# How male sound pressure level influences phonotaxis in virgin female Jamaican field crickets (*Gryllus assimilis*)

Understanding female mate preference is important for determining the strength and direction of sexual trait evolution. The sound pressure level (SPL) acoustic signalers use is often an important predictor of mating success because higher sound pressure levels are detectable at greater distances. If females are more attracted to signals produced at higher sound pressure levels, then the potential fitness impacts of signalling at higher sound pressure levels should be elevated beyond what would be expected from detection distance alone. Here we manipulated the sound pressure level of cricket mate attraction signals to determine how female phonotaxis was influenced. We examined female phonotaxis using two common experimental methods: spherical treadmills and open arenas. Both methods showed similar results, with females exhibiting greatest phonotaxis towards loud sound pressure levels relative to the standard signal (69 vs. 60 dB SPL) but showing reduced phonotaxis towards very loud sound pressure level signals relative to the standard (77 vs. 60 dB SPL). Reduced female phonotaxis towards supernormal stimuli may signify an acoustic startle response, an absence of other required sensory cues, or perceived increases in predation risk.

1 **How male sound pressure level influences phonotaxis in virgin female Jamaican field**

2 **crickets (*Gryllus assimilis*)**

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11   **Abstract**

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

13   direction of sexual trait evolution. The sound pressure level (SPL) acoustic signalers use is often

14   an important predictor of mating success because higher sound pressure levels are detectable at

15   greater distances. If females are more attracted to signals produced at higher sound pressure

16   levels, then the potential fitness impacts of signalling at higher sound pressure levels should be

17   elevated beyond what would be expected from detection distance alone. Here we manipulated the

18   sound pressure level of cricket mate attraction signals to determine how female phonotaxis was

19   influenced. We examined female phonotaxis using two common experimental methods: spherical

20   treadmills and open arenas. Both methods showed similar results, with females exhibiting

21   greatest phonotaxis towards loud sound pressure levels relative to the standard signal (69 vs. 60

22   dB SPL) but showing reduced phonotaxis towards very loud sound pressure level signals relative

23   to the standard (77 vs. 60 dB SPL). Reduced female phonotaxis towards supernormal stimuli may

24   signify an acoustic startle response, an absence of other required sensory cues, or perceived

25   increases in predation risk.

## Introduction

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

Sound pressure level (SPL) 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 more intense 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 males that signal at higher sound levels. Female wax moths (*Achroia grisella)* also prefer males that produce ultrasound signals that contain higher acoustic energy and power (Jang & Greenfield, 1996).

Males that produce signals at a higher sound pressure level should be detectable at greater distances; males that signal with high sound pressure level should therefore receive a selective advantage over males that signal with low sound pressure level and subsequently have a shorter detection distance (Forrest & Green, 1991; Forrest & Raspet, 1994). However, if females also prefer higher sound pressure level males over lower sound pressure level males then the potential fitness impacts of higher sound pressure level signalling should be elevated beyond what would be expected from detection distance alone. It is therefore important to quantify female preference

50  functions for sound pressure level to gain insights into the evolutionary consequences and

51  patterns of selection on this trait.

52      Female preference functions for sound pressure level have been understudied in crickets

53  despite extensive studies on female preference for other male signal components. Adult male

54  field crickets produce long distance acoustic mate attraction signals (also known as calls) by

55  rubbing their modified forewings together (Alexander, 1962). When a male closes his wings, the

56  scraper of one wing hits the teeth of the file on the other wing causing the harp to resonate and

57  produce a single pulse of sound (Bennet-Clark, 2003). Males concatenate these pulses into chirps

58  (Alexander, 1962; Bennet-Clark, 2003) and females use these long distance acoustic signals to

59  both orient towards signalling males (phonotaxis) and choose between potential mates

60  (Alexander, 1962). To date, only a handful of the experiments quantifying female cricket

61  preference for sound pressure level have explored a broad range of sound levels (Table 1). Even

62  fewer have explored the extremes of the natural range of sound pressure levels available to

63  females. Further, most studies examining female preference for sound pressure level often

64  simultaneously manipulate other signalling parameters, making it difficult to tease apart the

65  influence that sound pressure level alone has on female preference. For example, Stout and

66  McGhee (1988) examined the relative importance of pulse + interpulse duration (syllable period),

67  chirp rate and signal sound pressure level on female mate preference. Unfortunately, Stout and

68  McGhee (1988) made no reference to the natural range of sound pressure levels found in nature.

69  Instead, they quantified female phonotactic response at a 2 dB, 5 dB and 10 dB SPL increase

70  above their standard male signal of 65 dB SPL. By presenting female European house crickets

71  (*Acheta domesticus*) with a pair of male signals differing in one or more of these parameters,

72  Stout and McGhee (1988) concluded that sound pressure level was more important than chirp

73  rate, but that syllable period was more important than sound pressure level in influencing female

74  mate choice. While there is emerging interest in determining how multiple signal parameters

75 interact to influence patterns of selection and the evolutionary consequences of signalling

76 (Brooks et al., 2005), it is first worth examining how sound pressure level alone shapes female

77 preference functions.

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

79 to variation in male long distance acoustic mate attraction signal sound pressure level using two

80 standard research methods, a spherical treadmill and open arena. These two methods fall within

81 two broader categories of testing: open-loop and closed-loop methods, respectively. Open-loop

82 methods (Kramer spherical treadmill also called a trackball or locomotion compensator) tether a

83 female in one place, so that she does not experience changes in sound pressure level as she walks

84 'toward' the mate attraction signal (Kramer, 1976; Weber, Thorson & Huber, 1981; Doherty,

85 1985; Doherty & Pires, 1987; trackball: Hedwig & Poulet, 2005; Hedwig, 2006). Closed-loop

86 methods (open arena) allow the female to move within the enclosed space, thereby allowing her

87 to experience natural changes in sound pressure level as she approaches the mate attraction

88 signal. Quantifying female preference for the sound pressure level of the acoustic mate attraction

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

90 more effectively than the other because sound pressure level is one of the primary components

91 that differ between the two techniques. The focus of our study was therefore two-fold: 1) to

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

93 sound pressure level across the natural range of this species, and 2) to compare female phonotaxis

94 on the spherical treadmill to the open arena.

95 **Methods**

96 ***Cricket Rearing***

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

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

99 24 September, 2008. We did not require specific permits for collecting invertebrates because

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

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

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

103 certified (Canadian Food Inspection Agency permit # P-2012-03836). Our study was conducted

104 in accordance with the guidelines of the Canadian Council on Animal Care.

105 The crickets were reared in communal plastic bins (L x W x H = 64 cm x 40 cm x 42 cm)

106 with a 14:10 h L:D illumination period (lights on at 0600 h, off at 2000 h) at 28 ± 2 °C. They

107 were fed *ad libitum* food (Harlan Teklad Rodent diet 8604M, Harlan Laboratories, Indianapolis,

108 IN, USA: 24.3% protein, 40.2% carbohydrate, 4.7% lipid, 16.4% fiber, 7.4% ash). Water was

109 provided in plastic containers filled with marbles to provide a surface to perch on to minimize

110 drowning. In late 2012 (12-16 generations after field collection) we haphazardly collected 30 4th

111 instar females from the communal cricket bins (no wing bud development; ovipositor had just

112 become visible). These juveniles were housed together in a separate communal bin (same

113 conditions as described above) and monitored daily for imaginal eclosion. Within 24 hours of

114 imaginal eclosion we transferred the adult females to individual 520mL (D x H = 11 cm x 7 cm)

115 clear plastic circular containers with screened lids (4 cm x 4 cm). Each female was provided with

116 a small piece of cardboard egg container for shelter, *ad libitum* food, and 4-5 water gels every 2-3

117 days (created by soaking 2-4 mm sized water polymer crystals in distilled water for 4-6 hours;

118 Feeder Source, Cleveland, GA, USA). The light cycles and temperatures were identical to the

119 communal rearing environment.

120 ***Standard and Focal Mate Attraction Signals***

121 We created our standard and focal mate attraction signals using Adobe Audition CS5.5

122 software (Adobe Systems Incorporated, San Jose, California, USA). We fashioned our signals

123 after results published in Whattam and Bertram (2011). Because Whattam and Bertram's (2011)

124 study revealed that long distance acoustic mate attraction signaling was influenced by diet, we

125    selected our signals' parameters to reflect the mean from a population of males reared on high

126    quality food (recordings made at 26°C). The signaling parameters we used for both standard and

127    focal signals were: carrier frequency = 3719 Hz, pulse duration = 10.14 ms, interpulse duration =

128    15.21 ms, pulses per chirp = 8, and interchirp duration = 1055 ms (signal available at Figshare

129    doi: 10.6084/m9.figshare.1037378). Whattam and Bertram (2011) revealed that well-fed male

130    crickets signal at $60.60 \pm 8.45$ dB SPL ($\bar{X} \pm 1$ SD), ranging 34 - 72 dB SPL. We therefore always

131    set our standard signal to broadcast at 60.6 dB SPL (re: 20μPa RMS). We used four focal sound

132    pressure levels which we labelled very quiet, quiet, loud, and very loud, broadcast at 43 dB $\cong \bar{X}$-

133    2 SD, 52 dB $\cong \bar{X}$-1 SD, 69 dB $\cong \bar{X}$+1 SD, and 77 dB $\cong \bar{X}$+2 SD SPL, respectively. Our focal

134    sound pressure levels thus spanned the natural range and slightly beyond that which females are

135    accustomed to hearing. We measured sound pressure level using an EXTECH Digital Sound

136    Level Meter (Model # 407768; FLIR Systems, Waltham, MA, USA).

137    ***Preference Trials***

138    Each female's phonotaxis was tested using two-choice trials where the standard signal

139    was simultaneously presented against one of the four focal signals. Each female was tested across

140    all four focal signals in both the arena and the spherical treadmill, for a total of 8 tests. These

141    comparisons occurred across four consecutive days, 10-13 days post imaginal moult (hereafter

142    referred to as 10-13 days old) because this is the age range when female *G. assimilis* are most

143    phonotactically responsive (Pacheco et al., 2013). On a single test day, an individual female was

144    tested once on the spherical treadmill and once in the arena, each to randomly assigned focal

145    signals. Females were always given at least 1 hour of rest prior to switching methods. The order

146    of the method the female was tested on first was randomized.

147    In both spherical treadmill and open arena trials females were given 60 seconds to

148    acclimatize in silence (detailed below). Each signal was then broadcast on its own for 30 seconds

149 (order and speaker side randomized) to ensure the female heard both the focal and standard

150 signals prior to starting the trial. Both signals were then broadcast simultaneously from the

151 speakers with the signal chirps interleaved (alternating), so that a female had the potential to

152 identify which signal was coming from which speaker. Once both focal and standard signals were

153 being broadcast, the trial began and female phonotaxis was recorded (detailed below). Every trial

154 ran a total of 5 minutes. All trials were run in the evening (between 5:00 PM and 8:00 PM) under

155 a 75 wattage red light.

156 *Spherical Treadmill Preference Trials*

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

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

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

160 across from each other. Prior to mounting the female onto the spherical treadmill we ensured the

161 standard and focal signal would broadcast at the correct sound pressure level by pointing the

162 sound level meter probe directly at the active speaker, directly above the spherical treadmill, 17

163 cm from the active speaker, and setting the volume of the standard or focal signal accordingly.

164 To mount the female on the spherical treadmill we attached a coil- (micro-compression)

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

166 using low melting point wax. Coil springs were attached on day 9 post final moult (one day prior

167 to the first trial, *sensu* Pacheco et al., 2013). We mounted each focal female on the treadmill by

168 attaching her spring to a magnetic rod above the treadmill. This mounting ensured that while the

169 female cricket was held firmly in place on the top of the polystyrene ball, she could freely turn

170 360 degrees, as well as walk or run using natural motions (photos in Pacheco et al., 2013). We

171 adjusted the air pressure flowing to the polystyrene ball such that, with the cricket in position, the

172 ball was able to rotate freely in all directions in response to the cricket's walking, running, or

173    turning movements. We oriented all females in the same neutral position at the start of their trial:

174    tethered on the spherical treadmill facing straight ahead between the two speakers.

175          Once the female was mounted on the spherical treadmill, but prior to the start of data

176    collection, the female experienced 60 seconds of silence, then 30 seconds of the focal sound and

177    30 seconds of the standard sound (order and speaker side randomized). The female was free to

178    walk during this acclimatization period. We then broadcast both the focal and standard signals,

179    with the signal chirps interleaved. Data collection on female phonotaxis began as soon as both the

180    focal and the standard signals were being broadcast. Female phonotaxis was recorded relative to

181    the focal speaker. Both standard and focal signals were presented continuously throughout the 5

182    min trial, and the female's locomotor behavior was recorded from the treadmill at a sample rate

183    of 20 samples per second. Each 5 minute trial yielded a total of 6000 samples of cricket X, Y

184    positions. Temperature was held at 22-23°C in the trial room and was monitored with a Fisher

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

186    Ontario, Canada).

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

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

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

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

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

192    vector length of every recorded leg movement:

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

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

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

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

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

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

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

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

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

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

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

203  *Open Arena Preference Trials*

204      We conducted the open arena trials in the same room and temperature conditions as

205  spherical treadmill trials. We ran all trials in a chamber (L x W x H = 111.7 cm x 50 cm x 25 cm)

206  with the walls lined with sound-attenuating foam. Speakers were located at opposite ends of the

207  arena (lengthwise). We ensured the standard and focal signal would broadcast at the correct

208  sound pressure level by pointing the sound level meter probe directly at the active speaker from

209  the center of the arena, 55 cm from the active speaker, and setting the volume of the standard or

210  focal signal accordingly. We demarcated a semicircle "choice zone" (radius = 28 cm) in front of

211  each speaker. We then placed the female in the center of the arena (exactly 55 cm from each

212  speaker) under an opaque plastic container (L x W x H = 10 cm x 8.5 cm x 11 cm). Prior to the

213  start of data collection, the female experienced 60 seconds of silence, then 30 seconds of the focal

214  sound and 30 seconds of the standard sound (order and speaker side randomized). We then

215  broadcast both the focal and standard signals simultaneously, with the signal chirps interleaved.

216  As soon as both signals were being broadcast, we carefully and silently raised the opaque plastic

217  container by pulling on its attached string. Data collection on female phonotaxis began as soon as

218  the opaque container was removed.

219      Each trial ran for a total of 5 minutes. The following female responses were recorded: the

220  amount of time spent stationary in the middle of the arena without moving after the opaque

221    plastic container was removed, time spent in the arena outside of the choice zones (no-choice

222    zone) after moving from the acclimatization location, time spent in focal choice zone, time spent

223    in standard choice zone, and number of switches made between focal and standard choice zones.

224    The base of the arena was vacuumed and wiped down after each trial.

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

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

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

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

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

230    preference we calculated the proportion of time spent in the focal choice zone relative to the

231    proportion of time spent in both choice zones combined. A number close to one indicates a strong

232    phonotaxis response from the female with much more time spent in the focal zone than the

233    standard zone; conversely, a number close to zero indicates much more time was spent in the

234    standard zone than in the focal zone.

235    ***Statistical Analysis***

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

237    Campus Drive, Cary, North Carolina, USA). Female phonotaxis data (net vector scores from the

238    spherical treadmill and proportion of time spent in focal zone from the open arena) were analyzed

239    using repeated measures general linear mixed models (GLMM) that included sound pressure

240    level, age, and the interaction between sound pressure level and age as fixed effects; body size

241    (maximum head width) was included as a covariate; individual was treated as a random effect. To

242    compare experimental methods, we converted all phonotaxis data into z scores. Z scores were

243    then analyzed using a repeated measures GLMM. We included experimental method, sound

244    pressure level, age, experimental method x sound pressure level, sound pressure level x age,

245    experimental method x age, and experimental method x sound pressure level x age as fixed

246 effects; body size was included as a covariate; we treated individual as a random effect. Because

247 none of the interactions were significant in any of the models we re-ran all models without

248 interactions per Engqvist (2005). We only present the results of the partial models (excluding

249 interactions) because partial F-tests revealed that the full models did not explain significantly

250 more variation in female phonotaxis. We used one sample t-tests to determine which sound

251 pressure levels invoked significantly different female phonotaxis levels relative to the standard.

252 We used Tukey's post-hoc HSD tests to compare phonotaxis across different focal sound pressure

253 levels.

254 **Results**

255 Females in the spherical treadmill trials exhibited highly variable phonotaxis in response

256 to the different focal sound pressure levels. Some females walked substantially farther than

257 others, with distance covered ranging from 60 cm to 4900 cm. Females had over a 60-fold

258 difference in their velocity magnitudes, with the slowest moving at only 0.12 cm/s and the fastest

259 moving at 8.22 cm/s. Females also moved in all directions during trials relative to the focal

260 speaker. There was a 4-fold difference in net vector scores, indicating large variation in the

261 strength of female phonotaxis on the spherical treadmill. Sound pressure level significantly

262 influenced net vector scores, with females exhibiting highest phonotaxis towards the loud 69 dB

263 $\cong \bar{X}+1$ SD SPL treatment relative to all other focal sound pressure level treatments (Figure 1;

264 Table 2). Age and size did not significantly influence female phonotaxis. Further, although

265 individuals were included as a random effect in the GLMM, they did not explain any of the

266 variation in phonotaxis. Post hoc one-way comparisons revealed that phonotaxis towards the loud

267 69 dB $\cong \bar{X}+1$ SD SPL was significantly higher than towards the standard 60.6 dB SPL (t=3.66,

268  p=0.0010). Phonotaxis did not significantly differ relative to the standard sound pressure level in

269  the other three focal treatments (all p>0.25).

270     Females in the open arena trials also exhibited highly variable phonotaxis in response to

271  the different focal sound pressure levels. In 17/120 of the open arena trials (14%) females failed

272  to move out of the no-choice zone; these trials were excluded from analysis. Females usually

273  spent time in both standard and focal choice zones in the remaining open arena trials. Sound

274  pressure level significantly influenced female phonotaxis (relative time spent in focal choice

275  zone). Females were most phonotactic towards the loud 69 dB $\cong \bar{X}$+1 SD SPL, relative to all

276  other focal sound pressure level treatments (Figure 2; Table 2). Age and size did not significantly

277  influence proportion of time spent in focal choice zone. Further, individuals being included in the

278  GLMM as a random effect did not explain any of the variation in phonotaxis. Post hoc one-way

279  comparisons of the open arena data revealed that females spent significantly more time in the

280  loud 69 dB $\cong \bar{X}$+1 SD SPL choice zone than the standard 60.6 dB SPL choice zone (t=2.47,

281  p=0.0197). Conversely, when the sound pressure level was very loud 77 dB $\cong \bar{X}$+2 SD SPL,

282  females avoided the focal choice zone, instead spending significantly more time in the standard

283  60.6 dB SPL choice zone (t=-4.20, p=0.0003). Female time in the focal choice zone did not differ

284  significantly relative to time in the standard sound pressure level choice zone in the quiet and

285  very quiet treatments (all p>0.70).

286     We used z scores to compare phonotaxis across the two methods (spherical treadmill and

287  open arena). Experimental method, age and size did not significantly influence female

288  phonotaxis. Further, individuals being included in the GLMM as a random effect did not explain

289  any of the variation in phonotaxis. Female phonotaxis was only influenced by sound pressure

290  level, with females exhibiting highest phonotaxis towards the loud 69 dB $\cong \bar{X}$+1 SD SPL

291  treatment relative to all other focal sound pressure level treatments (Figure 3; Table 2).

**Discussion**

292

293    The loud focal signal (69 dB $\cong \bar{X}$+1 SD SPL) invoked greater female phonotaxis than any

294    other sound pressure level investigated (Figures 1-3; Table 2). Overall, our findings suggest that

295    males that signal at high sound pressure levels should experience a fitness advantage over males

296    that signal at lower sound pressure levels, provided the sound pressure level is within the natural

297    range of the species. This caveat is important, given females showed reduced phonotaxis towards

298    the very loud focal signal (77 dB $\cong \bar{X}$+2 SD SPL), suggesting female preference for elevated

299    sound pressure level is not open ended.

300    The very loud focal signal (77 dB $\cong \bar{X}$+2 SD SPL) may represent a novel supernormal

301    stimulus as it falls beyond *G. assimilis'* natural range of 34-71 dB SPL (Whattam & Bertram,

302    2011). Many of the females exhibited negative phonotaxis when exposed to this supernormal

303    stimulus (Figures 1-3). Weber, Thorson and Huber (1981) showed a similar response in *Gryllus*

304    *campestris* L, with females avoiding mate attraction signals above 70 dB SPL. This negative

305    response to a supernormal stimuli has been observed previously, such as when female crickets

306    exhibit negative phonotaxis to the number of syllables (pulses) in a chirp (Hedrick & Weber,

307    1998; Ryan & Keddy-Hector, 1992). Female negative phonotaxis to very loud signals may be an

308    acoustic startle response (Hoy, Nolen & Brodfuehrer, 1989), or the females may have a perceived

309    increases in predation risk (Moiseff, Pollack & Hoy, 1978; Zuk & Kolluru, 1998). It is also

310    possible that absence of other sensory cues (Crapon de Caprona & Ryan, 1990) invoke this

311    negative phonotaxis. In some species, such as *Xiphophorus nigrensis* and *X. pygmaeus*

312    (Poeciliidae) swordtails, mate selection is dependent on the dual effect of both visual and

313    olfactory cues.

314     Surprisingly, females did not exhibit significant phonotaxis towards the standard signal

315  when the focal signal's sound pressure level was less than that of the standard signal (quiet 52 dB

316  $\cong \overline{X}$-1 SD SPL or very quiet 43 dB $\cong \overline{X}$-2 SD SPL vs. 60.6 SPL; Figures 1-3). These findings

317  suggest females may not discriminate between sound levels unless one of the signals presents a

318  higher than average sound pressure level. Regardless, given low sound levels may go undetected

319  in nature due to elevated background noise levels, males should be selected to signal with high

320  sound pressure levels whenever possible. It is unknown whether females can discern low sound

321  pressure levels from the background noise level in the testing chamber (42±2 dB SPL); future

322  tests should determine whether females exhibit phonotaxis towards very quiet (43 dB $\cong \overline{X}$-2 SD

323  SPL) signals when the alternative is silence (Brown & Handford 1996).

324     Our study compared open arena and spherical treadmill methods. Quantifying female

325  preference for signal sound pressure level provides a powerful comparison of whether one

326  experimental method quantifies female phonotaxis more effectively than the other because sound

327  pressure level is one of the primary components that differ between the two methods. We found

328  that the two methods produced virtually identical female phonotaxis results for almost all of the

329  sound pressure levels examined. The only sound pressure level that evoked a slightly different

330  phonotactic response between the two methods was the very loud treatment (77 dB $\cong \overline{X}$+2 SD

331  SPL). Females in the open arena avoided the very loud focal sound pressure level, spending

332  significantly more time in choice zone of the standard signal. While females in the spherical

333  treadmill also exhibited somewhat reduced phonotaxis towards the very loud focal signal,

334  phonotaxis did not significantly differ from the standard signal. Overall, our findings are

335  consistent with the handful of other studies that compare these experimental methods. Walikonis

336  et al. (1991), Stout, Atkins and Zacharias (1991) and Pires and Hoy (1992) revealed female

337  phonotaxis was the same in the open arena and on the spherical treadmill. Similar to our study,

338  Stout, Atkins and Zacharias (1991) investigated female *Acheta domesticus* response to differing

339  signal intensities. Conversely, Walikonis et al. (1991) investigated female *A. domesitcus* response

340  to differing syllable periods while Pires and Hoy (1992) investigated female *G. firmus* response

341  to natural calls recorded songs at different temperatures. Given sound pressure level, syllable

342  period, and temperature are important predictors of mating preference, our joint findings suggest

343  that open arena and spherical treadmill methods may be used interchangeably to quantify

344  phonotaxis to acoustic signals.

345        Caution is warranted, however, when signals are broadcasted at unnaturally high sound

346  pressure levels because of fundamental methodological differences between the open arena and

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

348  enabling her to experience natural changes in sound pressure level. When individuals in the open

349  arena run away from very loud signals, they experience a ~6 dB drop in amplitude with every

350  doubling of their distance from the very loud signal (assuming a spherical, radiating sound source

351  without obstructions). However, when individuals on the spherical treadmill run away they do not

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

353  place on the spherical treadmill. Future research should explore how important sound pressure

354  level is relative to other signalling parameters.

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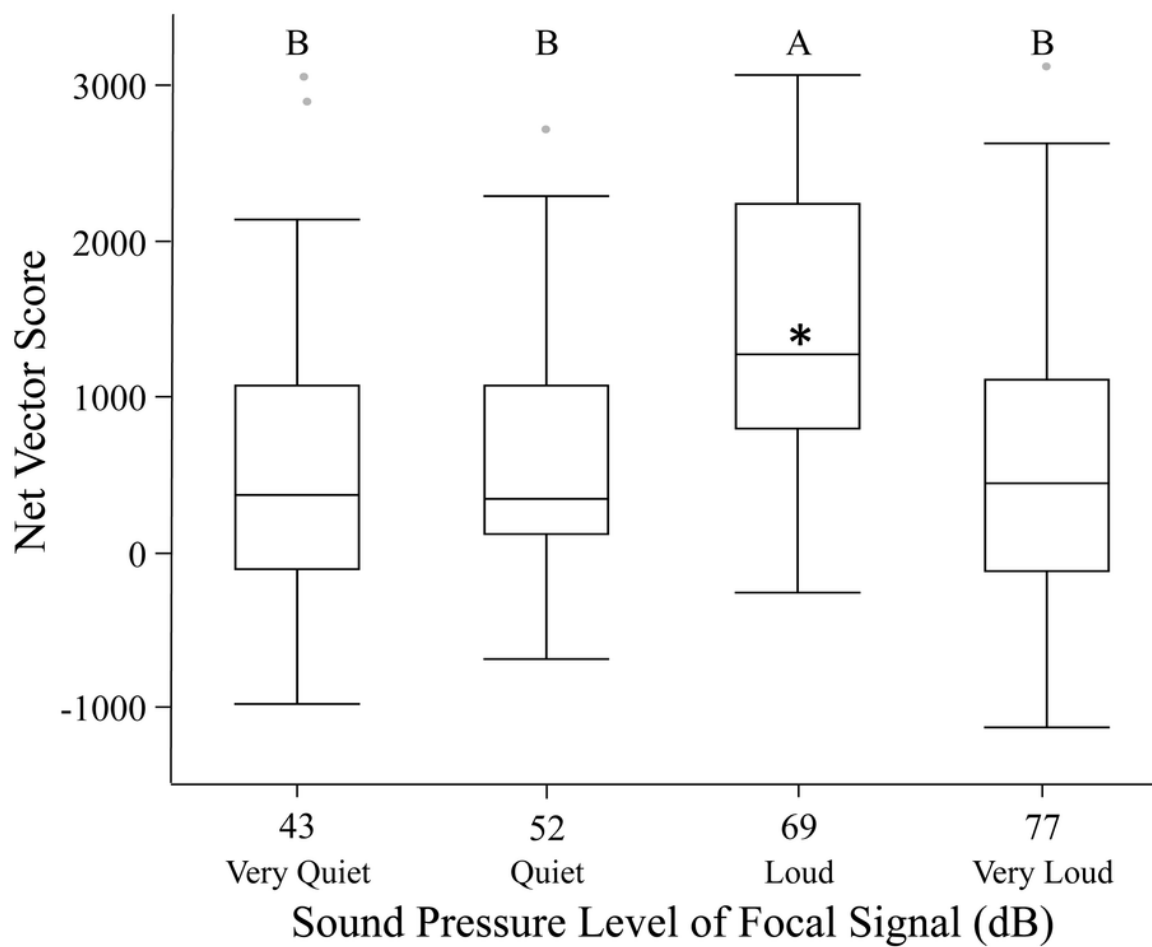
# Figure 1

Female phonotaxis under different sound pressure levels on the spherical treadmill

Phonotaxis was quantified using net vector scores (net movement toward or away from the focal signal; direct movement towards the focal speaker resulted in positive vector scores while direct movement away from the focal speaker resulted in negative vector scores). Letters represent significant differences across sound pressure levels (Tukey's post-hoc HSD analysis). Asterisks represent significant differences between the focal and standard signal (above median = phonotaxis toward focal signal; below median = phonotaxis toward standard signal). Box plots: horizontal line within each box = median phonotaxis measure; top of each box = 3$^{rd}$ (75%) quartile phonotaxis measure; bottom of each box = 1$^{st}$ (25%) quartile phonotaxis measure; whiskers represent the outermost data point that falls within the distances 1$^{st}$ quartile – 1.5*interquartile range or 3$^{rd}$ quartile + 1.5*interquartile range; whiskers are determined by the upper and lower data point values (excluding outliers) when the data points do not reach the above computed ranges; light grey dots represent outliers, when present.

Variation in the sound pressure levels used in female field cricket preference studies.

| Study | Species | SPL (dB) | Speaker Distance (cm) |
|---|---|---|---|
| Scheuber et al. 2004 | *Gryllus campestris* | 80 * | 10.0 |
| Lickman et al. 1998 | *Gryllus texensis* | 82-85 * | 35.4 |
| Bailey 2008 | *Teleogryllus oceanicus* | 70 | |
| Prosser et al.1997 | *Gryllus texensis* | 83 * | 35.4 |
| Wagner et al. 1995 | *Gryllus texensis* | 84.6 | 35.5 |
| Hedrick et al. 2007 | *Gryllus integer* | 72 | |
| Olvido & Wagner 2004 | *Allonemobius socius* | 66±2 | 5.0 |
| Gray & Cade 2000 | *Gryllus texensis* | 84 | |
| Gray & Cade 2000 | *Gryllus rubens* | 84 | |

* reference is made to the natural range of sound pressure levels

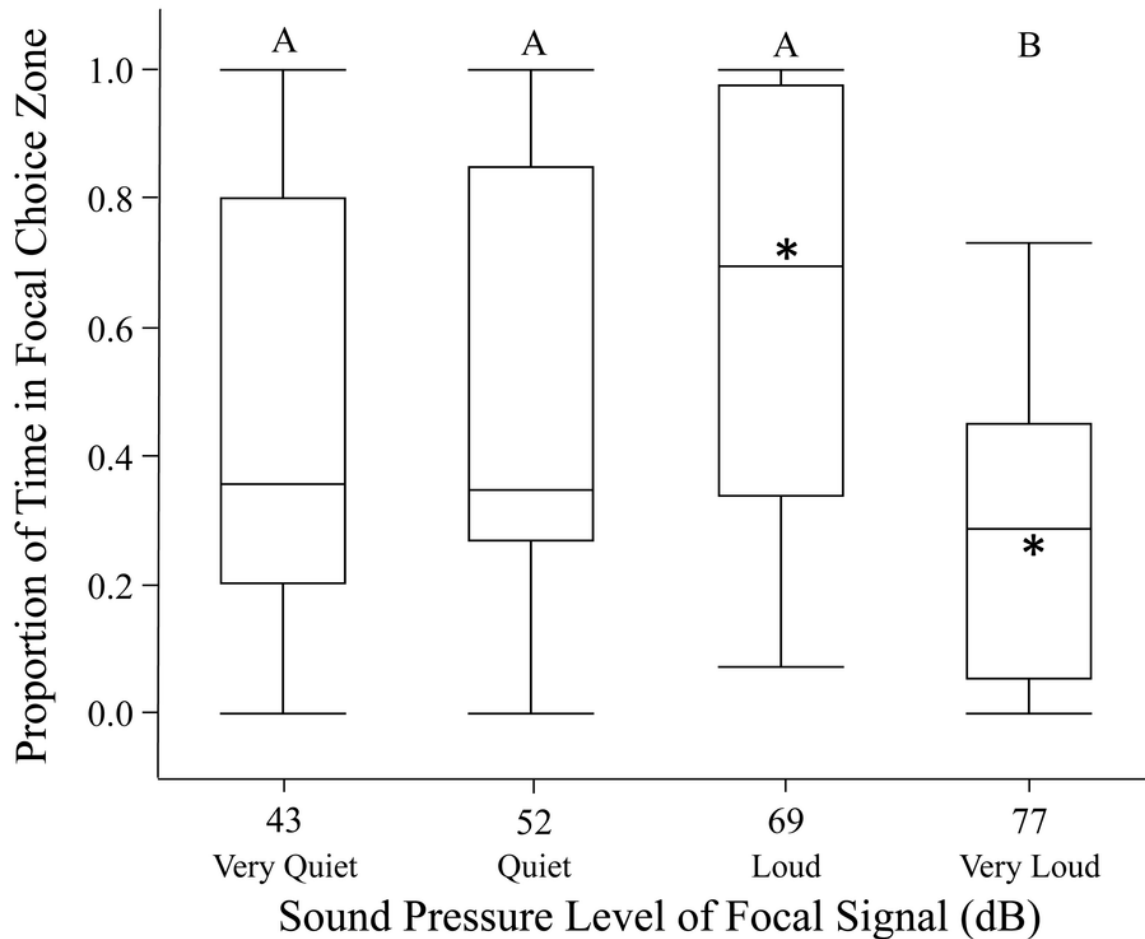
Repeated measures GLMM results for factors affecting female phonotaxis.

The spherical treadmill model used net vector scores to quantify phonotaxis and had an $R^2_{adj}$ = 0.0495. The open model used proportion of time spent in focal choice zone relative to time spent in both focal and standard choice zones to quantify phonotaxis and had an $R^2_{adj}$ = 0.0356. The method comparison model used z scores to quantify phonotaxis and had an $R^2_{adj}$ = 0.0626. Female phonotaxis was significantly influenced by sound pressure level but was not influenced by female age, female body size, or experimental method. Individual was identified as a random effect in all models, but did not explain any of the phonotaxis variation.

| Method | Source | DF | F | P |
|---|---|---|---|---|
| Spherical Treadmill | Age | 3,84 | 0.8947 | 0.4475 |
| | Sound Pressure Level | 3,84 | 5.7035 | 0.0013 |
| | Body Size | 1,28 | 0.1983 | 0.6595 |
| Open Arena | Age | 3,79 | 0.7662 | 0.5164 |
| | Sound Pressure Level | 3,79 | 5.3900 | 0.0020 |
| | Body Size | 1,29 | 0.3029 | 0.5864 |
| Both Methods | Age | 3,192 | 0.7747 | 0.5094 |
| | Sound Pressure Level | 3,192 | 9.5844 | <0.0001 |
| | Technique | 1,193 | 0.0392 | 0.8433 |
| | Body Size | 1,29 | 0.4584 | 0.5035 |

# Figure 2

Female phonotaxis under different sound pressure levels in the open arena.

Phonotaxis was quantified using proportion of time the female spent in the focal choice zone relative to the total amount of time spent in both the focal and standard choice zones. Box plot, asterisk, and letter descriptions provided in Figure 1.

# Figure 3

Female phonotaxis under different sound pressure levels in both the spherical treadmill (white bars) and open arena (grey bars).

Phonotaxis was compared across the two experimental methods by converting all phonotaxis data into z scores. Box plot, asterisk, and letter descriptions provided in Figure 1.