How male signaling intensity influences phonotaxis in virgin female Jamaican field crickets

(*Gryllus assimilis*)

Karen Pacheco and Susan M. Bertram

Department of Biology, Carleton University, Ottawa, Ontario, Canada

Corresponding Author:

Susan M. Bertram
Carleton University
1125 Colonel By Drive, Ottawa, ON, Canada
4631 CTTC Building
sue_bertram@carleton.ca

1   **Abstract**

2   Understanding female mate preference is important for determining the strength and the

3   direction of sexual trait evolution. Male signalling intensity is often an important predictor of

4   mating success because higher intensity (louder) signallers are detectable at greater distances.

5   However, if females are simultaneously more attracted to higher signalling intensities, then the

6   potential fitness impacts of higher intensity signalling should be elevated beyond what would be

7   expected from detection distance alone. Here we manipulated the signal intensity of cricket mate

8   attraction signals to determine how female phonotaxis was influenced. We examined female

9   phonotaxis using two common methodologies: spherical treadmills and open arenas. Both

10   methodologies showed similar results, with females exhibiting highest phonotaxis towards loud

11   ($\overline{X}+1$ SD = 69 dB) mate attraction signals but showing reduced phonotaxis towards the loudest

12   ($\overline{X}+2$ SD = 77 dB) signals. Reduced phonotaxis towards supernormal stimuli may occur for

13   several reasons including elevating the females' perceived predation risk, invoking females'

14   acoustic startle response, or exceeding females' perceptual limits.

Introduction

Acoustic communication in most anurans and insects is an essential part of courtship and reproductive behaviour (Alexander, 1975; Wells, 1977; Gerhardt, 1991; Gerhardt & Huber, 2002). In many species, acoustic signalling is the main sexual display and serves to attract receptive females from a distance (Walker, 1957; Alexander, 1962; Gerhardt, 1991; Ryan & Keddy-Hector, 1992). Behavioural studies have identified several specific components of male acoustic signals used for species recognition and to confer attractiveness (Rand & Ryan, 1981; Gerhardt, 1982; Simmons & Zuk, 1992; Wilczynski, Rand & Ryan, 1995). A broad assessment of the literature reveals that females generally prefer components of male acoustic signals that lie above the population mean (reviewed by Ryan & Keddy-Hector, 1992).

Signal intensity (also called loudness, amplitude, or sound pressure level) can be an important predictor of mate choice (Stout & McGhee, 1988; Castellano et al., 2000; Klappert & Reinhold, 2003; Hedwig & Poulet, 2005) with females tending to prefer louder signals when given the choice (Whitney & Krebs, 1975; Sullivan, 1983; Wells & Schwartz, 1984; Ryan, 1985; Gerhardt, 1991). For example, Arak (1988) revealed that female natterjack toads (*Bufo calamita*) can perceive small differences in male sound pressure levels and typically prefer louder males. Female wax moths (*Achroia grisella)* also prefer males produce louder ultrasound signals that contain higher acoustic energy and power (Jang & Greenfield, 1996).

Males that produce louder signals should be detectable at greater distances; louder males should therefore receive a selective advantage over quieter males that have a shorter detection distance (Forrest & Green, 1991; Forrest & Raspet, 1994). However, if females also prefer louder males over quieter males then the potential fitness impacts of loud signalling should be elevated beyond what would be expected from detection distance alone. It is therefore important

38   to quantify female preference functions for signal intensity to gain insights into the evolutionary

39   consequences and patterns of selection on this trait. Unfortunately, female preference functions

40   for signal intensity have been understudied in crickets despite extensive studies on female

41   preference for other male signal components.

42   Adult male field crickets produce long distance acoustic mate attraction signals (also

43   known as calls) by rubbing their modified forewings together (Alexander, 1962). When a male

44   closes his wings, the scraper of one wing hits the teeth of the file on the other wing the harp

45   resonates and produces a single pulse of sound (Bennet-Clark, 2003). Males concatenate these

46   pulses into chirps (Alexander, 1962; Bennet-Clark, 2003) and females use these long distance

47   acoustic signals to both orient towards signalling males (phonotaxis) and choose between

48   potential mates (Alexander, 1962).

49   To date, only a handful of the experiments quantifying female preference for signal

50   intensity have explored a broad range of signalling intensities (Table 1). Even fewer have

51   explored the extremes of the natural range of intensities available to females. Further, most

52   studies examining female preference for signal intensity often simultaneously manipulate other

53   signalling parameters, making it difficult to tease apart the influence that intensity alone has on

54   female preference. For example, Stout and McGhee (1988) examined the relative importance of

55   pulse + interpulse duration (syllable period), chirp rate and signal intensity on female mate

56   preference. Unfortunately, Stout and McGhee (1988) made no reference to the natural range of

57   signal intensity found in this species. Instead, they quantified female phonotactic response at a 2

58   dB, 5 dB and 10 dB increase above their standard male signal intensity of 65 dB sound pressure

59   level (SPL). By presenting female European house crickets (*Acheta domesticus*) with a pair of

60   male signals differing in one or more of these parameters, Stout and McGhee (1988) concluded

61   that signal intensity was more important than chirp rate, but that syllable period was more

62   important than signal intensity in influencing female mate choice. While there is emerging

63   interest in determining how multiple signal parameters interact to influence patterns of selection

64   and the evolutionary consequences of signalling (Brooks et al., 2005), it is first worth examining

65   how signal intensity alone shapes female preference functions.

66   Here we quantify female Jamaican field crickets (*Gryllus assimilis*) phonotactic response

67   to variation in male's long distance acoustic mate attraction signal intensity using two standard

68   research methodologies, a spherical treadmill and open arena. These two methodologies fall

69   within two broader categories of testing: open-loop and closed-loop methods, respectively.

70   Open-loop methods (Kramer spherical treadmill also called a trackball or locomotion

71   compensator) tether a female in one place, so that she does not experience changes in sound

72   intensity as she walks 'toward' the mate attraction signal (Kramer, 1976; Weber, Thorson &

73   Huber, 1981; Doherty, 1985; Doherty & Pires, 1987; trackball: Hedwig & Poulet, 2005; Hedwig,

74   2006). Closed-loop methods (open arena) allow the female to move within the enclosed space,

75   thereby allowing her to experience changes in sound intensity as she approaches the mate

76   attraction signal. Quantifying female preference for the intensity of the acoustic mate attraction

77   signal provides a powerful comparison of whether one technique quantifies female phonotaxis

78   more effectively than the other because sound intensity is one of the primary components that

79   differ between the two techniques. All signal parameters of the acoustic mate attraction signal

80   were held constant except signal intensity, which varied 1 or 2 standard deviations above or

81   below the average male signal intensity. The focus of our study was therefore two-fold: 1) to

82   examine female phonotaxis toward long distance acoustic mate attraction signals that vary in

83   signal intensity across the natural range of this species, and 2) to compare female phonotaxis on

84   the spherical treadmill to female phonotaxis in the open arena.

85

86   **Methods**

87   ***Cricket Rearing***

88   Our founding population of *Gryllus assimilis* was originally collected in Bastrop County,

89   Texas, United States (latitude ~ 30° 17' N, longitude ~ 97° 46' W, elevation ~145 m) from 15 to

90   24 September, 2008. We did not require specific permits for collecting invertebrates because

91   these crickets are neither endangered nor protected. We imported adult crickets and eggs to the

92   greenhouse laboratories at Carleton University, Ottawa, Canada (Canadian Food Inspection

93   Agency permit # 2007-03130). Our greenhouse facilities are Plant Pest Containment Level 1

94   certified (Canadian Food Inspection Agency permit # P-2012-03836). While we did not require

95   specific permits to conduct our cricket experiments, our study was conducted in accordance with

96   the guidelines of the Canadian Council on Animal Care.

97   The crickets were reared in communal plastic bins (64 cm x 40 cm x 42 cm) with a 16:8 h

98   L:D illumination period at 28±2 °C and fed *ad libitum* water and food (Harland's Tekland

99   Rodent diet 8604M; 24.3% protein, 40.2% carbohydrate, 4.7% lipid, 16.4% fiber, 7.4% ash). In

100   late 2012 (12-16 generations after field collection) we collected 30 4th instar females at random

101   from the communal cricket bins (no wing bud development; ovipositor had just become visible).

102   These juveniles were housed together in a separate communal bin (same conditions as described

103   above) and monitored daily for imaginal eclosion. Within 24 hours of imaginal eclosion we

104   transferred the adult females to individual 520mL (11 cm diameter x 7 cm height) clear plastic

105   containers with screened lids (4cm x 4cm mesh screens). Each female was provided with a small

106   piece of cardboard egg container for shelter and *ad libitum* water and food; light cycles and

107   temperatures were identical to the communal rearing environment. All females were tested daily

108   from 10-13 days post imaginal moult (hereafter referred to as 10-13 days old) because this is the

109   age range when female *G. assimilis* are most phonotactically responsive (Pacheco et al., 2013).

110   ***Standard and Focal Mate Attraction Signals***

111   We created a standard long distance mate attraction signal (hereafter standard signal)

112   using Adobe Audition CS5.5 software (Adobe Systems Incorporated, San Jose, California,

113   USA). We fashioned this standard signal after results published in Whattam and Bertram (2011).

114   This study showed that male acoustic mate attraction signaling is affected by diet; we selected

115   parameters that reflect the mean signal parameters from a population of males reared on high

116   quality food (recordings made at 26°C). The standard signal's parameters were: carrier frequency

117   = 3719 Hz, pulse duration = 10.14 ms, interpulse duration = 15.21 ms, pulses per chirp = 8,

118   interchirp duration = 1055 ms; broadcast at an intensity of 60.6 dB SPL (re: 20μPa RMS) as

119   measured using an EXTECH Digital Sound Level Meter (Model # 407768; FLIR Systems,

120   Waltham, Massachusetts, USA) with probe pointing directly at the active speaker, directly above

121   the spherical treadmill, 17 cm from the active speaker.

122   Focal signals only differed from the standard signal in their signal intensity. The range of

123   focal signal intensities used came from Whattam and Bertram (2011), who revealed that the

124   population's signal intensity was 60.6±8 dB ($\overline{X}$±1 SD), with a range from 34.2 dB to 71.9 dB.

125   We therefore used 43 dB ($\overline{X}$-2 SD), 52 dB ($\overline{X}$-1 SD), 69 dB ($\overline{X}$+1 SD) and 77 dB ($\overline{X}$+2 SD) as

126   our focal signals. This range of focal signals ensured that the loudest signals played ($\overline{X}$+2 SD)

127   were louder than the natural range females are accustomed to hearing, while the quietest signals

128   played ($\overline{X}$-2 SD) were within the natural range.

129   *Preference Trials*

130   Each female's phonotaxis was tested using a two-choice trial where the standard signal

131   (60.6 dB) was simultaneously presented against one of the four focal signals. Each female was

132   tested across all four possible focal signals in both the arena and the spherical treadmill, for a

133   total of 8 tests. These comparisons occurred across four consecutive days (10-13 days old). On a

134   single test day, an individual female was tested once on the spherical treadmill and once in the

135   arena to a randomly assigned signal treatment. There was always at least 1 hour of rest prior to

136   switching methods. The order of the method the female was tested on first was randomized.

137   We ran all spherical treadmill and open arena trials in the dark under red light. In both

138   spherical treadmill and open arena trials females were given 60 seconds to acclimatize in silence.

139   Each signal was then broadcast on its own for 30 seconds (order and speaker side randomized) to

140   ensure the female heard both the focal and standard signals prior to starting the trial. Both signals

141   were then broadcast simultaneously from the speakers with the signal chirps interleaved

142   (alternating), so that a female had the potential to identify which signal was coming from which

143   speaker. Once both focal and standard signals were being broadcast, the trial began and female

144   phonotaxis was recorded. Every trial ran a total of 5 minutes. All trials were run in the evening

145   (between 5:00 PM and 8:00 PM), and each female was tested on four consecutive days.

146   We ran all spherical treadmill trials in a chamber (L x W x H = 86 x 87 x 57 cm) lined

147   with sound-attenuating foam. The spherical treadmill was located in the middle of the chamber

148   with two speakers on either side of it, each 17 cm from the center of the sphere and directly

149   across from each other. We used low melting point wax to attach a coil- (micro-compression)

150   spring (diameter: 3mm, length: 8mm; spring constant: 210.15 N/m) to each female's pronotum

151   on day 9 post final moult (one day prior to the first trial, *sensu* Pacheco et al., 2013). Just prior to

152   the start of the spherical treadmill trial, we mounted each focal female on the treadmill by

153   attaching her spring to a magnetic rod above the treadmill. This mounting ensured the cricket

154   was held in position with her feet on the polystyrene ball (photos in Pacheco et al., 2013). Air

155   pressure was adjusted such that the polystyrene ball, with the cricket in position, was able to

156   rotate freely in all directions as the cricket moved. All females were oriented in the same neutral

157   position at the start of their trial: tethered on the spherical treadmill facing straight ahead

158   between the two speakers.

159   Once both signals were being broadcast simultaneously from the speakers with the signal

160   chirps interleaved, the trial began and female phonotaxis was recorded relative to the focal

161   speaker. Both standard and focal signals were presented continuously throughout the 5 min trial,

162   and the female's locomotor behavior was recorded from the treadmill at a sample rate of 20

163   samples per second. Each 5 minute trial yielded a total of 6000 samples of cricket X, Y positions.

164   Temperature was held at 22-23°C in the trial room and was monitored with a Fisher Scientific

165   Traceable Digital Thermometer (Model # 15-077-20; Fisher Scientific, Toronto, Ontario,

166   Canada).

167   We calculated instantaneous displacement (cm) and velocity (cm/s) vectors from the

168   positional data (X, Y coordinates). Total path length was calculated as the sum of all vector

169   lengths over the 6000 samples. Female preference was quantified using net vector score (after

170   Huber et al., 1984). Net vector score is the female's net movement toward or away from the focal

171   signal during the 5 min trial and takes into account the female's direction (vector angle) and the

172   vector length of every recorded leg movement:

$$\text{Net Vector Score} = \sum_{t=1}^{6000} [\cos(vector\ angle(t)) \times vector\ length(t)]$$

173   We defined the angle of the focal speaker as 0°. Females moving directly toward the

174   focal speaker (0°) had positive vector scores [cos(0°) = 1], females moving directly away from

175   the focal speaker (180°) had negative vector scores [cos(180°) = -1], and females moving

176   perpendicular to the focal speaker (90° or 270°) had vector scores of 0 [cos(90° or 270°) = 0]. By

177   multiplying this value by each vector length, and summing over the trial duration, we quantified

178   the female's relative attraction to the focal signal. A large positive net vector score indicated that

179   the female moved quickly toward the focal speaker, a small positive score indicated the female

180   moved slowly toward the focal speaker, a large negative score indicated the female moved

181   quickly away from the focal speaker, and a small negative score indicated the female moved

182   slowly away from the focal speaker (Huber et al., 1984).

183   Open arena trials were conducted in the same room and temperature conditions as

184   spherical treadmill trials. We ran all open arena trials in a chamber (111.7 cm[l] x 50 cm[w] x 25

185   cm[h]) with the walls lined with sound-attenuating foam. The base of the arena was vacuumed

186   and wiped down before each trial order. Speakers were located at opposite ends of the arena

187   (lengthwise). We marked a semicircle "choice zone" (each 28 cm in radius) in front of each

188   speaker. At the start of the trial the female was placed in the center of the arena under an opaque

189   plastic container (10 cm[l] x 8.5 cm[w] x 11 cm[h]) attached to a string. After the 60-second

190   silent acclimatization period and 60 seconds of individual signal exposure (described above), the

191   string was pulled, resulting in the opaque plastic container being carefully and silently lifted up

192   away from the test female. As soon as the cup was lifted the trial began and ran for a total of 5

193   minutes. The following female responses were recorded: the amount of time spent stationary in

194   the middle of the arena without moving after the opaque plastic container was removed, time

195   spent in the arena outside of the choice zones (no-choice zone) after moving from the

196   acclimatization location, time spent in focal choice zone, time spent in standard choice zone, and

197   number of switches made between focal and standard choice zones.

198   Because of the varying amount of time spent in focal and standard choice zone, females

199   were classified as 'preferring' a particular focal signal treatment if, over the course of the trial,

200   she spent relatively more time in the zone with the focal signal than in the zone with the standard

201   signal (i.e. time outside choice zones were not included to determine female choice) *sensu*

202   Bischoff, Gould and Rubenstein (1985) and Dugatkin and Godin (1992). To quantify female

203   preference we subtracted the time spent in standard choice zone from the time spent in the focal

204   choice zone. A large positive number indicates a strong phonotaxis response from the female

205   with much more time spent in the focal zone than the standard zone; conversely, a large negative

206   number indicates much more time was spent in the standard zone than in the focal zone.

207   ***Statistical Analysis***

208   All data were analyzed using JMP 10.0.0 statistical software (SAS Institute Inc., 100 SAS

209   Campus Drive, Cary, North Carolina, USA). Total path length and average instantaneous

210   velocity magnitude from the spherical treadmill data were Box-Cox transformed to meet

211   assumptions of normality. Female preference data (net vector scores from the spherical treadmill

212   data and relative time spent in focal zone from the open arena data) were analyzed using repeated

213   measures general linear mixed models (GLMM) that took into account all the fixed effects (age,

214   signal intensity, method order, and focal speaker side) and covariates (female size and weight)

215   that could affect female preference; individual was treated as a random effect. Tukey's HSD

216   post-hoc tests were conducted to identify significant differences among the treatments.

217   To compare female preference on the spherical treadmill with the open arena, we

218   converted the spherical treadmill net vector scores and arena choice zone times into z scores

219 $(z = \frac{(x-\bar{x})}{s})$. Z scores taken from both methods were then combined and analyzed using a

220 repeated measures general linear mixed model design, where individual was treated as a random

221 effect. The following fixed effects were included in the model: age, signal intensity, female size,

222 female weight, experimental method (spherical treadmill or open arena) and method order

223 (which method was presented first). We used Tukey's HSD tests to ascertain significant

224 differences among the signal intensities when GLMM results were significant.

225

226 **Results**

227   Females on the spherical treadmill exhibited highly variable phonotaxis in response to the

228 different signal intensities. Some females walked substantially farther than others, with distance

229 walked ranging from 60 cm to 4900 cm. Females had over a 60-fold difference in their velocity

230 magnitudes, with the slowest moving at only 0.12 cm/s and the fastest moving at 8.22 cm/s.

231 Females also moved in all directions during trials relative to the focal speaker. There was a 4-

232 fold difference in net vector scores, indicating large variation in the strength of female

233 phonotaxis on the spherical treadmill. Signal intensity significantly influenced female phonotaxis

234 (net vector scores), explaining 9% of the variation in female phonotaxis (Table 2). Females were

235 most phonotactic towards the $\bar{X}+1$ SD = 69 dB signal intensity (Figure 1). All other signal

236 intensities evoked significantly reduced phonotaxis. Female phonotaxis was not significantly

237 influenced by method order (spherical treadmill or open arena), side the focal signal played from,

238 female age, size and weight (Table 2). The repeated measures GLMM revealed that ** % of the

239 variation was attributed to the random effects of individual.

240   Females in the open arena usually spent time in both standard and focal choice zones.

241 The handful of trials (17/120, 14%) where the female failed to move out of the no-choice zone

242   were excluded from analysis. Signal intensity significantly influenced female phonotaxis

243   (relative time spent in focal zone), explaining 11% of the variation (Table 2). Females were most

244   phonotactic towards the $\overline{X}+1$ SD = 69 dB signal intensity (Figure 2). All other signal intensities

245   evoked significantly reduced phonotaxis, with females exhibiting negative phonotaxis towards

246   the $\overline{X}+2$ SD = 77 dB signal intensity (Figure 2). Female phonotaxis was not significantly

247   influenced by method order (spherical treadmill or open arena), side the focal signal played from,

248   female age, size or weight (Table 2). The repeated measures GLMM revealed that ** % of the

249   variation was attributed to the random effects of individual.

250   We compared phonotaxis results across the two methodologies using z scores. Female

251   phonotaxis was significantly influenced by signal intensity, explaining 16% of the variation

252   (Table 2; Figure 3). All other factors, including experimental methodology, method order, the

253   side the focal signal was presented from, female age, size or weight did not significantly

254   influence female phonotaxis. Further, there was no significant interaction between signal

255   treatment and experimental method, focal speaker side and experimental method, or method

256   order and experimental method. The repeated measures GLMM revealed that ** % of the

257   variation was attributed to the random effects of individual.

258

259   **Discussion**

260   Female crickets exhibited positive phonotaxis towards loud male acoustic mate attraction

261   signals ($\overline{X}+1$ SD = 69 dB) while ignoring or avoiding quiet signals ($\overline{X}-2$ SD = 43 dB and $\overline{X}-1$ SD

262   = 52 dB; Figures 1-3). Our results suggest females may be unable to differentiate low decibel

263   sounds from the background noise. While few studies have examined female preference in

264   response to signal intensities lower than 50 dB (Table 1), our findings suggest quiet signals ($\overline{X}-2$

265   SD = 43 dB and $\overline{X}$-1 SD = 52 dB) may go undetected in nature because abiotic and biotic sounds

266   would likely elevate background noise levels beyond that of our controlled laboratory study

267   (44±2 dB testing room, 42±2 dB testing chambers).

268   Interestingly, female preference for elevated signal intensity is not open ended. Females

269   showed reduced phonotaxis towards the loudest mate attraction signals ($\overline{X}$+2 SD = 77 dB). The

270   77 dB treatment presents a signal intensity that falls well beyond *G. assimilis'* natural range of

271   34-71 dB (Whattam & Bertram, 2011), making it a novel supernormal stimulus. Females often

272   exhibited negative phonotaxis to this supernormal stimulus, moving away from it (Figures 1-3).

273   Weber, Thorson and Huber (1981) showed a similar response in *Gryllus campestris* L, with

274   females avoiding mate attraction signals above 70 dB. Negative phonotaxis may be indicative of

275   perceptual limits (Magnus, 1958; Hedrick & Weber, 1998), elevated predation risk (Moiseff,

276   Pollack & Hoy, 1978; Zuk & Kolluru, 1998), an acoustic startle response (Hoy, Nolen &

277   Brodfuehrer, 1989), signal rejection due to the absence of other sensory cues (Crapon de

278   Caprona & Ryan, 1990), or a 'stopping to look and listen' tactic (Weber, Thorson & Huber,

279   1981).

280   Our study also compared open arena and spherical treadmill methodologies. Quantifying

281   female preference for signal intensity provides a powerful comparison of whether one technique

282   quantifies female phonotaxis more effectively than the other because sound intensity is one of

283   the primary components that differ between the two techniques. We found that the two

284   methodologies produced virtually identical female phonotaxis results for each of the signal

285   intensities. Our findings are consistent with the handful of other studies that compare these

286   methodologies. Walikonis et al. (1991), Stout, Atkins and Zacharias (1991) and Pires and Hoy

287   (1992) revealed female phonotaxis was the same in the open arena and on the spherical treadmill.

288   Similar to our study, Stout, Atkins and Zacharias (1991) investigated female *Acheta domesticus*

289   response to differing signal intensities. Conversely, Walikonis et al. (1991) investigated female

290   *A. domesitcus* response to differing syllable periods while Pires and Hoy (1992) investigated

291   female *G. firmus* response to natural calls recorded songs at different temperatures. Given

292   intensity, syllable period, and temperature are important predictors of mating preference, our

293   joint findings suggest that open arena and spherical treadmill methodologies may be used

294   interchangeably to quantify phonotaxis to acoustic signals.

295   Caution is warranted, however, when signals are broadcasted at unnaturally loud

296   intensities because of fundamental methodological differences between the open arena and the

297   spherical treadmill. The open arena lets the female to move within the enclosed space, thereby

298   enabling her to experience changes in sound intensity. When individuals in the open arena run

299   away from very loud signals, they experience a ~6 dB drop in amplitude with every doubling of

300   their distance from the very loud signal (assuming a spherical, radiating sound source without

301   obstructions). However, when individuals on the spherical treadmill run away they do not

302   experience a drop in amplitude regardless of the distance they run because they are tethered in

303   place on the spherical treadmill.

304   Overall, our findings suggest that louder males should experience a fitness advantage

305   over quieter males. Using both open arenas and spherical treadmills we demonstrated that signal

306   intensity actively influences female phonotaxis, as females exhibited highest phonotaxis towards

307   loud mate attraction signals. Loud male signallers should, therefore, receive elevated fitness

308   advantages beyond what would be expected from detection distance alone. Female preference for

309   elevated signal intensity is not, however, open ended as females exhibit reduced phonotaxis

310   towards novel supernormal stimuli ($\overline{X}$+2 SD = 77 dB). Future research should explore how

311   important signal intensity is relative to other signalling parameters.

312

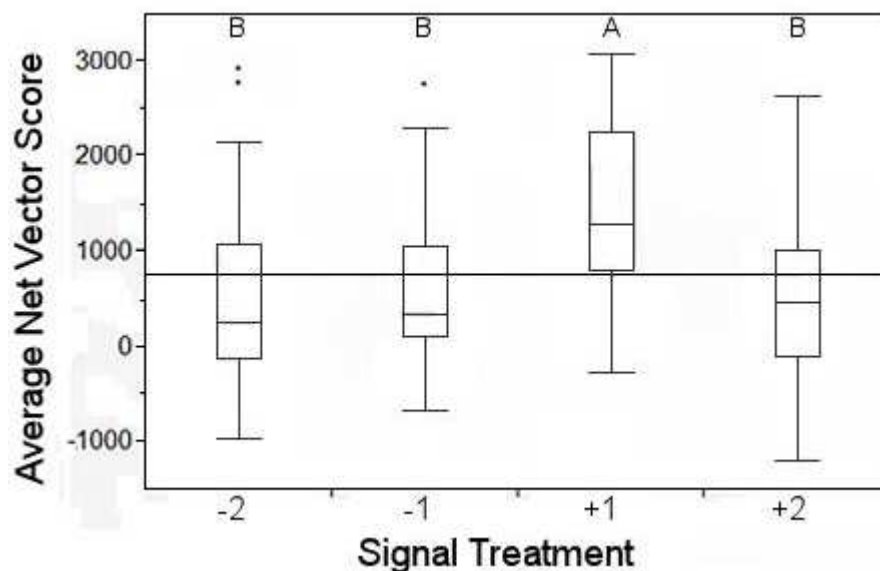313   **Acknowledgements**

325

326

Figure 1. Female phonotaxis on the spherical treadmill (average net vector scores) for each

signal intensity treatment ($\overline{X}$-2 SD = 43 dB, $\overline{X}$-1 SD = 52 dB, $\overline{X}$+1 SD = 69 dB, and $\overline{X}$+2 SD =

77 dB). Letters above each box plot were taken from a Tukey's post-hoc HSD analysis; signal

intensities with different letters are significantly different. The line within each box represents

the median. Error bars indicate the standard error above and below each mean and the dots
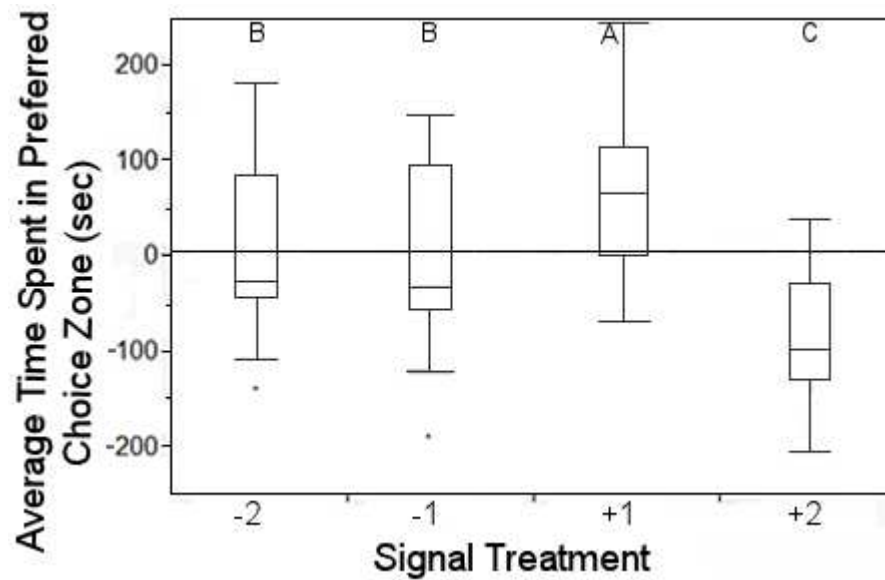
represent outliers.

333

Figure 2. Female phonotaxis in the open arena (relative time spent in the preferred choice zone: time in focal zone – time in standard zone) for each signal intensity treatment ($\overline{X}$-2 SD = 43 dB, $\overline{X}$-1 SD = 52 dB, $\overline{X}$+1 SD = 69 dB, and $\overline{X}$+2 SD = 77 dB).
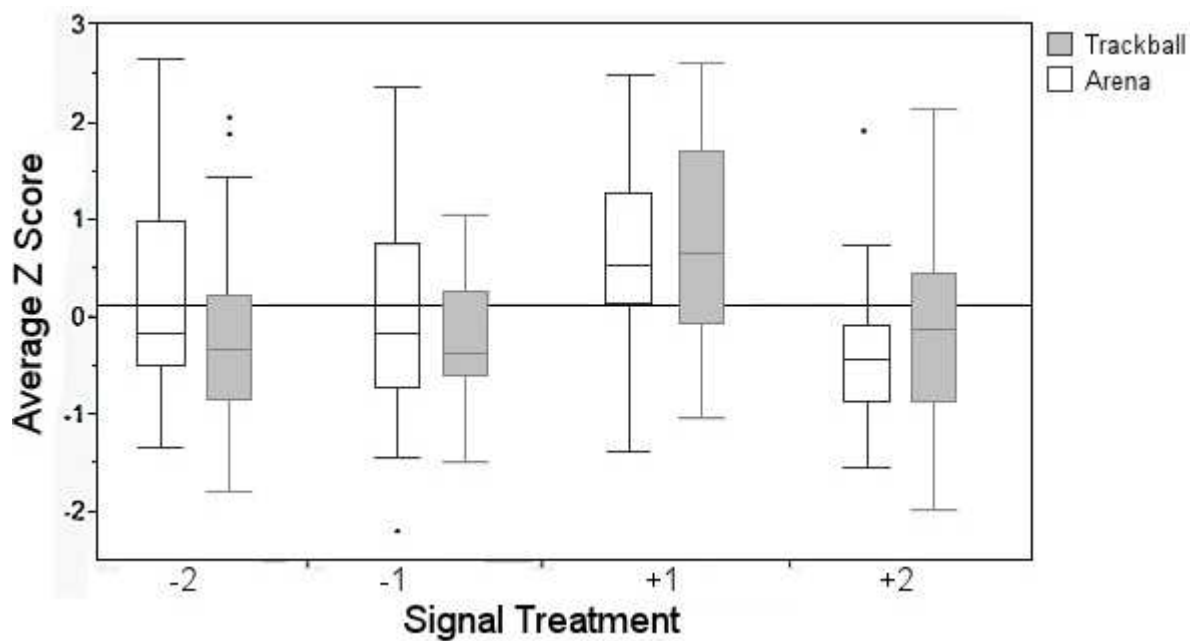
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338   Figure 3. Female phonotaxis (z scores) on the spherical treadmill and in the open arena in

339   response to four different signal treatments ($\overline{X}$-2 SD = 43 dB, $\overline{X}$-1 SD = 52 dB, $\overline{X}$+1 SD = 69 dB,

340   and $\overline{X}$+2 SD = 77 dB).

341 Table 1. Variation in the signal intensity (dB SPL) used in cricket female preference studies

Study	Species	Signal dB SPL tested
Scheuber, Jacot & Brinkhof 2004	*Gryllus campestris*	80 *
Lickman, Murray & Cade 1998	*Gryllus integer*	82-85 *
Gray & Cade 2000	*Gryllus texensis*	84
	Gryllus rubens	84
Bailey 2008	*Teleogryllus oceanicus*	70
Prosser, Murray & Cade 1997	*Gryllus integer*	83 *
Wagner, Murray & Cade 1995	*Gryllus integer*	84.6
Hedrick, Hisada & Mulloney 2007	*Gryllus integer*	72
Ritchie 1992	*Ephippiger ephippiger*	55
Olvido & Wagner 2004	*Allonemobius socius*	66±2

342
343 *reference is made to the natural range of signal intensity in the species

344   Table 2. Repeated measures GLMM outputs for factors affecting time spent in preferred open

345   arena zone, average net vector scores from the spherical treadmill, and z scores converted from

346   both methods.

Methodology	Source	DF	F ratio	P value
Spherical Treadmill	Age	3, 106.0	0.8268	0.4819
	Signal Intensity	**3, 105.3**	**5.5851**	**0.0014**
	Method Order	1, 109.0	0.0079	0.9295
	Focal Speaker Side	1, 100.4	2.3205	0.1308
	Size	1, 108.8	0.3154	0.5756
	Weight	1, 107.5	0.4602	0.4990
Open Arena	Age	3, 87.1	1.9207	0.1321
	Signal Intensity	**3, 87.3**	**7.1322**	**0.0002**
	Method Order	1, 91.5	0.0814	0.7760
	Focal Speaker Side	1, 91.9	0.2827	0.5962
	Size	1, 91.8	0.0332	0.8559
	Weight	1, 91.5	2.1915	0.1422
Comparing Methodologies	Age	3, 217.3	0.7478	0.5247
	Signal Intensity	**3, 216.8**	**10.2569**	**<0.0001**
	Method Order	1, 224.3	0.0000	0.9958
	Focal Speaker Side	1, 226.9	0.8380	0.1765
	Methodology	1, 217.9	0.8588	0.3551
	Size	1, 224.8	0.1548	0.6944
	Weight	1, 225.0	1.3575	0.2452

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