different level, Huber et al. (1984) (see also Schmitz et al., 1983, Schmitz, 1985; Schmitz et al., 1988; Weber and Thorson, 1989) examined the effect of the removal of one leg in the cricket *Gryllus bimaculatus.* Although affected by this ablation, the female was able to track the target source on a movement compensation wheel. Supporting this evidence, complete blocking of the spiracle of the bushcricket *Pachysagella australis* produced an identical effect to that in the cricket, where the insect spiralled to the intact side but the net movement was still towards the target (Bailey and Stephen, 1984).

Given the evidence from both simultaneous bilateral recording of paired auditory interneurons and experiments with severe disruption to the natural auditory symmetry, three hypotheses emerge that may explain, at least in part, the asymmetrical insect's ability to find a sound source. First, nocturnal insects undoubtedly rely on more than one sensory cue to locate a sound source. Visual cues in association with sound strongly influenced the walking paths of both the cricket *Acheta domesticus* (Weber et al., 1987) and, importantly for these experiments, the bushcricket *Poecilimon affinis* (Helversen and Wendler, 2000). Therefore, it is conceivable that under the conditions of low red light used in our experiments females could have taken a visual fix on the perceived sound source. This alternative explanation, however, should be treated with some caution as the spiracle-blocked insects were first used in the phonotaxis trials and so any initial experience should have set up an integrated neural bias left or right, and if the females associated visual cues with the direction of this sound, right-blocked females should have found the target from the right side and *vice versa* for left-blocked insects. This was not the case. Furthermore, if this spatial information had been retained from one trial to the next, as soon as the females were unblocked the error should have persisted. We found no such left–right error.

A second hypothesis suggests that the interaural null may be

so close to the mid-line that left–right differences created by bias in the peripheral auditory input are only influenced by gross changes (Römer and Krusch, 2000). In other words, shifting the null requires a significant manipulation, such as ablation or complete blocking of the auditory system on one side. Hence, due to its stochastic nature, internal neuronal noise will automatically create some discharge differences in ascending interneurones even with symmetrical inputs. Huber et al. (1984) suggest such a hypothesis, where background neural activities of contra-lateral neurones arguably provide a cricket with sufficient sense of direction to move to the sound source. Experimentally produced biases that merely increase interaural differences by 2–5 dB may have had an insignificant effect on the position of this null. But linked to this problem are signal-to-noise levels the experienced by the system. Thus, female *R. verticalis* in the first series of experiments, oriented to a signal close to 30 dB above auditory threshold and at these sound levels intensity gradients may simply take over from more 'refined' binaural information. In this way, left–right asymmetry may only affect sound localisation for distant males or for where the sound field is complex due reflection and absorption of surrounding vegetation (*vide* Rheinlaeder and Römer, 1986). It was for these reasons that we ran a second series of experiments in the following season to establish if left–right acuity could be maintained at much lower intensities. We chose 51 dB as a level close to threshold and much closer to noise levels measured between 10–25 dB of the anechoic room. Contrary to expectation, there appeared to be no difference in performance between the two intensities.

Finally, Faure and Hoy (2000) point out that as in most vertebrates, the central nervous system simply compensates for sensory asymmetry. For example, young owls show behavioural plasticity to experimentally induced hearing asymmetry, while adults fail to do so (Knudsen et al., 1982). Similarly, human subjects over time easily compensate for significant hearing asymmetries (Florentine, 1976). Therefore, these authors (Faure and Hoy, 2000) suggest that leg amputation experiments on larval crickets may lead to an adjustment at the central level of the adult, but there is no experimental proof of this. Interestingly, Weber and Thorson (1989), in reviewing the case on single-input orientation in crickets, note that the ability to find the male on a movement-compensation wheel varies between crickets, and in one case, a cricket, which they presumed to have re-grown a foreleg but without the tympana, had no problem in finding the male. Faure and Hoy (2000) state that further experimentation directed at the insect nervous system's plasticity may indicate the insect's ability to compensate for any manipulation on hearing symmetry, as in birds and mammals. In line with these ideas, we envisaged that time compensation might occur, particularly where there was a long time-gap between manipulation and testing. For this reason, females were subject to blocking at least 24 h prior to experimentation and many received their cotton block days in excess of this. However, as indicated above, as soon as the insect had completed a successful trial the block was quickly and simply removed (a

non-intrusive operation) and, within minutes, the insect was run again on the arena. We argue that if time-influenced neural compensation was a factor then restoration to the original symmetrical condition should have had immediate effect; there was simply no time to adjust. This was not the case.

Simmons at al. (1999b), following a meta-analysis of literature between 1990 and 1998, provide a cautionary note for the general applicability of empirical studies of fluctuating asymmetry in regard to sexual selection. Although these authors do not go so far as to say FA has no role in sexual selection, they do question its generality. In regard to acoustic systems, the fact that female *Gryllus campestris* prefer calls made by males with symmetrical wings appears to be the only evidence of the role of symmetry as a potential for mating success (Simmons and Ritchie, 1996). Studies on frogs (Ryan et al., 1995) and insects (Hunt and Allen, 1998, Faure and Hoy, 2000, including this study), provide no convincing evidence of any relationship between FA and either signalling, in the case of frogs and bushcrickets attacked by parasitoid flies (Hunt and Allen, 1998), or indeed signal detection in bushcrickets (Faure and Hoy, 2000; this study). But the experiments described here also question the interpretation of often elegant and now fully accepted models provided by physiological studies on sound lateralisation in crickets and bushcrickets (e.g. Lewis, 1974; Larsen and Michelsen, 1978; Hill and Oldfield, 1981; Larsen et al., 1989). Perhaps we should be more cautious when interpreting data obtained from insects restrained under neurophysiological experiment, because such experiments may create no more than an indicative model to explain the behaviour of the insect in nature. These often-complex models, involving a balance between multiple inputs, imply a precise comparison between perceived sound levels from bilateral input close to the insect's mid-line. It would appear that such precision is not required.

References

Bailey, W. J. (1985). Acoustic cues for female choice in bushcrickets (Tettigoniidae). In *Acoustic and Vibrational Communication in Insects* (ed. K. Kalmring and N. Elsner), pp. 101-110. Berlin and Hamburg: Paul Parey.

Bailey, W. J. (1990). The ear of the bushcricket. In *The Tettigoniidae: Biology. Systematics and Evolution* (ed. W. J. Bailey and D. C. F. Rentz), pp. 217-247. Bathurst: Crawford House Press.

Bailey, W. J. (1993). The tettigoniid (Orthoptera: Tettigoniidae) ear: Multiple functions and structural diversity. *Int. J. Insect Morph. Embryol.* **22**, 185-205.

Bailey, W. J. (1998). Do large bushcrickets have more sensitive ears? Natural variation in hearing thresholds within populations of the bushcricket *Requena verticalis* (Listroscelidinae: Tettigoniidae). *Physiol. Entomol.* **23**, 105-112.

Bailey, W. J. and Thomson, P. (1977). Acoustic orientation in the cricket *Teleogryllus oceanicus* (Le Guillou). *J. Exp. Biol.* **67**, 61-75.

Bailey, W. J. and Stephen, R. O. (1984). Auditory acuity in the orientation behaviour of the bushcricket *Pachysagella australis* Walker (Orthoptera, Tettigoniidae, Saginae). *Anim. Behav.* **32**, 816-829.

Bailey, W. J. and Yeoh, P. B. (1988). Female phonotaxis and frequency discrimination in the bushcricket *Requena verticalis*. *Physiol. Entomol.* **13**, 363-372.

Bailey, W. J., Withers, P. C., Endersby, M. and Gaull, K. (1993). The energetic costs of calling in the bushcricket *Requena verticalis* (Orthoptera: Tettigoniidae: Listroscelidinae). *J. Exp. Biol.* **178**, 21-37

Bailey, W. J. and Kamien, D. (2001). Hearing dimorphism, trait variation and conflicts over space in the thorax of the bushcricket *Requena verticalis* (Listroscelidinae: Tettigoniidae: Orthoptera). *J. Comp. Physiol. A* **187**, 647-652.

Bennet-Clark, H. C. (1984). Insect Hearing: Acoustics and transduction. In *Insect Communication* (ed. T. Lewis), pp. 49-82. London: Academic Press.

Cranford, T. W. (1992). Directional asymmetry in the odontocete forehead. *Am. Zool.* **32**, 140A.

Faure, P. A. and Hoy, R. R. (2000). Auditory symmetry analysis. *J. Exp. Biol.* **203**, 3209-3223.

Florentine, M. (1976). Relationship between lateralisation and loudness in asymmetrical hearing losses. *J. Am. Aud. Soc.* **1**, 243-251.

Forrest, T. G. and Green, D. M. (1991). Sexual selection and female choice in mole crickets *Scapteriscus* (Gryllotalpidae): Modelling the effects of intensity and male spacing. *Bioacoustics* **3**, 93-109.

Gerhardt, H. C. (1987). Evolutionary and neurobiological implications of selective phonotaxis in the green treefrog, *Hyla cinerea*. *Anim. Behav.* **35**, 1479-1489.

Gwynne, D. T. and Bailey, W. J. (1999). Female-female competition in katydids: sexual selection for increased sensitivity to a male signal? *Evolution* **53**, 546-551.

Helversen, D. V. and Wendler, G. (2000). Coupling of visual to auditory cues during phonotactic approach in the phaneropterine bushcricket *Poecilimon affinis*. *J. Comp. Physiol. A* **186**, 729-736.

Hill, K. G. and Oldfield, B. P. (1981). Auditory function in Tettigoniidae (Orthoptera: Ensifera). *J. Comp. Physiol. A* **142**, 169-180.

Huber, F., Kleindienst, H.-U., Weber, T. and Thorson, J. (1984). Auditory behavior of the cricket. III. Tracking of male calling song by surgically and developmentally one-eared females, and the curious role of the anterior tympanum. *J. Comp. Physiol. A* **155**, 725-738.

Hunt, J. and Allen, G. R. (1998). Fluctuating asymmetry, call structure and the risk of attack from phonotactic parasitoids in the bushcricket *Sciarasaga quadrata* (Orthoptera: Tettigoniidae). *Oecologia* **116**, 356-364.

Knudsen, E. I., Knudsen, P. F. and Esterly, S. D. (1982). Early auditory experiences modifies sound localization in barn owls. *Nature* **295**, 238-240.

Knudsen, E. I., Esterly, S. D. and Knudsen, P. F. (1984). Monaural occlusion alters sound localisation during a sensitive period in the barn owl. *J. Neurosci.* **4**, 1001-1011.

Larsen, O. N. and Michelsen, A. (1978). Biophysics of the ensiferan ear. III. The cricket ear as a four-input system. *J. Comp. Physiol. A* **123**, 217-227.

Larsen, O. N., Kleindienst, H.-U. and Michelsen, A. (1989). Biophysical aspects of sound reception. In *Cricket Behavior and Neurobiology* (ed. F. Huber, T. E. Moore and W. Loher), pp. 364-390. Ithaca: Cornell University Press.

Latimer, W. and Sippel, M. (1987). Acoustic cues for female choice and male competition in *Tettigonia cantans*. *Anim. Behav.* **35**, 887-900.

Lewis, D. B. (1974). The physiology of the tettigoniid ear. IV. A new hypothesis for acoustic orientation behaviour. *J. Exp. Biol.* **60**, 861-869.

Liggett, A. C., Harvey, I. F. and Manning, J. T. (1993). Fluctuating asymmetry in *Scatophaga stercoraria* L.: successful males are more symmetric. *Anim. Behav.* **45**, 1041-1043.

Møller, A. P. (1991). Sexual ornament size and the cost of fluctuating asymmetry. *Proc. R. Soc. Lond. B* **245**, 59-62.

Møller, A. P. (1992). Female swallow preference for symmetrical male sexual ornaments. *Nature* **357**, 238-240.

Møller, A. P. and Swaddle, J. P. (1997). *Asymmetry, Developmental Stability and Evolution*. Oxford: Oxford University Press.

Murphey, R. K. and Zaretsky, M. D. (1972). Orientation to calling song by female crickets, *Scapsipedus marginatus* (Gryllidae). *J. Exp. Biol.* **56**, 335-352.

Nocke, H. (1975). Physical and physiological properties of the tettigoniid ('Grasshopper') ear. *J. Comp. Physiol. A* **100**, 25-57.

Palmer, A. R. and Strobeck, C. (1986). Fluctuating asymmetry; measurement, analysis, patterns. *Ann. Rev. Ecol. Syst.* **17**, 391-421.

Payne, R. S. (1971). Acoustic localisation of prey by barn owls. *J. Exp. Biol.* **54**, 535-573.

Prager, J. and Larsen, O. N. (1981). Asymmetrical hearing in the water bug *Corixa punctata* observed with laser vibrometry. *Naturwissenschaften* **68**, 579-580.

Prager, J. and Streng, R. (1982). The resonance properties of the physical gill of *Corixa punctata* and their significance in sound reception. *J. Comp. Physiol. A* **148**, 323-335.

Prestwich, K., Brugger, K. E. and Toppong, M. (1989). Energy and communication in three species of hylid frogs: Power input, power output and efficiency. *J. Exp. Biol.* **144**, 53-80.

Regen, J. (1924). Über die Anlockung des Weibchens von *Liogryllus campestris* L. nach dem Stridulationsschall des Männchens. *Akad. Wiss. Math. Kl. Abt. I (Wien)* **132**, 81-88.

Rheinlaender, J. and Römer, H. (1980). Bilateral coding of sound direction in the CNS of the bushcricket *Tettigonia viridissima* L. (Orthoptera, Tettigoniidae). *J. Comp. Physiol. A* **140**, 101-111.

Rheinlaender, J. and Römer, H. (1986). Insect hearing in the field. I. The use of identified nerve cells as 'biological microphones'. *J. Comp. Physiol. A* **158**, 647-651.

Rheinlaender, J. and Römer, H. (1990). Acoustic cues for sound localisation and spacing in orthopteran insects. In *The Tettigoniidae: Biology. Systematics and Evolution* (ed. W. J. Bailey and D. C. F. Rentz), pp. 248-264. Bathurst: Crawford House Press.

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

Römer, H. and Krusch, M. (2000). A gain-control mechanism for processing of chorus sounds in the afferent auditory pathway of the bushcricket *Tettigonia viridisimma* (Orthoptera; Tettigoniidae). *J. Comp. Physiol. A* **186**, 181-191.

Ryan, M. J., Warkentin, K. M., McClelland, B. E. and Wilczynski, W. (1995). Fluctuating asymmetries and advertisement call variation in the cricket frog *Acris crepitans*. *Behav. Ecol.* **6**, 124-131.

Schatral, A. and Bailey, W. J. (1991). Song variability and the response to conspecific song and to song models of different frequency content in males of the bushcricket *Requena verticalis* (Orthoptera: Tettigoniidae). *Behavior* **116**, 163-179.

Schmitz, B. (1985). Phonotaxis in *Gryllus camp*estris L. (Orthoptera, Gryllidae). III. Intensity dependence of the behavioural performance and relative importance of tympana and spiracles in directional hearing. *J. Comp. Physiol. A* **156**, 165-180.

Schmitz, B., Kleindienst, H.-U., Schildberger, K. and Huber, F. (1988). Acoustic orientation in adult female crickets (*Gryllus bimaculatus* de Geer) after unilateral foreleg amputation in the larva. *J. Comp. Physiol. A* **162**, 715-728.

Schmitz, B., Scharstein, H. and Wendler, G. (1983). Phonotaxis in *Gryllus campestris* L. (Orthoptera, Gryllidae). II. Acoustic orientation of female crickets after occlusion of single sound entrances. *J. Comp. Physiol. A* **152**, 257-264.

Seymour, C., Lewis, D. B., Larsen, O. and Michelsen, A. (1978). Biophysics of the ensiferan ear. II. The steady-state gain of the hearing trumpet in bushcrickets. *J. Comp. Physiol. A* **123**, 205-216.

Simmons, L. W. and Ritchie, M. G. (1996). Symmetry in the songs of crickets. *Proc. R. Soc. Lond. B* **263**, 305-311.

Simmons, L. W., Beesley, L., Lindhjem, D., Newbound, J., Norris, J. and Wayne, A. (1999a). Nuptial feeding by male bushcrickets: an indicator of male quality? *Behav. Ecol.* **10**, 263-269.

Simmons, L. W., Tomkins, J. L., Kotiaho, J. S. and Hunt, J. (1999b). Fluctuating paradigm. *Proc. R. Soc. Lond. B* **266**, 593-595.

Stout, J. F. and McGhee, R. (1988). Attractiveness of the male *Acheta domestica* calling song to females I. The relative importance of syllable period, intensity and chirp rate. *J. Comp. Physiol. A* **164**, 277-287.

Weber, T. and Thorson, J. (1989). Phonotactic behavior of walking crickets. In *Cricket Behavior and Neurobiology* (ed. F. Huber, T. E. Moore and W. Loher), pp. 310-339. Ithaca: Cornell University Press.

Weber, T., Atkins, G., Stout, J. F. and Huber, F. (1987). Female *Acheta domesticus* track acoustical and visual targets with different walking modes. *Physiol. Entomol.* **12**, 141-147.

Zaretsky, M. D. (1972). Specificity of the calling song and short term changes in the phonotactic response by female crickets *Scapsipedus marginatus* (Gryllidae). *J. Comp. Physiol. A* **79**, 153-172.