Distribution of sound pressure around a singing cricket: radiation pattern and asymmetry in the sound field

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Abstract

Male field crickets generate calls to attract distant females through tegminal stridulation: the rubbing together of the overlying right wing which bears a file of cuticular teeth against the underlying left wing which carries a sclerotised scraper. During stridulation, specialised areas of membrane on both wings are set into oscillating vibrations to produce acoustic radiation. The location of females is unknown to the calling males and thus increasing effective signal range in all directions will maximise transmission effectiveness. However, producing an omnidirectional sound field of high sound pressure levels may be problematic due to the mechanical asymmetry found in this sound generation system. Mechanical asymmetry occurs by the right wing coming to partially cover the left wing during the closing stroke phase of stridulation. As such, it is hypothesised that the sound field on the left-wing side of the animal will contain lower sound pressure components than on the right-wing side as a result of this coverage. This hypothesis was tested using a novel method to accurately record a high resolution, three dimensional mapping of sound pressure levels around restrained *Gryllus bimaculatus* field crickets singing under pharmacological stimulation. The results indicate that a bilateral asymmetry is present across individuals, with greater amplitude components present in the right wing side of the animal. Individual variation in sound pressure to either the right or left-wing side is also observed. However, statistically significant differences in bilateral sound field asymmetry as presented here may not affect signalling in the field.

Keywords: Sound field, asymmetry, field cricket, stridulation
Introduction

The males of the field cricket *Gryllus bimaculatus* de Geer (Orthoptera; Gryllidae) generate acoustic signals for communication using tegmental stridulation (Pierce 1948; Ewing 1989). One wing is endowed with a series of cuticular teeth along a modified wing vein known as the stridulatory file or *pars stridens* (known as the file-bearing wing, hereafter FBW). The other wing lies below the FBW and has along its medial edge a hardened region known as the scraper or plectrum (the plectrum-bearing wing - PBW). As the scraper of one wing is passed over the file of the other, the tooth-scraper interactions produce vibrations which excite special areas of membrane on the wings that oscillate to radiate sound (Pierce 1948). Male field crickets stridulate conventionally with the right wing on top although in rare cases the left wing will be on top (Masaki et al. 1987). During stridulation, the wings open and close in a cyclical manner with the acoustic energy being generated on the closing stroke (Koch 1980; Elliot and Koch 1985). Male *Gryllus bimaculatus* typically produce three distinct types of acoustic signal. A long range ‘calling song’ to females, a close range ‘courtship song’ to females to induce copulation and a loud ‘aggressive song’ used in interactions with conspecific males (Frings and Frings 1958; Wagner and Reiser 2000; Gray and Eckhardt 2001). The calling song is performed to attract distant females who detect the signal and move towards the source of the sound, behaviourally known as phonotaxis (Huber and Thorson 1985). As such, of paramount importance is effective transmission of the signal from sender to receiver.

Information in an acoustic signal can be encoded in the parameters of intensity, frequency and time, and these can change during propagation (Gerhardt and Huber 2002). The parameter of intensity is of importance for both the information encoded therein and its relation to signal propagation (Wiley and Richards 1978; Naguib and Wiley 2001). Acoustic signals undergo attenuation with increased distance (Forrest 1994) with the effects of ground and atmospheric absorption reducing intensity across greater distances (Simmons 1988; Römer 1993). A louder signal carrying higher amplitude components further may effectively reach more conspecifics (Forrest and Raspet 1994). Besides the
effect that louder signals travel further, evidence exists that a louder call is a reliable indicator of the
individual’s quality (Searcy and Andersson 1986). In crickets, increased age and body size co-vary as
reliable indicators of male quality (Simmons 1995) and larger males produce louder calls which,
along with other call parameters indicating body size, are preferred by females (Simmons 1988).
Without passive attraction to the nearest male occurring (Forrest and Raspet 1994), then males
producing a louder call will attract more females than males producing a less intense signal (Pacheco
and Bertram 2014).

The production of such acoustic signals at high intensities has an associated metabolic cost
(Prestwich 1994; Prestwich 2005). Beyond their function in the resonant system (Elliot and Koch
1985; Koch et al. 1988; Bennet-Clark 1999; Bennet-Clark and Bailey 2002), the radiating membranes
on the wings of stridulating crickets have limitations for efficient sound production. The radiating
cells act as dipole radiator discs with sound produced on both sides of the membrane (Forrest 1982).
Unlike monopole sound radiators, dipoles do not radiate sound equally in all directions having their
maxima at 0° and 180° with no radiation along their edge at 90° and 270° due to destructive
interference along the edge of the membrane (Russel et al. 1999). This reduced net force on the
fluid medium (air) which makes dipoles less efficient than monopoles at radiating sound, especially
at low frequencies (Bennet-Clark 1998; Russell et al. 1999). Increasing calling efficiency through
reducing destructive interference can be achieved through the use of a baffle which acts as a
physical barrier along the edge of the disc to prevent the sound waves from one side affecting the
waves from the other (Forrest 1982). This is seen in tree crickets that make baffles for their wings
from leaves (Forrest 1991; Mhatre et al. 2011; Mhatre et al. 2012) and mole crickets (Bennet-Clark
1987; Forrest 1991) that use burrows as both an infinite baffle and an exponential horn to increase
output amplitude (Daws et al. 1996). Field crickets do not use external baffles, burrows or a
resonating chamber (Prestwich 2000) and sing freely in the environment. As such it appears that free
singing field crickets utilise an efficient resonant system for sound production from sound generator structures operating with the associated physical limitations of forewing membrane morphology.

The radiating cells on the wings of tree crickets and short-tailed crickets (acting as dipole radiators) produce a directional dumbbell shaped sound field along the anteroposterior axis of the animal due to the destructive interference (Forrest 1982; Forrest 1991). The dumbbell shaped sound field is a product of the physical properties of the membranes and omnidirectional transmission cannot be increased except through behavioural changes of body position during bouts of singing to effectively beam the signal across different directions (e.g. rotating through angles of azimuth). However, there is no evidence for this behaviour in field crickets. Within the limits of the system, maximum effectiveness of signal transmission can be expected to be facilitated across all axes of sound transmission. Yet what is particular to tegminal stridulation in crickets, as opposed to animals using vocalisations, is a mechanical asymmetry during sound production.

In bush crickets, the morphological asymmetries of the wings are acute (Montealegre-Z et al. 2003; Montealegre-Z and Mason 2005), while the wings of field crickets exhibit comparatively high levels of morphological symmetry (Pitchers et al. 2014). Yet functionally, in field crickets, there are still differences between the left and the right wing. Evidence exists demonstrating the relationship between frequency modulation and morphological asymmetry of the wings (Simmons and Ritchie 1996) as well the differing amplitude responses from each wing (Montealegre-Z et al. 2011). Beyond such investigations into morphological asymmetry of the wings, the effect which the mechanical asymmetry in the sound generation system has on the sound field has received relatively little attention. In field crickets the mechanical asymmetry in stridulation occurs as a result of the FBW coming to partially cover the PBW during each closing stroke. Despite the PBW having a greater amplitude response (Montealegre-Z et al. 2011) this coverage suggests that the levels of sound pressure from the underlying PBW (left-wing side in field crickets), should be lower than those from
the right-wing side (the side of the animal with the overlying FBW) as the radiating cells of the PBW will be increasingly covered during each wing closure. In field crickets, this would occur as a greater intensity on the animal’s right side as it is the right wing which lies on top during stridulation. Previous efforts to address the sound field around singing insects in terms of amplitude components have typically involved the use of multiple microphones. The work of Forrest (1991), Michelsen and Elsner (1999), and Michelsen and Fonesca (2000) all used a series of microphones arranged in an array which was manually manipulated about the insect in question. This technique places a limitation on the number of recording points which can be obtained simultaneously and raises the issue of variation in different microphone responses, although this can be corrected for post recording (Michelsen and Fonesca, 2000). The first attempt to quantify amplitude levels across a sound field of singing crickets was done by Forrest (1991). This work demonstrates the dumb-bell shaped sound field in the tree cricket and a short tailed cricket indicating the differences in the projected sound field between the differing generator morphologies as well as the use of leaf baffles in tree crickets. A limitation of this early work is the number of recording points obtained from the microphone array used by the author. Across the hemisphere of recording points, only 65 positions were recorded from which to reconstruct the sound field. A higher resolution of recording points across the sound field can only provide more information on the relative amplitude components, and this can now be facilitated with modern methodologies. Another limitation of Forest (1991) refers to the use of freely standing animals to take recordings. Accurate recordings of amplitudes relative to a sound source relies on the source being completely stationary, any changes of position, regardless of how small, may affect the amplitude in the recordings. Thus accuracy of recordings for relative amplitudes will be facilitated by the insect being completely restrained, something which has not been done before in crickets. Using pharmacological stimulation and a robotic arm controlling a microphone, this study presents a high resolution mapping of the sound field around a restrained and singing field cricket. We tested the hypothesis that the sound field around a singing cricket should be asymmetrical as a result of the
functional asymmetry in the sound generation system of *Gryllus bimaculatus* males (produced by
the right FBW overlying the left PBW), with amplitude components being greater on the right-wing
side of the animal. Results show that there are effectively some differences in the sound pressure
between left and right sides of the singing animal, however this different might not be of biological
importance.

**Materials and Methods**

Previous efforts to map sound radiation patterns in insects have relied on the use of calibrated
microphone arrays which are manipulated manually around a singing animal (Forrest 1991;
Michelsen and Elsner 1999; Michelsen and Fonesca 2000). Recording the sound field around a
singing animal will be facilitated if the sound source is stationary. Crickets, and many other acoustic
insects, rarely remain stationary during bouts of calling behaviour and if recordings are to be made
of these animals, the individuals will need to be restrained. However a restrained animal is unlikely
to produce any acoustic signals voluntarily. An established technique for the elicitation of
stridulatory behaviour in a restrained insect is through the use of neurochemical agents (Wenzel et
al. 1998). A descending brain neuron in the protocerebrum of *G. bimaculatus* has been identified as
a control neuron for stridulatory behaviour (Hedwig 1996). Localised microinjections of the
neurotransmitter acetylcholine and its agonists into the specific neuropile areas of the brain
(following the arborisation pattern of the descending neuron) will successfully elicit stridulatory
behaviour of the calling song (Wenzel and Hedwig 1999). The elicited calls of injected insects have
been shown to not differ from the natural calls in terms of call envelope, duration and frequency
modulation (Montealegre-Z et al. 2011). Therefore the process of pharmacological injection provides
a reliable method for the elicitation of stridulatory behaviour in restrained insects.

**Specimens**

Adult male crickets (*Gryllus bimaculatus*) were used from colonies maintained at the University of
Lincoln. Colonies were kept on a 12:12 light cycle and were fed ad lib with oats, dog biscuits and
water. Two breeding boxes were used, each containing about 40 animals. Egg cartons were present for hiding positions and these were removed for specimen selection. After the crickets had acclimatised to the egg box removal, individuals were chosen and kept in individual cages for 1-2 days prior to experimentation to ensure minimal damage to the wings from conspecific encounters. Individuals who exhibited more calling behaviour in the natural condition were chosen for the experiment preferentially as the calling song from these specimens was more easily recorded in the natural state and they responded better to the pharmacological elicitation process. Only young males (within ten days after the final moult) were used and specimens were chosen shortly after the majority of individuals in the colony had become imagos. The natural calling song of 33 males was recorded on the same day they were used for the pharmacological elicitation process (see details below). All males recorded exhibited the conventional wing overlap of RW over LW.

**Mounting specimens**

To obtain accurate acoustic recordings at equal distances from a singing animal, the sound source cannot move in relation to the microphone. As such, males must be restrained as this prevents the individual from moving and allows for easy dissection in preparation for the treatment. The males whose calling songs had been previously recorded were cooled to immobilisation in a domestic fridge for 4-6 min at 5-6°C. Each cooled animal was then placed on a block of Blu-Tack and gently clamped down with staple clamps over the legs and the abdomen. The insect was positioned so the prothorax was angled downwards to allow the wings to open and close in the normal position used for stridulation (Montealegre-Z et al. 2011). The head of the animal was immobilised by waxing it to a larger clamp to provide stability during the injection procedure. The Blu-Tack blocks were affixed to brass clasps which were attachable to an articulated rod allowing accurate manipulation of the animal’s position. Using a dissection microscope, a small area of cuticle was removed from the head of the insect to allow access to the brain, leaving the antennae intact. Dissection was performed using standard razor blades and dissection tools. Four incisions on the head of the animal allowed a small square of cuticle to be removed below the central ocelli and between the antennae. The fatty
tissues beneath the cuticle were manually removed with a custom made small metal hook. Ringer’s solution (Fielden 1960) was used to rinse away clotting haemolymph and prevent desiccation. A pedal activated suction pump attached by tube to a small pipette tip was also used to remove haemolymph and excess tissues in the process of exposing and clearing out the brain surface.

Pharmacological elicitation of stridulation

Stridulation can be elicited by the pharmacological stimulation of descending neurons in the anterior protocerebrum in the neuropile area between the mushroom body and the α-lobe (Wenzel et al. 1998). To achieve this, preparations were followed as described by Wenzel and Hedwig (1999) and Montealegre-Z et al. (2011). Microcapillaries were pulled from borosilicate glass tubing (external diameter: 1.2 mm, internal diameter: 0.9 mm; B120-69-8, Linton Instruments, Norfolk, England) using a micropipette puller (P30; Sutter Instruments, Novato, CA, USA) and a DMZ-Universal Puller (Zeitz-Instruments, Martinsried, Germany) to produce tips with a width of ~10 µm. The microcapillaries were then filled with eserine salicylate and nicotine diluted in cricket Ringer’s solution (10⁻² mol·l⁻¹ for both eserine salicylate and nicotine, Sigma-Aldrich Company Ltd, Dorset, UK) and connected to a nitrogen driven pico-pump (World Precision Instruments PV820, Sarasota, FL, USA). This allowed the administration small amounts of the neuroactive agent into the brain in the range of ~5-10 nl.

The electrode holder was mounted in a micromanipulator allowing accurate movement of the electrode into the protocerebrum using the brain locations as provided by Wenzel et al. (1998) as a guide. Usually one injection was sufficient to elicit stridulatory behaviour. If the first injection was unsuccessful then a second was administered to the other side of the brain. Stridulation occurred a few seconds to a few minutes after a successful injection. As reported before (Wenzel and Hedwig 1999) some animals exhibited the courtship or aggressive song. In these cases, if the song did not change to a reliable calling song after a few minutes, those animals were not used for the recordings. Animals of unsuccessful injection procedures were disposed of within two hours of
injection. Mounted specimens who exhibited typical calling song stridulation were used for the recordings (see Supplementary Material, Movie 1).

Recording the sound field

To obtain acoustic data at different points in relation to a sound source, recordings must be taken across a range of points around the specimen while maintaining a uniform distance and aspect of the microphone (Forrest 1991; Michelsen and Elsner 1999). The use of robot controllers allows for highly accurate manipulation of data acquisition instruments. A manoeuvrable robotic arm was used to move a microphone around a singing animal and take recordings at multiple points across three dimensions with a consistent distance and aspect of the microphone to the sound source. A quarter-sphere of points equidistant to a central position (Figure 1) was constructed using LabVIEW (National Instruments, Austin, TX, USA). The quarter-sphere consisted of 137 points separated by angles of 11.25° covering 17 points on each transect through 180° on the horizontal plane and 90° along the vertical plane to the final position at the pole (Figure 1). The angular positions of this quarter-sphere of points were then traced in a raster fashion (around the origin of the quarter-sphere) with a KUKA robot (KUKA Robotics, Germany) to which a GRAS type 40DD 1/8 inch condenser microphone (G.R.A.S. Sound and Vibration, Holte, Denmark) was attached. The microphone was connected to a GRAS type 12AA preamplifier which was connected to a sound board (USB-6259, National Instruments, Austin, TX) and then to the controlling computer. The robot was programmed using KRL (KUKA Robot Language; KUKA Robotics, Germany) and the controller was set to output a voltage pulse of 100 ms at every point in the quarter-sphere and trigger the microphone to record for one second at 50 kHz sampling rate. All recordings were performed using a custom LabVIEW program. The microphone was positioned at 28 cm from the animal at all recordings points and the articulation of the robot arm allowed that the microphone always faced the singing animal at the core of the sphere with its frontal aspect (Figure 2). A second microphone was positioned as a reference on the off-side of the recording hemisphere at 15 cm from the animal. Calibration of the microphones was performed prior to the recordings using a Brüel and Kjaer type 4321 calibrator.
(Brüel & Kjær, Nærum, Denmark) and the data was converted from volts to Pascal during recording. Sound pressure values were converted to dB SPL (re 20 µPa) where necessary. The mounted specimens were placed on a tripod at the central point of the half hemisphere facing forward (0° horizontal microphone position being frontal to the animal, 90° position vertically being directly above the animal) and then facing backward for separate recordings of the same animal. As natural stridulation occurs with a wing angle of ~30° from horizontal (Koch 1980), little acoustic energy would be captured in the rear-facing recordings as performed here. To achieve a measure of sound pressure levels both anteriorly and posteriorly to the singing animal the wings were positioned at ~60° from horizontal in both the front and rear-facing recordings. Frontal recordings were taken first, followed by rear-facing recordings for each specimen (see Supplementary Material, Movie 2). More than one recording for front and back were taken if the animal continued to sing reliably after the first set of recordings.

**Analysis**

To achieve a measure of the sound pressure of the signal, the peak amplitude of each pulse in the chirps recorded at every position were used. The signal from the robot-controlled microphone was filtered (Butterworth filter: bandpass, first cut-off frequency: 3000 Hz, second cut-off frequency: 6000 Hz) and the chirps were identified using custom written Matlab scripts (all signal analysis was performed using MATLAB and Signal Processing Toolbox 6.21 – version R2014a; The MathWorks Inc, Natick, MA, USA). The data from the robot microphone was normalised against the reference microphone as a control giving a relative amplitude value for every position of the quarter-sphere. Intermittent singing behaviour of the injected crickets during the recording process resulted in some points of the quarter-spheres containing little or no data.

To measure levels of bilateral asymmetry, the sound fields for both the front and back recordings were split laterally to provide data from the points on the right-wing side of the animal and the left-wing side (ignoring the recording points directly frontal to the animal – Figure 3). For every
specimen, the mean relative amplitudes were taken from all the points and each point was paired with its corresponding point on the other side of the animal. To examine if the sound pressure was greater on one side across all specimens, a permutation test was performed incorporating all paired right and left side data points from every animal. The permutation test was conducted as follows: for each individual, the mean of the differences between each paired relative amplitude recordings (left and right wings side) was calculated. The paired data from all specimens were compiled and the mean differences were compared to randomly permuted mean differences from all recorded sound pressure points using 10,000 iterations with each specimen’s data being permuted only within its own data set. $p$-values were calculated from the proportion of mean differences that were lower than the original mean difference and bootstrap confidence intervals were calculated using random samples from the left or right-wing side from each individual with replacement (10,000 repetitions) (Snijders and Borgatti 1999). Additionally, the same permutation test was performed on the paired data from every animal independently to investigate left or right-side bias on an individual level. For the individual permutation tests, $p$-values were corrected for multiple testing using the false discovery rate (FDR, Crawley 2005). To account for any point in a recording where no data was present (due to gaps in the singing behaviour of the singing insect), interpolation from data points surrounding the gaps in that particular recording was performed. Interpolation was performed using the ‘inpaint_nans’ function in Matlab using the least squares approach. All data visualisation was performed in Matlab.

**Results**

Thirty-three adult male crickets were injected with 20 exhibiting singing behaviour. Of these, 11 individuals performed the calling song reliably and their sound field was recorded. The 11 individuals recorded allowed for 18 front-facing and 12 rear-facing recordings to be taken. Front and back-facing recordings were analysed separately as not all specimens sang reliably enough to perform both recordings. Through the interpolating measure, the sound field recordings were completed from eight specimens for the front-facing recordings and for the backward-facing recordings the
sound fields of six specimens were completed. The remaining recordings could not be completed through interpolation due to the high prevalence of missing data points.

Radiation pattern

Radiation patterns averaged from all completed sound fields indicate a directionality of sound pressure frontal to the animal (Figure 4). The front-facing recordings contained higher amplitude components than the rear-facing recordings; mean sound pressure of all points in the front recordings was 76.1 ± 3.38 dB SPL (n=8) with the rear-facing recordings being 73.8 ± 2.94 dB SPL (n=6). Furthermore, the data suggests a trend towards the dumbbell shaped radiation pattern as previously reported for other stridulating cricket species (Forrest 1991). In this way, amplitude maxima are expected to occur at 90° normal to the oscillating membranes. Allowing for some variation in the positioning of the wings during stridulation, the greatest amplitude components are observed roughly 90° to the angle of the wings in the frontal recordings (wings positioned at 60°, Figure 6). Relative amplitudes in the rear-facing recordings increase in amplitude with lower elevations of recording with the maxima being on the horizontal.

Asymmetry in sound pressure radiation

Analysis of asymmetry was performed only on the frontal recordings to test the hypothesis of wing coverage affecting amplitude projection. From the analysis incorporating all recorded specimens together (n=8), there was a significant difference in the mean relative amplitudes between the left and right-wing side, with the right-wing side having higher amplitudes than the left-wing side (permutation test, p <0.0001, Figure 5). Average right-wing side sound pressure (all points) for the frontal recordings was 76.58± 3.22 dB SPL (n=8) with the left-wing side average being 76.05 ± 3.623 dB SPL (n=8). From the eight specimens whose frontal sound field was completed, the individual analysis shows that six specimens had significant differences in relative amplitudes between the points on the left-wing side of the quarter-sphere and the points on the right-wing side (permutation
test results presented in Table 1). Four of these specimens had higher pressure levels in the sound field on their right-wing side (average difference between sides from only robot controlled microphone, 1.58 dB) and two on their left (average difference between sides from only robot controlled microphone, 1.2 dB).

Discussion

The observed sound fields in this study agree with the dumb-bell shaped sound fields demonstrated in free singing crickets by Forrest (1991), in particular the short tailed cricket Anurogryllus arboreus, and confirm this effect in the field cricket G. bimaculatus. Furthermore, and in addition to this characterisation, the results indicate that stridulating crickets have higher amplitude components in the sound field on their right-wing side in relation to their left-wing side ($P<0.0001$) as predicted. This is in agreement with the hypothesis that the coverage of the underlying wing creates a mechanical bias to sound output on the right-wing side of the animal. However, as the difference between the two sides was so small, we suggest that the asymmetrical effect of the mechanical asymmetry is mediated by other processes within the system. Montealegre-Z et al. (2011) demonstrated that the radiating cells of the underlying left wing of G. bimaculatus vibrate with greater amplitude than those on the right wing. This amplitude response of the underlying PBW is shown to be between 1.6 to 2-fold higher than the overlaying FBW (see Figure 10 in Montealegre-Z et al. 2011). This increased amplitude from the underlying wing may compensate for the coverage of the overlaying wing and result in the low levels of asymmetry in the sound field as demonstrated here.

Furthermore, on the individual level, differences are observed in the levels of sound pressure output across the lateral sides of the sound field during stridulation. Variation in directionality of sound projection between individuals may be a result of morphological asymmetry between the two wings. Deviation from symmetry in acoustic parameters as a result of morphological asymmetry has been
presented by Simmons and Ritchie (1996) who suggest that frequency modulation in the signal is a result of bilateral asymmetry between the harps on each wing, however, vibrational response in relation to sound output was not measured in that study. The variation between individuals in amplitude response from the left wing demonstrated by Montealegre-Z et al. (2011) could affect the projection of pressure across the sound field. In this way, specimens who exhibit a comparatively higher amplitude underlying left wing may have a reduced effect on sound pressure projection from the right-wing coverage. Therefore, the increased amplitude from the left wing would not only compensate for any loss of intensity caused by being covered over by the overlaying FBW, but could potentially cause a bias of increased sound pressure on the left wing-side of the animal.

Under the hypothesis that bilateral output in the sound field will be caused by coverage of the left-wing by the overlaying right wing, any asymmetry can be expected to increase throughout the duration of each syllable as the radiating membranes of the underlying wing become increasingly covered. It has been suggested that each wing contributes sound to one half of the pulse only (Simmons and Richie 1996), however, a more recent analysis of the vibrational response of both wings indicates that they both contribute sound components for the duration of each pulse (Montealegre-Z et al. 2011). Maximum amplitude of each pulse occurs at around 0.4 mm of wing displacement; around the midpoint of the wing closure (Montealegre-Z et al. 2011) and amplitude decreases thereafter. This decrease in pulse amplitude has been attributed to different levels of mechanical excitation along the length of the file (Bennet-Clark 2003), however, if this reduction in amplitude is in part a result of the underlying left wing coverage by the right wing is unknown. Identifying the lateral intensity levels at different stages throughout the pulse, and accounting for varying wing positioning throughout each closing stroke (Koch 1980), could highlight any increasing asymmetry present as a result of increasing left-wing coverage.
As has been reported previously (Forrest 1991), the data indicates a focused directional dumbbell shaped sound field which lies perpendicular to the radiating cells as is suggested for the wings of stridulating crickets acting as dipole radiators (Forrest 1982). Models of wing membranes have been previously modelled as a circular disc vibrating without a baffle. Assuming the disc diameter is small in relation to the wavelength of the sound, the pressure field \( P_\theta \) of the dipole is proportional to

\[ P_\theta \approx |\sin(\theta)| \]

where \( \theta \) is the angle from the plane of the piston (Forrest 1991). The radiating harp (and immediate surrounding area) of the cricket wings producing the main amplitude components of the call has a diameter of \( \approx 4.7 \) mm which fits the assumption (such that wavelength \( \lambda = 7.2 \) cm and diameter = 0.059 \( \lambda \)). This predicted pattern was scaled so that the radial distance exactly perpendicular to the disc was equal to the averaged relative amplitude value of the closest point on the central line of the observed values (Figure 6). Contrary to the prediction, the relative amplitudes observed in this study do not approach 0 in the points of the rear facing recordings where the area of recording points is parallel to the wing membranes (blue dashed line – Figure 6.) although a reduction of amplitude is apparent in this area. In this study the wings of the specimens were positioned at \( \approx 60^\circ \) from the horizontal plane. As the recordings here occurred no lower than horizontal in elevation, the area of high pressure 90° from the ventral surface of the membranes would occur beneath the quarter-sphere as observed here and thus was only observed on the frontal recordings (Figure 4). Equally, the relatively low pressure levels in the backward-facing recordings observed in this study are likely due to the focus of sound on the rear side of the membranes being below the lowest angle of recording (Figure 6). The area of greatest vibrational amplitude is the harp (Montealegre-Z et al. 2011) and it is possible that the areas of tegmen surrounding the harp and mirror act as a partial baffle to facilitate efficient signal production (Forrest 1982). The baffling effect of the surrounding wing areas on acoustic radiation to both dorsal and ventral sides of the membranes merits further investigation.
The minor asymmetry observed in the projection pattern of sound pressure may not constitute a biologically relevant finding. Female detection of an acoustic trait affected by morphological asymmetry in crickets has only been demonstrated thus far in the discrimination of varying frequency modulation (Hirtenlehner et al. 2013). Asymmetries in sound pressure projection may not be distinguishable from natural amplitude fluctuations in the field (Römer and Lewald 1992) as the females move towards the male (Hirtenlehner et al. 2014). Furthermore, the low differences in relative sound pressure between the two sides are unlikely to be of sufficient magnitude to affect female phonotactic choice in the field (Hirtenlehner and Römer 2014). Aggregations of males may attract distant females collectively and other parameters of the calling song would be subject to female preference when in hearing range of the calls of multiple individuals (Simmons 1988). As such, a positive selection for omnidirectional equality of the calling song intensity as a trait in males seems to be unlikely. The frontal directionality of sound pressure demonstrated in this study may be favourable to field crickets while performing the aggressive song to conspecific males who conventionally interact directly facing each other during antagonistic encounters (Adamo and Hoy 1995), therefore making minor asymmetries irrelevant to signal function. While temporally different from the calling song (Alexander 1961), the aggressive song utilises the same mechanical acoustic generation process and as such the directional output of the wing cells is unlikely to differ between song types. The frontal projection of high amplitudes may convey reliable information about the individual’s size (Gray 1997) and prevent fights from escalating (Alexander 1961). Bilateral asymmetry in the sound field is unlikely to have a major effect on communication on the ground where field crickets sing, however, not all tegminal stridulators sing on this level. Mole crickets, for example, sing from within horn shaped burrows (Bennet-Clark 1970) with the sound field around the mouth of the burrow being hemispherical in seemingly equal amplitude components (see Forrest 1991). It is unlikely that bilateral asymmetry at the point of sound production (the singing insect) will affect the sound projecting from the mouth of the burrows of mole crickets. A further interesting example of tegminal stridulation comes from the short tailed cricket *Anurogryllus arboreus* who,
unlike similar species, sings above the ground on trees, shrubs and low vegetation (Paul and Walker 1979). Similarly is the case of tree crickets who sing from leaf cover and using baffles (Mhatre et al. 2012). In these cases, the three dimensional projection of sound, including the area ventral to the animals position (which we did not investigate here), may be more affected by bilateral asymmetry than those species singing close to the ground where excess attenuation and absorption may play a greater role. Further research into the sound fields of crickets should therefore include species from diverse habitats and with varying singing behaviours to investigate and compare the effects of sound field asymmetry under more natural conditions.

The method presented here offers an opportunity to collect high-resolution data for sound pressure levels in the sound field around a singing cricket. This allows for accurate identification of the signal amplitude from multiple positions from both the left-wing side and the right-wing side of the animal in three dimensions to investigate differences in sound pressure. This study indicates that field crickets produce an asymmetrical sound field during stridulation but variation between individuals exist in the natural projection of sound pressure levels to one side of the insect whilst calling. The observed individual variation suggests that mechanical asymmetry and coverage of the underlying wing cannot be concluded as a major cause of lateral sound field asymmetry and furthermore that minor asymmetries in sound field projection are unlikely to have an impact on signal function. Further investigations of symmetry in the projected sound field should focus on angular position and superposition of the wings throughout each closing stroke. Understanding the effect of lateral sound field asymmetry in relation to female phonotaxis and the response of conspecifics at close range encounters (e.g. females during courtship behaviours or males during aggressive interactions) will help elucidate any selection pressures which could select for directional signal transmission in stridulating crickets.
Supplementary material

Movie 1 A male adult *Gryllus bimaculatus* performing the calling song under pharmacological elicitation whilst restrained.

Movie 2 A singing male undergoing the recording procedure with the microphone maneuvered by the robotic arm. Animal positioned for a rear-facing recording.

Acknowledgements

Thanks go to Fabio Sarria-S for his practical assistance during the experiments. We also extend our thanks to Stefan Schöneich, Joaquim Pedro Jacob and Berthold Hedwig of Cambridge University for their helpful technical advice and instruction. Thanks go also to Tom Pike for his helpful comments on the study. We thank two anonymous reviewers for their comments which greatly improved the manuscript.
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Table 1. Sound pressure levels for front facing recordings for all specimens. Permutation test outputs for relative amplitudes testing all points on each lateral side for differences for each specimen (see text). For specimens with multiple recordings, the mean of each recording for every point was used. Pascal values from robot controlled microphone only. Pa, Pascals. dB, decibel re 20µPa. SPL, sound pressure level.

<table>
<thead>
<tr>
<th>Specimen</th>
<th>Mean of whole recording</th>
<th>Mean of right-wing side</th>
<th>Mean of left-wing side</th>
<th>P</th>
<th>Side of greatest SPL</th>
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<tr>
<td></td>
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<td>(Pa) (dB)</td>
<td>(Pa) (dB)</td>
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<tr>
<td>10(n=2)</td>
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<td>0.117 75.34</td>
<td>0.104 74.32</td>
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<td>13</td>
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<td>0.056 68.89</td>
<td>0.049 67.89</td>
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<td>15</td>
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<td>0.154 77.75</td>
<td>0.166 78.38</td>
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<tr>
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<td>0.131 76.30</td>
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<td>0.221 80.86</td>
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</tr>
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</table>
Figure captions

1. Figure 1. A Hemisphere of points equidistant to the centre of the same resolution used in the recording procedure. Only half the hemisphere is taken per recording. B Lateral view of the quarter-sphere comprising 136 points used for each recording. Each point represents a recording point of the microphone. Animal positioned as for frontal recordings. Image is not to scale.

2. Figure 2. Full experimental setup (not to scale). Animal positioned as for frontal recording. B&K, Brüel & Kjær; Mic, microphone.

3. Figure 3. Dorsal view of the quarter-sphere (from Fig.1:B; not to scale) indicating points designated as the left-wing side of the animal (red) and the right-wing side (black). Recording points frontal to the animal (blue) were not used for analysis of asymmetry. Image represents a front-facing recording.

4. Figure 4. Plots of averaged relative amplitude values as sound field patterns for front (n=8) and rear-facing (n=6) recordings. A Front-facing recordings. B Rear-facing recordings. Grid corners represent recording points. Colour scheme for relative amplitudes interpolated across the quarter-sphere. Arrows indicate positional facing of the animal. RWS, right wing side (overlying wing). LWS, left wing side (underlying wing).

5. Figure 5. Mean relative amplitudes of recording points on each side from all frontal recordings (black bars). Error bars on relative amplitudes indicates bootstrap confidence intervals (see text). ***=<0.001.

6. Figure 6. The stimated sound field. A Dorsal view of the recording quarter-sphere, red points indicate central line recording positions. B Lateral view of observed and predicted sound field projection shapes for recording points from the central line (elevation plane) for both frontal and rear-facing recordings. Red dashed line indicates predicted radiation pattern of a free piston and is angled to be at 90° from the wing angle in line with the observed values; blue dashed line indicates wing angle. Black solid line indicates observed average relative amplitude values for each central recording point. Black dashed lines indicate angle of elevation (11.5° increments).
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