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3 1 **Distribution of sound pressure around a singing cricket: radiation pattern and asymmetry**
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5 2 **in the sound field**
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1
2
3 25 **Abstract**
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5 26 Male field crickets generate calls to attract distant females through tegminal stridulation: the
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7 27 rubbing together of the overlying right wing which bears a file of cuticular teeth against the
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10 28 underlying left wing which carries a sclerotised scraper. During stridulation, specialised areas of
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12 29 membrane on both wings are set into oscillating vibrations to produce acoustic radiation. The
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14 30 location of females is unknown to the calling males and thus increasing effective signal range in all
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16 31 directions will maximise transmission effectiveness. However, producing an omnidirectional sound
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18 32 field of high sound pressure levels may be problematic due to the mechanical asymmetry found in
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20 33 this sound generation system. Mechanical asymmetry occurs by the right wing coming to partially
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22 34 cover the left wing during the closing stroke phase of stridulation. As such, it is hypothesised that the
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24 35 sound field on the left-wing side of the animal will contain lower sound pressure components than
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26 36 on the right-wing side as a result of this coverage. This hypothesis was tested using a novel method
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28 37 to accurately record a high resolution, three dimensional mapping of sound pressure levels around
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30 38 restrained *Gryllus bimaculatus* field crickets singing under pharmacological stimulation. The results
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32 39 indicate that a bilateral asymmetry is present across individuals, with greater amplitude components
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34 40 present in the right wing side of the animal. Individual variation in sound pressure to either the right
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36 41 or left-wing side is also observed. However, statistically significant differences in bilateral sound field
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38 42 asymmetry as presented here may not affect signalling in the field.
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44 44 **Keywords:** Sound field, asymmetry, field cricket, stridulation
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3 49 **Introduction**
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5 50 The males of the field cricket *Gryllus bimaculatus* de Geer (Orthoptera; Gryllidae) generate acoustic
6 51 signals for communication using tegminal stridulation (Pierce 1948; Ewing 1989). One wing is
7 52 endowed with a series of cuticular teeth along a modified wing vein known as the stridulatory file or
8 53 *pars stridens* (known as the file-bearing wing, hereafter FBW). The other wing lies below the FBW
9 54 and has along its medial edge a hardened region known as the scraper or plectrum (the plectrum-
10 55 bearing wing - PBW). As the scraper of one wing is passed over the file of the other, the tooth-
11 56 scraper interactions produce vibrations which excite special areas of membrane on the wings that
12 57 oscillate to radiate sound (Pierce 1948). Male field crickets stridulate conventionally with the right
13 58 wing on top although in rare cases the left wing will be on top (Masaki et al. 1987). During
14 59 stridulation, the wings open and close in a cyclical manner with the acoustic energy being generated
15 60 on the closing stroke (Koch 1980; Elliot and Koch 1985). Male *Gryllus bimaculatus* typically produce
16 61 three distinct types of acoustic signal. A long range 'calling song' to females, a close range 'courtship
17 62 song' to females to induce copulation and a loud 'aggressive song' used in interactions with
18 63 conspecific males (Frings and Frings 1958; Wagner and Reiser 2000; Gray and Eckhardt 2001). The
19 64 calling song is performed to attract distant females who detect the signal and move towards the
20 65 source of the sound, behaviourally known as phonotaxis (Huber and Thorson 1985). As such, of
21 66 paramount importance is effective transmission of the signal from sender to receiver.
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68 Information in an acoustic signal can be encoded in the parameters of intensity, frequency and time,
69 and these can change during propagation (Gerhardt and Huber 2002). The parameter of intensity is
70 of importance for both the information encoded therein and its relation to signal propagation (Wiley
71 and Richards 1978; Naguib and Wiley 2001). Acoustic signals undergo attenuation with increased
72 distance (Forrest 1994) with the effects of ground and atmospheric absorption reducing intensity
73 across greater distances (Simmons 1988; Römer 1993). A louder signal carrying higher amplitude
74 components further may effectively reach more conspecifics (Forrest and Raspet 1994). Besides the

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3 75 effect that louder signals travel further, evidence exists that a louder call is a reliable indicator of the
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5 76 individual's quality (Searcy and Andersson 1986). In crickets, increased age and body size co-vary as
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7 77 reliable indicators of male quality (Simmons 1995) and larger males produce louder calls which,
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9 78 along with other call parameters indicating body size, are preferred by females (Simmons 1988).
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11 79 Without passive attraction to the nearest male occurring (Forrest and Raspet 1994), then males
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13 80 producing a louder call will attract more females than males producing a less intense signal (Pacheco
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15 81 and Bertram 2014).
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20 83 The production of such acoustic signals at high intensities has an associated metabolic cost
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22 84 (Prestwich 1994; Prestwich 2005). Beyond their function in the resonant system (Elliot and Koch
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24 85 1985; Koch et al. 1988; Bennet-Clark 1999; Bennet-Clark and Bailey 2002), the radiating membranes
25
26 86 on the wings of stridulating crickets have limitations for efficient sound production. The radiating
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28 87 cells act as dipole radiator discs with sound produced on both sides of the membrane (Forrest 1982).
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30 88 Unlike monopole sound radiators, dipoles do not radiate sound equally in all directions having their
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32 89 maxima at 0° and 180° with no radiation along their edge at 90° and 270° due to destructive
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34 90 interference along the edge of the membrane (Russel et al. 1999). This reduced net force on the
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36 91 fluid medium (air) which makes dipoles less efficient than monopoles at radiating sound, especially
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38 92 at low frequencies (Bennet-Clark 1998; Russell et al. 1999). Increasing calling efficiency through
39
40 93 reducing destructive interference can be achieved through the use of a baffle which acts as a
41
42 94 physical barrier along the edge of the disc to prevent the sound waves from one side affecting the
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44 95 waves from the other (Forrest 1982). This is seen in tree crickets that make baffles for their wings
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46 96 from leaves (Forrest 1991; Mhatre et al. 2011; Mhatre et al. 2012) and mole crickets (Bennet-Clark
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48 97 1987; Forrest 1991) that use burrows as both an infinite baffle and an exponential horn to increase
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50 98 output amplitude (Daws et al. 1996). Field crickets do not use external baffles, burrows or a
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52 99 resonating chamber (Prestwich 2000) and sing freely in the environment. As such it appears that free
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3 100 singing field crickets utilise an efficient resonant system for sound production from sound generator
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5 101 structures **operating with the associated physical limitations of forewing membrane morphology.**
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9 103 The radiating cells on the wings of tree crickets and short-tailed crickets (acting as dipole radiators)
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11 104 produce a directional dumbbell shaped sound field along the anteroposterior axis of the animal due
12
13 105 to the destructive interference (Forrest 1982; Forrest 1991). The dumbbell shaped sound field is a
14
15 106 product of the physical properties of the membranes and omnidirectional transmission cannot be
16
17 107 increased except through behavioural changes of body position during bouts of singing **to effectively**
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19 108 **beam the signal across different directions (e.g. rotating through angles of azimuth).** However, there
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21 109 is no evidence for this behaviour in **field** crickets. Within the limits of the system, maximum
22
23 110 effectiveness of signal transmission can be expected to be facilitated across all axes of sound
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25 111 transmission. Yet what is particular to tegminal stridulation in crickets, as opposed to animals using
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27 112 vocalisations, is a mechanical asymmetry during sound production.
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31 114 In bush crickets, the morphological asymmetries of the wings are acute (Montealegre-Z et al. 2003;
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33 115 Montealegre-Z and Mason 2005), while the wings of field crickets exhibit comparatively high levels
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35 116 of morphological symmetry (Pitchers et al. 2014). Yet functionally, in field crickets, there are still
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37 117 differences between the left and the right wing. Evidence exists demonstrating the relationship
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39 118 between frequency modulation and morphological asymmetry of the wings (Simmons and Ritchie
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41 119 1996) as well the differing amplitude responses from each wing (Montealegre-Z et al. 2011). Beyond
42
43 120 such investigations into morphological asymmetry of the wings, the effect which the mechanical
44
45 121 asymmetry in the sound generation system has on the sound field has received relatively little
46
47 122 attention. In field crickets the mechanical asymmetry in stridulation occurs as a result of the FBW
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49 123 coming to partially cover the PBW during each closing stroke. Despite the PBW having a greater
50
51 124 amplitude response (Montealegre-Z et al. 2011) this coverage suggests that the levels of sound
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53 125 pressure from the underlying PBW (left-wing side in field crickets), should be lower than those from
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3 126 the right-wing side (the side of the animal with the overlying FBW) as the radiating cells of the PBW
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5 127 will be increasingly covered during each wing closure. In field crickets, this would occur as a greater
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7 128 intensity on the animal's right side as it is the right wing which lies on top during stridulation.
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9 129 Previous efforts to address the sound field around singing insects in terms of amplitude components
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11 130 have typically involved the use of multiple microphones. The work of Forrest (1991), Michelsen and
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13 131 Elsner (1999), and Michelsen and Fonesca (2000) all used a series of microphones arranged in an
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15 132 array which was manually manipulated about the insect in question. This technique places a
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17 133 limitation on the number of recording points which can be obtained simultaneously and raises the
18
19 134 issue of variation in different microphone responses, although this can be corrected for post
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21 135 recording (Michelsen and Fonesca, 2000). The first attempt to quantify amplitude levels across a
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23 136 sound field of singing crickets was done by Forrest (1991). This work demonstrates the dumb-bell
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25 137 shaped sound field in the tree cricket and a short tailed cricket indicating the differences in the
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27 138 projected sound field between the differing generator morphologies as well as the use of leaf baffles
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29 139 in tree crickets. A limitation of this early work is the number of recording points obtained from the
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31 140 microphone array used by the author. Across the hemisphere of recording points, only 65 positions
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33 141 were recorded from which to reconstruct the sound field. A higher resolution of recording points
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35 142 across the sound field can only provide more information on the relative amplitude components,
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37 143 and this can now be facilitated with modern methodologies. Another limitation of Forest (1991)
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39 144 refers to the use of freely standing animals to take recordings. Accurate recordings of amplitudes
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41 145 relative to a sound source relies on the source being completely stationary, any changes of position,
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43 146 regardless of how small, may affect the amplitude in the recordings. Thus accuracy of recordings for
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45 147 relative amplitudes will be facilitated by the insect being completely restrained, something which
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47 148 has not been done before in crickets.
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53 149 Using pharmacological stimulation and a robotic arm controlling a microphone, this study presents a
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55 150 high resolution mapping of the sound field around a **restrained and singing field** cricket. We tested
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57 151 the hypothesis that the sound field around a singing cricket should be asymmetrical as a result of the
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3 152 functional asymmetry in the sound generation system of *Gryllus bimaculatus* males (produced by
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5 153 the right FBW overlying the left PBW), with amplitude components being greater on the right-wing
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7 154 side of the animal. Results show that there are effectively some differences in the sound pressure
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9 155 between left and right sides of the singing animal, however this different might not be of biological
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11 156 importance.

157 **Materials and Methods**

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

174 *Specimens*

175 Adult male crickets (*Gryllus bimaculatus*) were used from colonies maintained at the University of
176 Lincoln. Colonies were kept on a 12:12 light cycle and were fed ad lib with oats, dog biscuits and

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3 177 water. Two breeding boxes were used, each containing about 40 animals. Egg cartons were present
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5 178 for hiding positions and these were removed for specimen selection. After the crickets had
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7 179 acclimatised to the egg box removal, individuals were chosen and kept in individual cages for 1-2
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9 180 days prior to experimentation to ensure minimal damage to the wings from conspecific encounters.
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11 181 Individuals who exhibited more calling behaviour in the natural condition were chosen for the
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13 182 experiment preferentially as the calling song from these specimens was more easily recorded in the
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15 183 natural state and they responded better to the pharmacological elicitation process. Only young
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17 184 males (within ten days after the final moult) were used and specimens were chosen shortly after the
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19 185 majority of individuals in the colony had become imagos. The natural calling song of 33 males was
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21 186 recorded on the same day they were used for the pharmacological elicitation process (see details
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23 187 below). All males recorded exhibited the conventional wing overlap of RW over LW.
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27 188 *Mounting specimens*

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29 189 To obtain accurate acoustic recordings at equal distances from a singing animal, the sound source
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31 190 cannot move in relation to the microphone. As such, males must be restrained as this prevents the
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33 191 individual from moving and allows for easy dissection in preparation for the treatment. The males
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35 192 whose calling songs had been previously recorded were cooled to immobilisation in a domestic
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37 193 fridge for 4-6min at 5-6 °C. Each cooled animal was then placed on a block of Blu-Tack and gently
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39 194 clamped down with staple clamps over the legs and the abdomen. The insect was positioned so the
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41 195 prothorax was angled downwards to allow the wings to open and close in the normal position used
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43 196 for stridulation (Montealegre-Z et al. 2011). The head of the animal was immobilised by waxing it to
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45 197 a larger clamp to provide stability during the injection procedure. The Blu-Tack blocks were affixed
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47 198 to brass clasps which were attachable to an articulated rod allowing accurate manipulation of the
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49 199 animal's position. Using a dissection microscope, a small area of cuticle was removed from the head
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51 200 of the insect to allow access to the brain, leaving the antennae intact. Dissection was performed
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53 201 using standard razor blades and dissection tools. Four incisions on the head of the animal allowed a
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55 202 small square of cuticle to be removed below the central ocelli and between the antennae. The fatty
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3 203 tissues beneath the cuticle were manually removed with a custom made small metal hook. Ringer's
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5 204 solution (Fielden 1960) was used to rinse away clotting haemolymph and prevent desiccation. A
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7 205 pedal activated suction pump attached by tube to a small pipette tip was also used to remove
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9 206 haemolymph and excess tissues in the process of exposing and clearing out the brain surface.

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12 207 *Pharmacological elicitation of stridulation*

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14 208 Stridulation can be elicited by the pharmacological stimulation of descending neurons in the anterior
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16 209 protocerebrum in the neuropile area between the mushroom body and the α -lobe (Wenzel et al.
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18 210 1998). To achieve this, preparations were followed as described by Wenzel and Hedwig (1999) and
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20 211 Montealegre-Z et al. (2011). Microcapillaries were pulled from borosilicate glass tubing (external
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22 212 diameter: 1.2 mm, internal diameter: 0.9 mm; B120-69-8, Linton Instruments, Norfolk, England)
23
24 213 using a micropipette puller (P30; Sutter Instruments, Novato, CA, USA) and a DMZ-Universal Puller
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26 214 (Zeitz-Instruments, Martinsried, Germany) to produce tips with a width of $\sim 10 \mu\text{m}$. The
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28 215 microcapillaries were then filled with eserine salicylate and nicotine diluted in cricket Ringer's
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30 216 solution ($10^{-2} \text{ mol} \cdot \text{l}^{-1}$ for both eserine salicylate and nicotine, Sigma-Aldrich Company Ltd, Dorset,
31
32 217 UK) and connected to a nitrogen driven pico-pump (World Precision Instruments PV820, Sarasota,
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34 218 FL, USA). This allowed the administration small amounts of the neuroactive agent into the brain in
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36 219 the range of $\sim 5\text{-}10 \text{ nl}$.

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42 221 The electrode holder was mounted in a micromanipulator allowing accurate movement of the
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44 222 electrode into the protocerebrum using the brain locations as provided by Wenzel et al. (1998) as a
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46 223 guide. Usually one injection was sufficient to elicit stridulatory behaviour. If the first injection was
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48 224 unsuccessful then a second was administered to the other side of the brain. Stridulation occurred a
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50 225 few seconds to a few minutes after a successful injection. As reported before (Wenzel and Hedwig
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52 226 1999) some animals exhibited the courtship or aggressive song. In these cases, if the song did not
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54 227 change to a reliable calling song after a few minutes, those animals were not used for the
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56 228 recordings. Animals of unsuccessful injection procedures were disposed of within two hours of
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3 229 injection. Mounted specimens who exhibited typical calling song stridulation were used for the
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5 230 recordings (see Supplementary Material, Movie 1).
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8 231 *Recording the sound field*

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10 232 To obtain acoustic data at different points in relation to a sound source, recordings must be taken
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12 233 across a range of points around the specimen while maintaining a uniform distance and aspect of
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14 234 the microphone (Forrest 1991; Michelsen and Elsner 1999). The use of robot controllers allows for
15
16 235 highly accurate manipulation of data acquisition instruments. A manoeuvrable robotic arm was used
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18 236 to move a microphone around a singing animal and take recordings at multiple points across three
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20 237 dimensions with a consistent distance and aspect of the microphone to the sound source. A quarter-
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22 238 sphere of points equidistant to a central position (Figure 1) was constructed using LabVIEW (National
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24 239 Instruments, Austin, TX, USA). The quarter-sphere consisted of 137 points separated by angles of
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26 240 11.25° covering 17 points on each transect through 180° on the horizontal plane and 90° along the
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28 241 vertical plane to the final position at the pole (Figure 1). The angular positions of this quarter-sphere
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30 242 of points were then traced in a raster fashion (around the origin of the quarter-sphere) with a KUKA
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32 243 robot (KUKA Robotics, Germany) to which a GRAS type 40DD 1/8 inch condenser microphone
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34 244 (G.R.A.S. Sound and Vibration, Holte, Denmark) was attached. The microphone was connected to a
35
36 245 GRAS type 12AA preamplifier which was connected to a sound board (USB-6259, National
37
38 246 Instruments, Austin, TX) and then to the controlling computer. The robot was programmed using KRL
39
40 247 (KUKA Robot Language; KUKA Robotics, Germany) and the controller was set to output a voltage
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42 248 pulse of 100 ms at every point in the quarter-sphere and trigger the microphone to record for one
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44 249 second at 50 kHz sampling rate. All recordings were performed using a custom LabVIEW program.
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46 250 The microphone was positioned at 28 cm from the animal at all recordings points and the
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48 251 articulation of the robot arm allowed that the microphone always faced the singing animal at the
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50 252 core of the sphere with its frontal aspect (Figure 2). A second microphone was positioned as a
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52 253 reference on the off-side of the recording hemisphere at 15 cm from the animal. Calibration of the
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54 254 microphones was performed prior to the recordings using a Brüel and Kjaer type 4321 calibrator
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3 255 (Brüel & Kjaer, Nærum, Denmark) and the data was converted from volts to Pascal during recording.
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5 256 Sound pressure values were converted to dB SPL (re 20 μ Pa) where necessary. The mounted
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7 257 specimens were placed on a tripod at the central point of the half hemisphere facing forward (0°
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9 258 horizontal microphone position being frontal to the animal, 90° position vertically being directly
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11 259 above the animal) and then facing backward for separate recordings of the same animal. As natural
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13 260 stridulation occurs with a wing angle of $\sim 30^\circ$ from horizontal (Koch 1980), little acoustic energy
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15 261 would be captured in the rear-facing recordings as performed here. To achieve a measure of sound
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17 262 pressure levels both anteriorly and posteriorly to the singing animal the wings were positioned at
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19 263 $\sim 60^\circ$ from horizontal in both the front and rear-facing recordings. Frontal recordings were taken first,
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21 264 followed by rear-facing recordings for each specimen (see Supplementary Material, Movie 2). More
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23 265 than one recording for front and back were taken if the animal continued to sing reliably after the
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25 266 first set of recordings.
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267 *Analysis*

268 To achieve a measure of the sound pressure of the signal, the peak amplitude of each pulse in the
269 chirps recorded at every position were used. The signal from the robot-controlled microphone was
270 filtered (Butterworth filter: bandpass, first cut-off frequency: 3000 Hz, second cut-off frequency:
271 6000 Hz) and the chirps were identified using custom written Matlab scripts (all signal analysis was
272 performed using MATLAB and Signal Processing Toolbox 6.21 – version R2014a; The MathWorks Inc,
273 Natick, MA, USA). The data from the robot microphone was normalised against the reference
274 microphone as a control giving a relative amplitude value for every position of the quarter-sphere.
275 Intermittent singing behaviour of the injected crickets during the recording process resulted in some
276 points of the quarter-spheres containing little or no data.
277

278 To measure levels of bilateral asymmetry, the sound fields for both the front and back recordings
279 were split laterally to provide data from the points on the right-wing side of the animal and the left-
280 wing side (ignoring the recording points directly frontal to the animal – Figure 3). For every

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3 281 specimen, the mean relative amplitudes were taken from all the points and each point was paired
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5 282 with its corresponding point on the other side of the animal. To examine if the sound pressure was
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7 283 greater on one side across all specimens, a permutation test was performed incorporating all paired
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9 284 right and left side data points from every animal. The permutation test was conducted as follows: for
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11 285 each individual, the mean of the differences between each paired relative amplitude recordings (left
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13 286 and right wings side) was calculated. The paired data from all specimens were compiled and the
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15 287 mean differences were compared to randomly permuted mean differences from all recorded sound
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17 288 pressure points using 10,000 iterations with each specimen's data being permuted only within its
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19 289 own data set. *p*-values were calculated from the proportion of mean differences that were lower
20
21 290 than the original mean difference and bootstrap confidence intervals were calculated using random
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23 291 samples from the left or right-wing side from each individual with replacement (10,000 repetitions)
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25 292 (Snijders and Borgatti 1999). Additionally, the same permutation test was performed on the paired
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27 293 data from every animal independently to investigate left or right-side bias on an individual level. For
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29 294 the individual permutation tests, *p*-values were corrected for multiple testing using the false
30
31 295 discovery rate (FDR, Crawley 2005). To account for any point in a recording where no data was
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33 296 present (due to gaps in the singing behaviour of the singing insect), interpolation from data points
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35 297 surrounding the gaps in that particular recording was performed. Interpolation was performed using
36
37 298 the 'inpaint_nans' function in Matlab using the least squares approach. All data visualisation was
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39 299 performed in Matlab.

300 **Results**

301 Thirty-three adult male crickets were injected with 20 exhibiting singing behaviour. Of these, 11
302 individuals performed the calling song reliably and their sound field was recorded. The 11 individuals
303 recorded allowed for 18 front-facing and 12 rear-facing recordings to be taken. Front and back-
304 facing recordings were analysed separately as not all specimens sang reliably enough to perform
305 both recordings. Through the interpolating measure, the sound field recordings were completed
306 from eight specimens for the front-facing recordings and for the backward-facing recordings the

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3 307 sound fields of six specimens were completed. The remaining recordings could not be completed
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5 308 through interpolation due to the high prevalence of missing data points.
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9 310 *Radiation pattern*

10 311 Radiation patterns averaged from all completed sound fields indicate a directionality of sound
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12 312 pressure frontal to the animal (Figure 4). The front-facing recordings contained higher amplitude
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14 313 components than the rear-facing recordings; mean sound pressure of all points in the front
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16 314 recordings was 76.1 ± 3.38 dB SPL ($n=8$) with the rear-facing recordings being 73.8 ± 2.94 dB SPL
17
18 315 ($n=6$). Furthermore, the data suggests a trend towards the dumbbell shaped radiation pattern as
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20 316 previously reported for other stridulating cricket species (Forrest 1991). In this way, amplitude
21
22 317 maxima are expected to occur at 90° normal to the oscillating membranes. Allowing for some
23
24 318 variation in the positioning of the wings during stridulation, the greatest amplitude components are
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26 319 observed roughly 90° to the angle of the wings in the frontal recordings (wings positioned at 60° ,
27
28 320 Figure 6). Relative amplitudes in the rear-facing recordings increase in amplitude with lower
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30 321 elevations of recording with the maxima being on the horizontal.
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35 323 *Asymmetry in sound pressure radiation*

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37 324 Analysis of asymmetry was performed only on the frontal recordings to test the hypothesis of wing
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39 325 coverage affecting amplitude projection. From the analysis incorporating all recorded specimens
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41 326 together ($n=8$), there was a significant difference in the mean relative amplitudes between the left
42
43 327 and right-wing side, with the right-wing side having higher amplitudes than the left-wing side
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45 328 (permutation test, $p < 0.0001$, Figure 5). Average right-wing side sound pressure (all points) for the
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47 329 frontal recordings was 76.58 ± 3.22 dB SPL ($n=8$) with the left-wing side average being 76.05 ± 3.623
48
49 330 dB SPL ($n=8$). From the eight specimens whose frontal sound field was completed, the individual
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51 331 analysis shows that six specimens had significant differences in relative amplitudes between the
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53 332 points on the left-wing side of the quarter-sphere and the points on the right-wing side (permutation
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3 333 test results presented in Table 1). Four of these specimens had higher pressure levels in the sound
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5 334 field on their right-wing side (average difference between sides from only robot controlled
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7 335 microphone, 1.58 dB) and two on their left (average difference between sides from only robot
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9 336 controlled microphone, 1.2 dB).

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12 338 **Discussion**

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15 339 **The observed sound fields in this study agree with the dumb-bell shaped sound fields demonstrated**
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17 340 **in free singing crickets by Forrest (1991), in particular the short tailed cricket *Anurogryllus arboreus*,**
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19 341 **and confirm this effect in the field cricket *G. bimaculatus*. Furthermore, and in addition to this**
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21 342 **characterisation, the** results indicate that stridulating crickets have higher amplitude components in
22
23 343 the sound field on their right-wing side in relation to their left-wing side ($P=<0.0001$) as predicted.
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25 344 This is in agreement with the hypothesis that the coverage of the underlying wing creates a
26
27 345 mechanical bias to sound output on the right-wing side of the animal. However, as the difference
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29 346 between the two sides was so small, we suggest that the asymmetrical effect of the mechanical
30
31 347 asymmetry is mediated by other processes within the system. Montealegre-Z et al. (2011)
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33 348 demonstrated that the radiating cells of the underlying left wing of *G. bimaculatus* vibrate with
34
35 349 greater amplitude than those on the right wing. This amplitude response of the underlying PBW is
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37 350 shown to be between 1.6 to 2-fold higher than the overlaying FBW (see Figure 10 in Montealegre-Z
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39 351 et al. 2011). This increased amplitude from the underlying wing may compensate for the coverage of
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41 352 the overlaying wing and result in the low levels of asymmetry in the sound field as demonstrated
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43 353 here.

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48 355 Furthermore, on the individual level, differences are observed in the levels of sound pressure output
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50 356 across the lateral sides of the sound field during stridulation. Variation in directionality of sound
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52 357 projection between individuals may be a result of morphological asymmetry between the two wings.
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54 358 Deviation from symmetry in acoustic parameters as a result of morphological asymmetry has been
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3 359 presented by Simmons and Ritchie (1996) who suggest that frequency modulation in the signal is a
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5 360 result of bilateral asymmetry between the harps on each wing, however, vibrational response in
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7 361 relation to sound output was not measured in that study. The variation between individuals in
8
9 362 amplitude response from the left wing demonstrated by Montealegre-Z et al. (2011) could affect the
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11 363 projection of pressure across the sound field. In this way, specimens who exhibit a comparatively
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13 364 higher amplitude underlying left wing may have a reduced effect on sound pressure projection from
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15 365 the right-wing coverage. Therefore, the increased amplitude from the left wing would not only
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17 366 compensate for any loss of intensity caused by being covered over by the overlaying FBW, but could
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19 367 potentially cause a bias of increased sound pressure on the left wing-side of the animal.
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24 369 Under the hypothesis that bilateral output in the sound field will be caused by coverage of the left-
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26 370 wing by the overlaying right wing, any asymmetry can be expected to increase throughout the
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28 371 duration of each syllable as the radiating membranes of the underlying wing become increasingly
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30 372 covered. It has been suggested that each wing contributes sound to one half of the pulse only
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32 373 (Simmons and Richie 1996), however, a more recent analysis of the vibrational response of both
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34 374 wings indicates that they both contribute sound components for the duration of each pulse
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36 375 (Montealegre-Z et al. 2011). Maximum amplitude of each pulse occurs at around 0.4 mm of wing
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38 376 displacement; around the midpoint of the wing closure (Montealegre-Z et al. 2011) and amplitude
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40 377 decreases thereafter. This decrease in pulse amplitude has been attributed to different levels of
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42 378 mechanical excitation along the length of the file (Bennet-Clark 2003), however, if this reduction in
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44 379 amplitude is in part a result of the underlying left wing coverage by the right wing is unknown.
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46 380 Identifying the lateral intensity levels at different stages throughout the pulse, and accounting for
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48 381 varying wing positioning throughout each closing stroke (Koch 1980), could highlight any increasing
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50 382 asymmetry present as a result of increasing left-wing coverage.
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3 384 As has been reported previously (Forrest 1991), the data indicates a focused directional dumbbell
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5 385 shaped sound field which lies perpendicular to the radiating cells as is suggested for the wings of
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7 386 stridulating crickets acting as dipole radiators (Forrest 1982). Models of wing membranes have been
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9 387 previously modelled as a circular disc vibrating without a baffle. Assuming the disc diameter is small
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11 388 in relation to the wavelength of the sound, the pressure field (P_θ) of the dipole is proportional to
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13 389 $P_\theta \approx |\sin(\theta)|$ where θ is the angle from the plane of the piston (Forrest 1991). The radiating harp
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15 390 (and immediate surrounding area) of the cricket wings producing the main amplitude components of
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17 391 the call has a diameter of ~ 4.7 mm which fits the assumption (such that wavelength (λ) = 7.2 cm and
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19 392 diameter = 0.059λ). This predicted pattern was scaled so that the radial distance exactly
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21 393 perpendicular to the disc was equal to the averaged relative amplitude value of the closest point on
22
23 394 the central line of the observed values (Figure 6). Contrary to the prediction, the relative amplitudes
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25 395 observed in this study do not approach 0 in the points of the rear facing recordings where the area
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27 396 of recording points is parallel to the wing membranes (blue dashed line – Figure 6.) although a
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29 397 reduction of amplitude is apparent in this area. In this study the wings of the specimens were
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31 398 positioned at $\sim 60^\circ$ from the horizontal plane. As the recordings here occurred no lower than
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33 399 horizontal in elevation, the area of high pressure 90° from the ventral surface of the membranes
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35 400 would occur beneath the quarter-sphere as observed here and thus was only observed on the
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37 401 frontal recordings (Figure 4). Equally, the relatively low pressure levels in the backward-facing
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39 402 recordings observed in this study are likely due to the focus of sound on the rear side of the
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41 403 membranes being below the lowest angle of recording (Figure 6). The area of greatest vibrational
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43 404 amplitude is the harp (Montealegre-Z et al. 2011) and it is possible that the areas of tegmen
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45 405 surrounding the harp and mirror act as a partial baffle to facilitate efficient signal production
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47 406 (Forrest 1982). The baffling effect of the surrounding wing areas on acoustic radiation to both dorsal
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49 407 and ventral sides of the membranes merits further investigation.
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3 409 The minor asymmetry observed in the projection pattern of sound pressure may not constitute a
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5 410 biologically relevant finding. Female detection of an acoustic trait affected by morphological
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7 411 asymmetry in crickets has only been demonstrated thus far in the discrimination of varying
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9 412 frequency modulation (Hirtenlehner et al. 2013). Asymmetries in sound pressure projection may not
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11 413 be distinguishable from natural amplitude fluctuations in the field (Römer and Lewald 1992) as the
12
13 414 females move towards the male (Hirtenlehner et al. 2014). Furthermore, the low differences in
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15 415 relative sound pressure between the two sides are unlikely to be of sufficient magnitude to affect
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17 416 female phonotactic choice in the field (Hirtenlehner and Römer 2014). Aggregations of males may
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19 417 attract distant females collectively and other parameters of the calling song would be subject to
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21 418 female preference when in hearing range of the calls of multiple individuals (Simmons 1988). As
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23 419 such, a positive selection for omnidirectional equality of the calling song intensity as a trait in males
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25 420 seems to be unlikely. The frontal directionality of sound pressure demonstrated in this study may be
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27 421 favourable to field crickets while performing the aggressive song to conspecific males who
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29 422 conventionally interact directly facing each other during antagonistic encounters (Adamo and Hoy
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31 423 1995), therefore making minor asymmetries irrelevant to signal function. While temporally different
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33 424 from the calling song (Alexander 1961), the aggressive song utilises the same mechanical acoustic
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35 425 generation process and as such the directional output of the wing cells is unlikely to differ between
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37 426 song types. The frontal projection of high amplitudes may convey reliable information about the
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39 427 individual's size (Gray 1997) and prevent fights from escalating (Alexander 1961). **Bilateral**
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41 428 **asymmetry in the sound field is unlikely to have a major effect on communication on the ground**
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43 429 **where field crickets sing, however, not all tegminal stridulators sing on this level. Mole crickets, for**
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45 430 **example, sing from within horn shaped burrows (Bennet-Clark 1970) with the sound field around the**
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47 431 **mouth of the burrow being hemispherical in seemingly equal amplitude components (see Forrest**
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49 432 **1991). It is unlikely that bilateral asymmetry at the point of sound production (the singing insect) will**
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51 433 **affect the sound projecting from the mouth of the burrows of mole crickets. A further interesting**
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53 434 **example of tegminal stridulation comes from the short tailed cricket *Anurogryllus arboreus* who,**
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3 435 unlike similar species, sings above the ground on trees, shrubs and low vegetation (Paul and Walker
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5 436 1979). Similarly is the case of tree crickets who sing from leaf cover and using baffles (Mhatre et al.
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7 437 2012). In these cases, the three dimensional projection of sound, including the area ventral to the
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9 438 animals position (which we did not investigate here), may be more affected by bilateral asymmetry
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11 439 than those species singing close to the ground where excess attenuation and absorption may play a
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13 440 greater role. Further research into the sound fields of crickets should therefore include species from
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15 441 diverse habitats and with varying singing behaviours to investigate and compare the effects of
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17 442 sound field asymmetry under more natural conditions.

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20 443 The method presented here offers an opportunity to collect high-resolution data for sound pressure
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22 444 levels in the sound field around a singing cricket. This allows for accurate identification of the signal
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24 445 amplitude from multiple positions from both the left-wing side and the right-wing side of the animal
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26 446 in three dimensions to investigate differences in sound pressure. This study indicates that field
27
28 447 crickets produce an asymmetrical sound field during stridulation but variation between individuals
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30 448 exist in the natural projection of sound pressure levels to one side of the insect whilst calling. The
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32 449 observed individual variation suggests that mechanical asymmetry and coverage of the underlying
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34 450 wing cannot be concluded as a major cause of lateral sound field asymmetry and furthermore that
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36 451 minor asymmetries in sound field projection are unlikely to have an impact on signal function.

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39 452 Further investigations of symmetry in the projected sound field should focus on angular position and
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41 453 superposition of the wings throughout each closing stroke. Understanding the effect of lateral sound
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43 454 field asymmetry in relation to female phonotaxis and the response of conspecifics at close range
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45 455 encounters (e.g. females during courtship behaviours or males during aggressive interactions) will
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47 456 help elucidate any selection pressures which could select for directional signal transmission in
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49 457 stridulating crickets.

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3 459 **Supplementary material**
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6 460 **Movie 1** A male adult *Gryllus bimaculatus* performing the calling song under pharmacological
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8 461 elicitation whilst restrained.
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11 462 **Movie 2** A singing male undergoing the recording procedure with the microphone maneuvered by
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13 463 the robotic arm. Animal positioned for a rear-facing recording.
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19 465 **Acknowledgements**
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27
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30 470 **manuscript.**
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1
2
3 474 **References**

4
5
6 475 Adamo SA, Hoy RR. 1995. Agonistic behaviour in male and female field crickets, *Gryllus bimaculatus*,
7
8 476 and how behavioural context influences its expression. *Anim. Behav.* 49:1491-1501.

9
10
11 477 Alexander RD. 1961. Aggressiveness, territoriality, and sexual behavior in field crickets (Orthoptera:
12
13 478 Gryllidae). *Behaviour.* 17:130-223.

14
15
16
17 479 Bennet-Clark H. 1970. The mechanism and efficiency of sound production in mole crickets. *J. Exp.*
18
19 480 *Biol.* 52:619-652.

20
21
22 481 Bennet-Clark HC. 1987. The tuned singing burrow of mole crickets. *J. Exp. Biol.* 128:383-409.

23
24
25
26 482 Bennet-Clark HC. 1998. Size and scale effects as constraints in insect sound communication. *Philos.*
27
28 483 *T. R. Soc. B.* 353:407-419.

29
30
31 484 Bennet-Clark HC. 1999. Resonators in insect sound production: how insects produce loud pure-tone
32
33 485 songs. *J. Exp. Biol.* 202:3347-3357.

34
35
36
37 486 Bennet-Clark HC. 2003. Wing resonances in the Australian field cricket *Teleogryllus oceanicus*. *J. Exp.*
38
39 487 *Biol.* 206:1479-1496.

40
41
42 488 Bennet-Clark HC, Bailey WJ. 2002. Ticking of the clockwork cricket: the role of the escapement
43
44 489 mechanism. *J. Exp. Biol.* 205:613-625.

45
46
47 490 Crawley MJ. 2005. *Statistics: an introduction using R*. West Sussex, UK: Wiley.

48
49
50
51 491 Daws A, Bennet-Clark HC, Fletcher N. 1996. The mechanism of tuning of the mole cricket singing
52
53 492 burrow. *Bioacoustics* 7:81-117.

54
55
56 493 Elliott C, Koch U. 1985. The clockwork cricket. *Naturwissenschaften.* 72:150-153.
57
58
59
60

- 1
2
3 494 Ewing AW. 1989. *Arthropod bioacoustics: neurobiology and behaviour*. Edinburgh: Edinburgh
4
5 495 University Press.
6
7
8 496 Fielden A. 1960. Transmission through the last abdominal ganglion of the dragonfly nymph, *Anax*
9
10 497 *imperator*. *J. Exp. Biol.* 37:832-844.
11
12
13 498 Forrest T. 1982. Acoustic communication and baffling behaviors of crickets. *Fla. Entomol.* 65:33-44.
14
15
16
17 499 Forrest T. 1991. Power output and efficiency of sound production by crickets. *Behav. Ecol.* 2:327-
18
19 500 338.
20
21
22 501 Forrest T. 1994. From sender to receiver: propagation and environmental effects on acoustic
23
24 502 signals. *Am. Zool.* 34:644-654.
25
26
27
28 503 Forrest T, Raspet R. 1994. Models of female choice in acoustic communication. *Behav. Ecol.* 5:293-
29
30 504 303.
31
32
33 505 Frings H, Frings M. 1958. Uses of sounds by insects. *Annu. Rev. Entomol.* 3:87-106.
34
35
36
37 506 Gerhardt HC, Huber F. 2002. *Acoustic communication in insects and anurans: common problems and*
38
39 507 *diverse solutions*. Chicago, University of Chicago Press.
40
41
42 508 Gray DA. 1997. Female house crickets, *Acheta domesticus*, prefer the chirps of large males. *Anim.*
43
44 509 *Behav.* 54:1553-1562.
45
46
47
48 510 Gray DA, Eckhardt G. 2001. Is cricket courtship song condition dependent? *Anim. Behav.* 62:871-877.
49
50
51 511 Hedwig B. 1996. A descending brain neuron elicits stridulation in the cricket *Gryllus bimaculatus* (de
52
53 512 Geer). *Naturwissenschaften* 83:428-429.
54
55
56
57
58
59
60

- 1
2
3 513 Hirtenlehner S, Kung S, Kainz F, Römer, H. 2013. Asymmetry in cricket song: female preference and
4
5 514 proximate mechanism of discrimination. *J. Exp. Biol.* 216:2046-2054.
6
7
8 515 Hirtenlehner S, Römer H. 2014. Selective phonotaxis of female crickets under natural outdoor
9
10 516 conditions. *J. Comp. Physiol. A.* 200:239-250.
11
12
13 517 Hirtenlehner S, Römer H, Schmidt AK. 2014. Out of phase: relevance of the medial septum for
14
15 518 directional hearing and phonotaxis in the natural habitat of field crickets. *J. Comp. Physiol. A.*
16
17 519 200:139-148.
18
19
20
21 520 Huber F, Thorson J. 1985. Cricket auditory communication. *Scient. Am.* 253:60-68.
22
23
24 521 Koch UT. 1980. Analysis of cricket stridulation using miniature angle detectors. *J. Comp.*
25
26 522 *Physiol.* 136:247-256.
27
28
29
30 523 Koch UT, Elliott CJ, Schöffner K, Kleindienst H. 1988. The mechanics of stridulation of the cricket
31
32 524 *Gryllus campestris*. *J. Comp. Physiol. A.* 162:213-223.
33
34
35 525 Masaki S, Kataoka M, Shirato K, Nakagahara M. 1987. Evolutionary differentiation of right and left
36
37 526 tegmina in crickets. In *Evolutionary biology of orthoperoid insects*. (ed. B. Baccetti), pp. 347-357.
38
39 527 Chichester: Ellis Horwood.
40
41
42
43 528 Mhatre N, Bhattacharya M, Robert D, Balakrishnan R. 2011. Matching sender and receiver:
44
45 529 poikilothermy and frequency tuning in a tree cricket. *J. Exp. Biol.* 214:2569-2578.
46
47
48
49 530 Mhatre N, Montealgre-Z F, Balakrishnan R, Robert D. 2012. Changing resonator geometry to boost
50
51 531 sound power decouples size and song frequency in a small insect. *PNAS.* 109:E1444-E1452.
52
53
54 532 Michelsen A, Elsner N. 1999. Sound emission and the acoustic far field of a singing acridid
55
56 533 grasshopper (*Omocestus viridulus* L.). *J. Exp. Biol.* 202:1571-1577.
57
58
59
60

- 1
2
3 534 Michelsen A, Fonseca P. 2000. Spherical sound radiation patterns of singing grass cicadas,
4
5 535 *Tympanistalna gastrica*. *J. Comp. Physiol. A*. 186:163-168.
6
7
8 536 Montealegre-Z F, Guerra PA, Morris GK. 2003. *Panoploscelis specularis* (Orthoptera: Tettigoniidae:
9
10 537 Pseudophyllinae): extraordinary female sound generator, male description, male protest and calling
11
12 538 signals. *J. Orthopt. Res.* 12:173-181.
13
14
15
16 539 Montealegre-Z F, Mason, AC. 2005. The mechanics of sound production in *Panacanthus pallicornis*
17
18 540 (Orthoptera: Tettigoniidae: Conocephalinae): the stridulatory motor patterns. *J. Exp. Biol.* 208:1219-
19
20 541 1237.
21
22
23
24 542 Montealegre-Z F, Jonsson T, Robert D. 2011. Sound radiation and wing mechanics in stridulating field
25
26 543 crickets (Orthoptera: Gryllidae). *J. Exp. Biol.* 214:2105-2117.
27
28
29 544 Naguib M, Wiley R. 2001. Estimating the distance to a source of sound: mechanisms and adaptations
30
31 545 for long-range communication. *Anim. Behav.* 62:825-837.
32
33
34 546 Pacheco K, Bertram SM. 2014. How male sound pressure level influences phonotaxis in virgin female
35
36 547 Jamaican field crickets (*Gryllus assimilis*). *PeerJ*. 2:e437.
37
38
39
40 548 Paul R, Walker T. 1979. Arboreal singing in a burrowing cricket, *anurogryllus arboreus*. . *J. Comp.*
41
42 549 *Physiol.* 132:217-223.
43
44
45 550 Pierce GW. 1948. *The Songs of Insects*. Cambridge, Massachusetts: Harvard University Press.
46
47
48
49 551 Pitchers W, Klingenberg C, Tregenza T, Hunt J, Dworkin I. 2014. The potential influence of
50
51 552 morphology on the evolutionary divergence of an acoustic signal. *J. Evol. Biol.* 27:2163-2176.
52
53
54 553 Prestwich K. 1994. The energetics of acoustic signalling in anurans and insects. *Am. Zool.* 34:625-643.
55
56
57
58
59
60

- 1
2
3 554 Prestwich K, Lenihan KM, Martin DM. 2000. The control of carrier frequency in cricket calls: a
4
5 555 refutation of the subalar–tegmental resonance/auditory feedback model. *J. Exp. Biol.* 203:585-596.
6
7
8 556 Prestwich K, O’Sullivan K. 2005. Simultaneous measurement of metabolic and acoustic power and
9
10 557 the efficiency of sound production in two mole cricket species (Orthoptera: Gryllotalpidae). *J. Exp.*
11
12 558 *Biol.* 208:1495-1512.
13
14
15
16 559 Römer H, Lewald J. 1992. High-frequency sound transmission in natural habitats: implications for the
17
18 560 evolution of insect acoustic communication. *Behav. Ecol. Sociobiol.* 29:437-444.
19
20
21 561 Römer H. 1993. Environmental and biological constraints for the evolution of long-range signalling
22
23 562 and hearing in acoustic insects. *Phil. Trans. Roy. Soc. B-Biol. Sci.* 340:179-185.
24
25
26
27 563 Russell DA, Titlow JP, Bemmen Y. 1999. Acoustic monopoles, dipoles, and quadrupoles: An
28
29 564 experiment revisited. *Am. J. Phys.* 67:660-664.
30
31
32 565 Searcy WA, Andersson M. 1986. Sexual Selection and the Evolution of Song. *Annu. Rev. Ecol. Syst.*
33
34 566 17:507-533.
35
36
37 567 Simmons L. 1988. The calling song of the field cricket, *Gryllus bimaculatus* (de geer): constraints on
38
39 568 transmission and its role in intermale competition and female choice. *Anim. Behav.* 36:380-394.
40
41
42
43 569 Simmons L. 1995. Correlates of male quality in the field cricket, *Gryllus campestris* L.: age, size, and
44
45 570 symmetry determine pairing success in field populations. *Behav. Ecol.* 6:376-381.
46
47
48
49 571 Simmons L, Ritchie M. 1996. Symmetry in the songs of crickets. *P. Roy. Soc. B-Biol. Sci.* 263:1305-
50
51 572 1311.
52
53
54 573 Snijders TA, Borgatti SP. 1999. Non-parametric standard errors and tests for network
55
56 574 statistics. *Connections.* 22:161-170.
57
58
59
60

- 1
2
3 575 Wagner Jr WE, Reiser MG. 2000. The importance of calling song and courtship song in female mate
4
5 576 choice in the variable field cricket. *Anim. Behav.* 59:1219-1226.
6
7
8 577 Wenzel B, Elsner N, Hedwig B. 1998. Microinjection of neuroactive substances into brain neuropil
9
10 578 controls stridulation in the cricket *Gryllus bimaculatus* (de Geer). *Naturwissenschaften.* 85:452-454.
11
12
13
14 579 Wenzel B, Hedwig B. 1999. Neurochemical control of cricket stridulation revealed by
15
16 580 pharmacological microinjections into the brain. *J. Exp. Biol.* 202:2203-2216.
17
18
19 581 Wiley RH, Richards DG. 1978. Physical constraints on acoustic communication in the atmosphere:
20
21 582 implications for the evolution of animal vocalizations. *Behav. Ecol. Sociobiol.* 3:69-94.
22
23
24
25 583
26
27
28
29
30
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584 **Table 1.** Sound pressure levels for front facing recordings for all specimens. Permutation test outputs
 585 for relative amplitudes testing all points on each lateral side for differences for each specimen (see
 586 text). For specimens with multiple recordings, the mean of each recording for every point was used.
 587 Pascal values from robot controlled microphone only. Pa, Pascals. dB, decibel re 20 μ Pa. SPL, sound
 588 pressure level.

Specimen	Mean of whole recording		Mean of right-wing side		Mean of left-wing side		<i>P</i>	Side of greatest SPL
	(Pa)	(dB)	(Pa)	(dB)	(Pa)	(dB)		
10(<i>n</i> =2)	0.111	74.85	0.117	75.34	0.104	74.32	0.1278	right
13	0.053	68.46	0.056	68.89	0.049	67.89	<0.001	right
15	0.162	78.16	0.154	77.75	0.166	78.38	0.0034	left
16	0.117	75.34	0.131	76.30	0.101	74.03	<0.001	right
19(<i>n</i> =2)	0.143	77.07	0.128	76.14	0.157	77.91	0.0001	left
20	0.139	76.81	0.156	77.81	0.116	75.3	<0.0001	right
21	0.111	74.85	0.114	75.10	0.107	74.56	<0.0001	right
24	0.225	81.02	0.224	80.96	0.221	80.86	0.4539	right

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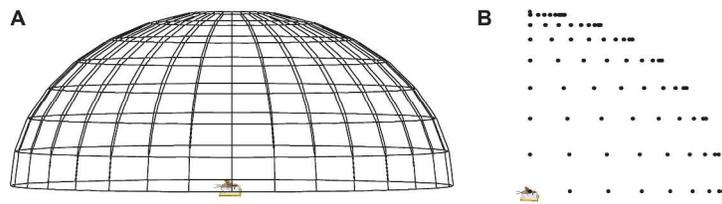
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606 **Figure captions**

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5 607 1. **Figure 1. A** Hemisphere of points equidistant to the centre of the same resolution used in
6 608 the recording procedure. Only half the hemisphere is taken per recording. **B** Lateral view of
7 609 the quarter-sphere comprising 136 points used for each recording. Each point represents a
8 610 recording point of the microphone. Animal positioned as for frontal recordings. Image is not
9 611 to scale.
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14 613 2. **Figure 2.** Full experimental setup (not to scale). Animal positioned as for frontal recording.
15 614 B&K, Brüel & Kjær; Mic, microphone.
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19 616 3. **Figure 3.** Dorsal view of the quarter-sphere (from Fig.1:B; not to scale) indicating points
20 617 designated as the left-wing side of the animal (red) and the right-wing side (black).
21 618 Recording points frontal to the animal (blue) were not used for analysis of asymmetry.
22 619 Image represents a front-facing recording.
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27 621 4. **Figure 4.** Plots of averaged relative amplitude values as sound field patterns for front (n=8)
28 622 and rear-facing (n=6) recordings. **A** Front-facing recordings. **B** Rear-facing recordings. Grid
29 623 corners represent recording points. Colour scheme for relative amplitudes interpolated
30 624 across the quarter-sphere. Arrows indicate positional facing of the animal. RWS, right wing
31 625 side (overlying wing). LWS, left wing side (underlying wing).
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35 627 5. **Figure 5.** Mean relative amplitudes of recording points on each side from all frontal
36 628 recordings (black bars). Error bars on relative amplitudes indicates bootstrap confidence
37 629 intervals (see text). ***= <0.001 .
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- 39 631 6. **Figure 6.** The estimated sound field. **A** Dorsal view of the recording quarter-sphere, red points
40 632 indicate central line recording positions. **B** Lateral view of observed and predicted sound
41 633 field projection shapes for recording points from the central line (elevation plane) for both
42 634 frontal and rear-facing recordings. Red dashed line indicates predicted radiation pattern of a
43 635 free piston and is angled to be at 90° from the wing angle in line with the observed values;
44 636 blue dashed line indicates wing angle. Black solid line indicates observed average relative
45 637 amplitude values for each central recording point. Black dashed lines indicate angle of
46 638 elevation (11.5° increments).
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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.
279x361mm (300 x 300 DPI)

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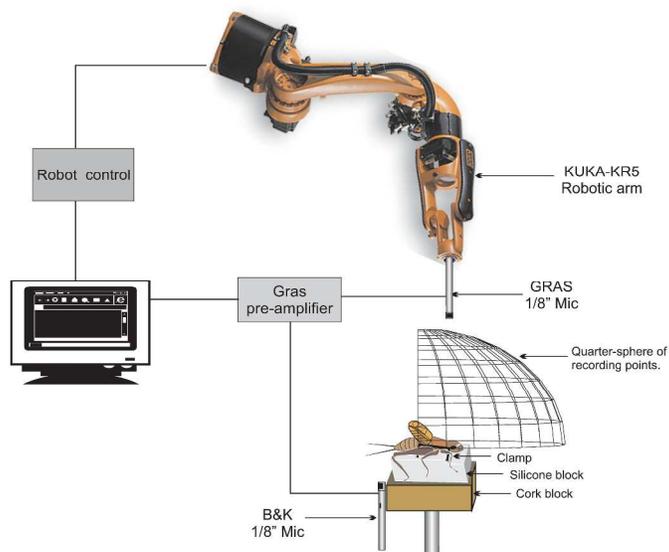


Figure 2. Full experimental setup (not to scale). Animal positioned as for frontal recording. B&K, Brüel & Kjær; Mic, microphone.

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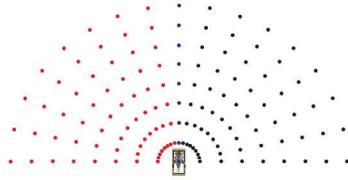


Figure 3. Dorsal view of the quarter-sphere (from Fig.1:B; not to scale) indicating points designated as the left-wing side of the animal (red) and the right-wing side (black). Recording points frontal to the animal (blue) were not used for analysis of asymmetry. Image represents a front-facing recording.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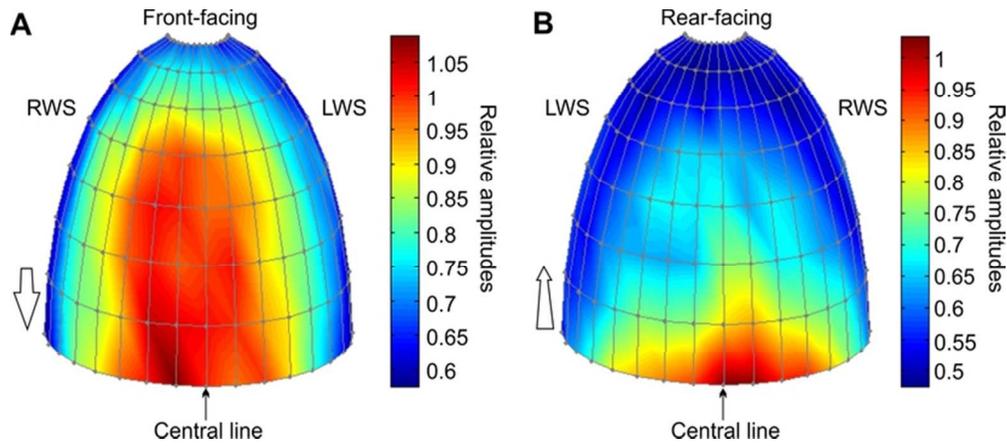


Figure 4. Plots of averaged relative amplitude values as sound field patterns for front ($n=8$) and rear-facing ($n=6$) recordings. A Front-facing recordings. B Rear-facing recordings. Grid corners represent recording points. Colour scheme for relative amplitudes interpolated across the quarter-sphere. Arrows indicate positional facing of the animal. RWS, right wing side (overlying wing). LWS, left wing side (underlying wing). 69x29mm (300 x 300 DPI)

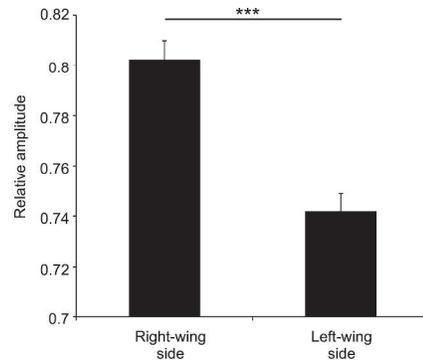


Figure 5. Mean relative amplitudes of recording points on each side from all frontal recordings (black bars). Error bars on relative amplitudes indicates bootstrap confidence intervals (see text). ***= <0.001 .
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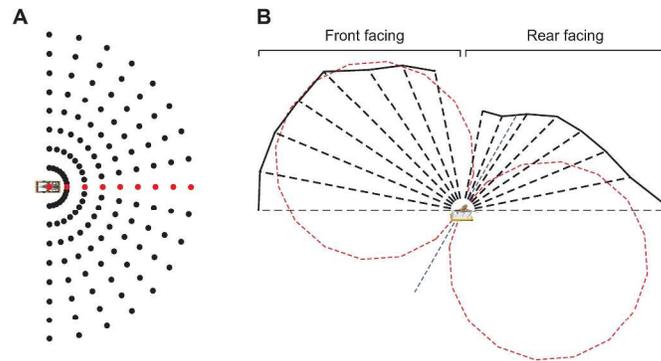


Figure 6. The estimated sound field. A Dorsal view of the recording quarter-sphere, red points indicate central line recording positions. B Lateral view of observed and predicted sound field projection shapes for recording points from the central line (elevation plane) for both frontal and rear-facing 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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