

Female preferences for male calling songs in the bladder grasshopper *Bullacris membracioides*

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Summary

Male acoustic signals and the information they convey are often critical determinants of female mate choice. Bladder grasshoppers are one of numerous orthopteran taxa utilizing sound as the basis of courtship and ultimately mating. However, despite the extreme specializations for long-distance acoustic communication in this family, female mating preferences for male calls have not been previously investigated. Here we examine female acoustic responses to playbacks of male calls in *Bullacris membracioides*. Females were tested in three separate contexts, *viz.* response to conspecific calls of different individuals, response to degraded conspecific calls, and response to the calls of two heterospecifics. Female response was significantly correlated with seven of eight measured call features within *B. membracioides*, indicating sexual selection to be operating in this species. Females also responded to conspecific calls with degradation levels equivalent to a male calling 150 m away, but intensity equivalent to one at 25 m, identifying call amplitude rather than degradation as the factor limiting female response. However, as response decreased with increasing call degradation, signal quality remains a factor in female preference. Calls of the sister taxon *B. intermedia* were equally attractive to *B. membracioides* females as were conspecific calls, while the more distinct calls of *B. serrata* were less preferred than those of both *B. membracioides* and *B. intermedia*. This indicates a lack of discriminatory ability against a similar sounding heterospecific.

Keywords: acoustic communication, female preference, Pneumoridae.

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Introduction

Acoustic signals have long been recognized to form an integral part of the courtship and mating rituals of numerous orthopteran insects. There is mounting evidence from a broad range of Orthoptera that male song prominently functions as a female choice cue through the provision of information regarding condition (Wagner & Hoback, 1999), proximity (Gwynne & Bailey, 1988), quality (Brown et al., 1996), or species identity (Gray & Cade, 2000), resulting in differential male mating success. The complex nature of female mate choice necessitates an examination of preference for multiple cues, and the interactions thereof, to fully understand the processes at work. Male song may simultaneously relay multiple messages to females, each of which may influence her decision to respond.

The function of male song can be assessed at several levels. Females may use song as a mate recognition tool to avoid the potentially costly penalties of erroneously mating with a heterospecific. Additionally, females may assess male calls at the finer scale of the individual to secure the best possible mate as a sire for their offspring. In order to gain a comprehensive insight into the role of sexual selection in shaping the evolution of male song, we need to know which song characteristics are influencing females' decisions, and at what level.

Conflicts may arise when sexual selection and species recognition cues overlap. Sexual selection and species recognition both deal with female response to variation in male characters, and these two processes may be viewed as variations of the same phenomenon rather than antithetical (Ryan & Rand, 1993; Gergus et al., 1997). It is often the case that characters that are sexually selected within a species serve a dual purpose in mate recognition to distinguish between closely related species (Ryan & Rand, 1993). Overlapping signals could therefore result in mistaken species identity. However, in other instances, sexual selection and species recognition cues may be more easily teased apart, with characters under sexual selection within a species having no bearing on mate recognition between species (Boake et al., 1997). Furthermore, species recognition cues may not always override intrasexual selection cues (Hankison & Morris, 2002; Wymann & Whiting, 2003).

In Orthoptera, the acoustic properties of the male signal that most commonly feature in female preferences, include carrier frequency (Gwynne &

Bailey, 1988), syllable rate (Hedrick & Weber, 1998), syllable length (Shaw & Herlihy, 2000), syllable number (Hedrick & Weber, 1998), signal duration (Stumpner & von Helversen, 1994) and signal rate (Doherty & Howard, 1996). Females generally tend to prefer intermediate values of carrier frequency and syllable rate, indicating that these may be used as species identity cues, while more extreme values of the remaining signal properties are usually preferred (Gerhardt & Huber, 2002). It may be beneficial for females to base their preferences on acoustic properties if these are reliable indicators of other desirable male traits. Signal properties that translate into elevated acoustical energy, such as faster rates of signaling and shorter inter-syllable intervals, may be preferred by females as they are more energetically costly to produce and may therefore signify increased male vigour (Brown, 1999). In addition, a higher number of syllables per chirp, a trait preferred by females, has been linked to a favourable immune function in house crickets (Ryder & Siva-Jothy, 2000). The correlation between male body size and call frequency is well documented (Bailey & Yeoh, 1988; Gwynne & Bailey, 1988; Simmons & Zuk, 1992). Females preferring songs with lower carrier frequencies may therefore be selecting males with larger body sizes, which may signify a competitive advantage. Preferentially mating with larger males has also been linked to increased fecundity in tree crickets (Brown et al., 1996).

Bladder grasshoppers (Orthoptera; Pneumoridae) are an intriguing family that has evolved extreme exaggerations of morphology in pursuit of maximizing sound communication ability. Males develop an inflated abdominal bladder functioning as a sound resonator during their final moult into adulthood, allowing them to achieve a sound output of ca. 98 dB SPL (at 1 m) (van Staaden & Römer, 1997). Pair formation occurs via reciprocal dueting and male phonotaxis. Receptive females indicate their willingness to mate by acoustically responding to the advertisement calls of males, thereby allowing flighted males to locate philopatric females through signal tracking. Once the male has successfully located the female, there is no further courtship and mating inevitably follows.

The acoustic signaling system of pneumorids incorporates a discrepancy between the transmission distance and the active space of a signal. This discrepancy arises from a substantially lower hearing threshold (ca. 32 dB SPL) than behavioural threshold (ca. 65 dB SPL) (van Staaden & Römer, 1998). As a result, sensitive hearing enables females to detect calls at a distance

of 1.9 km, but behavioural motivation requires sound intensities equivalent to a caller 50 m away (van Staaden & Römer, 1997). Long-range signaling results in significant call degradation along the transmission channel (Couldridge & van Staaden, 2004), which may result in decreased signal recognition. Males' use of increasing call amplitude as a tactic to encourage female behavioural response at greater distances may therefore be negated by the perceived loss of quality. Examining female responses to degraded calls in the absence of intensity cues enables the effects of degradation and intensity to be teased apart. It is not known how effectively males are able to perform phonotaxis over distances greater than 50 m, in which case it may not be beneficial for the female to respond, and risk revealing her location to unintended receivers, until the male is relatively close-by.

Despite the observed dependency of bladder grasshoppers on acoustic signals for mate location, very little is known about the capacity of females to discriminate among males, both within and between species. The pneumorid mating system presents a prime opportunity for females to exercise mate choice by responding selectively to the calls of males. Individual variation among male calls, as well as a correlation between acoustic and anatomical characters in males, has recently been documented (Myers & van Staaden, *subm.*). Moreover, songs may be reliable indicators of male quality due to their costly production. Males calling more frequently throughout their lifespan have a significantly earlier mortality (V. Couldridge, *unpubl. obs.*).

We conducted a series of acoustic playback experiments to examine female mating preferences in the bladder grasshopper *Bullacris membracioides*. Females were presented with three different choice scenarios. Firstly, a selection of conspecific calls representing the natural variation present in the population established any biases in response for particular acoustic traits. Secondly, a choice between the same call recorded at various distances tested response to degraded calls. Thirdly, we examined interspecific preferences by presenting the conspecific call along with calls belonging to two closely related species with overlapping or parapatric distributions, *Bullacris intermedia* and *Bullacris serrata*, respectively. Investigating female preference in the above three contexts allowed us to gain a more comprehensive understanding of the many complex factors that may be contributing to mate choice.

Materials and methods

Animal collection and rearing

Individuals of *Bullacris membracioides* were collected by hand from stands of *Berkheya* sp. in the Inchanga region of KwaZulu-Natal, South Africa, during January 2003 and March 2004. Animals were subsequently transported to a USDA-APHIS inspected laboratory in the United States, where all experimental procedures were carried out. Females were either captured in the field as adults, or as nymphs that were reared to adulthood in the laboratory, and were housed separately from males to maintain sexual naiveté; however, the virginal status of females collected as adults could not be ascertained. Females were kept in the laboratory in groups of up to 10 individuals per glass aquarium (400 × 250 × 300 mm) and fed *ad libitum* on a diet of organically grown romaine lettuce. Animals were kept at a constant temperature of 25°C and maintained on a 12-hour photoperiod.

Sound stimuli

Male *B. membracioides* produce an advertisement call consisting of five short introductory syllables followed by a final, longer, resonant syllable with a carrier frequency of approximately 1.7 kHz (Figure 1). The call of *B. intermedia* (Figure 1) is very similar to that of *B. membracioides*, but has an additional short syllable at the start of the call and a slightly higher carrier frequency of 1.8 kHz. *B. serrata* produces a notably shorter call, with only two very brief introductory syllables preceding the final resonant syllable, and a carrier frequency of around 2.0 kHz (Figure 1). Females of *B. membracioides* reply to male songs with a broadband signal of between 4 and 10 kHz (Figure 1), with the number of syllables produced per response varying from one to ten.

The male advertisement calls used in female response trials were exemplars of laboratory recordings selected from a previous sound library. Songs were digitized at a sampling rate of 44.1 kHz onto an Apple Macintosh G4 via the built-in 16-bit A/D soundboard. SoundEdit™ 16 was used to create sound files for playbacks, in which the order of call presentation was randomized and 30 seconds of silence separated each call. Male calls were played sequentially, rather than simultaneously, since females only respond acoustically to male calls and are not phonotactic, making it impossible to

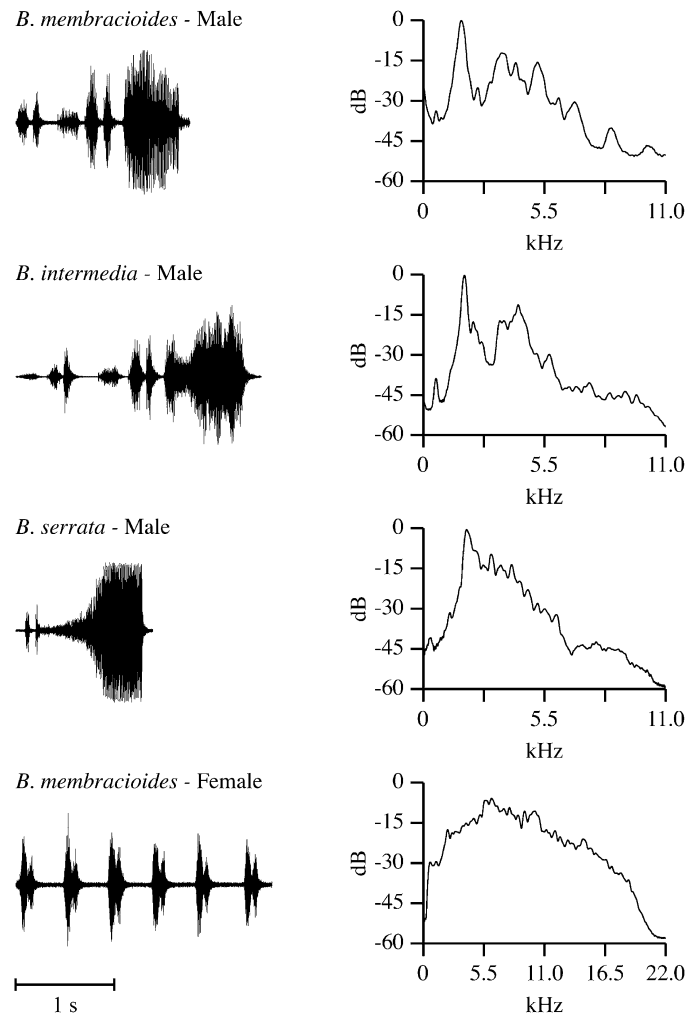


Figure 1. Oscillogram (left) and power spectrum (right) exemplars of male advertisement calls of *B. membracioides*, *B. intermedia* and *B. serrata*, and female response of *B. membracioides*.

determine which call a female is responding to in a two-way design. All calls were broadcast at the same peak intensity of 70 dB SPL, measured at the position of the female with a Larson Davis DSP83 sound level meter (A weighting, RMS fast). This intensity is well above the female behavioural response threshold of ca. 65 dB SPL, but below that at which receptor response is saturated (van Staaden et al., 2003).

Playback experiments

All playback experiments were performed at least four hours after the onset of the animals' dark cycle. Trials were conducted in a small semi-anechoic room containing a square arena ($1.2 \times 1.2 \times 0.3$ m) constructed of PVC piping and white fabric. Sounds were broadcast via a Macintosh G4 running SoundEdit™ 16 to one of four randomly selected speakers (RadioShack AMX-21) positioned in each corner of the arena. The speakers played back the full frequency range of the male calls. A single vertical wooden rod (185 mm in height), located in the center of the arena, served as a perch for the test subject. At the start of the trial, a female was placed on the perch and allowed a 10 minute acclimation period before playbacks began. A Sony video recorder (DCR-TRV140) equipped with infra-red capabilities was trained on the female and sent images to a television screen in the adjoining room, allowing the acoustic responses of females to male calls to be observed and recorded. If females were unresponsive, they were removed from the arena and tried again the following night, so that only responsive females were used.

Subjects were tested in three separate experimental contexts. The first experiment measured preference for signal characteristics within conspecific *B. membracioides*. Females were presented with one call belonging to each of 12 individual males, with each call repeated 10 times (120 calls in total). The 120 calls were split into two sessions of 60 calls each, separated by a break of at least 10 minutes, to sustain female interest. In the second experimental design, females were presented with a choice of variously degraded and non-degraded conspecific calls. Females were given six repetitions of the same call recorded in the species' natural savanna habitat at six different distances (1, 5, 25, 50, 100, 150 m) from the sound source (36 calls in total). The third experiment examined female response to heterospecific calls. Females received calls belonging to each of three species — conspecific *B. membracioides* and heterospecific *B. intermedia* and *B. serrata*. Six different calls from each species were used, with each call repeated three times (total of 56 calls). The same individuals were tested in each of the three experiments above, with the order of experiments randomized for each female.

Analysis

Three separate measures of female response were used as indicators of preference: firstly, the proportion of stimuli that elicited a response; secondly, the

number of syllables per response; and thirdly, the number of syllables per response for only those calls that elicited a response. For each trial, individual female response to multiple presentations of the same call was averaged to obtain a mean response value that was used in further analyses. To obtain normal distributions, arcsine transformations were applied to the proportion of calls to which females responded.

The 12 calls presented to females during the conspecific preference trials were analyzed using Canary 1.2.4 (Cornell Laboratory of Ornithology, 1995) and various measurements taken. These characters were selected based on a previous study that identified them as varying significantly between individuals (Myers & van Staaden, *subm.*), and included carrier frequency of the whole call (FC) and of the introductory syllables (FI), length of the whole call (LT), final syllable (LF) and introductory syllables (LI), time interval between the final and second last syllables (ISI), ratio of the final syllable to the entire call length (RL), and rise time of the final syllable (RT). Each of these signal characteristics was then included in a multiple regression model with female preference as the dependent variable.

Pearson correlations were used to test for a relationship between female preference and distance at which the calls were recorded.

One-way ANOVAs with Tukey multiple comparison tests were used to test for differences in female response to each of the three species. In addition, a Discriminant Function Analysis (DFA) was performed on 16 calls of each species. The same characters were measured from the calls as above, with the exception of RL, to identify which of these features are the most important in species categorization.

Results

A total of 24 females were tested for their responses to male calls. However, due to loss of responsiveness or death, sample sizes were uneven over the three experimental protocols. The average age at testing for females of known age was 15.4 ± 7.1 days (mean \pm SD; $N = 16$) after the final moult. This age corresponded to peak acoustic responsiveness. There was no significant difference in response, in either the number of calls responded to (Mann-Whitney test; $U_{7,11} = 35$; $p = 0.785$) or the number of syllables per response (Mann-Whitney test; $U_{7,11} = 30$; $p = 0.479$), between females collected as nymphs and those collected as adults.

Intraspecific preference

Female response ($N = 18$) ranged from a mean of 54.48 % for the calls of the least preferred male to a mean of 96.85 % for the calls of the most preferred male. Multiple regression identified intersyllable interval (ISI) as the only call characteristic not significantly correlated with female preference (Table 1). Shorter length of the introductory syllables (LI) was significantly related to the number of syllables per response, for both all calls and only those cases where females responded, but not the percentage of calls responded to. The remaining six call characters were significant across all three measures of preference (Table 1), with female response being negatively correlated with carrier frequency (FC), call length (LT), rise time (RT), and ratio of the final syllable to total call length (RL), and positively correlated with peak frequency of the introductory syllables (FI) and final syllable length (LF). Final syllable length was the character most strongly associated with female preference, as indicated by high standardized regression coefficients across all three measures of preference.

Table 1. Standardized partial regression coefficients (β) and their associated significance from multivariate regression analyses conducted on three separate measures of female preference (percentage of calls responded to, number of syllables per response, and number of syllables per response for only calls responded to) on male call characteristics.

Character	% Response		Number of syllables (All)		Number of syllables (Responded)	
	β	p	β	p	β	p
FC	-0.477	0.008	-0.658	< 0.001	-0.852	< 0.001
FI	0.363	< 0.001	0.506	< 0.001	0.515	< 0.001
LT	-2.576	0.005	-2.753	< 0.001	-3.085	< 0.001
LI	-0.278	0.141	-0.460	0.002	-0.665	< 0.001
LF	4.169	0.008	4.639	< 0.001	5.623	< 0.001
RT	-0.239	< 0.001	-0.261	< 0.001	-0.242	< 0.001
RL	-2.251	0.023	-2.395	0.003	-2.919	0.003
ISI	0.010	0.884	0.056	0.318	0.065	0.314
	$r^2 = 0.631$		$r^2 = 0.767$		$r^2 = 0.703$	

FC = carrier frequency, FI = frequency of introductory syllables, LT = total call length, LI = length of introductory syllables, LF = length of final syllable, RT = rise time of final syllable, RL = ratio: final syllable/total length, ISI = intersyllable interval.

There were relatively few correlations among individual call characteristics. Carrier frequency was negatively correlated with introductory syllable length, total call length was positively correlated with final syllable length, and ratio of the final syllable to total call length was necessarily correlated negatively with total length and positively with final syllable length (Table 2).

Degraded signal preference

Female response decreased significantly with increased recording distance from the sound source (Table 3). Distance was negatively correlated with the percentage of calls responded to (Pearson correlation: $r = -0.393$; $N = 10$; $p = 0.002$), the number of syllables per response for all calls (Pearson correlation: $r = -0.390$; $N = 10$; $p = 0.002$), and the number of syllables per response for only those calls eliciting a response (Pearson correlation: $r = -0.287$; $N = 10$; $p = 0.031$).

Two of the eight call characteristics were found to vary significantly with distance. As distance from the signal increased, there was an observed decrease in both the peak frequency of the introductory syllables (Spearman Rho = -0.604 ; $N = 18$; $p = 0.008$), and the total call length (Spearman Rho = -0.680 ; $N = 18$; $p = 0.002$).

Interspecific preference

Females responded to an average of 73.82% of the calls of conspecific *B. membracioides*, 85.60% of the calls of *B. intermedia*, and 46.13% of the calls of *B. serrata*. The mean number of syllables per response was 3.99 for *B. membracioides*, 5.33 for *B. intermedia*, and 2.19 for *B. serrata*. When cases where females did not respond to a call were excluded from the data set, the mean number of syllables per response increased to 5.38 for *B. membracioides*, 5.94 for *B. intermedia*, and 4.12 for *B. serrata* (Figure 2). Female response to the calls of *B. membracioides* and *B. intermedia* was significantly higher than to the calls of *B. serrata* for the percentage of calls eliciting a response (ANOVA: $F_{2,48} = 7.333$; $p = 0.002$), the number of syllables per response for all calls (ANOVA: $F_{2,48} = 9.582$; $p < 0.001$) and the number of syllables per response for calls eliciting a response (ANOVA: $F_{2,48} = 6.438$; $p = 0.004$). However, there was no significant difference between response to *B. membracioides* and *B. intermedia*.

Table 2. Partial correlation coefficients between eight male call characteristics ($N = 12$).

	FC	FI	LT	LI	LF	RT	RL
FI	0.612						
LT	-0.484	0.100					
LI	-0.896*	0.612	-0.559				
LF	0.573	-0.144	0.991**	0.650			
RT	-0.322	0.173	-0.263	-0.367	0.310		
RL	-0.481	0.068	-0.995**	-0.576	0.993**	-0.287	
ISI	0.340	-0.707	0.054	0.480	-0.090	0.096	0.045

FC = carrier frequency, FI = frequency of introductory syllables, LT = total call length, LI = length of introductory syllables, LF = length of final syllable, RT = rise time of final syllable, RL = ratio: final syllable/total length, ISI = intersyllable interval.

* $p < 0.05$; ** $p < 0.001$.

Table 3. Mean female responses ($N = 10$) to a male advertisement call of *B. membracioides* recorded at different distances from the source.

Recording distance (m)	% Calls eliciting response	Syllables per response (all responded)	Syllables per response
1	100.0	6.0	6.0
5	91.7	5.7	5.8
25	73.3	3.5	4.6
50	70.0	3.5	4.6
100	88.3	4.8	5.2
150	48.3	2.4	4.3

The species-specific signals of the three grasshopper taxa (Table 4) differed significantly from each other (Wilks' Lambda $p < 0.001$). The canonical centroid plot (Figure 3) distinguished species on the basis of temporal rather than frequency properties of the calls. *B. serrata* was separated very clearly from the two other species along the first canonical axis. The main characters contributing to this axis were LT, ISI and LF. The second canonical axis separated *B. membracioides* from *B. intermedia*, and the major contributing character to this axis was LI. Using these call characteristics, all but three calls were correctly classified as their respective species. One *B. membracioides* and two *B. intermedia* calls were misclassified as belonging to the reciprocal species.

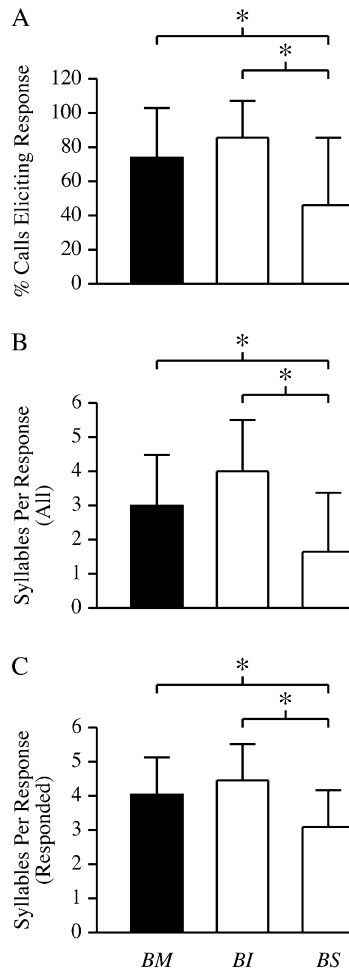


Figure 2. Response of female *B. membracioides* (Mean + SD) to the male advertisement calls of *B. membracioides* (BM), *B. intermedia* (BI), and *B. serrata* (BS). (A) The percentage of male calls that elicited a response, (B) the number of syllables per response and (C) the number of syllables per response for only those calls responded to. Shaded bars represent the conspecific male and unshaded bars heterospecific males. Asterisks indicate a significant difference at the 5% level. In A, B, and C, females responded significantly less to the calls of *B. serrata* than to the calls of both *B. membracioides* and *B. intermedia*. There were no significant differences between response to *B. membracioides* and *B. intermedia*.

Discussion

The playback experiments reveal that female *B. membracioides* discriminate amongst the calls of conspecific males, with all but one measured call char-

Table 4. Mean values \pm SD of the call characteristics of three pneumorid species, based on 16 calls of each, of which the calls used in the preference trials were a subset.

Character	<i>B. membracioides</i>	<i>B. intermedia</i>	<i>B. serrata</i>
FC (Hz)	1783 \pm 121	1861 \pm 123	1953 \pm 83
FI (Hz)	1848 \pm 267	2864 \pm 625	2680 \pm 639
LT (ms)	1989 \pm 148	2322 \pm 167	1304 \pm 54
LI (ms)	1193 \pm 171	1526 \pm 160	174 \pm 9
LF (ms)	746 \pm 38	778 \pm 82	1126 \pm 50
RT (ms)	497 \pm 106	534 \pm 88	953 \pm 110
RL	0.377 \pm 0.034	0.335 \pm 0.028	0.864 \pm 0.010
ISI (ms)	73 \pm 15	62 \pm 14	0

FC = carrier frequency, FI = frequency of introductory syllables, LT = total call length, LI = length of introductory syllables, LF = length of final syllable, RT = rise time of final syllable, RL = ratio: final syllable/total length, ISI = intersyllable interval.

acteristic (ISI) being significantly associated with preference. This suggests non-random mating in nature and supports the potential role of sexual selection in the evolution of male calls in this group. Signal carrier frequency, total length, final syllable length, and ratio of the latter two have all been inversely linked with aspects of male body size in *B. membracioides* (Myers & van Staaden, subm.). Thus, females who responded preferentially to calls with a lower carrier frequency, shorter total length, and smaller ratio would, directly or indirectly, be exercising a preference for larger males. However, females also preferred male calls with a longer final syllable length, indicative of smaller body size.

Female *B. membracioides* discriminated against degraded calls, with decreased response closely mirroring an increase in the distance at which the male call was recorded. In addition to loss of amplitude due to excess attenuation, the passage of sound through the transmission channel results in changes to the frequency and temporal domains of a signal, which further diminishes the potential for recognition (Simmons, 1988; Lang, 2000). The observed decline in female response with progressively increasing call degradation suggests a loss of recognition ability at greater transmission distances that is independent of amplitude, and identifies the importance of signal integrity as a determinant of female response. However, females do respond, albeit at lower levels, to calls with degradation levels approximating three times the normal communication distance, but played back at an intensity

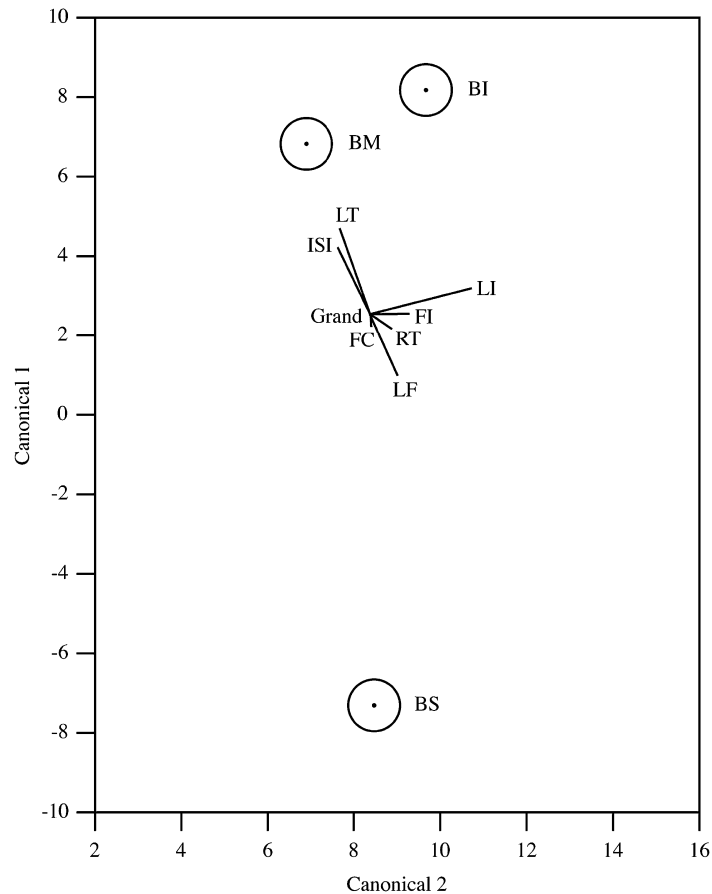


Figure 3. Canonical centroid plot of the discriminant function analysis showing the separation of male advertisement calls according to species. Circles represent 95% confidence limits for the mean. The first canonical axis very clearly separates the calls of *B. serrata* from those of the two other species. The major features contributing to this axis are total call length (LT), intersyllable interval (ISI), and length of the final syllable (LF). The second canonical axis separates *B. membracioides* and *B. intermedia* from each other on the basis of introductory syllable length (LI).

exceeding the behavioural response threshold. This points to call amplitude, rather than quality, as the critical factor limiting female acoustic response and it supports the notion that there is selective pressure on males to increase call amplitude.

False recognition, or even preference of, heterospecifics as mates, while deviating from the norm, is documented in a growing number of taxa (e.g.,

Morris et al., 1978; Ryan & Wagner, 1987; Doherty & Howard, 1996; Jones & Hunter, 1998; Deering & Scriber, 2002; Diego-Rasilla & Luengo, 2004). Preexisting sensory biases for novel or supernormal stimuli, or the presence of desirable conspecific traits in heterospecifics, are often employed as explanations for this puzzling phenomenon. Females of derived species may also retain relics of ancient preferences and consequently fail to discriminate against heterospecific males bearing ancestral traits (Ryan & Rand, 1995).

On presentation of heterospecific songs, female *B. membracioides* discriminated against *B. serrata*, but not against *B. intermedia*. In fact, female preference for *B. intermedia* was slightly higher than that for conspecific males, although not significantly so. This lack of discrimination may be attributed to the subtle differences between the two species' calls. Most of the call characteristics under examination overlapped between the species; the only discernable difference being the extra initial syllable in *B. intermedia*, resulting in a longer introductory syllable length and consequently a longer signal length. Preference for *B. intermedia* may therefore reflect a transfer of conspecific preference criteria to a rather similar sounding species.

The resemblance in the calls of *B. membracioides* and *B. intermedia* is not unexpected considering the general similarity and recency of divergence of these two species. Preliminary molecular analysis places *B. intermedia* as the most closely related species to *B. membracioides*, and this the most recent split in the pneumorid family tree (van Staaden, in prep.). However, whether this split represents a true species distinction or populations under divergence remains unclear. While Dirsh (1965) upheld the specific status of *B. intermedia* in the most recent revision of this family based purely on morphological distinctions, he conceded that these individuals could be simply a local race of *B. membracioides*.

B. serrata is the next closely related species in this group (van Staaden, in prep.), with calls which are distinctly different in temporal characteristics to those of the other two species. That females responded at all to *B. serrata* was unanticipated in light of these large differences in the species-specific acoustic signals. Furthermore, due to an absolute reliance on acoustic signals for pair formation, it is reasonable to expect that the nature of the pneumorid mating system allows little room for error in species recognition based on calling song. It is extremely unlikely in this taxon that species recognition rests with the male, as female calls are indistinguishable to the human ear and males of species belonging to different genera are known to simultaneously

phonotactically track the same crude approximation of the female call (M. van Staaden, pers. obs.). It is possible that limited overlap in geographic distribution coupled with low population density and slight temporal shifts in breeding, sufficiently restricts gene flow to maintain species integrity. In addition, the cost of female response is expected to be low, so an ambiguous call may still elicit a response if a female is highly motivated.

A further explanation as to why female *B. membracioides* respond to songs of heterospecific males may be sought in the population under study existing in isolation from all other pneumorid species. Under natural conditions, the females of this population would only encounter conspecific males, and therefore face no selective pressure to mate assortatively. Differential mating preferences for male calls in areas of allopatry versus sympatry, indicative of reproductive character displacement, have been reported in both anurans (Marquez & Bosch, 1997; Höbel & Gerhardt, 2003) and insects (Marshall & Cooley, 2000). However, this is not always the case, and it should be noted that geographic proximity does not necessarily dictate avoidance of heterospecifics (Gregory et al., 1998). While *B. membracioides* and *B. serrata* appear to be parapatric, areas of sympatry between *B. membracioides* and *B. intermedia* are documented in the southernmost part of the geographic range of *B. membracioides*, approximately 235 km from the study population. It remains to be seen whether females under sympatry would continue to respond so eagerly to the calls of heterospecific males.

Female acoustic response to male song appears to be the sole means of courtship and pair formation in the Pneumoridae. Detailed observations of matings reveal no additional discrimination by either sex once the male has successfully located the female, and that copulation is initiated in a matter of seconds after physical contact is made (V. Couldridge, pers. obs.). This underscores the critical nature of the acoustic signals per se and the far-reaching consequences that expressed female preferences have on the evolution of calling song and species distinctions in this family.

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