ACOUSTIC COMMUNICATION IN THE DUETTING KATYDID, *SCUDDERIA PISTILLATA* (ORTHOPTERA: TETTIGONIIDAE: PHANEROPTERINAE)

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Duetting katydids (Orthoptera: Tettigoniidae) are unique in that both the male and the female use airborne sounds for mate attraction. The presence of a female response alerts the male to her location as well as provides a signal of her motivation or willingness to mate. The purpose of this study was to characterize the male advertisement call and the female tick response in the broad-winged bush katydid, *Scudderia pistillata,* to understand how differing contexts affect both sexes' calls, and the implications these findings might have on mate attraction and localization. Male *S. pistillata* produce a complex call of increasing syllables per phrase, ranging from a single-syllabled phrase to an eleven-syllabled phrase. Males are consistent in how they add syllables to phrases in a bout and the syllable number and phrase number produced relates to an index of condition, residual weight. They also exhibit plasticity in the length of their call, varying syllable number and phrase number depending upon whose acoustic signal they perceive. Male call is most highly influenced by the presence of a female; he increases his rate of calling and the total length of his bout when hearing female ticks. When males are presented with the call of another male, he synchronizes his call to theirs, but lowers his overall acoustic energy compared to a female encounter. The implications of this behavior on male chorus structure are discussed. Analysis of *S. pistillata* duetting behavior demonstrates that females alter their tick number with motivation as well as the number of syllables and sound intensity of the syllables she hears. When a male hears her response he performs phonotaxis; however the number of ticks he hears after each phrase in his bout does not assist in his ability to reach the female. The importance of variability in female tick number is therefore discussed. Males also exhibit a form of mate defense by producing sounds after his phrase in the time window in which female response would fall. This acoustic mate guarding serves to confuse the location of the female for eavesdropping males.

BIOGRAPHICAL SKETCH

Susan grew up in Kansas City, Missouri and from an early age knew that she would become a biologist. As a child she was inspired by people like Jane Goodall and Jack Hanna, because they showed her it was possible for someone to have a career around animals. She kept her general interested in biology through high school knowing she wanted to work with animals, but without a strong idea of where she wanted to concentrate. Susan entered Truman State University, a small liberal arts college in Missouri. During her stay at Truman, courses dealing with animal communication, invertebrate biology, as well as a field course in southern Texas highly influenced her current interests in biology. She learned that she had a strong interest in the behavior of insects and in field biology. After four years at Truman she graduated with a B.S. in Biology with honors and a concentration in organismal biology. She continued to graduate school at Cornell University in the department of entomology under her thesis advisor, Dr. Cole Gilbert. Cole Gilbert's interest primarily lies in beetle vision, but he is also generally interested in insect behavior and worked with Susan in deciding where she wanted to focus. After exploration into various acoustic communication systems she focused her graduate student research on a duetting katydid, *Scudderia pistillata*. In the project she has described the acoustics of both the male and the female call, as well as what acoustic inputs effect both male and female call, and characterized the phonotaxis behavior of male *S. pistillata*.

Her graduate project has provided her with extensive knowledge of performing field-based research, through many collecting trips and field-based experiments. Also, because little was known about *S. pistillata* prior to the project, she now has a wealth of experience in designing experiments from the ground up, addressing questions which had no clear answer in the literature and coming up with logical predictions. Because Cole Gilbert's lab is primarily visual, he and Susan worked together to acquire the necessary equipment to do acoustic work, through acquiring grants as well as generating a unique microphone array to simultaneously record four individuals, all acoustically isolated, via hand-made sound dampening boxes.

In her time at Cornell she has had many opportunities to teach students, including organismal biology, genetics, ecology, etc. She has received two awards for teaching excellence because she used her interest in biology and her passion for research to foster interest in the subject matter for the students. In the evaluations from the general biology lab Susan taught they said her enthusiasm was motivating and that her personal interest in their success made a difference. This is the type of relationship between students and faculty which she plans to continue in her post-graduate career.

Once achieving her doctorate, Susan will explore other systems that address similar questions to that of her katydid work. She is interested in understanding what factors affect the structure of a chorus, what role variability in male call has for female response/male-male interaction, why males exhibit complexity in calling types and how this complexity affects female response, and finally if there is any effect of female response on subsequent male calls. Acoustic communication is a diverse field and she hopes to spend her career learning more about the many ways in which organisms interact sonically and teaching students about the various topics related to animal communication.

Dedicated to my parents for all their support

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Firstly, I’d like to thank my advisor, Dr. Cole Gilbert. He and I worked together to develop a project in a study system neither of us was very familiar with. Through all the miss-steps and unknowns of this project he was a supportive and intelligent advisor, truly aiding in the progress of this dissertation.

Secondly, I’d like to thank my committee, Dr. Jack Bradbury and Dr. Ron Hoy. Both are experts in their respective fields of animal communication and orthopteran acoustics. Their review of various manuscripts gratefully improved the writing and analysis of this dissertation. I’d also like to thank an honorary member of my committee, Lang Elliott, for introducing me to this marvelous katydid and being very helpful in finding these katydids as well as aiding in sound equipment selection.

Thirdly, I’d like to thank my family, my parents, and my fiancé for their constant support and love through this whole project. From the very beginning I knew I could accomplish whatever I set my mind to thanks to their encouragement.

There are a number of people who helped with the various field-aspects of this work. I would like to thank the Connecticut Hill Wildlife Preserve for letting me use their land. Also, I’d like to thank Elisa and John Miller for the generous use of their property for my Bald Hill site. I also had a number of helpers. Mike Orr, Allan Jilo and Jeffrey Clements helped mark and recapture katydids for my chorus structure analyses. A number of fellow grad students also lent a helping hand for collecting katydids from 2009 to 2012: Lauren Cator, Punita Juneja, Ginny Howick, Michelle Helinski, Mark Jandricic. Special mention goes to Sarah Jandricic for being my number one field helper, I couldn’t have done it without her. Sarah Short and Leo Stellwag were also quite helpful when I reared nymphs by supplying flies and pollen.

A special thanks also to the people at The Cornell Statistical Consulting Unit for their aid in various analyses, specifically Françoise Vermeylen and Jason Barry. This work was funded by the SUNY Diversity Fellowship, Sigma-Xi Grant-in-Aid of Research, and Cornell Entomology’s Grace Griswold Endowment and Rawlins Endowment Funds.

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CHAPTER 1

THE UNIQUE COUNTING CALL OF MALE *S. PISTILLATA*

Abstract

The broad-winged bush katydid, *Scudderia pistillata* Brunner, 1878 (Orthoptera: Tettigoniidae), is anecdotally called the counting katydid because the syllables produced from each wing closure of the male calling song are grouped into phrases, with each successive phrase in the first seven phrases of a calling bout typically possessing one more syllable than the previous phrase. Analysis of over 500 recorded male bouts showed that adding syllables to each phrase is stereotypic for the species. Although this aspect of the calls was stereotypical, other aspects of the calls exhibited variability, including the total numbers of syllables and phrases per bout, which were correlated with a male's nutritional condition, as indexed by residual weight. Potential behavioral functions of the counting sequence are discussed.

Introduction

Katydids (Orthoptera: Tettigoniidae) use acoustic communication for mate localization and pair formation, with males typically producing a song and females silently orienting toward conspecific calling males (Ewing 1989; Robinson & Hall 2002). The male creates the advertisement song by rubbing a file on his left forewing against a scraper on the right forewing. Each wing closure produces a sound called a phonotome, or syllable (Ragge & Reynolds 1998; Gerhardt & Huber 2002). Each katydid species produces a species-specific call, differing from other species in the temporal pattern of syllables and/or frequency spectrum. The simplest advertisement call consists of a single syllable repeated continuously for the length of calling time or multiple wing closures given in close succession to comprise a phrase. More complex calls contain more than one syllable type produced using different muscular movements or files with varying tooth structure to create diverse call spectra (Walker & Dew 1972).

The more complex katydid calls tend to be produced within the subfamily Phaneropterinae, the False Katydids (Walker & Dew 1972; Heller 1990; Korsunovskaya 2009). *Amblycorypha* spp. is particularly well documented in the literature for having complex calls, with many species in the genus producing multiple syllable types (Heller 1990; Walker 2004). *Amblycorypha longinicta* Walker (2005), for example, exhibits four syllable types. Their pattern of production exhibits long repetitions in a single syllable type and syllable types are not produced in a stereotypic order (Walker & Dew 1972).

Another Phaneropterinae katydid is the broad-winged bush katydid, *Scudderia pistillata* Brunner 1878, considered as the only counting katydid because males add a syllable to each successive phrase of sound (Elliott & Hershberger 2006; Walker 2008). Similarly to the other *Scudderia* spp., *S. pistillata* produces four distinct call types, only one of which is meant to be advertised to listening females (Spooner 1964). When a male starts his advertisement call, there are relatively few syllables produced (2 or 3) per phrase. But by the time the male has finished the bout of calling, syllables have been added to each phrase leading to a final phrase of 9 or 10 syllables. Such increase in length of phrases of sound in a call is unique among katydids. *Scudderia curvicauda*, a congener of *S. pistillata*, produces a similar call (Tuckerman et al. 1993), but it has not been analyzed in detail. The call of *S. pistillata* is anecdotally described (Spooner 1968a), but the sounds produced by *S. pistillata* and the regularity of the counting sequence have not been analyzed.

Singing Tettigoniidae males must not only attract a female with their songs, but also produce a nuptial gift to the female upon mating. There is a direct trade-off in energy allocation to the calling song and donation of the nuptial gift (Simmons et al. 1992). A meta-analysis on male investment in nuptial gifts shows, when controlling for body weight and phylogeny, a positive correlation exists between weight and sperm number transferred, as well as spermatophyllax size and sperm number. More sperm transferred induces a longer refractory period for female re-mating and may hasten oviposition (Vahed & Gilbert 1996; Vahed 2007). Sperm transfer is also dependent on nutritional state (i.e., body condition, Jakob et al. 1996), with low-diet males exhibiting a smaller spermatophyllax, and less sperm within a more watery spermatophores (Jia et al. 2000). If calling song attributes were correlated with physical aspects of the calling male, his song could provide information for the female to assess the condition of the caller in terms of ability to provide a sufficient nuptial gift. Because males in better condition provide a greater nuptial gift to the female, females would benefit by discriminating acoustic correlates of male condition. Analysis of male variability and any correlations of size indicators with calling song parameters could potentially demonstrate which attributes of the male call may be most informative for the female.

In this study, recorded bouts of male *S. pistillata* advertisement calls were analyzed to characterize the call. The purpose of the analysis was to answer the following questions: 1) Do successive bouts contain sequentially more syllables? 2) How long is a typical male bout? 3) Are there discernible differences between males in calling song, and are those attributes correlated with physical characteristics of the male? From this analysis, we demonstrate that the song is a stereotypic counting sequence, that there is significant variation in call parameters across males, and that some of these parameters are related to male body size.

Methods

Animal collection, care and housing

Male *Scudderia pistillata* (Tettigoniidae: Phaneropterinae) were collected from old fields, dominated by golden rod, *Solidago* spp., surrounded by mixed conifer and deciduous forests, on Bald Hill (42°21’11.28” N, 76°22’57.46” W) and Connecticut Hill (42°20’32.13” N, 76°39’42.50” W) near Ithaca, NY from June through September of 2007 and 2009. Males are nymphs until mid-June, and begin to sing approximately 1 wk. after molting to adults. Singing males were collected at night using their call for localization. Individuals were housed separately in 30.5 x 11.5 cm diameter wire mesh cylindrical cages spaced throughout a 3 x 3m large room. All were fed a varied diet including romaine lettuce, pollen, apples, and various herbaceous plants, including goldenrod and milkweed. They were provided water via cricket food (Fluker’s Orange Cube Complete Cricket Diet, Port Allen, LA), maintained at room temperature (75°-83°F) with natural ambient photoperiods.

Call recording and analysis

Individual recordings of singing males were obtained to characterize the species advertisement call. A focal male was acoustically isolated from the group of males and recorded with a Sennheiser ME66/K6 combo shotgun microphone

(Sennheiser Electronic Corp., Old Lyme, CT), placed ~30cm from the individual's cage, connected to a Tascam HD-P2 digital audio recorder (44 kHz sampling frequency, TEAC Corp., Japan). Individuals were recorded from 2 to 4 hr. on a single night, with temperatures ranging from 24-29°C. Each night a new focal male was selected. Multiple recordings were collected from each individual male. Recordings were transferred to a computer and analyzed using two software packages: temporal analysis using Audacity 1.3.5 Cross-Platform Sound Editor (http://audacity.sourceforge.net/) and frequency analysis using Raven Pro 1.3 (Bioacoustics Research Program, Cornell Laboratory of Ornithology, Ithaca, NY). These recordings resulted in 515 calling bouts from roughly 30 individual males that were analyzed for the number of syllables produced for each phrase in every bout. To assess whether the number of syllables increased over the duration of a bout, comparisons of syllables per phrase produced for the length of the bout, as well as conditional probabilities of syllable number given the preceding phrase were performed. Conditional probabilities were obtained by hand tabulating all recorded sequential syllables per phrase for all phrases recorded, regardless of their position in the bout.

From these recordings, the bouts of 26 identified males [9 from 2007, 17 from 2009] were used for analysis of inter-male variability. The following individual males' call parameters were compared: total syllables per bout, maximum syllables per phrase, and number of phrases per bout. These parameters were then used in analysis with 15 males to determine whether male song correlated with male size. Morphological measurements of hind tibia length, pronotum area, and forewing length were made using dial calipers to assess variability in size. Wet weight was also measured upon capture, using 0.01g precision portable digital balance. Due to no significant effect of year on the acoustic and morphological parameters measured, data from 2007 and 2009 were combined for analysis.

Statistical analysis

Statistical tests were performed using JMP statistical analysis software (SAS Institute 2009). Whenever possible, a Tukey-Kramer HSD (Honestly Significant Difference) test was used to determine significantly different means in various comparisons. When the normality assumption could not be satisfied, the non-parametric Van der Waerden test (VDW) was used. To determine any relationship between morphological features of a male and its acoustic parameters, and when multiple data points were collected from a single male, linear and nonlinear mixed effect models were performed with individual males as the random variable. In the event of multiple comparisons, a Bonferroni correction was performed.

Results

Call characteristics

From sound spectrogram analysis, the structure of the male *S. pistillata* advertisement call was found to be a noisy song of varying amplitude (Figure 1.1). The initial syllables are quiet, 30-40 dB SPL, but increase in amplitude within each phrase and over the length of the bout. The frequency spectra of male *Scudderia pistillata* bouts are broad band, with most power between 6 kHz and 12 kHz. Analysis of 45 phrases from nine individuals' power spectrum shows a peak in energy at 8147 Hz (±8066.4 Hz) with a bandwidth (-3dB SPL) of 7765 Hz (±781.0 Hz) to 8731 Hz

(±890.8 Hz) (Figure 1.1, Lower panel).

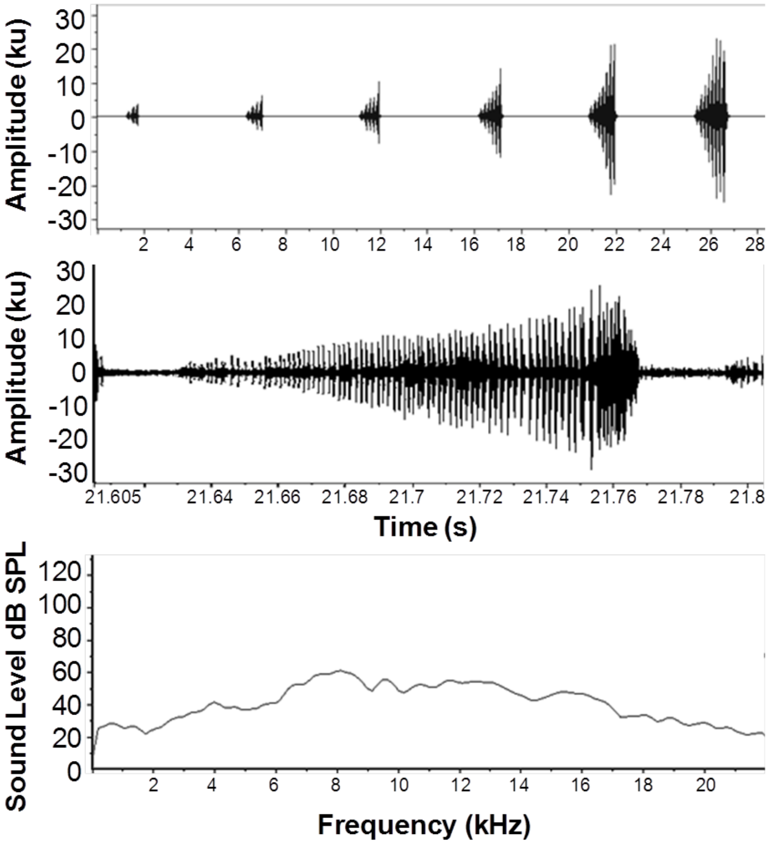


Figure 1.1. Exemplar bout of male *Scudderia pistillata* advertisement calling song. **TOP**: A bout consisting of six phrases, each incrementing the number of syllables by one is displayed. **MIDDLE**: A single syllable. **BOTTOM**: Sound spectrum from a single syllable (Hann Window; Window Length: 256 samples, 80.1% overlap).

To characterize the structure of the calling song, we examined all bouts for consistency in acoustic parameters. In this study, phrases varied from one syllable to ten syllables. A bout can vary from two phrases up to 14 phrases. Analysis of 515 bouts showed a significantly higher mean number of syllables for each successive phrase in a bout up to the seventh phrase (Mixed Effects Linear Regression: *Syllable number* = 1.66 + 1.17*phrase number; P* < 0.0001; RMSE=0.952) (Figure 1.2). After the seventh phrase, the mean number of syllables of each following phrase was not significantly different. However, these longer bouts are rare in the population (only 16% all bouts recorded have >7 phrases), with extremely long bouts (13-15 phrases) recorded from only two individuals.

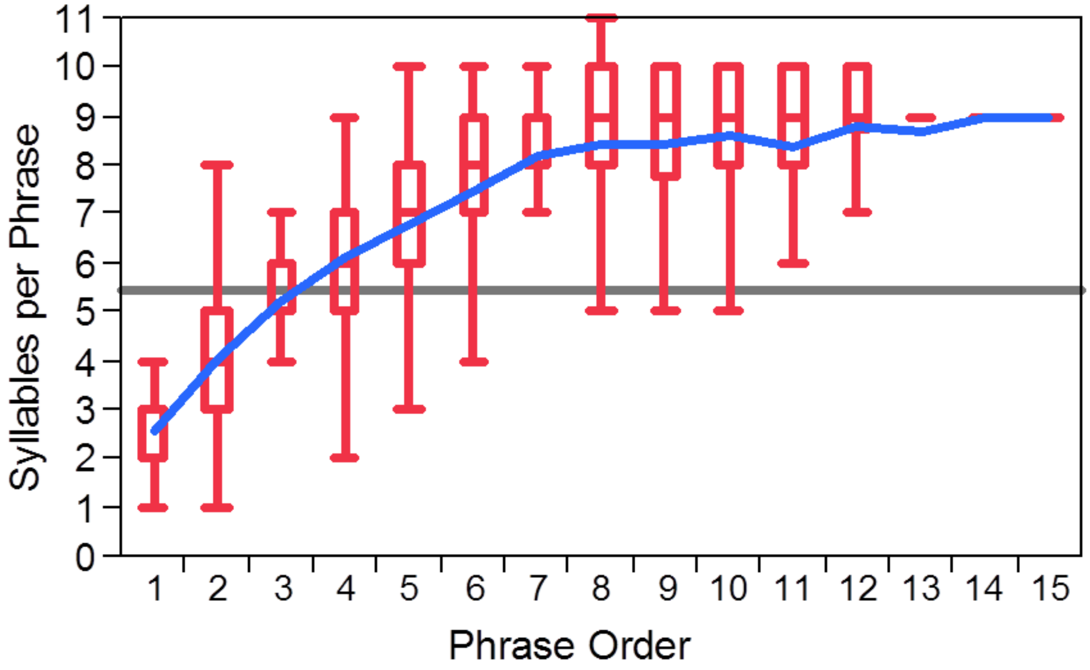


Figure 1.2. Quartile box plots depicting the median syllable number for each phrase in male *Scudderia pistillata* advertisement calling bouts. Grey line is the overall mean, blue line connects mean syllables per phrase. Whiskers indicate quartile range.

To determine the regularity of incremental counting, conditional probabilities of the number of syllables preceding each phrase were calculated (Figure 1.3). Almost half (46.8%) of all phrases show N+1 syllables per phrase in the subsequent phrase. Males count sequentially, that is they generally added one, or two (20% of all phrases), syllables to each subsequent phrase in a bout. N-1 syllables in subsequent phrases rarely occurred (7.3% of all phrases). This finding is confirmed with the slope of the mixed effects linear regression of 1.17, indicating an increase of roughly one syllable per phrase produced. As demonstrated in the phrase analysis of Figure 1.2, males eventually reach a plateau in the number of syllables per phrase, but they continue calling. This plateau accounts for 21.8% of all phrases analyzed, showing N syllables in the subsequent phrase. Figure 1.3 illustrates that males who produce eight, nine or ten syllable phrases show a higher occurrence of repeating the syllable number or, in the case of 10 syllable phrases, decrementing one syllable. Instead of males adding additional syllables to a ten syllable phrase, they repeat either nine or ten syllable phrases.

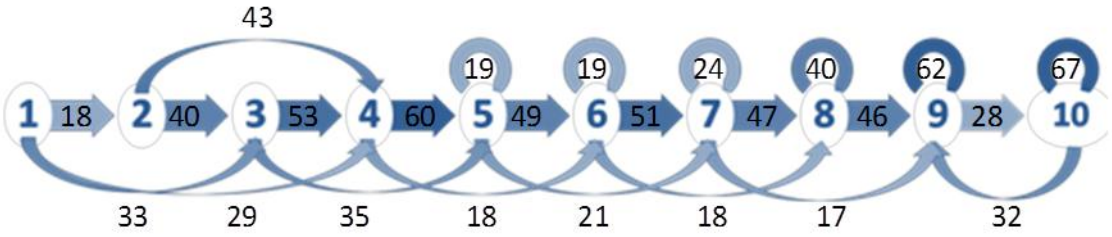


Figure 1.3. Conditional probabilities of incremental counting in male *Scudderia pistillata* calling bouts. Numbers in blue represent the number of syllables per phrase. Arrows indicate the probability that the subsequent phrase contained the syllable number at the arrow head. Intensity of arrows indicates strength of probability. Transitional probabilities less than 15% are omitted for clarity.

Inter-male variation in call parameters

To understand the variation between males, Van der Waerden tests were performed on various acoustic parameters. There were significant differences among males in: total syllables per bout (μ = 18.95 ± 12.06; F = 14.500; df = 24,205; P = 0.0003; coefficient of variation (CV) = 0.636), maximum syllables per phrase (μ = 6.33 ± 1.93; F = 2.941; df = 24,144; P = 0.0006; CV = 0.306), and the number of phrases per bout (μ = 4.27 ± 2.26; F = 8.541; df = 24,204; P = 0.0003; CV = 0.529).

Across all males, a correlation existed between how many syllables are produced and how many phrases are produced in a bout ($max\ syllables\ per\ bout = 2.31 + 0.94 phrases\ per\ bout - 0.09 phrases\ per\ bout^2$; $P < 0.0001$; RMSE = 1.137). However, when correlated within individual males, fewer than half (9 of the 26 males) exhibit even weak associations ($P < 0.05$), thus, the overall correlation is due to the influence of those 9 males. Some males with relatively few phrases per bout were still consistently able to produce phrases with a maximum of seven or eight syllables (Figure 1.4).

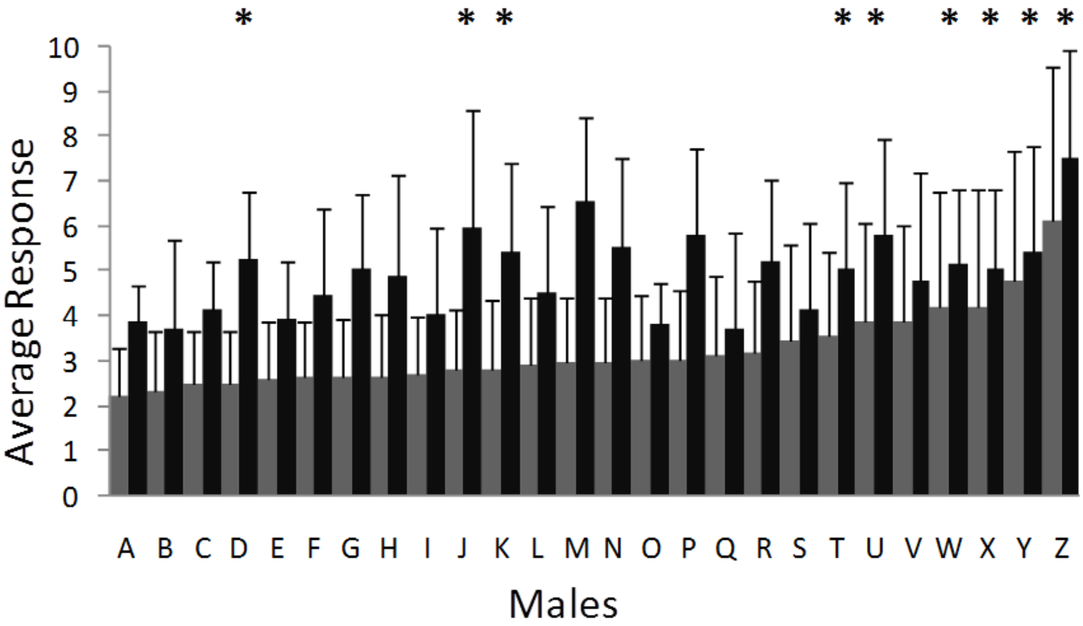


Figure 1.4 . Individual male variability in the average maximum number of syllables per phrase (dark grey) and the average number of phrases per bout (light grey) with standard deviations. The data from individual males have been ordered from smallest to largest average number of phrases per bout (light grey) to show the variability in how well these two variables correlate. Stars indicate those males (only 9 of 26) for which these variables were significantly correlated (P value < 0.05) within an individual male.

Relationship between acoustic and morphological parameters

Pairwise correlations among physical parameters showed all variables, except tibia length, are positively correlated with wet weight (Bonferroni correction for multiple comparisons, N = 15, Table 1.1). Weight and tibia length are therefore used in this analysis as independent predictors of size. Male body condition was estimated in subsequent analysis with acoustic parameters using the residuals from a regression of weight on pronotal area (e.g. Wagner and Hoback 1999 as an index).

Table 1.1. Morphological measurements and their correlations with male wet weight.

Variable	Equation	*P-value*	R^2	RMSE[a]
Right Hind Tibia Length	*0.728 + 0.448x*[b]	*0.3909*	0.167	0.052
Right Forewing Length	*1.023 + 0.569x*	*0.0213*	0.439	0.034
Pronotal Area	*0.020 + 0.036x*	*0.0042*	0.558	0.002

[a]RMSE = root mean square error, [b]x = male wet weight

No acoustic parameter of the male call correlated with any morphological measures that scaled directly with body size. However, residual weight predicted both the total syllables per bout (Mixed Effects Linear Regression: *Syllables per bout* = 25.12 + 142.29*residual weight*; *P* = 0.0264; RMSE = 12.1273) and the number of phrases per bout (*Number phrases* = 5.03 + 20.94*residual weight*; *P* = 0.0402; RMSE = 2.0041) (Figure 1.5).

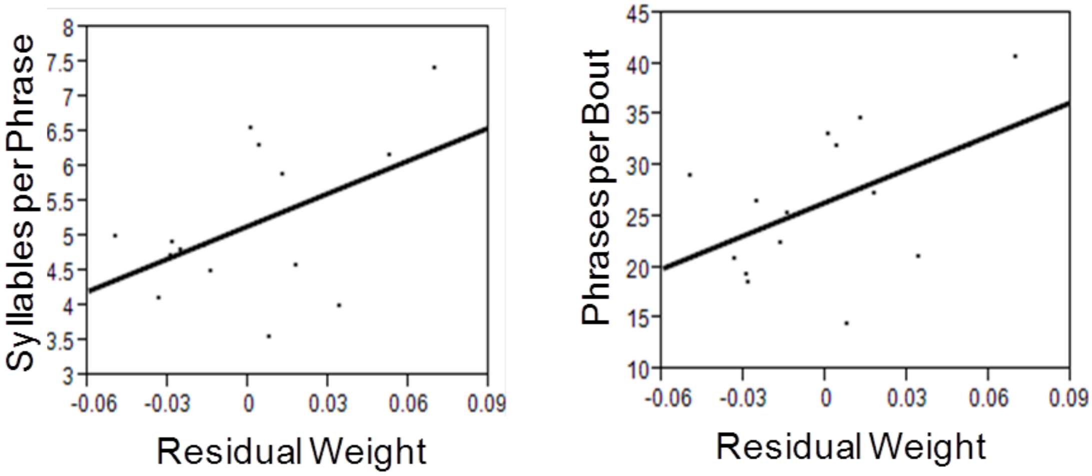


Figure 1.5. Relationship between the number of syllables produced per phrase as well as the number of phrases produced per bout with an index of male condition.

Discussion

This study is the first to document an unusual feature in the calling song of male *Scudderia pistillata,* in which males repeatedly produce a series of phrases comprised of increasing numbers of syllables. The males in the present study exhibited congruence in adding one or occasionally two syllables to each subsequent phrase in the first seven phrases in a bout. Within this species-specific signal, however, individual males show variation in a number of acoustic parameters. There also appears to be no constraint on the number of syllables and the number of phrases a male produces. Males with relatively few phrases in a bout can still produce a large number of syllables per phrase.

Also, we found that an aspect of the male song relates to a feature of male size unrelated to the direct production of the call. Heavier males produce songs with more phrases and more syllables in later phrases. Therefore, songs potentially contain information about male condition that the female could use to assess multiple calling males. Males that put more energy into their bouts could indicate a benefit to the female in terms of energy invested. As well, increased calling time associated with producing more phrases could be advantageous to a male in that there is a greater chance of the female hearing the call. Though males were variable in a number of acoustic and physical variables, it was unknown whether the variability among males can be assessed by females and is the topic of Chapter 2.

Variation in the male call

Singing in katydids is a highly energetic behavior (Stevens & Josephson 1977; Suarez 2000). Though the energetic cost of calling in *S. pistillata* is unknown, males of *Requena verticalis*, an intermittent caller like *S. pistillata*, exhibit a similar high rate of oxygen consumed per wing stroke as most trilling species (Bailey et al. 1993). Several studies have shown that females respond more strongly to the more energetically expensive parameters of a male's call: more syllables, longer periods of calling, and faster syllable rates (Bailey et al. 1990; Galliart & Shaw 1996; Gray 1997; Tauber et al. 2001; Orci 2007). A number of studies have also found that females prefer larger males (Gwynne 1982; Galliart & Shaw 1992; Gray 1997) and those heavier males called more continuously and did not lose as much weight while calling as did smaller males (Galliart & Shaw 1994). Because the size and quality of the spermatophore is contingent on the size and condition of the male producing it (Heller et al. 1998; Jia et al. 2000; Vahed 2007; Lehmann & Lehmann 2009), energetic information in the song is of potential value to listening females.

Though little energy may need to be invested to add single syllables to phrases, much energy would be invested to produce bouts of more phrases. In the present study, we demonstrated a significant correlation of the more energetically expensive parameters of syllables per bout and phrases per bout with an index of the male's condition, which in this study is approximated by weight once the effect of body size has been removed. Total energy invested does not appear to be related to a male's overall size, but to its current condition as indexed by residual weight. Total syllables per bout and phrases per bout had larger coefficients of variation among males and were significantly correlated with the male's condition. Therefore, females should prefer calls with higher values in these parameters. Analysis of female responses to experimentally manipulated songs is the focus of Chapter 2.

Why is the call complex?

For katydids, complex calling songs are typically defined as having multiple syllable types. Producing more than one syllable implies a more complex neural network as well as changing muscular movement to produce the various signals (Dobler et al. 1994; Walker & Dew 1972). Alexander (1960) also listed an irreversible pattern as another elaboration, i.e., syllables produced in a phrase which vary in length and/or intensity to produce a signal unlike an acoustic palindrome. This type of complexity is seen in *S. pistillata*'s calling song.

There are a number of convergent behavioral ecological traits that tend to be associated with complex calls. For instance, katydids exhibiting stronger territoriality tend to have more complex calls, with new syllable types added to mediate the interaction between rival males (Heller 1990; Korsunovskaya 2009). How chorusing males react to an acoustically calling neighbor male would indicate the potential competitive behavior between the individuals. Several studies have shown that males alter their temporal pattern in the presence of other males (Schatral et al. 1984; Dadour 1989; Morris and Mason 1995). Territoriality is not likely a driving force for complex calls in chorusing *S. pistillata* males due to their lack of site fidelity and homogeneous field conditions (Chapter 4). The effects of hearing a calling bout of a rival male on another male's song is the topic of Chapter 3.

Species in which both the males and the females produce sound also tend to produce more elaborate calls (Bailey 2006). The most complex katydid calls recorded are produced by males in the subfamily Phaneropterinae, a group also known for their duetting behavior (Walker & Dew 1972; Bailey & Hammond 2003). Non-duetting males must sing throughout the night in order for females to perform a silent phonotaxis. This requires large amounts of energy that if not used for singing could go towards spermatophore production. Duetting males, on the other hand, know when a female is present by her acoustic response and can therefore conserve energy when no female is present by not singing (Robinson 1990). By calling less, males can also decrease their risk of predation (Heller 1992).

Males potentially have selective pressure placed on them by females to show their quality in terms of energetic expenditure, yet many duetting katydids exhibit intermittent calling patterns limiting their calling rate. Sexual selection could act in favor of call complexity instead of calling rate (Bailey 2006), which could account for the unique counting call seen in *S. pistillata.* Information on energy investment is then conveyed through higher numbers of syllables produced over longer periods of time, yet still allowing for silences between phrases, during which the female responds.

Male *S. pistillata* that cannot invest much energy into long calls can still produce a large number of syllables per phrase as seen in Figure 1.4. In crickets, calling rate was effected by current body condition, whereas call duration and syllable number remained unchanged (Wagner & Hoback 1999; Scheuber et al. 2003a.). In this study it appears that regardless of condition, males can produce a large number of syllables per phrase, an acoustic parameter that other studies have found to be independent of current condition and more reliant on long term condition (Olvido & Mousseau 1995; Scheuber et al. 2003b.). Therefore, syllable number in *S. pistillata* could be an indicator of other effects not related to current condition, such as nymphal condition. They could also be dependent on other factors not accounted for in this study, such as age, or other genetic factors.

As mentioned previously, a potential elaboration of calling song in addition to multiple syllable types is the production of an irreversible pattern (Alexander 1960). In *S. pistillata*, the call is amplitude modulated, with syllables considerably more quiet at the start of the phrase and increasing in amplitude. Amplitude modulation could be the result of a mechanistic warming up of muscles responsible for the closing of the wing. Muscle activation for such an energetic behavior would require some warm-up before the muscles' potential is achieved (Heller 1986). A potential adaptive function of amplitude modulation could be to reduce eavesdropping from unintended receivers, including predators and rival conspecific males. By not broadcasting its call as loud initially, the quieter phrases would be perceived by the nearest female. From personal observations of *S. pistillata*, when a female is detected, the male quickly works to localize her call and moves in her direction. He also alters the call rate and amplitude, a behavior also witnessed by Spooner (1968b). Less movement would be required to reach a nearby female, and as a result, allows for fewer opportunities for any satellite males to intercept their duet or lessens the chance of being heard by a predator. With each added syllable, the male incrementally increases the active range of his signal, with louder syllables being perceived by more distant females.

Conclusions

In this study, we present the first analysis of a katydid exhibiting an elaboration of calling behavior in which syllables are regularly added to subsequent phrases. Some male pseudophylline katydids alter the number of pulses produced (Hebard 1941), but not in a specific sequence and in not as long of a series as produced by *S. pistillata*. Though the mechanism behind this counting behavior remains unknown, explorations into its adaptive function are currently underway. Analysis of female response to bouts with a series of incrementally increasing phrases should assist in our understanding of why this complex counting behavior has evolved.

CHAPTER 2

FEMALE ACOUSTIC REPLY TO VARIATION IN THE MALE CALL

Abstract

Female phaneropterinae katydids (Orthoptera: Tettigoniidae) commonly reply to conspecific male attraction calls with a brief, broadband acoustic tick. In this paper we describe the complex interaction of the katydid *Scudderia pistillata* female tick response with the variation in male call, which consists of a series of increasing length phrases. The female responds to each phrase in the bout with a variable number of ticks. She adjusts her tick response, ranging from 1 to 8, with the number of syllables the male presents, responding maximally to 7-9 syllables per phrase. Females also adjust their tick response in reply to various manipulations of a male bout when presented in playback. Because males are variable in the number of syllables per phrase and phrases per bout they produce, and both these attributes are associated with an index of condition, the female potentially demonstrates her preference for certain male bouts through her varied tick response. The latency to female reply is highly correlated with both the number of syllables she hears, as well as the number of ticks she produces. Therefore, there is no constant reply latency except with phrase lengths beyond what the males are known to produce.

Introduction

Katydids (Orthoptera: Tettigoniidae) in the subfamily Phaneropterinae and the group Ephippigerini (-inae) perform acoustic duets, in which both the male and the female interact sonically for the purpose of mate attraction and assessment (Bailey 2003). The male advertisement call always precedes the female acoustic response, followed by either one or both katydids approaching the other (Spooner 1968a, 1995). Acoustic interaction from both sexes is necessary for pair formation, especially for species at low density where the chance of randomly finding a mate is low (Heller & v. Helversen 1993). Pair-forming behavior in all non-duetting katydids typically relies on the silent assessment of male advertisement calls followed by female phonotaxis. In a duetting system, the male calls intermittently and then waits for a female reply, meaning less energy is needed for mate attraction and can therefore be used for other purposes, such as nuptial gift investment (Robinson 1990). Moreover, an acoustically responding female could indicate her level of interest with a quantifiable reply to an individual male's call, rather than simply signaling her presence and location (Bailey 2003).

The duet of the katydid *Scudderia pistillata* (Tettigoniidae: Phaneropterinae) provides an opportunity to understand how female response is affected by male advertisement call variability. *Scudderia pistillata*, the Broad-Winged Bush Katydid, forms acoustic duets with males exhibiting a complex call type. Male *S. pistillata* add more wing closures (syllables) to each subsequent burst of sound (phrase) over the length of their call (bout) thereby providing the female with a quantifiable male call (Villarreal & Gilbert 2011). Males of this species not only produce a series of phrases with increasing numbers of syllables in a stereotypic manner, individuals are also variable in the number of phrases produced per bout and the number of syllables produced per phrase, and that variability was associated with an index of condition (Villarreal & Gilbert 2011). Because males exhibit variability in advertisement call with discrete changes in phrase length, it provides easily quantifiable differences between males that the females could assess.

The typical female acoustic call is a “tick” sound, produced from the right forewing flicking against the ventral side of the left forewing (Nickle & Carlysle 1975; Heller & v. Helversen 1986). Only a few species of duetting katydids exhibit variability in the number of ticks a female gives in response to the male (Tuckerman et al., 1993; Bailey & Hammond, 2004). Through varying her response, a female could be indicating her willingness to mate and providing an indication of her preference for one male signal over another. The male also could benefit from hearing her increased acoustic response by receiving more information on her location. Females could benefit from producing an increased response by better enticing higher quality males. Therefore, just as the male *S. pistillata* signal is quantifiable, variability in female acoustic response associated with that variability between males could be an indicator of her preference for calls of certain male katydids.

The timing of the female response in an acoustic duet is also important, as it can act as a pre-mating isolation mechanism (Heller & v. Helversen 1986). By having a specific time window for the female’s signal to arrive, the male is silent and primed to listen and localize a calling conspecific female, thus reducing time with which the male is evaluating incoming signals (v. Helversen et al. 2001). The female latency to respond varies with species, with some species female latencies as short as 28 msec (Zimmermann et al. 1989) and others as long as 2 sec (Spooner 1968b). The trigger in the male call that initiates the female species-specific reply latency varies by species and can occur at the beginning, end or following a specific sequence within the male call (Korsunovskaya 2008). Therefore knowing what aspect of the male call triggers a female’s response is important in understanding the time window in which the female response must arrive.

In this study, female *S. pistillata* were presented with digitally manipulated playbacks of the male advertisement call and her acoustic response was recorded. Because male syllables per phrase vary over the length of the bout, females were presented with multiple bouts of varying acoustic properties. These altered playbacks also allow us to explore which aspects of the male call the female attended to as well as if females would show an altered acoustic response, in terms of the number of ticks produced, to particular male call attributes.

Methods

Study animals

Female Broad-Winged Bush Katydids, *Scudderia pistillata* Brunner 1878 (Tettigoniidae: Phaneropterinae), were collected during the summers of 2009 and 2010 from old fields (composed mostly of goldenrod, *Solidago* spp.,) near Ithaca, NY on Bald Hill (42°21’11.28” N, 76°22’57.46” W) and Connecticut Hill (42°20’32.13” N, 76°39’42.50” W). Most individuals were field-collected as adults from July through August by sight at night. Some individuals were collected as nymphs via sweep netting during the day and earlier in the season. All individuals were brought back to the laboratory and were housed in communal cages (0.5m in diameter, 0.75m in height) prior to experimentation and for the remainder of their life. They were fed an *ad libitium* diet of leafy greens and apples, and kept at room temperature (24°-28°C) with natural photoperiods.

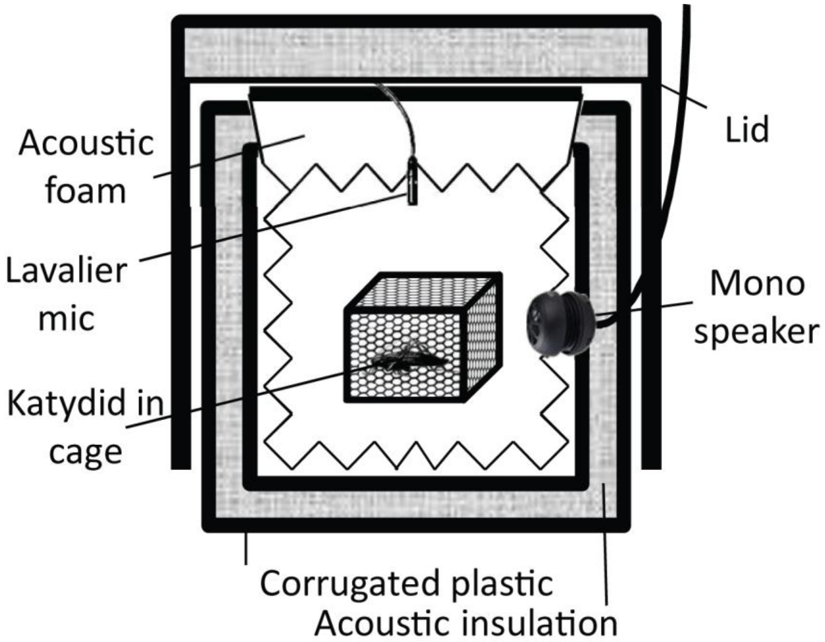


Figure 2.1. Diagram of the sound dampening box in which responses of female *S. pistillata* were recorded after stimulation with digitally manipulated male calls.

Playback experiments

Individual female katydids were acoustically isolated during playback trials via placement in an individual custom-built sound dampening box (30cm cubed) (Figure 2.1), which reduced noise coming from each box by approximately 40 dB SPL (sound pressure level). Playback files were presented to females through a speaker (75-85 dB SPL, 218Hz-20kHz, Omnitech portable mini speaker, Omnitech, Inc., Sioux Falls, SD) embedded in the boxes and her response to the playback was recorded using an Audio-Technica ATR-35S condenser microphone (Audio-Technica U.S., Inc., Stow, OH) also embedded in the box. In order to simultaneously record responses of four females to playback files, microphones from four separate sound isolation boxes were connected to a digital multi-track recorder (Fostex HD-P2, Foster Electric Co., Ltd., Tokyo, Japan). The captured sound files were transferred to a computer and female acoustic information was extracted using Audacity 1.3.5 Cross-Platform Sound Editor (http://audacity.sourceforge.net/). Sound imaging and frequency analysis (Figure 2.2) was performed using Raven Pro 1.3 (Bioacoustics Research Program, Cornell Laboratory of Ornithology, Ithaca, NY).

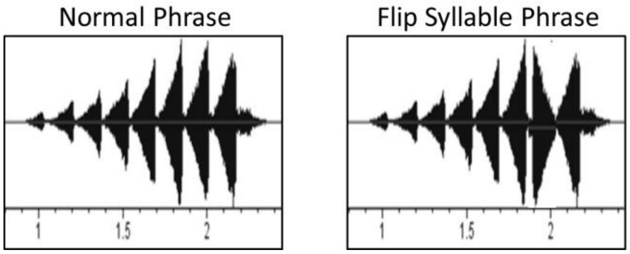
The male calling bouts in all playback files were digitally constructed from a single eight syllable phrase using Audacity software. The original male call was recorded using a Sennheiser ME66/K6 combo shotgun microphone (Sennheiser Electronic Corp., Old Lyme, CT), placed ~30cm from the individual's cage, connected to a Tascam HD-P2 digital audio recorder (44 kHz sampling frequency, TEAC Corp., Japan). Longer and shorter length phrases were artificially created by disassembling or repeating syllables of the eight syllable phrase. Male syllables naturally increase in amplitude over the course of the phrase (Villarreal and Gilbert 2011) therefore this amplitude modulation was maintained in the digitally constructed bout. Only one phrase from a single male was used to construct all playbacks in order to avoid confounding the female's response to each phrase with variation in male syllable parameters.

Eight digitally constructed bouts were used to assess a female's response to various male call types (Table 2.1). There were two goals to presenting females with differing bouts: first, to characterize the female *S. pistillata* tick response, both in the timing and the number of ticks she gives, and second, to assess female preference for different call structures. To characterize the female *S. pistillata* tick response, females were presented with an artificial bout composed of 20 phrases, each phrase with an increasing number of syllables from one to twenty. Phrases constructed with more than 11 syllables (supernumerary phrases) were presented to the female to determine how she would respond to calls that exceed those measured in natural populations. If a female shows preference for longer male phrases, then the number of female ticks produced will increase with the number of male syllables per phrase heard.

Table 2.1. Digitally manipulated male calling bouts presented to female *S. pistillata*. Below is an illustration of what a "flip syllable" phrase looks like, with the second to last syllable reversed.

Playback type	Sequence Syllables/Phrase	# Syllables Presented	# Bouts, with Response	N Females
20 Phrase	1,2,3,4,5,6,7,8,9,10,11,12 13,14,15,16,17,18,19,20	210	28	6
Typical Male Bout	3,4,5,6,7,8	33	87	14
8 Syllabled Phrases	8,8,8,8,8,8	48	63	12
Irregular Sequence	1,3,5,7,9,8	33	47	9
3 Min. IPI[a]	3,4,5,6,7,8	33	94	14
Plateau	3,4,5,6,7,8,9,9,9,9,9	78	39	8
Long Bout	3,4,5,6,7,8,9,10,11,12,13	88	61	14
Flip Syllable[b]	3,4,5,6,7,8	33	75	15

[a] *IPI = Inter-phrase interval;* [b] *Syllable manipulations were only performed on phrases with 6,7,8 syllables*



The remaining playback call types were used to characterize any shifts in female tick response associated with the differing call characteristics (Table 2.1). By altering the properties of the phrase itself, we can assess which attributes of the phrase a female attends to. First, a bout was created that represents a typical male advertisement call (Villarreal & Gilbert 2011) to compare against the responses to the remaining six playbacks. Each playback type addressed a different aspect of a male bout to which the female might attend and which could influence her response. Also, if females attend to more than just the current phrase she hears, then her response to each phrase in these manipulations should vary when previous syllables per phrase heard varies.

The "8 syllabled phrases" and "irregular sequence" playback types addressed changes in the sequence of phrase lengths. Males typically add n + 1 syllables to each subsequent phrase, but the importance of the regularity of this sequence is unknown. The "3 min. IPI" playback type addressed the timing of phrases in the bout. Male phrases in a bout are typically spaced 3-4 seconds apart. Increasing that interval to three minutes should simulate phrases heard in isolation. The "plateau" and "long bout" playback types addressed changes in bout length. Shorter bouts (≤7 phrases, 3-8 syllables per phrase) are most common in the population. A few males also produce longer phrases (8, 9, or 10 syllables), repeating the same number of syllables towards the end of their bout; i.e. the number of syllables per phrase reaches a plateau. The "flip syllable" playback type was constructed to assess if females noticed the within-phrase differences, versus attending to the total duration or energy of calling. For this playback the first three phrases were unaltered, whereas phrases with 6-8 syllables had the second to last syllable reversed (see Table 2.1 for an example of the manipulated 8 syllable phrase). On a given night, the female was presented with three playback types, each repeated three times, with 3 min. between each iteration and 10 min. between each playback type. The presentation order of the playbacks was randomized to account for any order effects.

Different parameters of the female *S. pistillata's* response were measured. To characterize her response, the following variables were analyzed: number of acoustic ticks produced by the female after each male phrase, latency to the first tick, latency to the last tick, and the time from the beginning of his call to the beginning and end of her response. If her tick response fell within his call, an average latency was recorded from the start of the call. The latency to the end of her acoustic response was recorded to determine the envelope of time the male might attend to a female signal. For preference tests, only the total number of response ticks given by the female was compared.

Statistical analysis

To characterize the female acoustic response, a mixed effect polynomial/linear regression (MEP/LR) was run on the female's tick response and her latency to reply respectively for the 20 phrase playback file. Females were presented with multiple male calling bouts and therefore bout was set as a fixed factor in this analysis, and was nested within female to account for any variance in tick response to later bout sequences. Females were also randomized for this analysis because they are field caught and therefore represent a random assortment of females that a male might encounter.

Two separate tests were performed to determine if tick response could be predicted by the remaining seven playback types presented. The first analysis was a mixed effect logistic regression by year in order to determine if the occurrence of a female response (present/absent) varied by playback type. Second, a mixed effect linear regression was performed on tick response, again with females randomized. There was no significant difference in response between 2009 and 2010, therefore these data were combined and analyzed together. Statistical tests were performed using JMP statistical analysis software, version 8 (SAS Institute Inc., Cary, NC), with the exception of the logistic regression, and the analysis comparing individual playbacks versus the typical male bout which were performed using SAS® 9.3 (SAS Institute Inc., Cary, NC).

Results

Female *Scudderia pistillata* produced 1-8 ticks in response to male phrases of 1-11 syllables in length. Each tick consisted of 1-5 subunits (defined as a clustered set of peaks less than 80 msec apart) 0.015s ± 0.009s in duration and each tick was spaced on average 0.1s apart (Figure 2.2). The frequency spectrum of female tick response is broad band, with most power between 4000 Hz and 16000 Hz.

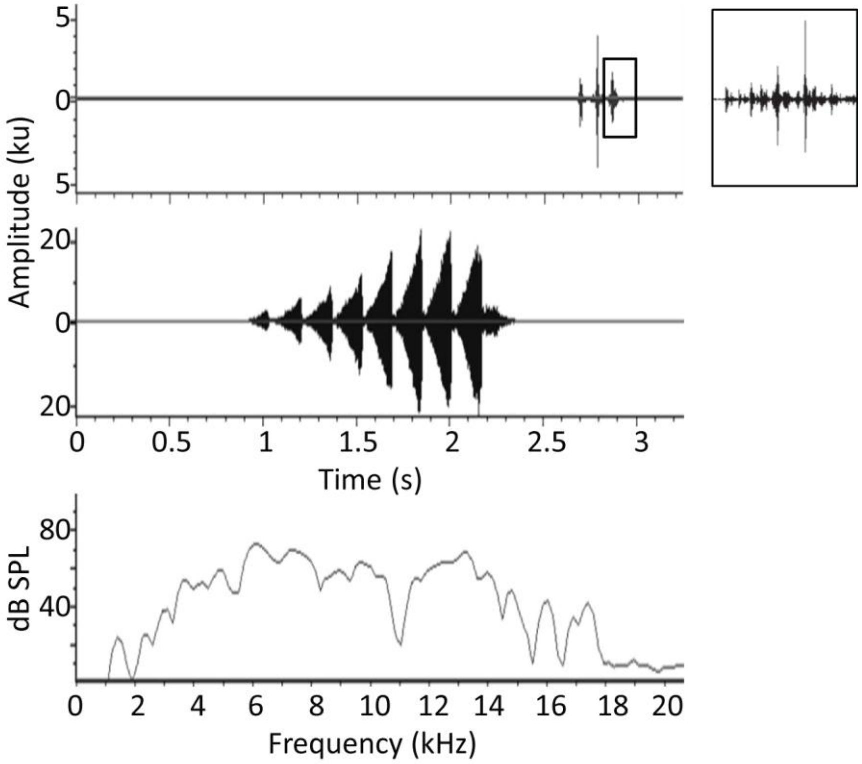


Figure 2.2. Waveform and spectrogram of *S. pistillata* duet. A female response of three ticks (top) to a male eight syllable phrase (middle). Female tick subunits are seen as the multiple peaks within a single tick, which can be seen in the detailed view of a single tick in the box on the right. Bottom panel is a spectrogram view of a single female 3 tick response (Hann Window, 256 samples, 80% overlap).

Female tick production

When presented with 20 phrases of increasing syllable number, females produced a variable number of ticks (MEPR: $F_{1,345} = 105.25$, $P < 0.0001$). For 1-7 syllabled phrases, females increased the number of ticks they produced as the number of syllables per phrase of the male call increased (Figure 2.3). Her tick response function reached a broad peak in response to male calls with 7-9 syllables per phrase and then decreased as additional syllables were added to each phrase. Her response to these supernumerary phrases was comparable to her response to phrases with few syllables, as there is no difference in her mean response per phrase between the first four (phrases 1-4, X ± SD = 2.047 ± 0.983) and last four phrases (phrases 17-20, X ± SD = 2.244 ± 1.003) presented in the 20 phrase bout (MELR: $F_{1,\,101} = 0.396$, $P = 0.531$). The female tick response also habituated. It was stronger for initial bouts and lower with subsequent bouts ($F_{6,347} = 13.25$; $P < 0.0001$), but there was no interaction with syllable number ($F_{6,341} = 1.927$; p = 0.0757).

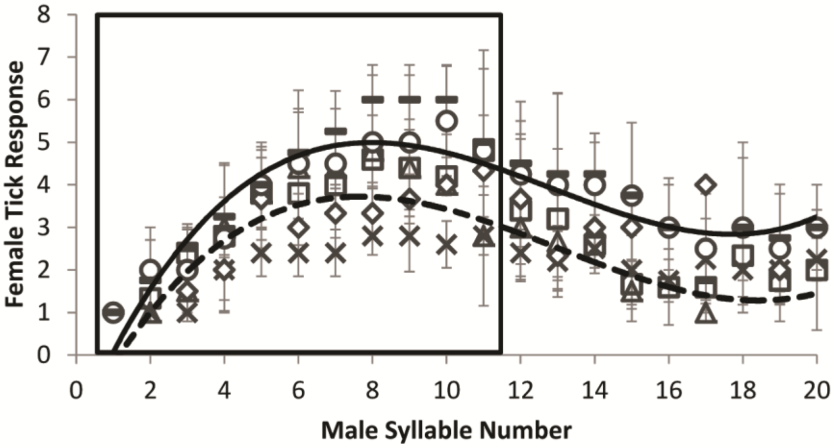


Figure 2.3. Female *S. pistillata* tick response to playback of a digitally manipulated male bout of 1-20 syllables per phrase. Each symbol indicates the mean (+/- 1 SD) response for each separate female (N=6) to phrases of 1 to 20 syllables. The lines through the data represent the best fit line based on the MEPR model for each bout presented. The lines are presented in grayscale, with her response to the first bout being lightest and last bout being darkest. The box around the data points indicates biologically relevant tick response to phrase lengths seen in nature.

The increased female tick response to higher numbers of syllables in subsequent phrases in the male bout could be due to a simple order effect within the bout rather than females responding to the number of syllables in the male phrase, per se. For instance, females could start out with a few ticks and increase their response as they warm up. Two lines of evidence argue against this interpretation. First, though females continue to respond over the length of long bouts (20 phrases) the number of ticks decreased as the number of syllables per phrase increased beyond 8-9 (Figure

2.3). Second, the eight syllable first phrase of the “8 syllabled phrases” playback (Table 2.1) elicited a larger female response than the three syllable first phrase in the “typical male bout” (MELR: $F_{1,602} = 5.000$, $P = 0.020$). Thus, the increased tick response to increased numbers of syllables per phrase up to 8-9 indicates that the female likely attends to the number of syllables a male produces on a phrase by phrase basis.

Female Latency

Almost all (90% CI) female ticks fall between 0.185 and 0.805 sec after the male call regardless of phrase length (Figure 2.4, A). Females respond after each phrase in a male call, but the latency from the end of his phrase to the beginning of the female response was not constant throughout the length of the bout (Figure 2.4, B). There is also no indication that the female adjusts the end of her tick response to correspond with some maximum value of the male time window (Figure 2.4, C). Female response latency was affected not just by number of syllables the female heard but also the number of ticks in her response (Figure 2.5). The shortest latency was 0.013s to the start of a three tick response to a 13 syllable phrase and the longest was 0.920s to a two tick response to a 2 syllable phrase. Female latency was negatively correlated with tick response (MELR: F 1,278 = 6.002, $P = 0.015$) and syllable number (F 1,258 = 480.081, $P < 0.0001$), as well as the interaction between the two (F 1,258 = 14.130, $P = 0.0002$). A female’s active calling time increased with increased number of syllables per phrase (F 1, 231 = 9.986, $P = 0.002$) likely because she responded with more ticks.

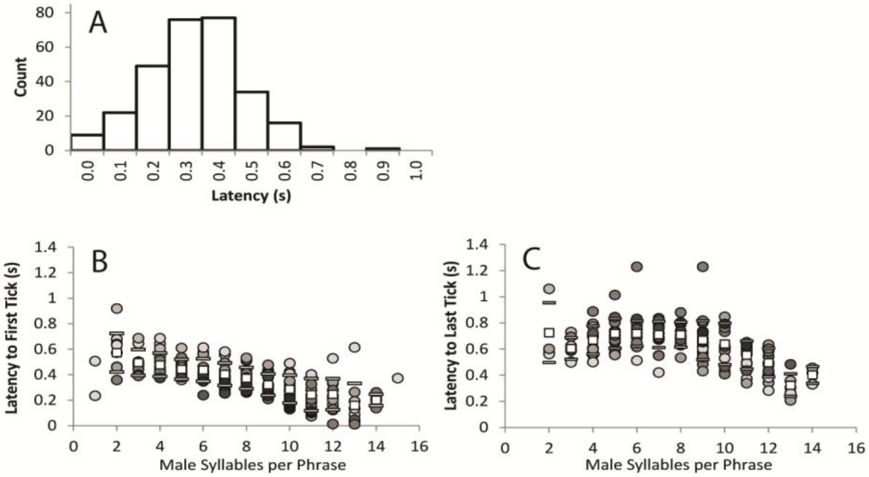


Figure 2.4. Latency of female response to the 20 phrase male bout. (A) All female latencies from the end of a male phrase to the onset of her tick response. Note histogram excludes all female responses to supernumerary phrases. The female latency to reply from the offset of the male phrase to the first female tick (B) or last tick (C) produced by the female. Mean latencies to each syllable number are indicated by the white open squares, with dashes indicting +/- 1 SD Grey scale circles represent the strength of the female response for each syllable number. Darker indicates more ticks. Notice that in panel B the darker dots (more ticks) are mostly the lowest points, indicating reduced onset latency when the female replies with an increased number of ticks.

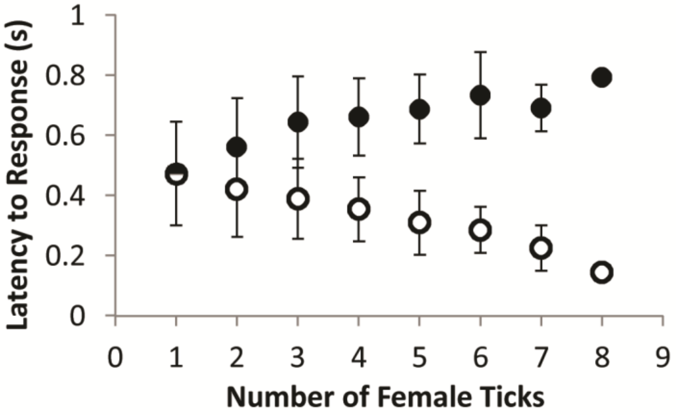


Figure 2.5. Mean latency (+/- 1 SD) from the end of a male phrase to the start (open circles) and to the end (closed circles) of the female response as it varies with the number of ticks a female produces. Lines indicate linear fit based on the MELR model for the start of her response (R^2 = 0.778, *P* = 0.0149) and the end of her response (R^2 = 0.753, *P* = 0.0001).

The offset of the male's call does not trigger the female's response with a constant latency as found in many other duetting systems. Therefore we investigated other triggers for her response, for instance the latency from the onset of the male's call rather than the offset, but found no consistent window (Figure 2.6). For most of the male phrases with supernumerary syllables (14 – 20) the female began her tick response while the digitally manipulated call was still playing and exhibit a constant latency. However, males have not been recorded producing these long phrases in nature.

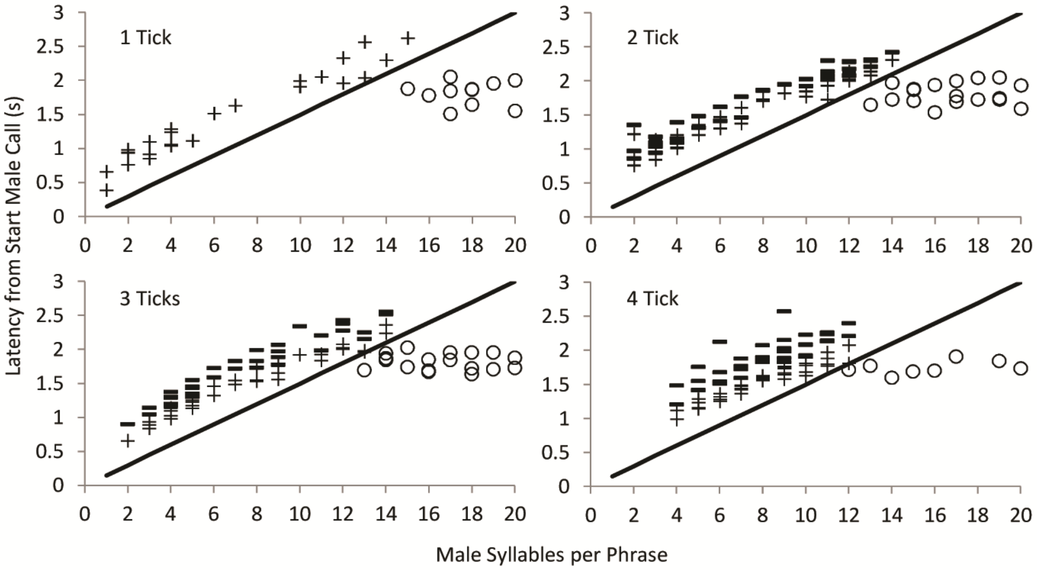


Figure 2.6. The timing of the female's response to the 1-20 syllables per phrase male bout, from the beginning of the male's call to the first (+) and to the last (-) female tick. Circles indicate average latency when female call fell within the male's phrase. The four panels illustrate how her latency changes with the number of ticks she gives. The solid lines indicate the mean offset of the male phrase of given syllable numbers. The four panels show the overall trend of no constant timing from the onset or offset of the male call regardless of the number of ticks produced when her call fell outside his phrase.

The effect of male call structure

To test several hypotheses about a female's perception of the structure of the male call, female tick responses were recorded to a variety of digitally manipulated playback calls (N = 27 females, responding to 466 total bouts). None of the seven playback call types was so manipulated that it was unrecognizable to the females. They gave at least one tick in response to each call type. There was no significant difference in the simple presence vs. absence of an acoustic response to any of the playback types for either 2009 (Mixed effect logistic regression: $X = 8.00$, $P = 0.433$) or 2010 ($X = 5.71$, $P = 0.680$). Similarly, there was no difference in the presence or absence of an acoustic response to bouts presented earlier or later during a trial for 2009 ($X = 0.61$, $p = 0.436$) or 2010 ($X = 1.34$, $P = 0.247$).

To determine whether the temporal structure of the male's call influenced the female's tick response, we compared the number of ticks a female gave in response to each phrase per bout for each playback type. Female tick responses varied with the number of syllables per phrase in the modified playbacks (MELR, $F_{1, 2226} = 26.476$, $P < 0.0001$), as would be expected from their responses to the playback of the 1-20 syllable bout previously described (Figure 2.3). The structure of the modified playback type also had a significant effect on female tick response ($F_{6, 2250} = 20.203$, $P < 0.0001$). There was also a significant interaction between playback type and syllables per phrase ($F_{6, 2232} = 24.877$, $P < 0.0001$). This is due to females having a more varied response to higher syllable numbers in the different playbacks.

Tick response comparisons were made to assess how the various manipulations differed from the typical male bout (syllables per phrase increasing from 3-8). Table 2.2 depicts the interaction of playback type with syllable number for all playbacks presented and their significance when compared to the typical male bout. Figure 2.7 depicts the average tick response for each phrase in a bout for each playback type versus the typical male bout. Note that the x axis displays position in bout and not syllable number. A female's tick response was not significantly affected by either an "8 syllabled phrases" (Figure 2.7a) or an "irregular sequence" (Figure 2.7b) when compared to the typical male bout. For the remaining playbacks, there was an interaction between playback and syllable number, as the effect of the playback type on female tick response was greatest for phrases with more syllables. At higher syllable numbers, "3 min IPI," "plateau," "long bout," and "flip syllable" playbacks (Figure 2.7c-f) all had a significantly decreased tick response (see also Table 2.2). The "long bout" playback (Figure 2.7e) contained an extended bout of up to thirteen syllables and showed the strongest effect on female tick response.

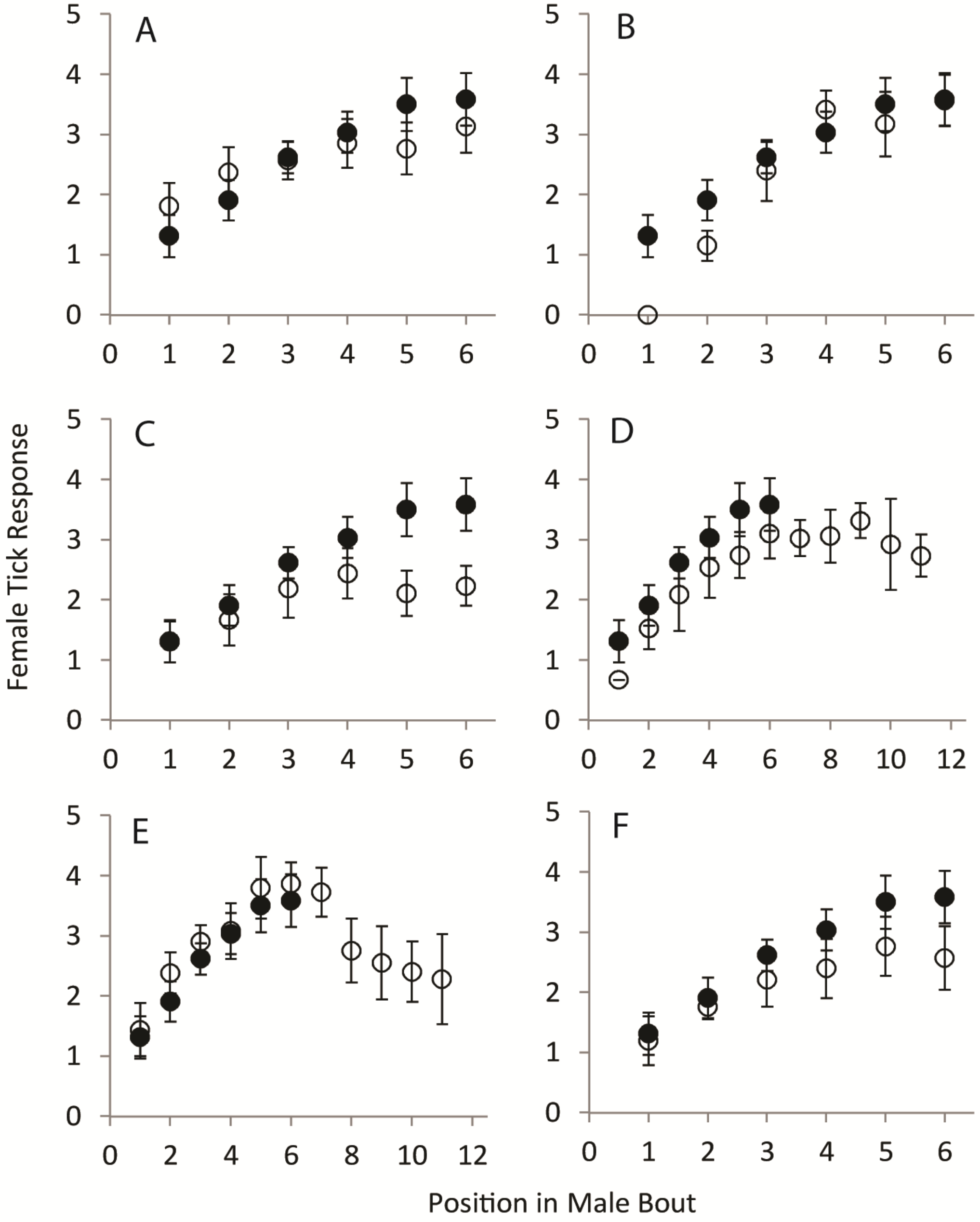


Figure 2.7. Average female tick response to various playbacks. Black circles indicate average female response to typical male bout playback, open circles indicates average female response towards playback: a. 8 Syllabled Phrases; b. Irregular Sequence; c. 3 min IPI; d. Plateau; e. Long Bout; f. Flip Syllable. Data are mean +/- 1 SD

Table 2.2. Parameter estimates for different playback types based on the MELR model. The response to each playback type was compared against the response to playbacks of the typical male bout (3-8 syllables per phrase). P-values indicate the probability that the female response varied from the typical male bout.

Parameter	Estimate	Std Error	DF	*t* Value	*P* Value
Intercept	0.117	0.403	130	0.40	0.689
Syllable Number	0.272	0.053	2191	11.55	**<0.0001**
8 Syllabled Phrases	-0.489	0.444	2205	-0.16	0.875
Irregular Sequence	-0.426	0.110	2202	-1.14	0.253
3 Min. IPI	0.389	0.121	2203	1.06	0.290
Plateau	0.665	0.100	2197	1.87	0.062
Long Bout	2.175	0.090	2007	7.88	<0.0001
Flip Syllable	0.550	0.112	2214	1.55	0.121
Interaction Terms:					
Syllable Number*8 Syllabled Phrases	-0.072	0.390	2204	-0.16	0.871
Syllable Number*Irregular Sequence	-0.036	0.053	2192	-0.47	0.637
Syllable Number*3 Min. IPI	-0.206	0.061	2195	-3.25	**0.001**
Syllable Number*Plateau	-0.206	0.050	2192	-4.57	**<0.0001**
Syllable Number*Long Bout	-0.396	0.042	2194	-9.18	**<0.0001**
Syllable Number*Flip Syllable	-0.164	0.057	2204	-2.88	**0.004**

Discussion

Variable female response to male advertisement call

Our results demonstrate that female variability in call duration (number of ticks) is associated with the male call parameter of syllables per phrase. With more syllables per male phrase, females increased their acoustic responses, giving as many as 8 ticks in response to 7-9 syllables. However, the response increase is not monotonic. With longer phrase lengths (>10 syllables) females decreased their tick response, though still continuing to respond to a 20 syllable phrase even though such supernumerary phrases do not occur in natural male populations (Villarreal & Gilbert 2011). Only a few species of duetting females exhibit variability in the number of ticks she gives in response to the male (Tuckerman et al. 1993; Bailey & Hammond 2004). Female variability in the duration of their reply signal theoretically allows for testing of sexual selection pressures on the structure of the male call (Bailey 2003). This variability in female response could therefore be an indication that females are assessing male syllable number. Individual male *S. pistillata* exhibit inter-male variability in the number of syllables they produce in a bout and that variability is correlated with an attribute of male condition (as indexed by residual weight) (Villarreal & Gilbert 2011). Therefore females could be assessing male advertisement call in terms of syllables per phrase as an indicator of condition which could be driving males to produce longer phrases in this species. However, female choice of males based on this syllable variability has yet to be examined.

From the perspective of the male, a variable female response benefits the listening male by providing a cue to female motivational (Bailey & Hammond 2004). Males that produce more syllables and/or more phrases will also receive more acoustic information for localizing the female. Considering that in *S. pistillata* males are the sex performing phonotaxis (Nickle 1976; unpublished data) this increased location information could help that male reach the female faster and is discussed in Chapter 3.

When presented with phrases sequentially, the females did not show any additional preference in terms of tick response to a male that produces 9 syllables per phrase versus a male that produces 7. Although female mate preference is often characterized by females preferring male traits of greater quantity (Ryan & Keddy-Hector 1992), females *S. pistillata* do not give maximal ticks in response to phrases with the maximum number of syllables. A similar pattern was seen in *S. curvicauda*, with the largest number of male syllables presented not receiving the largest tick response (Tuckerman et al. 1993). However, syllables per phrase greater than seven were only produced in approximately one quarter of male advertisement calls, with an average syllable number of 6 produced for the population (Villarreal & Gilbert 2011). Because females produce the most ticks to phrases higher than the male average, there could be some directional selective pressure on male call to produce more syllables if female tick number is an important mating parameter.

The gradual increase in female response could also serve to stimulate the male into producing more phrases, and therefore more syllables per phrase. Perhaps by varying tick response, the female motivates the male to produce a greater signal, similar to the behavior of satin bowerbirds, in which signals from the female modify the intensity of male displays (Patricelli & Krakauer 2009). Analysis of the effect of female response on male phonotaxis is currently underway. Additionally, females in this study were not allowed to assess multiple signals concurrently. Other studies on katydids have found that females assessing multiple signals concurrently exhibited a stronger preference than to a single signal (Berg & Greenfield 2005). Direct comparison between two phrases with 7 and 9 syllables could demonstrate a stronger directionally selective pressure than that indicated in the present study.

Some females are highly motivated and produce as many as 8 ticks in response to a male phrase, whereas others produce only 1-2 ticks to the same phrase. The females used in this study were not controlled for age or mating status and thus represent the variability of females in the field and therefore shows the range of motivation a male potentially encounters on any given night. In a separate analysis in which live males were paired with virgin females, they showed similar variability as the females in this study, with some females only producing up to three ticks and others as many as six (Chapter 3). How motivation in terms of perceived female ticks affects the behavior of the male is the topic of Chapter 3.

Female Latency

Within the duet of *S. pistillata*, shorter reply latencies are associated with longer phrases. *Caedicia sp. 10* is similar to *S. pistillata* in that females produce a large range of ticks in response to the male call and reduce their latency when responding with multiple ticks (Bailey & Hammond 2004). In duetting species, typically the male call length and female reply latency are positively correlated (Bailey & Hammond 2003). With *Caedicia sp. 10,* timing of the female's reply depended on her motivation, with highly motivated females (those responding with more ticks) requiring fewer syllables from the male to elicit her tick response, resulting in shorter latencies. These motivated females also shifted their timing to start from the beginning of the male call instead of the end (see Figure 6 in Bailey & Hammond 2004). Though female *S. pistillata* are similar to *Caedicia sp. 10* in how they respond to variation in male call, when measuring *S. pistillata* latency from the beginning of the male call there is no constant value with increased tick response for those phrase lengths experienced in nature (Figure 2.6).

Male *S. pistillata* exhibit modulation in the amplitude of their call; the modulations is both from within the phrase as well as over the length of the bout (Villarreal & Gilbert 2011). If females start their auditory time window from the beginning of the signal, then complex amplitude modulations are not necessary and could potentially confuse the initiation of the song (Heller et al. 1997) and therefore the timing of the female response (Tauber & Pener 2000). Both of these attributes lead to ambiguity about the onset of the male call. In playback experiments, female *S. pistillata* still respond to extreme phrase lengths of 11-20 syllables (Figure 2.3), but in phrases consisting of more than 13 syllables most females produce responses during the male phrase and her average latency appears to be constant. Tauber & Pener (2000) found in the bush cricket *Phaneroptera nana* a minimum male syllable number is necessary to elicit a female tick and a constant latency from that number until the onset of her response. However, even with extreme phrase lengths, female *S. pistillata* do not appear to be timing their response from a syllable at the beginning of the male's phrase, or a phrase with an adequate number of syllables, e.g., 8, as we would expect a plateau in latency for all subsequent phrases, but we do not see it (Figure 2.6).

A female *S. pistillata* likely reaches a threshold while listening to the longer phrases of the male advertisement call in which excitation is enough to initiate her response. However unlike in other duetting species discussed, her response does not have consistent latency from that trigger or threshold syllable number, but is delayed somewhat by hearing subsequent incoming syllables. With the supernumerary phrase presented, there likely reaches a point in which excitation is strong enough that the female acoustic response is no longer inhibited until the end of the phrase.

The beginning of the female response can range 600 msec (Figure 2.4, A), therefore the male has a rather large window in which he must listen for a conspecific female. Several studies of duetting katydids have demonstrated that male response to female ticks exhibited a larger time window when compared to female latency values (Heller & v. Helversen 1986; v. Helversen et al. 2001). By playing ticks at various intervals after the male call and determining the timing that elicits his phonotaxis, one would get a better understanding of the species-specific timing of her response. An interactive playback in which the female tick response can be stereotypically triggered (Hammond et al. 2003) would allow for assessment of the male's time window. Alternatively, males could respond with phonotaxis to any female tick that falls less than one second after the end of his call.

Female response to call structure

Here we present the first analysis in which a male call consisting of a series of increasing syllable number phrases was manipulated to determine how that series affected female response. Tuckerman et al. (1993) presented female *Scudderia curvicauda* with on average long-phrased and short-phrased male bouts and found that females preferred bouts with the higher average syllables per phrase. However, they did not consider the structure of the perceived bout and how that could have an effect on tick response. A typical male bout consists of a series of phrases increasing from 3 to 8 syllables (Villarreal & Gilbert 2011) and therefore served as a comparison for all other manipulations. In this study the difference in tick response to each of the playback types was most apparent for phrases with more syllables. For almost all call manipulations, the female was presented with the same syllables per phrases as in the typical male bout, yet females changed their tick response.

There was no difference in a female's response to the typical male bout versus the "8 syllabled phrases" and the "irregular sequence" bout. This indicates that the female assesses each phase and not the sequence as a whole. For acoustically calling invertebrates, little evidence exists for females assessing multiple phrases (Tauber et al. 2001). Assessing relative alternative male calls through memory of previous phrases would allow the female to have an enhanced ability to assess alternatives (Dukas 2006), as well as a reduced assessment time through maintained attraction (Akre & Ryan 2010).

The decreased response to playback “flip syllable” indicates the female noticed the within-phrase effect of a single flipped syllable. This is also a strong indication that her tick response to certain phrases goes beyond just the amount of energy in the call. If energy was of sole importance, there would be no difference in tick number when she was presented with the same number of syllables in different playbacks (Figure 2.7). Few studies have looked at the effect of within-phrase changes. Ritchie et al. (1995) altered the end of male *Ephippiger ephippiger* syllable by inserting a slip (missed teeth) within the wing stroke and found a decreased response of the female. Flipping an element within the phrase of a male call has not been studied, however Tauber & Pener (2000) found that in *Phaneroptera nana*, flipping the entire phrase did not affect the number of ticks female’s gave in response.

The decreased responses we measured to playback types “plateau” and “long bout” most likely reflects the decreased tick response to longer bouts as seen in Figure 2.3. If females were just driven by the total energy produced in a bout she should have a stronger response to these playbacks. It is not uncommon for duetting female katydids to exhibit their strongest response to phrase lengths less than the maximum males produce (Tuckerman et al. 1993; Dobler et al. 1994; Tauber & Pener 2000; Bailey & Hammond 2004). Her decreased response leads to the question of why males would produce longer bouts if they gain no added ticks in response. However, with increased calling time males will receive more information on the female’s location.

Overall, females totaled on average 22.95 ± 13.16 and 25.97 ± 12.78 ticks in response to the plateau and long bout respectively and only 14.14 ± 7.75 to the typical male bout. Therefore the production of longer bouts could serve to encourage female response and therefore allow better localization without the female having a preference for long bouts, *per se*.

Conclusions

The variability in latency of the female *S. pistillata* response may complicate a male's assessment of species identity through tick response timing. Because *S. pistillata* are found in fields with several congeneric duetting species (personal observation), species isolation through the timing of the female tick response should be highly selected for. However, the interaction between the motivation of the female and the number of syllables she hears per phrase obscures any central value of her tick response. Further analysis of the time window in which the male attends to female's ticks is paramount to understanding how this species copes with the problem of finding the right mate. Nevertheless, variability in tick response means the male can not only locate those highly motivated females faster, he can also assess her receptivity to mate. Therefore female preference through tick response serves as an indicator of the sexual selective pressures females place on the structure of male call.

CHAPTER 3

VARIATION IN MALE AND FEMALE ACOUSTIC CALLS

Abstract

Instead of producing strictly stereotyped signals, males and females of the duetting katydid *Scudderia pistillata* exhibit variability in their call structure and the effect of this variability on their duet has not previously been analyzed. Males vary their advertisement call over the length of their bout by increasing the number of syllables per phrase. Females respond to each phrase by producing a variable number of brief acoustic ticks, related to the perceived male calls. However all recordings of male and female behavior was from isolated individuals in sound dampening boxes. The purpose of this study is to ask two questions. 1. How do males respond to the calls of other males? 2. How does the interaction of live males and females in a duet alter both sexes' behaviors? To address aim one, males were recorded in isolation, in response to an artificial male call, and in response to a female call. Males were found to adjust syllable number when placed in differing contexts. To address aim two, males and females were paired in an arena and allowed to move and behave freely. Males called the most when in the presence of females and males that traveled farther were more likely to reach a female. Though there is some effect of female acoustics on the male's ability to reach the female, the effect is not large and was the opposite of what was predicted. Age had the largest impact on the success of male phonotaxis, with older katydids being significantly less successful, even with unmated females.

Introduction

Male katydids produce an advertisement call within aggregated choruses in order to attract receptive females, who perform silent phonotaxis toward the calling males (Gwynne 2001). In katydid species within the subfamily Phaneropterinae and the group Ephippigerini (-inae), females respond acoustically to these advertisement calls. The female's acoustic structure is independently derived from the male call (Nickle & Carlysle 1975), often consists of a brief click or tick, and occurs after a species-specific latency (Heller & v. Helversen 1986). The evolution of the female response has profound effects on interactions between neighboring males as well as on the call structure of the male when a female is perceived (Galliart & Shaw 2001).

The broad-winged bush katydid, *Scudderia pistillata*, is a duetting katydid whose males produce an advertisement call with a variable call structure (Villarreal & Gilbert 2011). Within a bout, males add syllables (wing closures) to subsequent phrases (bursts of sound) and produce as few as one or two syllabled phrases then increasing to as many as eleven syllables in a single phrase. There is some consistency in the overall structure of the bout, though individual males vary in how long they call and how many syllables they add to their phrases. Female *S. pistillata* produce a variable response in reply to the perceived male call (Chapter 2). A female adjusts the number of ticks in her response based on the number of syllables in the preceding male phrase, as well as to within-phrase and within-bout differences between advertisement calls. Both the male and female acoustic characteristics, however, have been described previously when both sexes were acoustically isolated from all other individuals. Whether either sex would adjust their calls during live duets is part of the focus of the current experimentations.

The male advertisement call is important not only in attracting a female, but also in interactions with other males. The advertisement call is used to attract males to aggregations and to maintain spacing between males (Thiele & Bailey 1980; Guerra & Mason 2005). Males have also been shown to alter their call based on the perceived calls of their neighbors and often synchronizing with the other caller, though the reason for the synchrony can often vary (Greenfield & Schul 2008). The call of male duetting katydids has been found to be affected by the calls of other males in the chorus, often with the focal male adjusting his duty cycle, amplitude, or length of calling (Dadour 1989; Galliart & Shaw 1991; Nityananda & Balakrishnan 2008). Duetting males have also been found to be affected by the calls of females, with males increasing their acoustic output in the presence of a responding female (Galliart & Shaw 1991; Tauber et al. 2001). When duetting males are the sex performing phonotaxis they have been also shown to lower their amplitude as well, believed to be a means of protecting the duet from listening neighbors (Spooner 1968b; Galliart & Shaw 1991). Few species of duetting katydids exhibit variability in the number of ticks the female presents to a male. Those that do have been shown to change their acoustic output based on the male call perceived (Tuckerman et al. 1993; Bailey & Hammond 2004), however these analyses were performed with females responding to playback of an artificial male call as opposed to a live male. In a more natural setting a male's call could be altered by a live encounter, as several taxa which perform sequential mate assessments have been shown to alter their tactics during male-female interactions (Patricelli et al. 2011).

Male *S. pistillata* call structure, either in number of syllables produced or in duty cycle, may be affected by whether he is calling in isolation, calling in response to the calls of other males, or calling in response to a perceived female. Therefore this study has two aims: 1. To determine how a male call is affected by the context in which he is calling, i.e., in a private duet, in response to advertisement calls of a rival male, or alone by himself. 2. To determine how males and females behave acoustically and phonotactically in a live duet interaction. A single male and female were placed in an arena and their acoustic performance and movements were recorded. Many variables of male and female condition and movement could be affecting the duet, and we predict that female acoustic output will have an impact on male behavior, with males being more successful at reaching a female at a given time when she presents him with more acoustic location information and/or information on her motivation.

Methods

Specimens

Broad-Winged Bush Katydids, *Scudderia pistillata* Brunner 1878 (Tettigoniidae: Phaneropterinae), were collected during the summer of 2011 from old fields composed mostly of goldenrod, *Solidago* spp., near Ithaca, NY on Bald Hill (42°21’11.28” N, 76°22’57.46”W) and Connecticut Hill (42°20’32.13” N, 76°39’42.50” W). All females (*N* = 22) were collected as nymphs via sweep netting the first week of July. Male *S. pistillata* (*N* = 18) were collected either as nymphs during that first week or as adults the second week of July. All nymphs were fed bee-collected pollen, apples, and romaine lettuce until they molted into adults. Once adult, males and females were separately housed by gender in communal mesh cages (50cm in diameter, 75cm in height) and acoustically isolated from the opposite sex. All adults were fed apples, romaine lettuce, and the leaves of the Japanese silverberry, *Elaeagnus* *umbellata*, and kept at room temperature (24°-28°C) with natural photoperiods. Males were used multiply in duet interactions with females. Prior to experimentation, an individual's age (days since molting) and weight (in cg) was recorded.

Male acoustics in varying contexts

Acoustic output was recorded from adult males 8-28 days past their final molt in three contexts to evaluate how his call was affected by various treatments: males in isolation, males in the presence of a calling "rival" male, and males in the presence of a calling female. To record males in isolation, individuals were placed in a sound dampening box, and their calls were recorded for 2-3 hours via a microphone embedded in the box (see Chapter 2 for detailed methods). Each male was recorded on at least two nights.

To record males in response to the call of another male, a male was played artificially generated male bouts through a speaker embedded in the box. The playbacks were of two types, each repeated 3 times, with 10 minutes between each of the iterations. One playback type consisted of a sequence of syllables per phrase sequentially increasing from 3 to 8. The second playback type consisted of a male calling bout composed of sequentially increasing syllables per phrase from 3-9, and then 9 syllable phrases repeated four additional times. These types simulated the "typical" and "plateau" call were created to expose males to two types of calling common in natural populations (Chapter 1). Where a male placed his call relative to the artificial bouts was also recorded to determine how males react in timing to the static playback. Finally, to record males in the presence of calling females the male's acoustic output was extracted from duet interactions (see section "*Male-female duet* *interaction*" for details).

In this study we tested the hypothesis that male acoustic output (syllables per phrase, syllables per minute, phrases per minute) would be affected by the contexts in which the male was calling (in isolation, in the presence of a calling male and in a duet with a calling female). We also examine the effects of his age status (young versus old), the type of playback heard ("typical" versus "plateau"), and whether or not the male successfully reached a female in duet trials. Of the 18 males in this study, some did not respond during certain experimental contexts and therefore were not included in those analyses.

All male acoustic output was recorded on a Fostex HD-P2 digital recorder (Foster Electric Co., Ltd., Tokyo, Japan), transferred to a computer and analyzed using Audacity 1.3.5 Cross-Platform Sound Editor (http://audacity.sourceforge.net/). Average acoustic output when a male was recorded in isolation, with the call of another male, and in a duet, was then compared using a repeated measures ANOVA using JMP statistical analysis software, version 9 (SAS Institute Inc., Cary, NC). Only those males that produced acoustic output under all contexts analyzed were used in the ANOVA. The Mauchly's Test was used to confirm that the assumption of sphericity was not violated. To assess if males altered their syllable production based on the length of time they were presented with either playback calls, a mixed effect linear regression (MELR) was performed on syllable number versus time, with males as random variables.

Male-female duet interaction

In order to determine how male and female behavior is affected by a live duet, a single male and female were placed in an arena and their acoustic and behavioral responses during the duet were recorded. Individuals were housed in small plastic mesh cages (15cm cubed). Each cage had a sliding door that was opened manually by the experimenter pulling a long string, allowing the katydid to exit. Male and female cages were placed 3m apart, on top of 1m tall perches. To record the acoustics of both individuals, at the base of each perch was a ME66/K6 combo shotgun microphone (Sennheiser Electronic Corp., Old Lyme, CT) providing input to separate channels of a Tascam HD-P2 digital audio recorder (44kHz sampling frequency; TEAC Corp., Tokyo, Japan). An observer seated equidistant from each cage (~4m) recorded movement data. All experimental duets were recorded from 21:00 to 1:00 hours from the 18th of July to the 19th of August, and ambient temperature was recorded (Taylor 2752 Wireless Weather Station, Sherman Instruments, Vancouver, Canada).

For each duet trial, a male and a female were placed in each of the two cages and allowed to acclimate for two minutes. Lone males produce calling songs infrequently (see Results) and had to be stimulated to duet. When a female produces her call, the focal male becomes aware of her presence and will begin calling. Consequently, after the two minute acclimation an artificial male call was played from a portable mini speaker (75-90 dB SPL, Omnitech Inc., Sioux Falls, SD) to provoke a duet. Once the male and female were interacting with each other acoustically – and not with the artificial male call – the cages were opened. The number of male syllables and the number of female acoustic ticks in response were recorded by the microphones and later extracted from the sound files for analysis via Audacity software. The observer recorded the non-acoustic behavior displayed during the duet, including: timing of male movement, distance a male traveled, direction of male movement (no movement, movement towards the female or movement in any direction not towards the female). Because females rarely exited their cage, and displayed no phonotaxis behavior when they did, female non-acoustic behavior was not a factor in this analysis. A trial was concluded when the male contacted the female cage, or was within 15cm of the female. If the male successfully reached the female, the total time of the trial (time to reach female) was recorded. If a male did not exit his half of the arena after 30 min., or did not reach the female's cage after 60 min. the trial was considered unsuccessful and acoustic and behavioral responses of such males were compared to successful trials.

Virgin females were first paired with males 8-13 days after the female's final molt. Females were paired with two individuals in one night in order to determine any order effects (whether the male was the first male heard, or the second) on the duet. Once females were recorded with two males, they were allowed to mate with a third random male 5-8 days after their first trial. The females were given 6-11 days to recuperate, and then were placed again in the arena with two new males sequentially to observe how their behavior changed after given the chance to mate. Because mating attempts were not monitored, a successful mating was recorded as fertilized eggs laid by the female. Although all females were allowed to mate, not all females laid fertilized eggs. Therefore whether or not a female laid fertilized eggs was considered in this analysis.

For each duet, female identity, male identity, age (young = 8-13 days, old = 19-28 days), temperature, female weight, male weight, male presentation order, time to reach female and success at reaching a female was recorded. A single value for each parameter was extracted for both male movement data and female acoustic data for each duetting pair to perform survival analysis. Male movement behavior was recorded as the number of times a male made a movement towards the female (positive phonotaxis), and the total distance traveled when moving towards the female (cm towards). Because females can vary in how often they reply as well as how strongly they reply (number of ticks per phrase), the number of female replies and the total number of ticks produced were both quantified in each duet. Separate analyses of the female acoustic parameters were performed.

Because the male time to reach a female is similar to the time to an event in survival analysis, a Cox proportional hazard survival analysis (Allison 1995) was performed with the time to reach the female as the event, and success at reaching a female as the censor. All data were type I right censored, with those males that "survived past the censoring time" not being observed reaching a female. The covariates used in the analysis included age as bimodal (old, young) male and female weight, male order as bimodal (first, second), female acoustic behavior, and male phonotaxis behavior of number of movements towards and distance in cm towards the female. Because there was an association between temperature and age ($F_{1, 65} = 20.82$, $P < 0.0001$), only age was added as a covariate to avoid collinearity. Only female acoustic information was considered in this analysis, due to the influence of syllable number on female tick number (Chapter 2). Survival analysis was performed using SAS® 9.3 (SAS Institute, Inc., Cary, NC).

Because male or female response could vary over the length of a duet interaction, a separate analysis was performed to determine if the sequence of phrases varied in the different contexts. Conditional probabilities of syllable number given the preceding phrase were obtained by hand tabulating all recorded sequential syllables per phrase for all phrases recorded, regardless of their position in the bout (Chapter 1). A similar method was used on this current analysis to compare the conditional probabilities for each experience transition in syllable number for subsequent phrases (e.g. 7 syllabled phrase to an 8, 9 or 10 syllabled phrase, etc.). Comparisons were made across contexts by using a matched pairs analysis and a Wilcoxon signed rank test, due to non-normality of the data. Conditional probabilities were obtained for males in the presence of females, males in the presence of males, and males recorded alone.

To determine if there was any effect of the time associated with a female on male call – if the male altered his calling pattern within a duet – conditional probabilities were obtained at three different time points within the duet: the first 30 phrases produced, the 45th-65th phrase produced, and the 85th-105th phrase produced. These bins were extracted based on the average phrases produced in the time a male took to reach a female (unpublished data). Also to determine if female tick number affected subsequent male phrase numbers, conditional probabilities were obtained for the phrase before and after a weaker female response was given (1-2 ticks) versus syllables given before and after a stronger female response was given (3-6 ticks) for roughly 400 randomly sampled phrases.

Results

The mean number of male syllables per phrase did not differ significantly between males collected at the two field sites (Independent Samples t test: $t_{17} = 1.07$, $P > 0.05$), nor did the mean number of female ticks per phrase (Independent Samples t test: $t_{20} = 1.10$, $P > 0.05$). Therefore individuals from both field sites were analyzed together.

Male acoustics in varying contexts

Male calls were compared across various contexts (alone, to "plateau" bout, to "typical" bout, in a successful duet, and an unsuccessful duet) to determine if males altered their calls depending on the acoustic environment. Males exhibited the highest mean number of syllables per phrase in a duet and lowest in response to the calls of other males (Figure 3.1; Repeated Measures ANOVA: $F_{2,\,9} = 22.85$, $P < 0.001$). Of the 18 males, only 11 responded acoustically in all 3 contexts. The male mean number of syllables per phrase did not vary significantly with male age (Paired Sample *t* test: $t_{14} = 1.32$, $P > 0.05$). There was also no effect of playback type on male mean syllables per phrase (Paired Sample *t* test: $t_9 = 0.51$, $P > 0.05$). The mean number of syllables per phrase also did not vary with successful versus unsuccessful duets (Paired Sample *t* test: $t_7 = 1.20$, $P > 0.05$). Males also did not appear to adjust their syllable output based on how long they were associated with either the "plateau" or "typical" bout calls (MELR: $F_{1,191} = 2.2620$, $P = 0.1342$ & $F_{1,182} = 0.2323$, $P = 0.6304$, respectively).

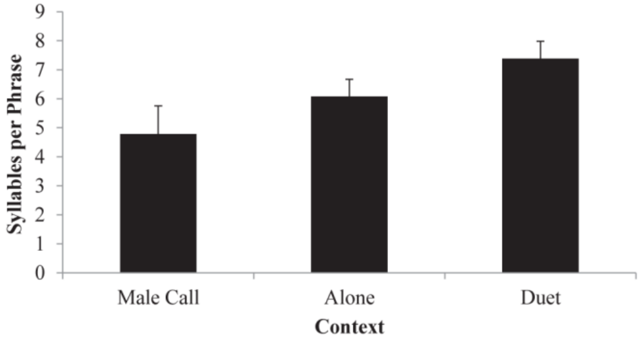


Figure 3.1. Average (+SD) of male syllables per phrase for males in three different contexts.

To assess the male’s rate of calling, the number of phrases per minute was used as a measure of acoustic output per unit time, as there is a significant positive relationship between the number of syllables per minute and the number of phrases per minute ($F_{1,16} = 154.07$, $P < 0.0001$; syllables/min = 0.45 + 5.98phrases/min; $r^2 = 0.89$). The male mean phrases per minute did not vary significantly with age (Paired Sample t test: $t_{14} = 1.93$, $P > 0.05$). However, phrases per minute does vary with playback type, with focal males producing fewer phrases to the longer, 3-9 syllable “plateau” call ($X = 4.2$) than to the 3-8 syllable “typical” call ($X = 6.8$; Paired Sample t test: $t_9 = 3.25$, $P < 0.05$). Males that reached the female also produce significantly more phrases per minute ($X = 4.8$), and reached the female than those males in unsuccessful duets ($X = 2.0$; Paired Sample t test: $t_7 = 4.21$, $P < 0.05$). Therefore, male mean phrases per minute (N = 5) were compared between the five treatments (alone, to “plateau” bout, to “typical” bout, in a successful duet, and an unsuccessful duet) and there was no overall significant difference in phrases per minute (Repeated Measures ANOVA: $F_{4,1} = 20.96$, $P = 0.1622$). This is likely due to the variability between males in how they responded in the different contexts as well as the reduced sample size (Figure 3.2).

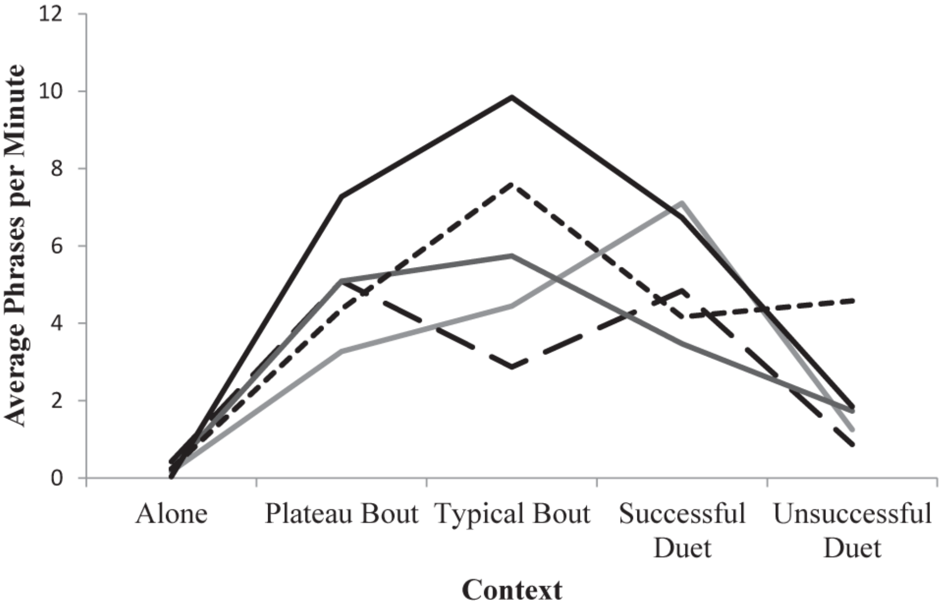


Figure 3.2. Average male phrases per minute for males in five different contexts. Each line indicates a single male's average response.

Though male phrase rate is quite low when the male is recorded in isolation, they also produce a separate acoustic call. Like other *Scudderia* spp. (Spooner, 1964), male *S. pistillata* have multiple call types that they use in different contexts. Males in the presence of another male as well as recorded when alone produced a second sound type. This sound type is a "rasp" call, consisting of 4-10 phonotomes produced in quick succession (duration ~ 0.04s phonotomes versus ~0.13s male syllable at 25°C). The "rasp" call was most often produced when the male was alone, with over 9000 rasps produced in total (N = 18). Rasps were produced during playbacks of another male's call only in the silence in between hearing bouts of another male. Because they only produced syllables in response to syllables heard, they produced far fewer rasps (around 800) during the male call stimulus. These rasp calls are also often heard from males in the field and in lab throughout the day. In the field on several occasions the rasp call was heard to be produced prior to males producing their call in synchrony with their neighbor male (unpublished data; see also Chapter 4).

Male-female duet interactions

Males typically initiated the duet while still in the cage. Once the cage was opened, females mostly remained inside the cage while males usually climbed to the top of the cage and produced a number of phrases. All males then jumped or flew off their cage to some intermediate point between the two cages. Once landing in his new location a male would usually produce a number of phrases before initiating his next flight/jump. If the male landed on a vertical surface, he often proceeded to walk upward while calling before flying or jumping away. Of the 563 recorded male movement events in all duetting trials, 72% occurred after a male finished an advertisement bout, and only 28% of male movements occurred after just one or two phrases. When the male was near (<0.5m) the base of the female cage he usually proceeded to walk upwards towards the responding female quickly and directly. On average, a male took 17 minutes (±8min) to reach a female in her cage during a successful trial, much faster than the 30 or 60 minute period (see Methods) a male was given to leave his half of the arena and reach a female, respectively, in unsuccessful trials.

The acoustics of both males and females varied with age (Figure 3.3). Older males produced more syllables per phrase than younger ones (Paired Sample *t* test: $t_{12} = 7.25$, $P < 0.001$) whereas older females responded with fewer ticks than younger ones (Paired Sample *t* test: $t_7 = 4.12$, $P = 0.003$). Those females whose acoustics were recorded when they were old were only paired with old males. Therefore, despite the increased average syllables heard, female tick response was lower. Note the sample size is reduced for both sexes. This is due to deaths as well as 13 of the katydids having no acoustic data for pairings when they were "old."

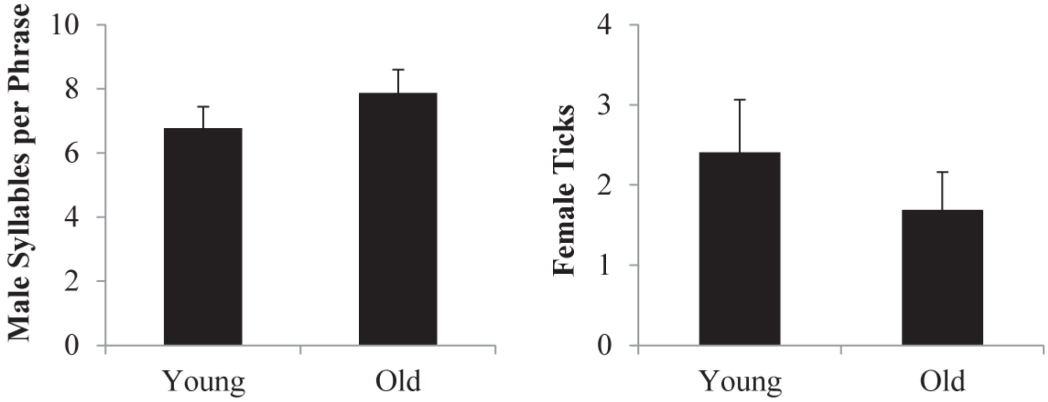


Figure 3.3. Difference in male (left) and female (right) average (+SD) acoustic output by age.

The age of the male and the female also had a significant effect on whether a male reached the female. Old males and old females had fewer successful pairings (2x2 Contingency Table: Yates' corrected $X^2_1 = 14.50$, $P < 0.001$; $X^2_1 = 5.35$, $P < 0.05$ respectively). However, success in reaching a female did not depend upon whether or not a female was fertilized (mated and laid eggs) (2x2 Contingency Table: Yates' corrected $X^2_1 = 0.37$, $P > 0.05$) or whether the male was the first or second to be presented to a female (2x2 Contingency Fable: Yates' corrected $X^2_1 = 0.0003$, $P > 0.05$).

Because age has an effect on male call, age could also affect the interaction between the male and the female. For example, an older and younger male might differ in how they allocate their energy over the length of a calling bout. However, comparing syllable number for phrases occurring at the start of a bout, in the middle of a bout, and at the end of a bout showed no difference in syllable number (Repeated Measures ANOVA: $F_{2,27} = 0.34$, $P = 0.7113$).

Two separate survival analyses were performed to determine what covariates had an effect on the likelihood of a male reaching a female. In the survival analysis performed here, a reduced time to an event indicates a higher probability of a male successfully reaching the female, rather than an increased risk of a hazard as in a typical survival analysis. Several factors were found to have a significant effect on increasing or lowering a male's success at reaching a female (Table 3.1). In both tests, there was consistency in which covariates significantly affected success as well as the predicted hazards. For those covariates that significantly affected the probability of a male reaching a female, most had small effects on the overall baseline survival, with only age showing a large effect on probability of success. The one significant positive effect was associated with male direction of travel; males that traveled toward the female had an increased probability of reaching her of 0.3% per centimeter of travel. All other significant factors had a negative effect on a male's probability of successfully reaching a female.

Table 3.1. Estimates and relative hazards, with 95% confidence intervals (CI), of a male reaching a female differentiated by covariates, with p-values indicating strength of significance. There were no significant interaction terms.

	Number of Female Replies			**Sum of Female Ticks Produced**		
	Estimate	“Hazards” Ratio (CI)	*P*	Estimate	“Hazards” Ratio (CI)	*P*
Towards (cm)	0.003	1.003 (1.000-1.005)	0.0114	0.003	1.003 (1.001-1.005)	0.0109
Age [old]	-1.448	0.235 (0.106-0.0499)	0.0002	-1.448	0.236 (0.105-0.509)	0.0003
Male Weight (cg)	-6.774	0.934 (0.972-0.992)	0.0229	-6.273	0.939 (0.884-0.997)	0.0408
Female Acoustic	-0.017	0.983 (0.972-0.993)	0.0012	-0.005	0.994 (0.991-0.998)	0.0028

It could be that individual male movements are influenced by the female tick number preceding them. If the male receives a single tick he might not move as far as with multiple ticks. Additionally, if the male receives multiple ticks it might reduce the latency between when a male perceives the ticks and when he moves. Analysis for both the distance of male movement and the latency until male movement showed neither of these to be predicted by female acoustics (MELR: $F_{1, 287} = 0.86$, $P = 0.35$ & $F_{1, 215} = 0.49$, $P = 0.48$, respectively).

Neither the regression nor survival can detect whether males alter their signal pattern within a context, i.e. if males change their syllables per phrase for subsequent phrase based on the type of signal they hear. To address this, the conditional probabilities were established for each possible transition in syllable number observed. For example, the probability that a male transitioned from a three syllabled phrase to a four syllabled phrase was calculated for males when they were calling alone, when they were calling in response to a male call playback, and when they were interacting with a live female. Results of matched pairs analyses demonstrated that no significant difference was observed in the overall probabilities for each transition between contexts, as well as over the length of a duet and with varying strengths of female tick response (Table 3.2). The only significant time when a male alters the structure of his bout is seen when you only consider the longer phrases (7 - 9 syllables). Figure 3.4 illustrates the probability of each transitional event for *S. pistillata* males when they were alone versus when they were with a female. There is a shift upward when a male is present with a female. In fact, of all the transitions possible, a male going from a 7, 8, or 9-syllabled phrase to any other phrase is 62.83% when the male is with a female, and only 26.47% when the male is alone.

Table 3.2: Matched pairs analysis comparing conditional probabilities of each syllabled phrase transition. P-values and test statistic S obtained from Wilcoxon Signed Rank test. N is the number of transitions compared.

	Conditional Probabilities:	N	S	*P*
Between Contexts:	*Alone v. With Male*	79	285	0.1028
	Alone v. With Female	79	308	0.0646
	With Female v. With Male	79	215	0.2963
7-9 syllabled phrases:	*With Female v. Alone*	40	294	< **0.0001**
Between phrases within the male-female duet:	*1st-30th v. 45th -65th*	64	266	0.075
	1st-30th v. 85th -105th	64	164	0.2404
	45th -65th v. 85th -105th	64	32	0.7738
Based on the strength of a female response:	*1-2 ticks v. 3-6 ticks*	45	110	0.2156

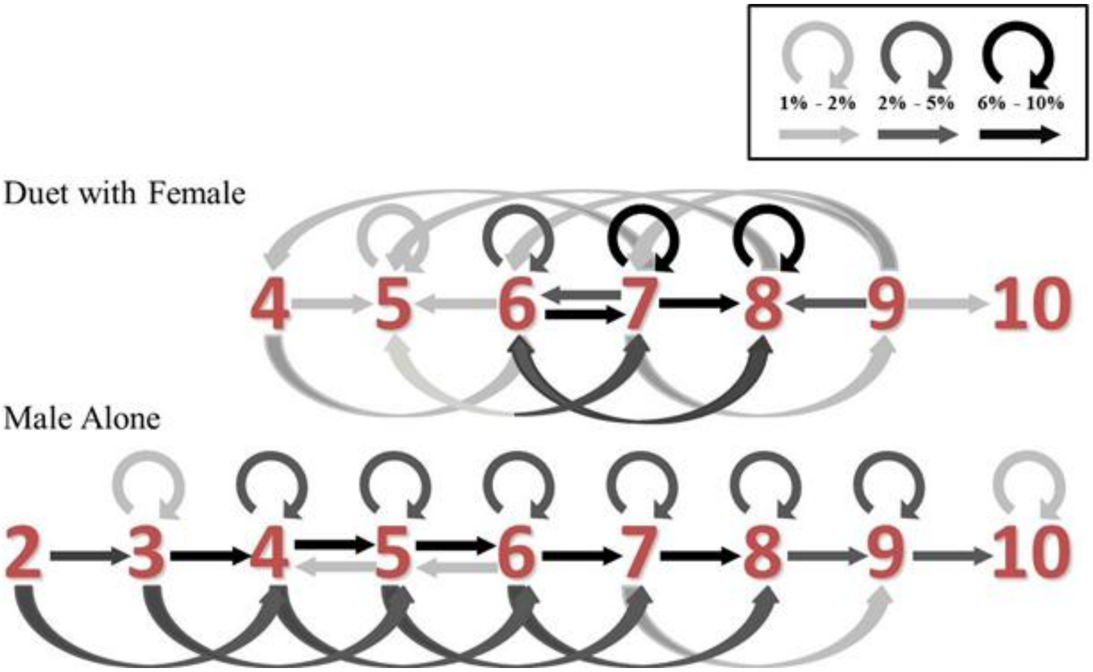


Figure 3.4. Conditional probabilities of incremental counting in male *Scudderia pistillata* calling bouts in two contexts. Numbers in red represent the number of syllables per phrase. Arrows indicate the probability that the subsequent phrase contained the syllable number at the arrow head. Intensity of arrows indicates strength of probability. Transitional probabilities less than 1% are omitted for clarity.

Discussion

Context-dependent variability in male advertisement calls

Male *Scudderia pistillata* alter their calling structure depending on the context with which they are presented. Our study was not designed to investigate the effect of interactions between males on male calling structure. However, when males were calling by themselves, they produced their advertisement call infrequently and when presented with playbacks of the advertisement call of a potential rival male, the focal male called more but produced fewer syllables per phrase, and how often he calls depends upon the stimulus presented. When presented with a typical advertisement call of 3-8 syllables per phrase, the focal male responded to nearly every perceived phrase, often producing their call in the midst of the perceived call. When presented with a more energetic rival call containing a plateau of repeated 9 syllable phrases, the focal male did not respond as frequently, only responding to four out of the thirteen phrases presented. When presented with a female, males increased their acoustic output, similar to the behavior seen in other duetting katydid males (Galliart & Shaw 1991; Tauber et al. 2001).

In this contrived set up, males reduce their syllable output when in the presence of calling males and this reduction was consistent over the length of the interaction with the playbacks. Both *Mygalopsis marki* and *Amblycorypha parvipennis* males were found to exhibit similar reduced acoustic output when another male playback was presented (Dadour 1989; Galliart & Shaw 2001). *A. parvipennis* alternates his call with his neighbor due to males inhibiting the phrase of neighboring males. When males were placed nearer to each other, the inhibition was enhanced (Galliart & Shaw 2001). Dadour (1989) proposed that males reduce their rate of calling to monitor the activity of neighboring males. In both these studies the male calls were perceived to be close based on sound level. *S. pistillata* playback presentations also were at a sound level which potentially indicated a close male. Therefore the reduced syllable output seen with male *S. pistillata* might be due to males assessing the playback to determine if they needed to move to a new location, and were unable to react in a way they might when in a more natural situation.

Static playbacks presentations can often differ from the behavior of live individuals. In túngara frogs, when males were presented with a static playback they did not add chucks to the end of their whine call based on the perceived complexity of the male call (Bernal et al. 2009). However when in a dynamic playback, males vary their chuck number with the variation in the perceived male's call strategy, decreasing their chuck production when the model exhibited a de-escalating strategy (Goutte et al. 2010). Live interactions between male katydids is also likely to be more dynamic than those illustrated as males could employ alternate tactics based on the perceived assets of their neighbors (Patricelli et al. 2011). Because male syllable response did not vary over the length of a context, it is likely that interactions do not play as strong a role in this species, but further dynamic interactions are necessary to determine how live males react in the field.

When males are alone, they produce advertisement calls infrequently, without lowering their average syllables per phrase. They also produce a second call type, the "rasp" call. Spooner (1964) also noted a similar call type in a congener, *S. texensis,* and hypothesized this "fast-pulsed song" served to attract distant females as well as stimulate other nearby males into producing their own "fast-pulsed song" or to produce a loud ticking noise important for male spacing. Many male katydids have been found to be attracted to neighboring males based on their acoustics to form a chorus (Guerra & Mason 2005). Male *S. pistillata* in the lab and chorusing in the field were heard producing this rasp call prior to initiating a calling bout with surroundings males (personal observation). Therefore, when male *S. pistillata* do not hear the calls of other males, they most likely produce the rasp call in order to goad other males into calling, or to find any nearby chorus of males. Because focal males produced this call type only with the call of another male and not in duets with females, it likely has little use in mate attraction.

Male *S. pistillata* synchronized their call with the call from the playback. Other katydids have been shown to synchronize with their neighbors. Greenfield and Schul (2008) proposed two mechanisms which promote synchrony. Males could be taking advantage of the precedence effect, in which females prefer the lead caller, and therefore males could be synchronizing with the playback in order to jam the other caller's signal. In *S. pistillata*, males often signaled after the initiation of the playback, resulting in their call falling within the call of the perceived neighbor male (unpublished data). Additionally, the male call increases in amplitude over the length of the bout, making the onset of the call less obvious for listening females, especially for those positioned farther away. Therefore precedence effect is likely not a strong factor promoting synchrony in *S. pistillata*. Alternatively, in the evolution of discontinuous song, a silent interval is required. Therefore synchrony is this case is a byproduct of maintaining this silent interval and males actively adjust their song compared to their neighbors for this purpose (Greenfield & Schul 2008). This explanation is most likely for *S. pistillata*, as the silent period is essential for the male to hear the female's response.

In a natural chorus, there is also potential for males hearing a duet to alter their acoustic behavior. In some grasshoppers, hearing a duet influences male behavior, though most males studied respond with silent positive phonotaxis towards the duet (Otte 1972; Donelson & v. Staaden 2005). In the katydid *Elephantodeta nobilis,* when a male hears a duet, he interjects his own acoustic call in order to steal away the responding female (Bailey & Field 2000). *Caedicia* sp. produces a masking "chirp" after their call to prevent eavesdropping males from interrupting their duet (Hammond

& Bailey 2003). Male *S. pistillata* also produce a call at the termination of an advertisement phrase when both male and female calls can be heard. The purpose of this behavior is explored in Chapter 5.

What makes a successful duet?

Female *S. pistillata* are known to be influenced by the number of male syllables she hears, and also by changes in the timing of phrases and within-phrase differences (Chapter 2). In an interactive duet in which both the male and the female are calling it is unclear how both sexes' calls would be further altered by the acoustic feedback of the other individual, and how this variability might contribute to finding their mate. Females when presented with a live male still varied their tick number with the male syllables perceived similar to those females recorded in response to playback (unpublished data). When compared with his call in other contexts, males increase their acoustic output when with a female. Males adjusting their syllable number up leads to females producing more ticks. Though male call was not directly included in the survival analysis, indirectly it can be seen in both the number of replies and the sum of replies, as both are contingent upon the male producing a call for females to respond to. It was found that for both the number and sum of female tick production a one unit increase leads to a reduced risk of the male reaching the female. This is contrary to what we believed would be the influence of increased acoustic energy of the female.

Males mostly performed flights towards the female when the distance between the two individuals was greater than one meter. The most flights were performed during successful duets (78%). However, male flights are very erratic. When males were observed prior to a flight movement, they were oriented towards the female. Once a male took to the wing, he exhibited an erratic, whirling and non-linear flight path, and his location once landed was often not in the direction he had initially pointed. Reinlander et al. (2007) found that walking male *Leptophyes punctatissima* accurately performed phonotaxis to a single female tick. When male *S. pistillata* walked instead of flew, their movements were more directed on a linear path toward the female. Perhaps in a more natural setting, in which more perching options were placed between male and a female, the male would chose to walk or perform small jumps towards the female. If he did, he would maintain the general orientation towards the sound source and then perhaps we would see a greater effect of female tick number on his orientation.

Because in both *L. punctatissima* and *S. pistillata*, females are stationary, each acoustic response provides further information on the location of the female. *L. punctatissima* accurately performed phonotaxis to playbacks of females which produced only a single tick. This would indicate that duetting males can accurately find a responding female regardless of how many ticks she gives. In *L. punctatissima*, approximately 36 female responses were needed for the male to travel 2m to reach the calling female, which he did in approximately in two minutes (Reinlander et al. 2007). In *S. pistillata*, males traveled on average 5 m (even though the shortest distance between male and female cages was 3 m), and the female responded on average 65 times, but males heard an average of 151 ticks while successfully reaching the female in approximately 18 min. *L. punctatissima* and *S. pistillata* appear to be displaying differing movement behaviors based on the differences of the environment. Because male *S. pistillata* are found in a heterogeneous environment in which there is no direct connection of vegetation between the two signalers, they are likely more reliant on flights and jumps, which serve to decrease the accuracy of localization.

Females seem not to fatigue or habituate to male calls, as she responded equally to the first and second male presented. Age however had a profound effect on whether the male successfully reached the female, as both the male and female have a higher rate of success when younger. Age seems to have more of a role in their acoustic mating behavior than whether or not they were fertilized, as there was no difference in the success of a bout between old females that laid eggs and old females that didn’t. The decreased response rate with older females lead to many duet trials not being initiated, due to males not being aware of the female’s presence. When a duet was initiated, most males were not successful in finding the female, most likely due to her decreased acoustic output either leading to reduced localization ability for the male, or the male attending to a motivational cue from the female and deciding not to perform phonotaxis to an unreceptive partner.

There are several reasons why a female’s strength of response would vary with age. An old, virgin female should be less choosy and expend more energy trying to solicit a mate to ensure leaving progeny. In many katydid species males have been shown to increase their nuptial gift mass as they get older (Lehmann & Lehmann 2009) though it is unclear of the quality of these gifts and what impact that could have on female response. In *Ephippiger ephippiger* females showed a decreased response to older males whose stridulatory files were showing signs of wear (Ritchie et al. 1995). Female *S. pistillata* might also have this bias towards younger males. In addition, a male’s advertisement call is for long distance mate attraction and there is a separate set of behaviors exhibited for courtship. An encounter with a male that is physically present is more likely to lead to fertilization compared to hearing a male that is acoustically present but some distance away. Old males were not allowed to perform courtship acts with old females and therefore her response to these encounters is unknown.

Conclusion

Both the male and female *S. pistillata* call is influenced by the context with which they are signaling. Males appear to conserve energy when they don't perceive another calling male, and then increase their acoustic energy when in the presence of another male, and synchronize. When a female is present, the male produces more syllables, which serves to increase the acoustic information from the female. In this situation, females giving increased numbers of ticks did not aid in males finding the female, potentially due to the orientation errors of male flight. There also appears to be a point in which unmated males increase acoustic output with age in order to attract more female attention; however females appear to wane in acoustic response with age, regardless of whether or not she lays fertilized eggs. Further studies in which males are allowed to acoustically interact with other males in the presence of a female should further illustrate the potential function of female tick variability and how males compete for mates.

CHAPTER 4

ACTIVE ACOUSTIC SPACE AND CHORUS STRUCTURE OF MALE *S. PISTILLATA*

Abstract

Male *Scudderia pistillata* (Orthoptera: Tettigoniidae: Phaneropterinae) call in choruses at night in fields to attract acoustically responding females. Two fields were analyzed for male spacing behavior; in 2007 a field was “seeded” with males and consists of heterogeneous flora, in 2008 a natural population in a field of homogeneous flora was studied. Analysis of the spatiotemporal aspects of the chorus shows males call mostly within 5-8m of their nearest neighbor males, though their spacing varied significantly between the two fields; Males were spaced farther apart in the heterogeneous field. Due to the attenuation of their calls, males most likely hear the calls of only 1-2 nearest neighbors. Females respond maximally to loud phrases composed of more syllables and would hear and respond to multiple males in the field. This potentially allows for preference for a particular male’s calls as well as some competition between neighboring males. However, neighboring males have been shown to be influenced by the calls of other males by synchronizing and lowering their syllable output. Because males are the sex which perform phonotaxis, their spacing is stochastic in nature with neighboring males varying night to night.

Introduction

Male katydids call in choruses in fields with a variety of other acoustic calling Orthoptera. Their advertisement call not only attracts responding females for the purpose of mating, but is also useful in maintaining the spatiotemporal structure of a chorus (Robinson & Hall 2002; Guerra & Mason 2005). Understanding how males interact acoustically in a chorus is important for understanding the influence of nearby males on responding females, as well as any competition between neighboring males.

Despite the myriad of noise surrounding them, males of *S. pistillata* manage to carve out their own acoustical space with their unique calling sequence. Their call is atypical in that instead of repeating the same phrase continuously over the course of the night, *S. pistillata* males add syllables to consecutive phrases in a bout. The bout usually starts with a 1-4 syllabled phrase and ends with a 7-11 syllabled phrase (Chapter 1).

The time interval between bouts is highly variable, with males being silent from one minute to one hour. If a male doesn't hear the call of another male, or the response of the female, he calls rather infrequently (Chapter 3). In the field and in laboratory trials, males often synch up with the onset of their neighbor's calls. This synchrony indicates that they listen to the calls of their neighbors, but no in-depth analysis of their chorus calling structure has been performed.

Male katydids often adjust the rhythm of their song to either synch up or alternate with their neighboring male (Greenfield & Snedden 2003). Interactions between males in a chorus are limited by two factors. First, the separation of males in an aggregation influences how many males can potentially hear each other in a chorus due to attenuation of the call in the biotope. Because of the small size of katydids their high frequency calls can attenuate greatly as they travel through the vegetation (Römer & Bailey 1986). Secondly, the ability of a male to adjust his call output with his neighbor is dependent upon the plasticity inherent in the call. Males have been shown to alter their call in interactions with other males in several katydid species (Dadour 1989; Schatral & Bailey 1991). Male *S. pistillata* have also been shown to alter their call output when presented with varying acoustic contexts. They respond by lowering their syllable production when hearing males, and adding phrases to their bout when hearing a long bout produced by another male (Chapter 3). Therefore spacing is the most likely factor that limits interactions between neighbors.

The acoustic response of the female *S. pistillata* adds complexity to the interactions between chorusing males. Females of most katydid species exhibit silent phonotaxis towards a calling male. Therefore none of the males in the chorus know of the presence of the female until she is within a very close range. On the other hand, female phaneropterine katydids, including those of *S. pistillata*, stridulate in response to the calls of conspecific males (Bailey 2003). Female stridulation is quite different from that of the males, consisting of a few broadband ticks generated from a poorly developed stridulatory apparatus located on the dorsal edges of the tegmina (Nickle & Carlysle 1975). How many males a female hears depends on how far apart males are spaced and at what intensity females will respond to male calls.

The purpose of this study is to determine the structure of the male *S. pistillata* chorus in two different habitats. If male spacing is based on males listening to the calls of their neighbors, then they should aggregate within the site, but show stereotypic distances between males based on how sound attenuates in the field. Because females are also acoustically present and in the chorus, it is important to understand the attenuation and audience of her call.

Methods

Specimens

Chorus structure of the Broad-winged bush katydid, *Scudderia pistillata* Brunner 1878 (Tettigoniidae: Phaneropterinae), was analyzed during the summers of 2007 and 2008 from old fields near Ithaca, NY at a 1853 m^2 site on Bald Hill (42°21’11.28” N, 76°22’57.46”W) and a 4027 m^2 site on Whitted Rd (42°25’14.43’’N, 76°25’41.62”W). Choruses of males were found steadily calling from sundown until approximately midnight. Though the composition of both fields’ foliage included grasses and forbs, such as milkweeds and goldenrod, there was variation in the flora between locations, with the Whitted Rd site including more perennial shrubs such as the Japanese silverberry, *Elaeagnus umbellata* and the Bald Hill site being more homogenous composed mostly of goldenrod, *Solidago* spp.

Chorus Spacing Analysis

In 2007 a study population was created in the Whitted Rd site by releasing eight captured males into the 71.4 m x 56.4 m field known to contain several *Scudderia* species including *S. pistillata*. In 2008 at the Bald Hill site chorus structure of a naturally occurring population of calling males was analyzed in a field 47.4 m x 39.1 m. For each night of fieldwork, males were localized from a distance by their calling song and then located amongst the foliage via flashlight. Once located, males were identified by writing a unique number on their forewing with a Sharpie pen (Sanford Corporation, IL), and their location and identification was marked with surveyor's tape (Presco Products, TX) tied onto the plant from which they had been calling. Males were then rereleased where they were captured. If males were repeatedly recorded on that field night their location was noted with minimum disturbance to the katydid.

During the following day distance and cardinal direction data were measured from each surveyor's tape tag to a focal point to create a spatial map of the previous night's calling males. The data were then scaled and recreated onto paper, converting cardinal directions into compass directions to determine the distances between each male and all others recorded that night in the chorus. Spatial distribution of male nearest-neighbor distances was analyzed via the modified R statistic derived by Clark and Evans (1954). The R statistic measures the degree to which observed distributions depart from random using the ratio of observed to expected mean nearest neighbor distances: $R=R_A/R_E$, with $R=1$ being a random distribution and $R=0$ when individuals are maximally aggregated. The significance of their departure from normal was tested by calculating a c test statistic, with a value of 1.96 and 2.58 indicating a 5% and 1% level of significance, respectively. Males whose locations were recorded across more than 2 field nights were also analyzed for site fidelity, the measure of their mobility from night to night.

Sound attenuation and female response to changing sound levels

Measurements of the sound level attenuation of the male advertisement call were also performed in the Bald Hill field site using two sound sources: natural male calls and an artificial male call. On a single night the sound levels of three males' calls were recorded. On a separate night a single phrase of an artificial male call (see

Chapter 2 for details on how the artificial male sound file was created) was played from various locations in the field from a .wav file played from a iPod docking station speaker (peak amplitude = 90 dB SPL at source; Logic3 plc, Hertfordshire, UK) ~1.5m off the ground, similar to the perch height of the naturally calling males. Measurement of both live and artificial male sound levels was performed in open areas without foliage and within the foliage at three different heights: 1.25m off the ground (top of the foliage), 0.65m off the ground (mid-foliage) and ground level. Sound level was recorded at each vertical position for increasing distances away from the sound source until the sound level from the source matched that of the background noise (~50dB SPL). How far the sound signal traveled before diminishing into the background was compared with the spacing of males in the field to determine the active chorus space around a male. All sound levels were measured with a 1982 precision sound level meter and analyzer set at an 8 kHz octave filter frequency to match the peak frequency of the male's call (Chapter 1; IET Labs, Inc, Westbury, NY).

To determine the amplitude of the male call to which the female responded, female *S. pistillata* were collected from field sites as adults from July through August by sight at night in 2009 (N = 5). All katydids were brought back to the laboratory and were housed in communal cages (0.5m in diameter, 0.75m in height) prior to experimentation and for the remainder of their life. They were fed an *ad libitium* diet of leafy greens and apples, and kept at room temperature (24°-28°C) with natural photoperiods. To record female acoustic response to changing sound levels, a single female was acoustically isolated and played the typical male advertisement call (Chapter 1) increasing from 3 syllables (wing closures) per phrase to 8 syllables per phrase. Male calls were constructed from a single eight syllable phrase using Audacity software (for details see Chapter 2). Females were placed 25 cm from the iPod docking station, with the sound level meter set 90° off axis and equidistant to the speaker. The amplitude of the sound file was adjusted randomly and the number of syllables heard, peak amplitude of the syllable, and number of female response ticks to the male phrase were recorded to compare against sound attenuation in the field.

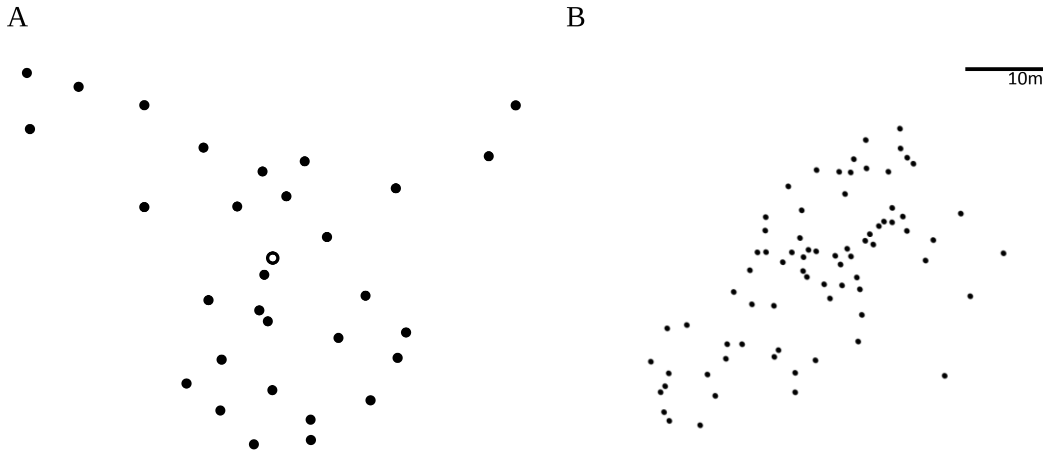
Results

Male Spacing in Chorus

In 2007, the males were released into the Whitted Rd field site on August 9th, and the locations of released males as well as local calling males were recorded from Aug. 9-28 for a total of six field nights (N = 14 males). Though eight males were released from a single central location, these individuals were recorded as far as 44 m from the original release location. Males in this field spaced as close as 0.9 m from their nearest neighbor and as far away as 36 m from their furthest. Nearest neighbor males were found to significantly aggregate on most nights, though some nights exhibited low sampling (Table 4.1). The distribution of males in the field, as well as average spacing for the three nearest neighbor males, is given in Figure 4.1a.

Table 4.1. Analysis of near neighbor distances (NND) for male *S. pistillata* katydids in two habitats. Asterisk indicates normally distributed data. Expected NND are extracted using the formula: [1/2√male density] (Clark & Evans 1954).

Field & Date	N	Male density (male/m^2)	Expected NND (m)	Observed NND (m)	R	c	*P*	Distribution
Whitted Rd								
8/13/2007	4	0.0010	15.86	6.10	0.38	-2.36	<0.01	Aggregated
8/14/2007	8	0.0020	11.22	5.52	0.49	-2.75	<0.01	Aggregated
8/19/2007	9	0.0022	10.58	9.41	0.89	-0.63	>0.05*	Random
8/22/2007	5	0.0012	14.19	21.28	1.50	2.14	<0.05	Uniform
8/23/2007	7	0.0017	11.99	8.62	0.72	-1.42	>0.05	Random
Bald Hill								
8/3/2008	9	0.0049	7.17	3.93	0.55	-2.60	<0.01	Aggregated
8/4/2008	12	0.0065	6.21	4.78	0.77	-1.53	>0.05*	Random
8/6/2008	13	0.0070	5.97	5.21	0.87	-0.88	>0.05*	Random
8/9/2008	10	0.0054	6.81	5.58	0.82	-1.09	>0.05	Random
8/12/2008	11	0.0059	6.49	5.57	0.86	-0.90	>0.05	Random
8/13/2008	9	0.0049	7.17	6.20	0.86	-0.78	>0.05*	Random
8/14/2008	5	0.0027	9.63	2.74	0.28	-3.06	<0.01	Aggregated



Parameter	Nearest Neighbor	Second Nearest	Third Nearest
Mean	9.7	15.1	22.9
SD	7.4	7.2	10.8
Range	3-36	5-46	12-52

Parameter	Nearest Neighbor	Second Nearest	Third Nearest
Mean	5	8.1	11
SD	3.8	4.2	4.8
Range	1-16	1.5-21	2-26

Figure 4.1. Distribution of male *S. pistillata* katydids for all recorded field nights within the two field sites, along with descriptive statistics of neighbor male distances (in meters). (a) Whitted Rd site. (b) Bald Hill site. Open circle indicates release point of males in Whitted Rd. site.

In 2008 at Bald Hill, spacing behavior was recorded from Aug. 3-14 with eight field nights (N = 41males). Males had already started calling at night in the field when the two week period began. Males were found great distances from each other, as far as 56 m in one night. Though the chorus occupied a larger area of the field, nearest neighbors were found as close as 1 m from each other. Analysis of the spacing of nearest neighbors demonstrated that males were randomly distributed on most nights (Table 4.1). There is also a significant difference in the distribution of nearest neighbor males between the two field sites (Welch's *t*-test: $t_{38} = 3.39$, $P = 0.0016$), with males at the Bald Hill site spacing closer. The distribution and neighbor male distances are given in Figure 4.1b.

Site fidelity

Males from the Whitted Rd field site were not recaptured frequently enough to assess site fidelity from night to night. Over a period of approximately one week, males traveled on average 27m (Figure 4.2). Of those males captured in the Bald Hill site, eight of 41 males (19%) were recaptured in repeated nights (more than two capture events). Males were highly variable in the total area traveled during the two weeks of recording as well as how far they traveled on consecutive nights, ranging from 2.4m to 14.6m per night. Males that didn't travel too far mostly remained within 10m from their initial capture site (fig 4.2, 4.3).

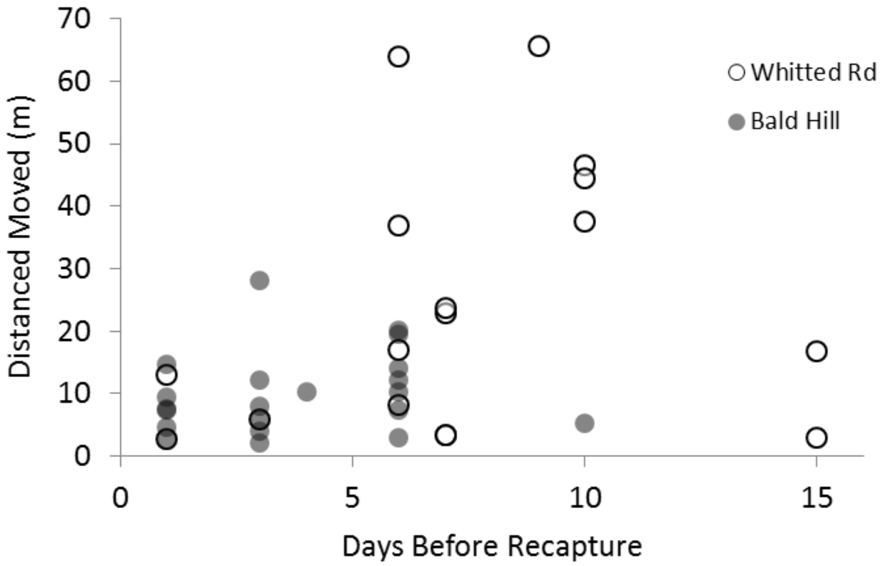


Figure 4.2. Average distance moved between days of recapture for both field sites.

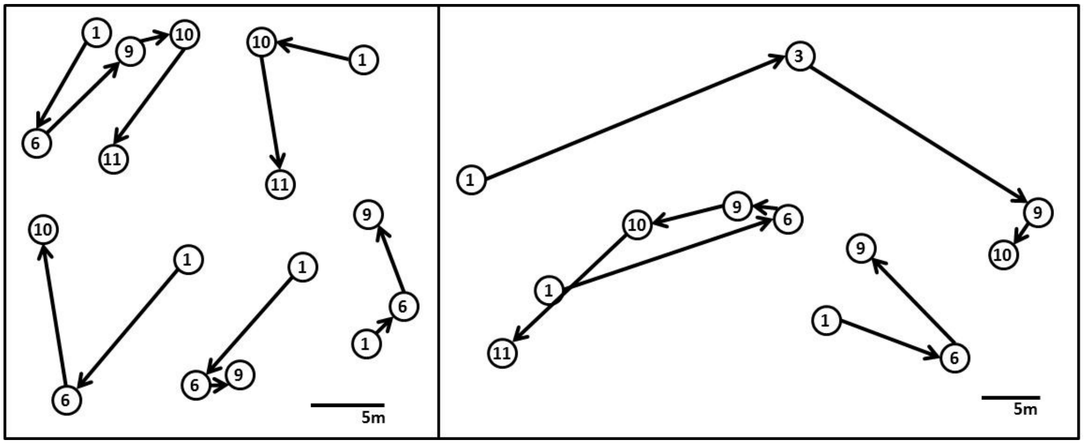


Figure 4.3. Eight male *S. pistillata* recapture data. Number in circles indicates days from first capture/marking, arrows indicate direction moved between sampling. Note separation of male spacing to account for different scales.

Call attenuation in the biotope

Sound attenuation from both live and artificial male advertisement calls showed similar patterns of attenuation in the Bald Hill field site (Figure 4.4). For reference, sound attenuation due to geometrical spread is given as a solid line, with dB

SPL at the source set depending on actual recorded values (85 dB from live male, 90 dB from artificial). Males in the laboratory respond to male call playbacks as low as 38dB SPL (unpublished data). However, in the field background noise of other calling Orthoptera was around 50 dB SPL at 8 kHz and the male call became indistinguishable from background noise at a distance around 10 m for the Bald Hill field site (Figure 4.4). Based on near neighbor distances, each male most likely hears conspecific calls of only the closest two males. Sound was attenuated approximate 20 dB SPL from the top of the plants to the ground. Males sing approximately 1.4m off the ground on goldenrod flowers and near the top of grass blades. When males called from shrubs, such as the silverberry, they mostly called from the exterior surface of the plant in a ring ~ 1.0 - 1.5 m off the ground. Therefore males appear to position themselves in the biotope in locations which allow their signals to travel further.

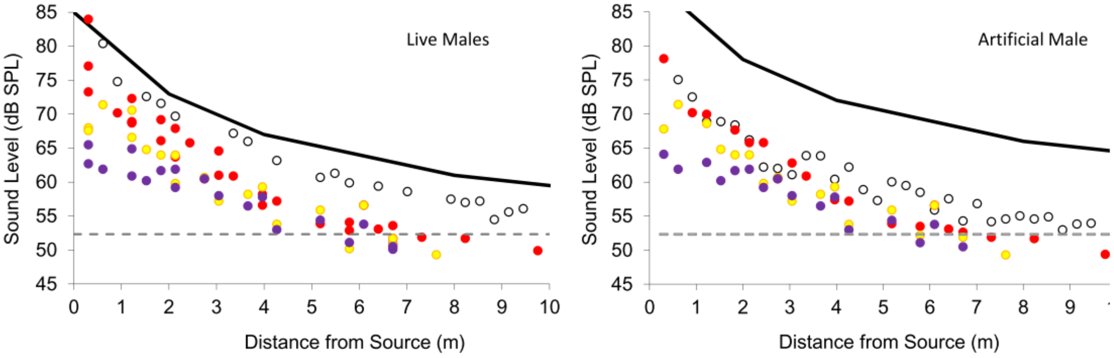


Figure 4.4. Male *S. pistillata* advertisement call attenuation for both live male calls and an artificial 90 dB SPL male call. Open circles indicate measurement along sound paths unobstructed by vegetation at a height of 1.5m. Closed circles indicate measurements taken within the biotope at 1.25m (red) at 0.65m (yellow) and at ground level (purple). Solid line indicates sound loss due to geometrical spread alone. Dashed line indicates average dB SPL of calling Orthoptera in the field.

Female response to sound level

The number of acoustic ticks the female gives in response to male phrases increases with increased syllable number in the advertisement call, as well as sound intensity (Figure 4.5). Females will respond to male calls as low as 32 dB SPL. Based on sound attenuation in the field, females would still respond to male calls when she is 10 m from the source for most male phrase lengths, however her strongest response would come with larger syllable numbers. Based on female response and male spacing, the female likely hears and responds to the calls of multiple males, and the strength of her response depends on the amplitude and length of the call. However, females are found at various levels within the biotope (personal observation) and her acoustic response is much quieter and briefer than the male advertisement call (~60-70 dB SPL at the source; unpublished data).Therefore only a few males would perceive her acoustic response.

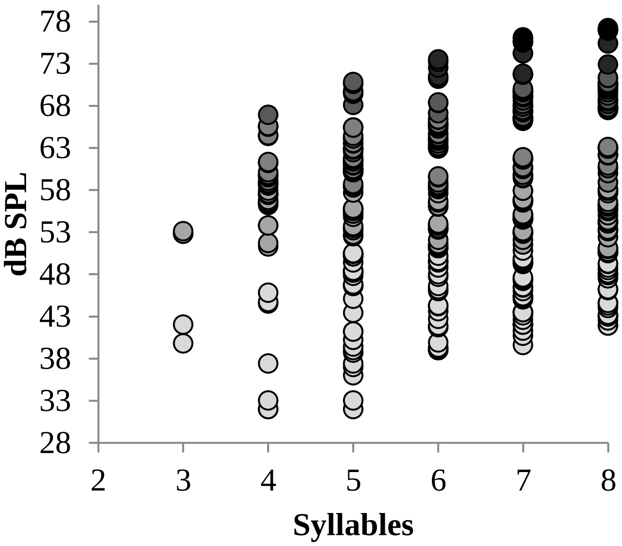


Figure 4.5. Female *S. pistillata* acoustic tick response to depends upon amplitudes of the male 3-8 syllabled phrase. Increasing darkness indicates an increase in tick number, from light gray indicating a one tick response, to the darkest gray indicating a 6 tick response.

Discussion

Male *S. pistillata* space roughly 5-8m from their closest male neighbor and likely can attend only to the call of their two closest neighbors due to the attenuated calls being lost in the noise. Though the identity of their neighbor varies from night to night and the spatial distribution of males within the field depends upon the structure of the local flora. Males were found to be aggregated within the more heterogeneous Whitted Rd field site and spaced randomly in the more homogenous Bald Hill site. The Whitted Rd site exhibited distinct areas of differing plant species which may have attracted males due to perch preferences and would explain their tendency to aggregate. In the bush cricket *Tettigonia viridissima* males spaced in a heterogeneous field site based on the distribution of the height of the vegetation and also exhibited aggregations (Arak & Eiriksson 1992). The random spacing in the Bald Hill site most likely reflects the homogeneous nature of the field, with many suitable perches spread throughout the field. Several katydids have been shown to aggregate based on the perceived sound levels of near neighbor males as seen in other chorusing katydids (Guerra & Mason 2005), and those that did tended to space randomly (Chamorro et al. 2007).

Male location in the biotope was mostly dependent on the available heights of the tall grass and goldenrod flowers. Preferred perch height is often found to be related to the height of vegetation for katydids that aren’t plant specific in their perch choice (Allen 1995; Chamorro-R et al. 2007). In the more homogenous of the biotopes studied here, all males were found to perch at the top of grasses and goldenrod. In the Whitted Rd site with a heterogeneous biotope, males were found on nearly all types of flora. In low vegetation areas, they were found primarily on the tallest foliage. When in large bushes and shrubby trees males rarely favored the highest point and remained approximately 1.5m off the ground. Because sound intensity lessened around ground level where foliage is most dense, males most likely favor remaining above or near the top of the average foliage height in order to suffer the least amount of attenuation of their advertisement call or the response of females. Due to the regularity in the vegetation of the Bald Hill site, the attenuation pattern around males is easier to predict. In a more complex environment as seen in the Whitted Rd site, attenuation properties are less predictable and highly dependent on the surrounding foliage (Thiele & Bailey 1980) which might explain why the largest deviation in neighbor distances was seen in this field.

In both fields most male *S. pistillata* called within 5m - 9m of their nearest neighbor male with variation in neighbor distance (±1-5 m) most likely due to behavioral and environmental reasons. Males receive cues on spacing distance based on dB SPL of either individual males or aggregate calls (Thiele and Bailey 1980) and there may be a threshold signal to noise ratio that males use to maintain certain distances (Greenfield & Snedden 2003; Guerra & Mason 2005; Chamorro et al. 2007). Males can also attend to differential attenuation of frequency components of the species' call to assess distance due to increased attenuation (Dadour 1989; Schatral and Bailey, 1991).

Males of *S. pistillata* increase the amplitude of syllables, both within a phrase and from the first phrases to the last in a bout. Later syllables in a phrase as well as later phrases will be perceived as louder by a receiver at a fixed distance. This, increased amplitude could simply be a result of the biomechanical mechanism that produces this unique counting sequence, but it could also be an active way for the male to successively sample for receivers at greater distances. Males or females responding to later phrase might inform the male of the distance at which the receiver is listening. Further explorations into the effects of amplitude on the active space around a male are necessary to understand the adaptive significance of increasing amplitude during a phrase and bout.

In the Bald Hill site, males exhibited low site fidelity and would often travel far from one night to the next. Though these recaptures are single time points, they illustrate the movement patterns and contrast with studies of some other species in which males were found to have stronger site fidelity (*Panacanthus pallicornis*: Chamorro-R 2007; *Docidocercus gigliotosi:* Lang & Römer 2008). In male *S. pistillata* the male is the sex that performs phonotaxis (Chapter 3), which may explain their low site fidelity. On one occasion, a female-male duetting pair was observed during a field night in the Whitted Rd field site. When the male heard the female calling from 4m away, he increased his rate of calling, and was very active, jumping from perch to perch. Although I couldn't hear the female, the male moved directly in her direction and found her on her perch. Once he found her, he stopped calling and proceeded to court the female. Therefore *S. pistillata* males do not defending a signaling position, but rather "chase down" responding females. This is similar to the behavior in other duetting species, *Amblycorpha parvipennis,* in which males also exhibit low site fidelity (Shaw et al. 1981).

To further complicate interactions among neighboring males, often males adjust their calls in the presence of females (Galliart & Shaw 1991). *S. pistillata* males alter their calls when in the presence of females by increasing their signal output in terms of number of syllables they produce per phrase (Chapter 3). Therefore if neighboring males perceived the call of a female there is likely an opportunity to compete both in terms of acoustic output as well as localization of the responding female. These interactions likely have a stochastic effect on the structure of the chorus. On one occasion in the Bald Hill field site three males were circling a single female, moving readily and signaling often. The female can discriminate among the callers based on their signal output, as females have been shown to vary the strength of her response to the male call (Chapter 2). An analysis in which a pair of males is subjected to the response of a female and their behavior is recorded is needed to determine if and how males compete in these situations.

Conclusion

Male *S. pistillata* were found to space in the field based on the vegetation height, perch availability, and sound attenuation of the neighboring calls. Males in a heterogeneous field site tend to aggregate more, and will chose perch sites which maintain the katydid’s height at ~1-1.5 m off the ground. Among more homogeneous vegetation, males space more regularly in the field and perch at the top of foliage, limiting the attenuation due to vegetation. In both fields males space so that they hear and interact with the two closest neighbor males. Female *S. pistillata* also produce a call which can be heard by several males and this leads to the chorus being stochastic with rival neighbor males changing from night to night.

CHAPTER 5

CHAPTER 5: EVIDENCE FOR EAVESDROPPING AND PRECOPULATORY ACOUSTIC MATE GUARDING PERFORMED BY *MALE S. PISTILLATA*

Abstract

Male *S. pistillata* produce an advertisement call consisting of a series of phrases, with an increasing number of syllables per phrase. A female responds with a call of her own by producing one to six brief ticks in a specific time window after his signal. When in an environment in which a male can hear both a female and male calling, he produces a previously un-described call consisting of a single tick after his calling phrase and during the time window that the female makes her acoustic response. Because male katydids have been shown to find it difficult to perform accurate phonotaxis to a female sound source when the sounds arrive from two different locations, a male tick might serve to confuse localization of the female for eavesdropping males. To test this prediction, males were first assessed for his ability attend to another male's duet and accurately locate the female. Once eavesdropping was established, we tested the possibility that the male tick served to mimic the female response and found that males exhibited a reduce ability to accurately locate the female. Therefore male ticks appear to act as a form of acoustic mate guarding of his temporary pair bond with the female.

Introduction

Alternative mating tactics are common amongst insects, especially conditional strategies in which males choose the alternative tactic according to their status, as a means of increasing their fitness. The most common alternative strategy is illustrated in the dung beetle, *Onthophagus* spp., in which large males exhibit the big-horned phenotype and fight for females, whereas small males lack the horns and instead sneak mating attempts (Cook 1990). Often in insects the variability found in phenotype is the result of larval diet and growth (Gross 1996).

Another alternative mating tactic common in orthopteran insects is interception, or eavesdropping. In this context the “fighter” male is the one broadcasting his signal, whereas the sneaker male is silent nearby the calling male and attempts to intercept any searching females. Though the males in the sneaker role can exhibit an alternative morph (Donelson & v. Staaden 2005), often the males lack any major phenotypic difference.

Systems in which both the male and female call in a duet are especially susceptible to their temporary pair bond being taken over by eavesdropping males. Female duetting katydids in the subfamily Phaneropterinae produce an acoustic tick in response to the male advertisement call (Chapter 2; Bailey 2003). The male call always precedes the female response and the timing of her response acts as a pre-mating isolation mechanism (Heller & v. Helversen 1986) and therefore the male is primed to listen and localize a female response falling in a specific time window (v. Helversen et al. 2001). Male duetting katydids have been shown to perform phonotaxis with high acuity for female signals arriving in this time window (Rheinlaender et al. 2007). The trigger in the male call that initiates the female reply varies by species (Korsunovskaya 2008) and in some species can be exploited by the eavesdropping male. For example, *Elephantodeta nobilis* are at the risk of a satellite interloper interjecting his own signal when the female expects the trigger in the focal male's call, which can shift her phonotaxis behavior (Bailey & Field 2000). In this species, the male exhibits no acoustic counter measures to circumvent the theft of his pair bond.

If the listener male can perceive the time window in which females respond to another calling male, he has as much information on the location of the female as the focal male and can move towards her. It has already been demonstrated that male katydids attend to the calls of their neighbors (Dadour 1989; Bailey & Field 2000; Guerra & Mason 2005). In several species of duetting orthopterans the male has been shown to perform phonotaxis to females responding to the calls of other males (katydid: Hammond & Bailey 2003; grasshopper: Otte 1972; Donelson & v. Staaden 2005). In situations such as these it would be highly beneficial for the male to exhibit some sort of pre-copulatory mate guarding to protect his investment.

Caedicia sp. is the first instance of recorded mate guarding via an acoustic signal in a katydid (Hammond & Bailey 2003). The male produces a series of ticks that have been shown to mask the location of the female from the eavesdropping male. Though males move toward females responding to the calls of other males, when the masking ticks occur the male performed no phonotaxis. Even when the female signal was 8 dB greater than the mask and occurred 50 msec after the masking tick, males were still unable to locate the female.

Species within the genus *Scudderia* also have the potential to exhibit acoustic mate guarding. There is evidence within the genus that males attend to the duets established by other males when the focal male was silent (Spooner 1964). Males of *S. curvicauda* and *S. texensis* respond to the calls of other conspecific males with a tick sound and there is preliminary evidence that the ticks serve to repel other males (Spooner 1964; 1968a). Males of *S. cuneata* and *S. furcata* both also produce ticks, except their ticks were occasionally produced after their own call, approximately when the female should tick (Spooner 1968a).

Male *Scudderia pistillata* also produce a sound around the same time as the female reply. During previous experiments (described in Chapter 3) in which males and female were allowed to interact, the male produce a new type of sound not heard in any other context. Males are stimulated to produce their advertisement call when they hear the call of another male (Chapter 3). However in the live interactions, the female can also hear and attend to a non-focal male sound. Occasionally when the focal male heard the female respond to other male's calls he produced a volley of tick sounds, during and after the advertisement phrase of the other caller. These sounds are similar to the "crackling" sound observed by Spooner (1964) which served to disperse aggregated males. These ticks were also heard with male *S. pistillata* when they are in close proximity and usually served to silence the calling male (unpublished data).

Once a duet was established and the male called to a responding female he occasional added a single tick during the time window in which a female was expected to respond. The male tick sounded similar to the female tick and to the observer it was hard to distinguish the two sources of sound without carefully attending to the direction of the source. Because of the timing and similarity of these acoustic ticks, we hypothesized that the male tick might serve to confuse localization of a female for eavesdropping males. Evidence for this function is seen in *Poecilimon ornatus* attending to the calls of two females. Males perform phonotaxis to a female tick when it arrives 40-140 msec from the beginning of his call. However, if two female ticks arrive during this time window, but from locations separated by 115°, the male chose an intermediate path between the two signals instead of attending to one or the other (v. Helversen et al. 2001).

In this study we first examine if males would eavesdrop on a duet and assessed the similarity between the male and female tick calls. Secondly, we tested the effectiveness of the male tick to act as a mimic for the female response and whether hearing a tick altered the behavior of the eavesdropping male. If male ticks serves to mask the signal (Hammond & Bailey 2003) or act as an aggressive display aimed towards eavesdropping males (Spooner 1968a), a focal male upon hearing these ticks should either not perform any directed movement towards the female or make a directed movement away from the male call. If the tick serves to confuse the localization of the female by serving as a mimic to the female tick, the male call should exhibit similar characteristics to the female call and should reduce an eavesdropping male's ability to successfully approach the responding female.

Methods

Specimens

Broad-Winged Bush Katydids, *Scudderia pistillata* Brunner 1878 (Tettigoniidae: Phaneropterinae), were collected during the summer of 2012 from old fields near Ithaca, NY on Connecticut Hill. A single female and seven males were collected as nymphs via sweep netting the first week of July. All individuals were raised and maintained similar to previous experiments (see Chapter 3) except each katydid was housed in its own mesh cage (12cm in diameter, 20cm in height). The seven males were allowed to interact acoustically and the female was maintained in isolation until experimentation.

Characterizing the male tick

Male acoustic ticks were recorded during male-female duets the previous summer (see Chapter 3). The characteristics of these ticks were analyzed using Audacity1.3.5 Cross-Platform Sound Editor (http://audacity.sourceforge.net/) except for sound imaging, which was performed using Raven Pro 1.3 (Bioacoustics Research Program, Cornell Laboratory of Ornithology, Ithaca, NY). Male call characteristics of frequency, duration, and timing from the offset of the male call was compared against these three characteristics of the female call, also recorded during male-female duets, via independent sample *t* tests in JMP statistical analysis software, version 10 (SAS Institute Inc., Cary, NC).

Male phonotaxis with and without male ticks

All phonotaxis experiments were performed in a 3m x 4m room on a circular paper arena marked with concentric circles radiating out from the center every 3 cm for a total radius of 36 cm. The arena was kept moist, as humidity was found to be important in releasing phonotaxis behavior. A male call was broadcasted from a speaker (80 dB SPL at source, 218Hz-20kHz, Omnitech portable mini speaker, Omnitech, Inc., Sioux Falls, SD) approximately 40 cm from the center of the arena. The female's cage was at a distance 115° away from the male speaker and their exact location around the edge of the arena varied from trial to trial. An observer sat nearby, and the position of the observer relative to the speakers was also randomized. Lighting was provided from a single dim red light positioned off the arena.

Each male *S. pistillata* was placed under a 25cm plastic dome in the center of the arena on a 10 cm tall paper perch. The male was allowed to acclimate under the dome for 5 minutes. A typical male 8 syllable advertisement call (Villarreal & Gilbert 2011) was played continuously with a 6 second delay between each presentation, to simulate the natural duty cycle of a male call, and the female was allowed to respond freely. The trial ended when a male crossed the perimeter of the arena and his location relative to the speakers and female was noted within 15°. Males that did not respond to the stimulus after 15 minutes were removed and retried again later.

Each male was subjected to two treatments. The first treatment was a synthetic male call played from a speaker with a live female responding, to assess if males exhibited eavesdropping behavior and performed phonotaxis to the live female. The second treatment, males were presented with a synthetic male call followed by a male tick from the same speaker. The treatments were randomized by flipping a coin for each male and every male was presented with both treatments in a single night. Because a female could respond to the focal male's call, the focal male was prevented from calling via misting from a water bottle. To ensure misting did not influence a male's behavior, males were also randomly misted in the other trial.

A male 8-syllable phrase was artificially created (see Chapter 2 for details) based on the average acoustic properties recorded during the male-female duets. To create the "with male tick" stimulus, a single male tick was extracted from recorded sound files and added to the male 8-syllabled phrase sound file. The male tick was placed in the sound file based on the average latency from the end of the male call and the average duration of the tick observed (0.507s latency with 0.049s duration).

For statistical analysis, the location of the female speaker was set at zero, and the male speaker set 250° for all trials regardless of their placement on the arena. The exit points relative to the female and male speaker, as well the time until exit, was recorded for each playback trial. The 15° bins were then re-binned to 45° for analysis due to the large number of zero bins. If males are eavesdropping on a duet, then we predict that males will chose to exit near the female signal, and used the V test to confirm if the males behaved as predicted (Zar 1996). If the male tick serves to confuse the location of the female for the eavesdropping male, then we predict the trial in which the male tick was present should confuse male phonotaxis – perhaps even attract males to the male generated tick – and therefore reduce the vector strength, leading to a non-significant vector length. The time it takes for a male to localize a sound could also be effected by the presence of a male tick, therefore the time parameter was compared between the two treatments using a non-parametric Wilcoxon Test using JMP statistical analysis software, version 9 (SAS Institute Inc., Cary, NC).

Results

Figure 5.1 provides example of a duet interaction in which a male produces a tick after his call. Of the 19 males recorded during live male-female duets, 17 produced at least one phrase that was followed by a tick. Of the 3907 total phrases produced in these interactions, 233 of them exhibited a male mimic tick (10%). Most males exhibited ~ 1-19% of their phrases followed by their own tick. Two katydid males were more prone to produce these ticks, occurring after 35% and 69% of their phrases. A male never responded with more than a single tick and the male tick rarely overlapped a female tick, regardless of how many ticks she gave.

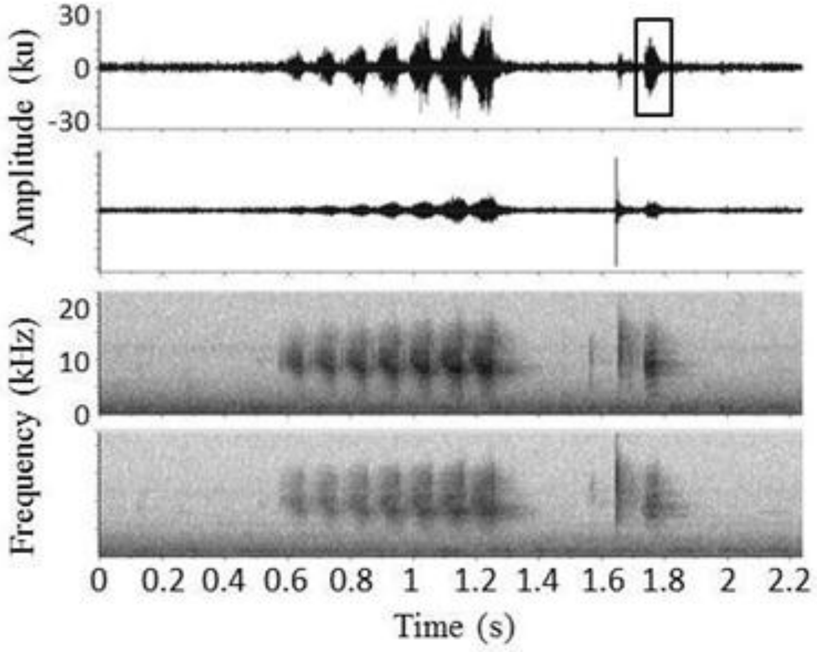


Figure 5.1. Mimic tick production by male *S. pistillata*. Upper waveform channel was recorded closer to the male caller and the lower waveform channel recorded closer to the female. The frequency spectrum is presented for each channel (Hann window; window length = 163 samples, 49.7% overlap). The black box indicates a male tick produced during the time window of a female response.

Both the male and female ticks are similar in their frequency spectra, both being broadband (Figure 5.1, 5.2). The timing of the male tick falls in the middle of the female response window (Figure 5.3). It is significantly after the first tick of the female response (Independent Samples *t* test: $t_{180} = 19.9$, $P < 0.0001$) and significantly before the last female tick (Independent Samples *t* test: $t_{391} = 9.7$, $P < 0.0001$). The duration of the male tick (0.049s ± 0.027s) was significantly longer than the female tick (0.015s ± 0.012s; Figure 5.4; Independent Samples *t* test: $t_{191} = 24.8$, $P < 0.0001$). Because on average female ticks are separated by 0.1 sec (Chapter 2), the male call typically fell between the first and second tick when the female ticked multiply.

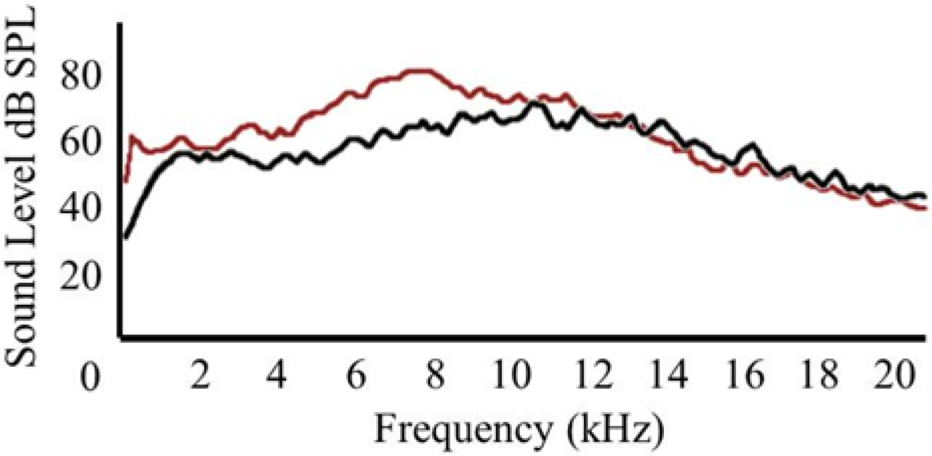


Figure 5.2. Frequency spectrum for male (red) and female (black) acoustic ticks.

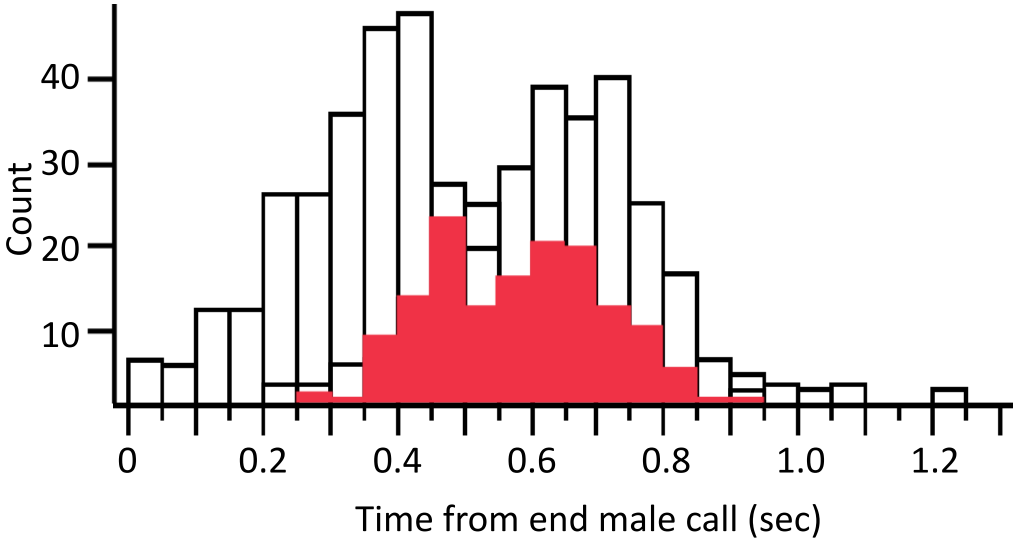


Figure 5.3. Histograms displaying the timing of the male tick (red) falling between the first tick and last tick of the female response (2 black histograms).

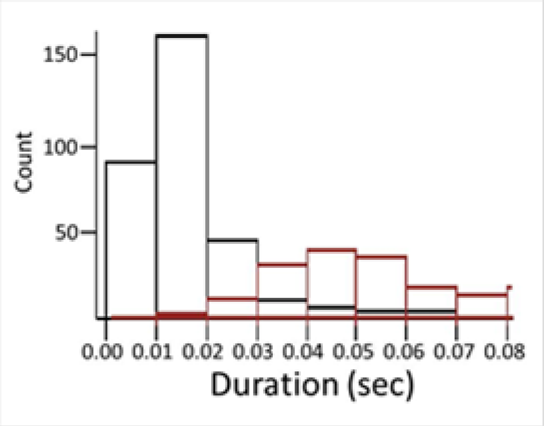


Figure 5.4. Duration of the male (red) and female (black) tick.

Male phonotaxis

Contrary to what Hammond and Bailey (2003) found for *Caedicia* listening to the male masking ticks, focal male S. pistillata in this study exited the arena regardless if he heard the female alone or with a male tick. Males orient to live females responding to the calls of another male (Figure 5.5, Left; V-test: N = 15, mean vector length = 0.69, $P < 0.005$). When the focal male hears a live female response as well as a male tick his orientation is disturbed (Figure 5.5, right; V-test: N = 16, mean vector length = 0.17, n.s.). The overall mean vector angle, though insignificant, points to an intermediate point between the male call and female response. Though the vector strength and angle is affected by the male mimic tick, there is no difference between the two treatments in the time it takes for the male to reach the edge of the arena (Wilcoxon Test: $X^2_1 = 0.13$).

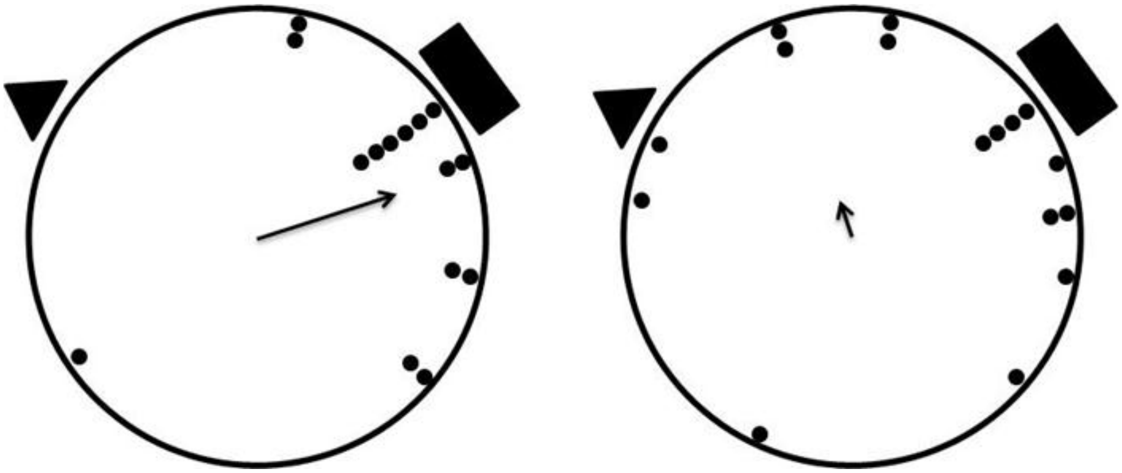


Figure 5.5. Location that males exited the arena relative to the male speaker (triangle) and live female (rectangle). Left panel shows trials in which the focal male heard a male call followed by a female response. The right panel shows trials in which the focal male heard a male call followed by a female response and a male tick. The arrow inside the circle indicates the mean phonotaxis vector. Each circle represents a single male exit point.

Discussion

Here we demonstrate the ability of male *Scudderia pistillata* katydids to eavesdrop on a conspecific courtship duet and assess the location of the female participant based on her response to the advertisement calling of the male participant. Only in *Caedicia* spp. has this ability been documented previously (Hammond & Bailey 2003). *S. pistillata* and *Caedicia* have previously been shown to have many sound characteristics in common, such as female variability in the number of ticks produced and latency until the onset of her response (Chapter 2). Hammond & Bailey (2003) hypothesize that *Caedicia* males were able to recognize the timing of the female's acoustic response to locate her, even though she was responding to another male. Male *S. pistillata* must also use a similar mechanism as a cue to female location.

Where *S. pistillata* and *Caedicia* differ is in the function of their acoustic tick.

The benefit of an acoustic mimic defense over a masking defense is that there is less of a cost in terms of the male masking not only the female signal for eavesdropping males but also from himself. During duetting, the situation in which these sounds were first discovered, males produced mimic ticks and were still able located the female. The male's mimic tick also rarely overlapped with the ticks of a female's response, leaving auditory time in which the male could hear her call. In male crickets, the tympanum responds to sounds not generated by the male while he is singing via sound processing in the peripheral auditory system (Poulet & Hedwig 2001). This mechanism along with the brevity of the male signal could explain how males can generate sound without risk of missing the female's response.

Male ticks are not a perfect mimic for the female tick response. Though the difference in duration between a male and female tick is small, the duration of his response is longer than it should be if he wanted to mimic the characteristics of the female. Males produce their tick sound by flicking their wings in such a way that only a few teeth are stroked (Spooner 1968a). Because the male stridulatory structure is more developed than the disorganized teeth typically present in the female (Nickle & Carlysle 1975), the subtle movement needed to produce a tick sound may be difficult for the male to perfect. Because the timing of the female tick is so important in recognition, it is likely that duration has little effect on male response, as long as the sound is brief. Spooner (1968b) attracted male katydids to himself by hitting the bottom of a glass jar with a knife at approximately the species-specific latency, therefore it is likely males are fairly relaxed in what they require out of the female tick response beyond its timing.

Why should a female allow a male to eavesdrop on her courtship conversation and then mate with her without expending any acoustic energy? Though work on eavesdropping as an alternative mating tactic often doesn't consider the response of the female, there are several reasons why the female might allow a sneaker, and her role in the maintenance and success of sneaker males can be substantial. There are situations in which a female might prefer to mate with a sneaker male to whose call she is not attending. For example, she might want competition between males arriving in response to her signal in order to assess their quality, or if the genetic diversity of her offspring is important she might want to mate with multiple males of varying phenotype (Reichard et al. 2007). However, because female katydids signal at night and at potentially large distances, it can be difficult for the female to know whether the male that is approaching her is also the male with whom she has been duetting. Further understanding how a female might respond to multiple suitors is therefore important in understanding how males might compete for a nearby female and how her behavior might influence their interaction.

Based on this study it appears as if a male produces a mimic tick in order to protect the temporary pair bond he has initiated with a female. The male is taking advantage of a maladaptive phonotactic response exhibited by males (v. Helversen et al. 2001) in order to confuse localization of the signaling female. The cost of producing these male ticks appears to be low, as they likely hear the female's response without interruption. The development of mate guarding tactics is some indication of the strength of the effect of this alternative tactic. However, how large an impact eavesdropping has on the mating system requires further analysis.

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