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How male signaling intensity influences phonotaxis in virgin female Jamaican field crickets
(*Gryllus assimilis*)

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Abstract

Understanding female mate preference is important for determining the strength and the direction of sexual trait evolution. Male signalling intensity is often an important predictor of mating success because higher intensity (louder) signallers are detectable at greater distances. However, if females are simultaneously more attracted to higher signalling intensities, then the potential fitness impacts of higher intensity signalling should be elevated beyond what would be expected from detection distance alone. Here we manipulated the signal intensity of cricket mate attraction signals to determine how female phonotaxis was influenced. We examined female phonotaxis using two common methodologies: spherical treadmills and open arenas. Both methodologies showed similar results, with females exhibiting highest phonotaxis towards loud ($\bar{X}+1$ SD = 69 dB) mate attraction signals but showing reduced phonotaxis towards the loudest ($\bar{X}+2$ SD = 77 dB) signals. Reduced phonotaxis towards supernormal stimuli may occur for several reasons including elevating the females' perceived predation risk, invoking females' 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

to quantify female preference functions for signal intensity to gain insights into the evolutionary consequences and patterns of selection on this trait. Unfortunately, female preference functions for signal intensity have been understudied in crickets despite extensive studies on female preference for other male signal components.

Adult male field crickets produce long distance acoustic mate attraction signals (also known as calls) by rubbing their modified forewings together (Alexander, 1962). When a male closes his wings, the scraper of one wing hits the teeth of the file on the other wing the harp resonates and produces a single pulse of sound (Bennet-Clark, 2003). Males concatenate these pulses into chirps (Alexander, 1962; Bennet-Clark, 2003) and females use these long distance acoustic signals to both orient towards signalling males (phonotaxis) and choose between potential mates (Alexander, 1962).

To date, only a handful of the experiments quantifying female preference for signal intensity have explored a broad range of signalling intensities (Table 1). Even fewer have explored the extremes of the natural range of intensities available to females. Further, most studies examining female preference for signal intensity often simultaneously manipulate other signalling parameters, making it difficult to tease apart the influence that intensity alone has on female preference. For example, Stout and McGhee (1988) examined the relative importance of pulse + interpulse duration (syllable period), chirp rate and signal intensity on female mate preference. Unfortunately, Stout and McGhee (1988) made no reference to the natural range of signal intensity found in this species. Instead, they quantified female phonotactic response at a 2 dB, 5 dB and 10 dB increase above their standard male signal intensity of 65 dB sound pressure level (SPL). By presenting female European house crickets (*Acheta domesticus*) with a pair of male signals differing in one or more of these parameters, Stout and McGhee (1988) concluded

that signal intensity was more important than chirp rate, but that syllable period was more important than signal intensity in influencing female mate choice. While there is emerging interest in determining how multiple signal parameters interact to influence patterns of selection and the evolutionary consequences of signalling (Brooks et al., 2005), it is first worth examining how signal intensity alone shapes female preference functions.

Here we quantify female Jamaican field crickets (*Gryllus assimilis*) phonotactic response to variation in male's long distance acoustic mate attraction signal intensity using two standard research methodologies, a spherical treadmill and open arena. These two methodologies fall within two broader categories of testing: open-loop and closed-loop methods, respectively. Open-loop methods (Kramer spherical treadmill also called a trackball or locomotion compensator) tether a female in one place, so that she does not experience changes in sound intensity as she walks 'toward' the mate attraction signal (Kramer, 1976; Weber, Thorson & Huber, 1981; Doherty, 1985; Doherty & Pires, 1987; trackball: Hedwig & Poulet, 2005; Hedwig, 2006). Closed-loop methods (open arena) allow the female to move within the enclosed space, thereby allowing her to experience changes in sound intensity as she approaches the mate attraction signal. Quantifying female preference for the intensity of the acoustic mate attraction signal provides a powerful comparison of whether one technique quantifies female phonotaxis more effectively than the other because sound intensity is one of the primary components that differ between the two techniques. All signal parameters of the acoustic mate attraction signal were held constant except signal intensity, which varied 1 or 2 standard deviations above or below the average male signal intensity. The focus of our study was therefore two-fold: 1) to examine female phonotaxis toward long distance acoustic mate attraction signals that vary in

signal intensity across the natural range of this species, and 2) to compare female phonotaxis on the spherical treadmill to female phonotaxis in the open arena.

Methods

Cricket Rearing

Our founding population of *Gryllus assimilis* was originally collected in Bastrop County, Texas, United States (latitude ~ 30° 17' N, longitude ~ 97° 46' W, elevation ~145 m) from 15 to 24 September, 2008. We did not require specific permits for collecting invertebrates because these crickets are neither endangered nor protected. We imported adult crickets and eggs to the greenhouse laboratories at Carleton University, Ottawa, Canada (Canadian Food Inspection Agency permit # 2007-03130). Our greenhouse facilities are Plant Pest Containment Level 1 certified (Canadian Food Inspection Agency permit # P-2012-03836). While we did not require specific permits to conduct our cricket experiments, our study was conducted in accordance with the guidelines of the Canadian Council on Animal Care.

The crickets were reared in communal plastic bins (64 cm x 40 cm x 42 cm) with a 16:8 h L:D illumination period at 28±2 °C and fed *ad libitum* water and food (Harland's Tekland Rodent diet 8604M; 24.3% protein, 40.2% carbohydrate, 4.7% lipid, 16.4% fiber, 7.4% ash). In late 2012 (12-16 generations after field collection) we collected 30 4th instar females at random from the communal cricket bins (no wing bud development; ovipositor had just become visible). These juveniles were housed together in a separate communal bin (same conditions as described above) and monitored daily for imaginal eclosion. Within 24 hours of imaginal eclosion we transferred the adult females to individual 520mL (11 cm diameter x 7 cm height) clear plastic containers with screened lids (4cm x 4cm mesh screens). Each female was provided with a small

piece of cardboard egg container for shelter and *ad libitum* water and food; light cycles and temperatures were identical to the communal rearing environment. All females were tested daily from 10-13 days post imaginal moult (hereafter referred to as 10-13 days old) because this is the age range when female *G. assimilis* are most phonotactically responsive (Pacheco et al., 2013).

Standard and Focal Mate Attraction Signals

We created a standard long distance mate attraction signal (hereafter standard signal) using Adobe Audition CS5.5 software (Adobe Systems Incorporated, San Jose, California, USA). We fashioned this standard signal after results published in Whattam and Bertram (2011). This study showed that male acoustic mate attraction signaling is affected by diet; we selected parameters that reflect the mean signal parameters from a population of males reared on high quality food (recordings made at 26°C). The standard signal's parameters were: carrier frequency = 3719 Hz, pulse duration = 10.14 ms, interpulse duration = 15.21 ms, pulses per chirp = 8, interchirp duration = 1055 ms; broadcast at an intensity of 60.6 dB SPL (re: 20µPa RMS) as measured using an EXTECH Digital Sound Level Meter (Model # 407768; FLIR Systems, Waltham, Massachusetts, USA) with probe pointing directly at the active speaker, directly above the spherical treadmill, 17 cm from the active speaker.

Focal signals only differed from the standard signal in their signal intensity. The range of focal signal intensities used came from Whattam and Bertram (2011), who revealed that the population's signal intensity was 60.6±8 dB ($\bar{X} \pm 1$ SD), with a range from 34.2 dB to 71.9 dB. We therefore used 43 dB ($\bar{X} - 2$ SD), 52 dB ($\bar{X} - 1$ SD), 69 dB ($\bar{X} + 1$ SD) and 77 dB ($\bar{X} + 2$ SD) as our focal signals. This range of focal signals ensured that the loudest signals played ($\bar{X} + 2$ SD) were louder than the natural range females are accustomed to hearing, while the quietest signals played ($\bar{X} - 2$ 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

the start of the spherical treadmill trial, we mounted each focal female on the treadmill by attaching her spring to a magnetic rod above the treadmill. This mounting ensured the cricket was held in position with her feet on the polystyrene ball (photos in Pacheco et al., 2013). Air pressure was adjusted such that the polystyrene ball, with the cricket in position, was able to rotate freely in all directions as the cricket moved. All females were oriented in the same neutral position at the start of their trial: tethered on the spherical treadmill facing straight ahead between the two speakers.

Once both signals were being broadcast simultaneously from the speakers with the signal chirps interleaved, the trial began and female phonotaxis was recorded relative to the focal speaker. Both standard and focal signals were presented continuously throughout the 5 min trial, and the female's locomotor behavior was recorded from the treadmill at a sample rate of 20 samples per second. Each 5 minute trial yielded a total of 6000 samples of cricket X, Y positions. Temperature was held at 22-23°C in the trial room and was monitored with a Fisher Scientific Traceable Digital Thermometer (Model # 15-077-20; Fisher Scientific, Toronto, Ontario, Canada).

We calculated instantaneous displacement (cm) and velocity (cm/s) vectors from the positional data (X, Y coordinates). Total path length was calculated as the sum of all vector lengths over the 6000 samples. Female preference was quantified using net vector score (after Huber et al., 1984). Net vector score is the female's net movement toward or away from the focal signal during the 5 min trial and takes into account the female's direction (vector angle) and the vector length of every recorded leg movement:

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

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We defined the angle of the focal speaker as 0° . Females moving directly toward the focal speaker (0°) had positive vector scores [$\cos(0^\circ) = 1$], females moving directly away from the focal speaker (180°) had negative vector scores [$\cos(180^\circ) = -1$], and females moving perpendicular to the focal speaker (90° or 270°) had vector scores of 0 [$\cos(90^\circ$ or $270^\circ) = 0$]. By multiplying this value by each vector length, and summing over the trial duration, we quantified the female's relative attraction to the focal signal. A large positive net vector score indicated that the female moved quickly toward the focal speaker, a small positive score indicated the female moved slowly toward the focal speaker, a large negative score indicated the female moved quickly away from the focal speaker, and a small negative score indicated the female moved slowly away from the focal speaker (Huber et al., 1984).

Open arena trials were conducted in the same room and temperature conditions as spherical treadmill trials. We ran all open arena trials in a chamber (111.7 cm[l] x 50 cm[w] x 25 cm[h]) with the walls lined with sound-attenuating foam. The base of the arena was vacuumed and wiped down before each trial order. Speakers were located at opposite ends of the arena (lengthwise). We marked a semicircle "choice zone" (each 28 cm in radius) in front of each speaker. At the start of the trial the female was placed in the center of the arena under an opaque plastic container (10 cm[l] x 8.5 cm[w] x 11 cm[h]) attached to a string. After the 60-second silent acclimatization period and 60 seconds of individual signal exposure (described above), the string was pulled, resulting in the opaque plastic container being carefully and silently lifted up away from the test female. As soon as the cup was lifted the trial began and ran for a total of 5 minutes. The following female responses were recorded: the amount of time spent stationary in the middle of the arena without moving after the opaque plastic container was removed, time spent in the arena outside of the choice zones (no-choice zone) after moving from the

acclimatization location, time spent in focal choice zone, time spent in standard choice zone, and number of switches made between focal and standard choice zones.

Because of the varying amount of time spent in focal and standard choice zone, females were classified as 'preferring' a particular focal signal treatment if, over the course of the trial, she spent relatively more time in the zone with the focal signal than in the zone with the standard signal (i.e. time outside choice zones were not included to determine female choice) *sensu* Bischoff, Gould and Rubenstein (1985) and Dugatkin and Godin (1992). To quantify female preference we subtracted the time spent in standard choice zone from the time spent in the focal choice zone. A large positive number indicates a strong phonotaxis response from the female with much more time spent in the focal zone than the standard zone; conversely, a large negative number indicates much more time was spent in the standard zone than in the focal zone.

Statistical Analysis

All data were analyzed using JMP 10.0.0 statistical software (SAS Institute Inc., 100 SAS Campus Drive, Cary, North Carolina, USA). Total path length and average instantaneous velocity magnitude from the spherical treadmill data were Box-Cox transformed to meet assumptions of normality. Female preference data (net vector scores from the spherical treadmill data and relative time spent in focal zone from the open arena data) were analyzed using repeated measures general linear mixed models (GLMM) that took into account all the fixed effects (age, signal intensity, method order, and focal speaker side) and covariates (female size and weight) that could affect female preference; individual was treated as a random effect. Tukey's HSD post-hoc tests were conducted to identify significant differences among the treatments.

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

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were excluded from analysis. Signal intensity significantly influenced female phonotaxis (relative time spent in focal zone), explaining 11% of the variation (Table 2). Females were most phonotactic towards the $\bar{X}+1$ SD = 69 dB signal intensity (Figure 2). All other signal intensities evoked significantly reduced phonotaxis, with females exhibiting negative phonotaxis towards the $\bar{X}+2$ SD = 77 dB signal intensity (Figure 2). Female phonotaxis was not significantly influenced by method order (spherical treadmill or open arena), side the focal signal played from, female age, size or weight (Table 2). The repeated measures GLMM revealed that ** % of the variation was attributed to the random effects of individual.

We compared phonotaxis results across the two methodologies using z scores. Female phonotaxis was significantly influenced by signal intensity, explaining 16% of the variation (Table 2; Figure 3). All other factors, including experimental methodology, method order, the side the focal signal was presented from, female age, size or weight did not significantly influence female phonotaxis. Further, there was no significant interaction between signal treatment and experimental method, focal speaker side and experimental method, or method order and experimental method. The repeated measures GLMM revealed that ** % of the variation was attributed to the random effects of individual.

Discussion

Female crickets exhibited positive phonotaxis towards loud male acoustic mate attraction signals ($\bar{X}+1$ SD = 69 dB) while ignoring or avoiding quiet signals ($\bar{X}-2$ SD = 43 dB and $\bar{X}-1$ SD = 52 dB; Figures 1-3). Our results suggest females may be unable to differentiate low decibel sounds from the background noise. While few studies have examined female preference in response to signal intensities lower than 50 dB (Table 1), our findings suggest quiet signals ($\bar{X}-2$

SD = 43 dB and $\bar{X}$ -1 SD = 52 dB) may go undetected in nature because abiotic and biotic sounds would likely elevate background noise levels beyond that of our controlled laboratory study (44±2 dB testing room, 42±2 dB testing chambers).

Interestingly, female preference for elevated signal intensity is not open ended. Females showed reduced phonotaxis towards the loudest mate attraction signals ($\bar{X}$ +2 SD = 77 dB). The 77 dB treatment presents a signal intensity that falls well beyond *G. assimilis*' natural range of 34-71 dB (Whattam & Bertram, 2011), making it a novel supernormal stimulus. Females often exhibited negative phonotaxis to this supernormal stimulus, moving away from it (Figures 1-3). Weber, Thorson and Huber (1981) showed a similar response in *Gryllus campestris* L, with females avoiding mate attraction signals above 70 dB. Negative phonotaxis may be indicative of perceptual limits (Magnus, 1958; Hedrick & Weber, 1998), elevated predation risk (Moiseff, Pollack & Hoy, 1978; Zuk & Kolluru, 1998), an acoustic startle response (Hoy, Nolen & Brodfuehrer, 1989), signal rejection due to the absence of other sensory cues (Crapon de Caprona & Ryan, 1990), or a 'stopping to look and listen' tactic (Weber, Thorson & Huber, 1981).

Our study also compared open arena and spherical treadmill methodologies. Quantifying female preference for signal intensity provides a powerful comparison of whether one technique quantifies female phonotaxis more effectively than the other because sound intensity is one of the primary components that differ between the two techniques. We found that the two methodologies produced virtually identical female phonotaxis results for each of the signal intensities. Our findings are consistent with the handful of other studies that compare these methodologies. Walikonis et al. (1991), Stout, Atkins and Zacharias (1991) and Pires and Hoy (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

towards novel supernormal stimuli ($\bar{X}+2$ SD = 77 dB). Future research should explore how important signal intensity is relative to other signalling parameters.

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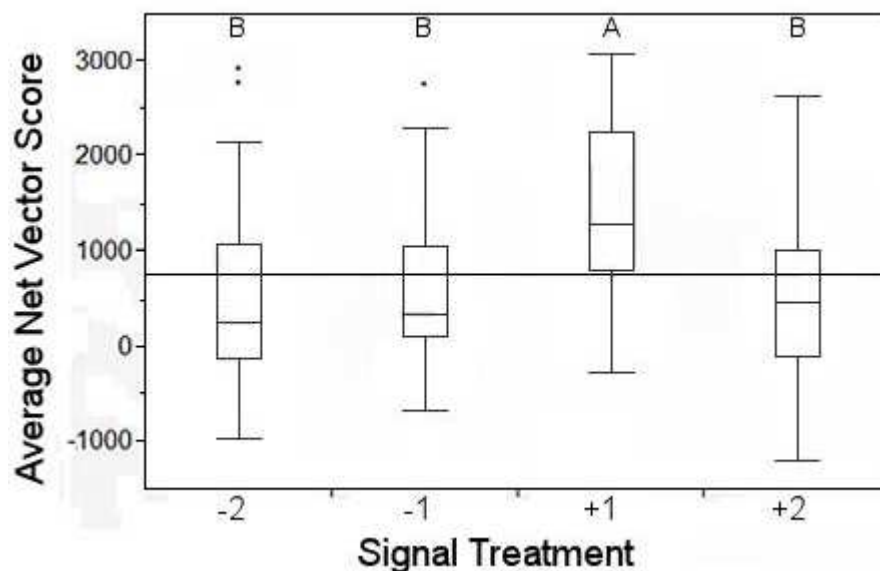


Figure 1. Female phonotaxis on the spherical treadmill (average net vector scores) for each signal intensity treatment ($\bar{X}-2$ SD = 43 dB, $\bar{X}-1$ SD = 52 dB, $\bar{X}+1$ SD = 69 dB, and $\bar{X}+2$ SD = 77 dB). Letters above each box plot were taken from a Tukey's post-hoc HSD analysis; signal 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.

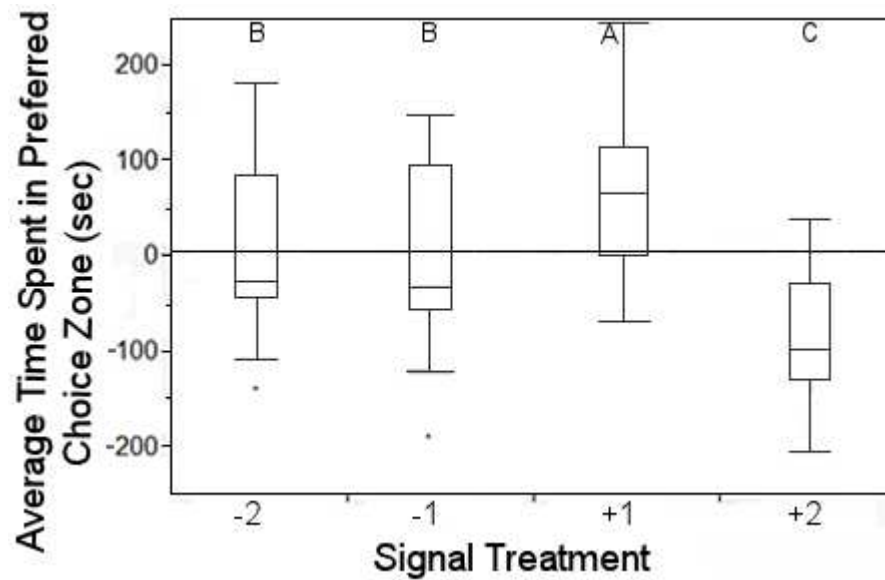


Figure 2. Female phonotaxis in the open arena (relative time spent in the preferred choice zone: time in focal zone – time in standard zone) for each signal intensity treatment ($\bar{X}$ -2 SD = 43 dB, $\bar{X}$ -1 SD = 52 dB, $\bar{X}$ +1 SD = 69 dB, and $\bar{X}$ +2 SD = 77 dB).

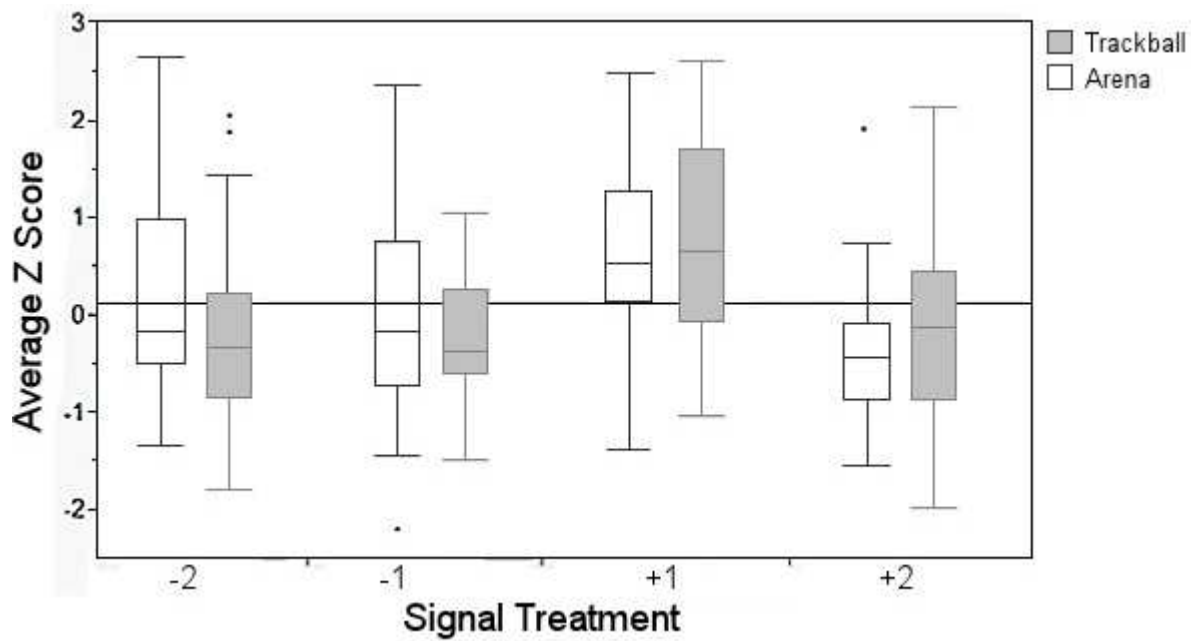


Figure 3. Female phonotaxis (z scores) on the spherical treadmill and in the open arena in response to four different signal treatments ($\bar{X}-2$ SD = 43 dB, $\bar{X}-1$ SD = 52 dB, $\bar{X}+1$ SD = 69 dB, and $\bar{X}+2$ SD = 77 dB).

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	<i>Gryllus campestris</i>	80 *
Lickman, Murray & Cade 1998	<i>Gryllus integer</i>	82-85 *
Gray & Cade 2000	<i>Gryllus texensis</i>	84
	<i>Gryllus rubens</i>	84
Bailey 2008	<i>Teleogryllus oceanicus</i>	70
Prosser, Murray & Cade 1997	<i>Gryllus integer</i>	83 *
Wagner, Murray & Cade 1995	<i>Gryllus integer</i>	84.6
Hedrick, Hisada & Mulloney 2007	<i>Gryllus integer</i>	72
Ritchie 1992	<i>Ephippiger ephippiger</i>	55
Olvido & Wagner 2004	<i>Allonemobius socius</i>	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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