

THE EVOLUTION OF COMPLEX CALLS

IN MEADOW KATYDIDS

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The undersigned, appointed by the dean of the Graduate School, have examined the dissertation entitled

THE EVOLUTION OF COMPLEX CALLS
IN MEADOW KATYDIDS

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and hereby certify that, in their opinion, it is worthy of acceptance.

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Professor Patricia Friedrichsen

For my family

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THE EVOLUTION OF COMPLEX CALLS

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ABSTRACT

Meadow Katydids (genera *Orchelimum* and *Conocephalus*) are a specious group often are found in habitats with several species within the group living in sympatry. They produce complex calls with two distinct phrases, “buzzing” and “ticking”. These two phrases are organized in a highly diverse way across species. This diversity of call patterns in Meadow Katydids provides an excellent opportunity to comparatively study the evolution of complex calls.

We tested the function of the two call phrases in male-male interactions. we examined the structure of the male call in the context of communities to identify candidate traits (i.e. traits likely involved in reproductive isolation). We constructed a molecular phylogeny from twenty species of Meadow Katydids, and examined the phylogenetic signal within call traits. The results of all of this taken together suggests ticking evolved in the context of male-male interaction, buzzing has been important for diversification, and in some species females have co-opted the tick to also function in reproductive isolation.

Importantly, we have also designed and field-tested a plan to use Meadow Katydids as tools in primary, secondary, and post-secondary classrooms/laboratories.

Chapter 1

THE FUNCTION OF MULTI-COMPONENT MEADOW KATYDID CALLS IN MALE-MALE INTERACTIONS

Introduction

Acoustic communication functions mainly in social contexts, particularly for attracting females for reproduction, and in male-male interactions (review in Gerhardt and Huber 2002). Insects and anurans encode the information relevant to these contexts in a multitude of signal components. The diversity of these components might, or might not, reflect their potential roles in these complex social situations (Greenfield 2002).

Advertisement calls, often highly stereotyped, function primarily in male-female communication. However, they also regularly play a role in male-male interactions. In crickets, for example, males show phonotaxis to other males' advertisement calls. This double function of the advertisement call is widespread, occurring in frogs (Sullivan and Wagner 1988, Wagner 1989, Rand and Ryan 1981, Ayre et al., 1984), toads (Harrison and Littlejohn 1985, Gerhardt 1994, Sullivan and Leek 1986), and crickets (Alexander 1961). In some groups, advertisement calls are even the only signal produced (e.g. Tettigoniidae, Shaw et al. 1982).

In many insect and frog groups, male-male interactions involve discrete signals or signal components intended primarily for other males. For example, in some systems aggressive calls are produced if other males approach (Wells & Schwartz 1984, Schwartz 1989, Adamo and Hoy 1995), or as part of elaborate dominance interactions (Simmons 1986, Littlejohn et al., 1985, Lopez et al., 1988, Schwartz 1989, Brenowitz and Rose 1999). In species with complex advertisement calls, specific signal components may function predominantly in male-male interactions. In the Coqui frogs' two-phrase 'Co-Qui' call, the first phrase 'Co' is used in male-male territorial encounters with the 'Qui' phrase being directed at females (Narins and Capranica 1978). *Ligurotettix planum* grasshoppers produce a two-phrase call, with one phrase ('rasps') used in territorial encounters and the other ('shucks') in attracting females (Greenfield and Minckley 1993).

These discrete call components intended for males may be co-opted into the male-female advertisement call, either increasing the attractiveness or even being necessary to elicit female responses (Berglund et al., 1996, Borgia 2006, Borgia and Coleman 2000, Greenfield 2002). Tungara frogs, for instance, have two distinct phrases. One phrase is necessary and sufficient for female phonotaxis, and the other enhances attractiveness to the call (Rand and Ryan 1981). Fisher (1930) suggested that complex signals like these can evolve by co-opting components from different behavioral contexts such as male-male interactions (War Propaganda Model). Ultimately, to reconstruct the evolutionary

history of these complex calls, elucidating the function of different call components is necessary (Laidre 2012).

Within katydids, (Orthoptera, Tettigoniidae), males typically produce only one simple call that is intended for both males and females (Gwynne 2001). Complex calls are, with few exceptions, limited to the subfamilies Phaneropterinae and Conocephalinae (Heller et al., 2015, Morris and Walker 1976). Simple calls have multiple functions, including advertisement calls for females, giving spacing cues to calling neighbors, and eliciting aggressive interactions between males (Brush et al, 1985, Nityananda and Balakrishnan, 2008, Shaw et al., 1982, Meixner and Shaw 1979, Shaw et al., 1981).

One subfamily with complex calls are Conocephalinae (Naskrecki 2000, Alexander 1975). The high diversity in their call structure suggests there could be diversity in call function. In this subfamily, Meadow Katydid (genera *Orchelimum* and *Conocephalus*) produce complex calls with two distinct phrases, “buzzing” and “ticking” (Fig. 1). These two phrases are organized in a highly diverse way across species. At one extreme, some species have highly stereotyped calls, such as *Orchelimum nigripes* which invariably produce 3-5 ticks before each buzzing phrase (Feaver 1983). These units are then repeated with high stereotypy. On the other hand, some species (e.g., *O. vulgare*), have a much more variable arrangement of ticking and buzzing, at times producing only buzzing phrases of varying duration, at other times buzzing phrases interspersed

with ticking, and often ticking alone. Most species lie somewhere on the continuum between these highly stereotyped and highly variable examples (Morris and Walker 1976, Walker 2010). The diversity of call patterns within this speciose group provides an excellent opportunity to study both the function and the evolution of complex calls.

Here, we evaluate whether one, or both, of these call phrases function in male spacing behavior. Meadow Katydid form dense aggregations (Morris et al., 2018, Morris 1967, Alexander 1972) and space from each other within these aggregations, often without using direct physical interaction (Guerra and Mason 2005, Feaver 1977, Feaver 1983). We test the function of the two call phrases, buzz and tick, in male-male spacing across several species. We used five species with varying degrees of call variability and a sixth species with calls that lack a ticking phrase. We examine how changing the phrase-composition of conspecific calls affects the distance males choose to settle from a neighbor.

The diversity in call structure across these species suggests the two phrases might function in diverse ways. If the stereotyped call of *O. nigripes* is an advertisement call that incorporates both phrases in attracting females, a stimulus including both phrases might elicit a different spacing strategy from males than in a species such as *O. vulgare*, with highly variable calls. We assume here, for sake of argument, that buzzing in Meadow Katydid is homologous to the simple call of most katydids, and the ticking phrase evolved

as aggressive calls that were co-opted into the advertisement calls. Based on this assumption we hypothesize that, in male-male interactions, (1) the buzzing phrase has attractive and repelling effects, (2) the ticking phrase has mostly repelling effects, and (3) that the relative role of the two components for spacing differs among the species, depending on their call structure. We quantify the distance males spaced from a speaker broadcasting various call models, and interpret the results in the context of the evolution of call complexity in this group.

Methods

Animals

We collected males of six species of Meadow Katydid (Tettigoniidae: Conocephalinae) as nymphs, or adults early in their breeding season.

Orchelimum vulgare Harris 1841, *O. nigripes* Scudder 1875, and *Conocephalus brevipennis* (Scudder 1862), were all collected in Boone County, Missouri, USA.

Orchelimum fidicinium Rehn and Hebard 1907 were collected in Cape May County, New Jersey, USA. *Orchelimum agile* (De Geer 1773) were collected in Somerset County, New Jersey, USA.

Conocephalus cinereus (Thunburg 1815) were collected from a population living in the Sachs Butterfly House in St. Louis, Missouri, USA. This population was likely introduced from Florida. Individuals

were identified after Rehn and Hebard (1915 a,b) and Walker (2010). Insects were kept in plexiglass/screen cages (40x40x60cm) in an environmental chamber at 22-26°C, 40% relative humidity, at a 14:10 h light:dark cycle. They

were provided with grass cut from local grasslands, apples, Fluker's high-calcium

cricket feed, and Purina Pro Plan Adult Cat Food. Collection took place during July and August in 2015 and 2016.

Stimulation

Stimuli were made from representative male calls of each species. Calls of ten males were recorded in an anechoic chamber at an ambient temperature of $25\pm 1^{\circ}\text{C}$. The specimens were placed in small screen cages 15cm in diameter. The microphone was placed 20cm dorsal of the calling male. Calls were recorded with a 1/4in (6.3mm) free field microphone (GRAS 40 BF; Holte, Denmark), amplified (GRAS 26 AC and 12 AA), high-pass filtered (1000Hz, Krohn Hite 3202; Brokton, MA, USA), and digitized using a custom-made A/D converter system (16 bit resolution, 250kHz sampling rate). This setup provided a flat ($\pm 1\text{dB}$) frequency response in the range from 2kHz to 70kHz. We selected 10s of continuous calling representative of each species' typical call, as revealed by our recordings and Walker (2010) (Fig.1).

Meadow Katydid produce two distinct call phrases. The sound pulses, produced during the closing movement of the forewings, are similar in envelope and spectrum during both phrases, but the temporal pattern differs distinctly between them. During buzzing phrases, pulses are repeated at a regular, predictable pulse rate. During ticking phrases, the pulse rate is slower and typically less regular. Values of temporal parameters (e.g. buzz duration, buzz period, number of ticks, etc.) can be highly variable (Walker 2010, Morris and Walker 1976,

Alexander et al., 1972, Naskrecki 2000). The selected calls of all species except for *O. fidicinium* included ticking and buzzing phrases and were used as stimuli in the 'buzz and tick' treatment. To generate stimuli for 'buzz only' and 'tick only' treatments we replaced either ticking or buzzing phrases with silence. We never observed ticking in *O. fidicinium* in the field or during our recordings. In this species, we selected the representative call ('buzz only') with a silent phrase similar to that of the other species. To build an artificial ticking phrase for this species we isolated pulses from their buzzes and inserted a silent interval that resulted in a tick period that was five times the pulse period found in the buzzing phrase. Males of all species were tested in four randomized situations: (1) no-stimulus (silence), (2) buzzes only, (3) buzzes and ticks, and (4) ticks only. Stimuli were continually looping and presented for 15 minutes.

Arena and experimental protocol

Experiments took place in a 120cm square arena. The arena walls were 9cm tall, and the arena was covered with fine fiberglass screen secured at the top edges of the walls. On top of this screen was a 5cm grid pattern made with fine thread. A loudspeaker was placed in one corner of the arena. We released individual males in the arena (under the screen) in the corner opposite to the loudspeaker position. Males moved along the underside of the screen top. Most males started to call during the 15 min of stimulation, indicating that they likely interacted with the stimulus. If the male was calling after 15min of stimulation, we considered him responsive and recorded his position within the arena. Otherwise, we did not

use the trial. We tested each male four times, once with each stimulus. Between trials, males had at least one hour of rest. Speaker position (always in one of the four corners, and always opposite the release point) and the stimulus sequence were randomized. We tested 20 individuals with the four stimuli in all species except for *N. cinereaus* (N=19).

Analysis

The position of the male at the end of the experiment (i.e. 15 minutes after release) was photographed and measured with an accuracy of ± 2 cm. To visualize the spacing of males in the arena, we aggregated the position data from each treatment and plotted heat maps (Fig. 2) of the Two-Dimensional Kernel Density Estimation, using the MASS Density package in R studio (Venables 2002). The Z-parameter was held constant for each output file so that the heat maps are comparable. We converted the XY positions to distance-from-loudspeaker values and ran an ordered logistic repeated measures model in SAS. This model allowed us to test if individuals of a given species in each treatment were at a significantly different distance from the speaker. The overall results were output as type II tests of fixed effects; post hoc tests for interaction results were given as simple differences of least squares means adjusted for multiple comparisons using a bonferroni correction. Tests for skewness and kurtosis did not reject normal distribution of the data. To be conservative, however, we present data as box and whisker plots.

Results

During the control (silence) treatment, males of the six species were widely distributed in the arena and did not aggregate at a specific distance to the loudspeaker (Fig. 2a-f, left panels). The mean distance to the loudspeaker was skewed slightly towards the corner they were released from, opposite the loudspeaker (Fig. 3).

Four species (*C. brevipennis*, *O. agile*, *C. cinereus*, and *O. vulgare*) showed a similar pattern of the aggregated x-y position data across the three treatments (Fig. 2, a-d): During stimulation with buzzes-only, males were closest to the loudspeaker. When stimulated with ticks only they were furthest away. The mean distance was intermediate during the buzz+tick treatment.

An ordered logistic regression using repeated measures (detailed test results in Table 2) indicated that positions of all four species during 'buzz-only' were significantly closer to the loudspeaker than during the control (32-64cm for the four species, Fig. 3). Distances during 'buzz+tick' were significantly farther away than during 'buzz only' stimulation (60-100cm, Fig. 3), but not farther than the control. During stimulation with ticking-only, males of these four species settled at significantly farther distances (118-145cm, Fig. 3) than during the buzz-only and buzz+tick treatments. In all species, except for *C. cinereus*, the median position during the tick-only situation was also significantly farther away from the loudspeaker than during no-stimulation.

Male calls of *O. nigripes* have a fixed pattern of short ticking phrases preceding buzzes. In this species, males settled closest to the loudspeaker during the buzz+tick treatment (median = 69 cm). During the 'buzz only' treatment, they settled a median of 119cm from the loudspeaker (Fig. 3) with a trend that looked similar to the 'tick-only' treatment (Fig. 2 e). During the 'tick-only' treatment males settled a median of 126cm from the loudspeaker (Fig. 3). The distances males settled from the loudspeaker did not differ significantly from the control treatments for either 'buzz-only' or 'tick-only' treatments (Table 2).

Males of *O. fidicinium* do not produce any noticeable ticks. They positioned farther away from the speaker during all three treatments than they did during silence (median distances of 118cm, 134cm, 129cm during buzz only, buzz+ tick, tick only, respectively). These differences, however, were only significant for the two stimuli that included ticks (i.e. buzz+ticks and tick-only, Table 2).

Discussion

We tested how the different call components influence male spacing behavior in Meadow Katydid species. The ticking phrase alone repelled males of all six species. Individuals of four species with typical call patterns (*C. brevipennis*, *O. agile*, *C. cinereus*, and *O. vulgare*) settled near to the speaker playing the buzzing phrase alone while the two phrases together produced an intermediate response. Spacing behavior of the two species with alternative call characteristics (one with high stereotypy, *O. nigripes*, one lacking ticks, *O. fidicinium*) differed.

Calling males may form aggregations for a variety of reasons: increasing their overall attractiveness to females (Alexander, 1975; Höglund & Alatalo, 1995), lowering the risk of predation (Brunel-Pons et al, 2011), and/or resulting from habitat structure (Eriksson et al.,2013). In Meadow Katydids, males aggregate densely by approaching conspecific calls (Morris & Fullard, 1983, Guerra & Morris, 2002, Guerra & Mason 2005). Within aggregations males settle at some distance from other males. This requires male calls to both attract and repel other males, with the distance they settle resulting from the collective outcome of the two functions. Attraction and repulsion can be caused by a single call component, as some species (e.g. Tettigonia) use a simple one-phrase call to determine spacing within groups (Arak and Eriksson 1992).

When interpreting the functions of different call components in species with complex calls (e.g. the two phrase calls of Meadow Katydids), it is often argued that different components have different intended receivers (Narins and Capranica 1978, Greenfield and Minckley 1993). For example, in Meadow Katydids the time spent ticking increased while buzzing decreased during male-male interactions, leading many to suggest that ticking is intended for males and buzzing for females (Feaver 1983, Feaver 1977, Alexander 1961, Gerhardt and Huber 2002). We, however, found that both phrases modified male behavior, suggesting a more complex picture than a simple dichotomy of function.

In four species, (*C. brevipennis*, *O. agile*, *C. cinereus*, and *O. vulgare*) buzzing was attractive to other males, suggesting an important role in forming aggregations. Males sat closest to the buzz only stimulus, but did not directly approach the speaker. Unlike female behavior to attractive calls, males didn't reach the speaker, but rather settled near to it. This indicates that these calls have an equilibrium of attraction and repulsion. In this context, the buzz is similar in function to the complete call of most other Tettigoniids.

We found that ticks function to repel males in all species tested. Adding ticks to the buzzing phrase modified male behavior causing an increased distance to the speaker. This is further highlighted by the tick only treatment, which led to the largest spacing from the speaker. It is not clear from our data whether ticks are also attractive from some distance. As the males settled near the arena wall opposite the speaker, we could not distinguish whether an equilibrium of attraction and repulsion exists beyond the arena walls or whether repulsion alone pushed males near to the wall. A larger arena is needed to assess this.

A trade-off exists between the benefits of calling in an aggregation and the costs of calling close to competitors (Brunel-Pons et al, 2011, Hartbauer et al., 2014). Whatever the benefits of forming an aggregation, calling males that are positioned very close to other calling males might experience lowered mating success. Being near to another caller lowers the area that a female perceives a

focal male as being the loudest. It can also make it impossible to adjust call timing, an important factor for females of some species that prefer chorus leaders, or even ablate important temporal parameters entirely (Arak 1988, Arak 1983, Nityananda and Balakrishnan, 2008). Ticking might have evolved as an aggressive signal to counter the aggregative effect of the buzz at this small spatial scale. At this scale Arak et al. (1990) have suggested individuals would benefit from spacing apart, rather than clumping together. So, a call trait such as ticks that function to repel neighbors would be selected for.

Orchelimum fidicinium, the species lacking a natural tick, spaced at a similar distance from the loudspeaker in all three treatments. Adding ticks to the buzzing phrase did not significantly change the spacing distance, but ticking alone also resulted in repulsion. This alternative response could have something to do with their unique natural history. They are a saltmarsh specialist living in a homogenous environment with little acoustic competition. This could reduce the pressure to aggregate; indeed we did not observe well defined groups at the collection site.

So, in a species without ticks, habitat relieves the need to form aggregations and males are found evenly spaced. This would reduce the selection pressure on increasing the distance to nearby neighbors that is needed at smaller scales and allow for the loss of the ticking part in their standard calls. The absence of ticks is thus in agreement with the phrase having evolved in the context of within-group

spacing.

Orchelimum nigripes, the second species with an alternative response, has incorporated the ticking phrase into a fixed call pattern with 3-7 ticks always preceding a fixed-duration buzz. The stereotypy of this fixed pattern suggests importance for selective phonotaxis in females (Gerhardt 1991, Helversen and Helversen 1994). Indeed, Miller (2010) showed that the ticks significantly increase attractiveness for females when added to a buzz-only call model. Our results suggest male attraction is strongest for the complete pattern – paralleling female preferences. While the ticking has seemingly become part of the 'advertisement' call to attract females (and males) it may still serve in male-male interactions; *O. nigripes* males do indeed produce ticking alone, however, buzzing always occurs together with ticks (Feaver 1983).

Here we found evidence that both call phrases contribute to male-male spacing. Ticks function to repel males in every species tested, even in a species lacking the phrase. Buzzing functions to attract males in most species. The interaction of these two phrases and the parity of their functions varied between species, depending on how their call is structured. The data presented here, paired with a deeper phylogenetic understanding and more extensive female-preference-tests, have the potential to reveal much about the evolutionary history of this complex trait.

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Figures

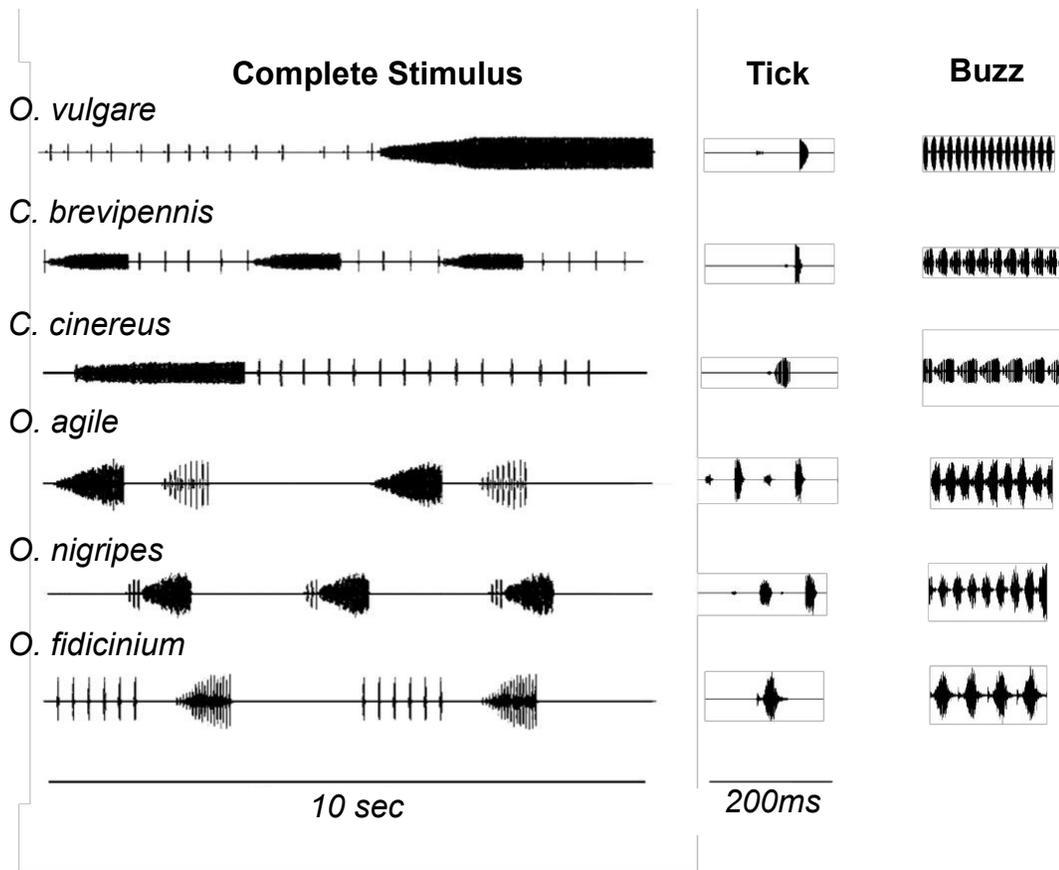


Figure 1

Temporal pattern of stimuli used during spacing experiments

Oscillograms on the left show the temporal pattern of the 10s stimulus selected from the call recordings. This was used as stimulus during the playback experiments with the six species. The stimuli shown were repeated without additional pause. For the Tick and the Buzz treatments, the appropriate phrase was replaced with silence. Oscillograms on the right show pulses during 200ms of the ticking and buzzing phrases. Note the different time scales left and right

Figure 2

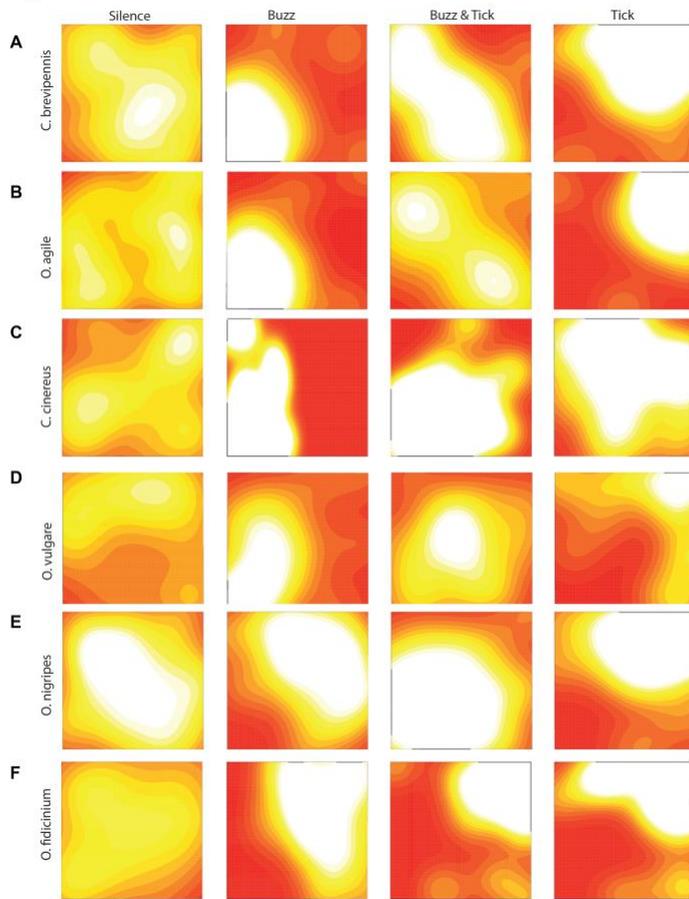


Figure 2

Distribution of males during spacing experiments

Heat maps constructed from aggregated position data for each species by treatment. White indicates individuals are found in higher density and red indicates lower density. In each map, the release point is in the top right corner and the loudspeaker in the bottom left corner.

Figure 3

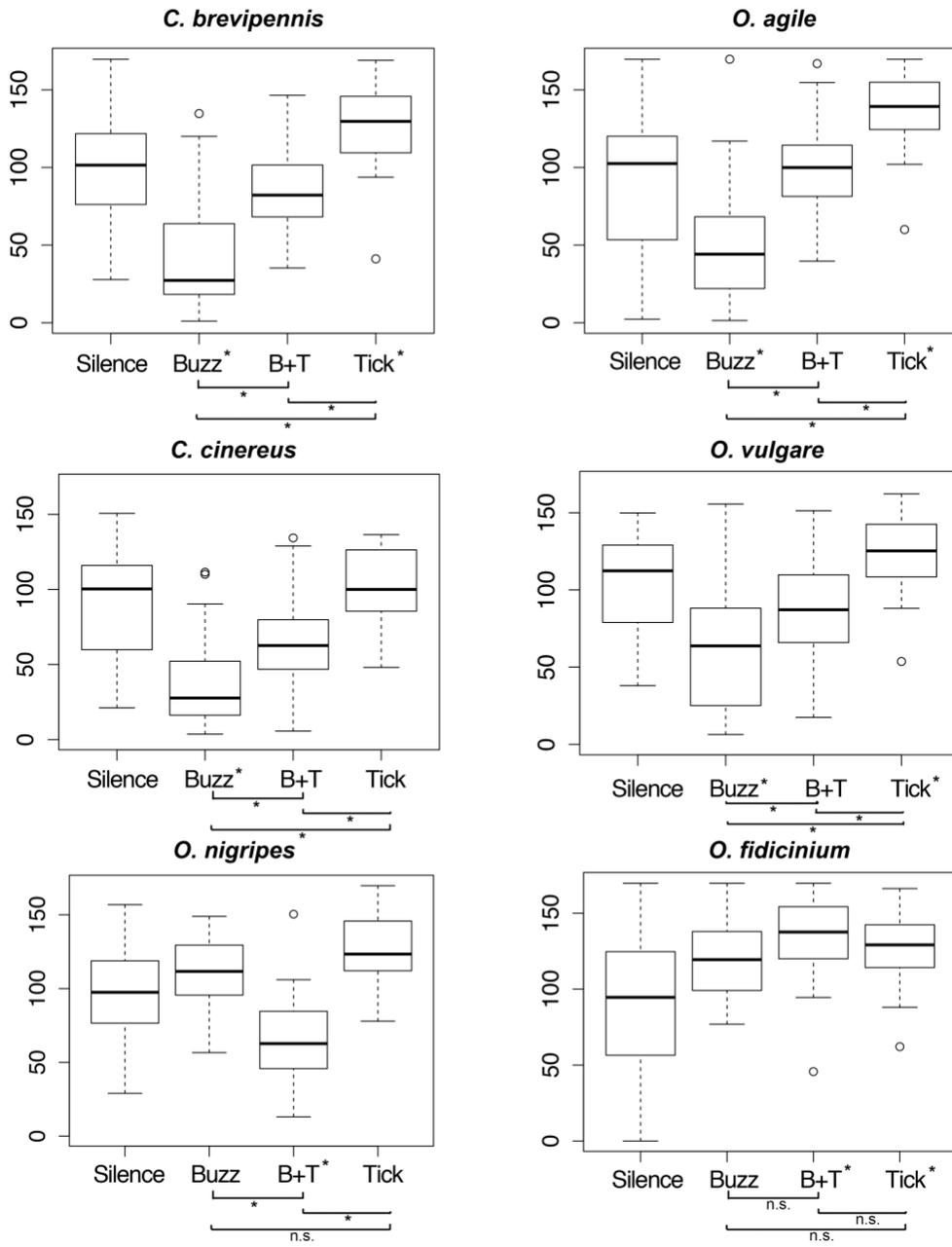


Figure 3

Distance-from-speaker plots

Box and whisker diagrams of the distances males settled from the loudspeaker during treatments denoting median, 25th-75th percentile and range. *asterisk next to treatment name denotes significant difference from the 'Silence' treatment (if no asterisk, no significant difference from Silence was found). On brackets, *asterisk denotes significant difference between the two bracketed treatments; 'n.s.' denotes no significant difference was found ($p > .05$).

Table 1

Call trait values

Actual values of the stimuli shown in bold. Average values for ten individuals are given in adjacent columns. *values that have *Coefficients of Variation* above 50%

	Pulse Period		Tick Period		Buzz Duration		Buzz Period	
<i>O. nigripes</i>	24	25	67.9	68.5	1360*	928	3015	3001
<i>C. brevipennis</i>	19	20	456	452	1,321	1381	3189	3211
<i>O. vulgare</i>	12	12	552*	424	4219*	4446	8905*	10000
<i>O. agile</i>	24	24	128*	93	1237	1138	5200	5242
<i>C. cinereus</i>	32	33	390	455	1605*	2589	878	858
<i>O. fidicinium</i>	49	51	----	258	964	935	4991	5006

Table 2

Significance of spacing results by species and treatment

Results of within-species treatment x treatment Ordered Logistics Regression using repeated measures run in SAS. P-values to the right of the diagonal, test statistic (t value) to the left. All reported P values are adjusted for multiple comparisons: Bonferroni.

<i>O. vulgare</i>	Silence	Buzz	Buzz + Tick	Tick
Silence		.0027	.7988	.0391
Buzz	3.53		.03316	<.0001
Buzz + Tick	.5	4.02		.004
Tick	3.09	5.36	3.43	

<i>O. agile</i>	Silence	Buzz	Buzz + Tick	Tick
Silence		.0003	.7944	.0002
Buzz	3.67		<.0001	<.0001
Buzz + Tick	.36	4.03		.0004
Tick	3.73	7.12	3.59	

<i>C. brevipennis</i>	Silence	Buzz	Buzz + Tick	Tick
Silence		<.0001	.9391	.0449
Buzz	4.66		.0033	<.0001
Buzz + Tick	-1.42	3.48		.0004
Tick	2.72	6.89	4.01	

<i>C. cinereus</i>	Silence	Buzz	Buzz + Tick	Tick
Silence		.0013	.9118	.3932
Buzz	3.73		.036	<.0001
Buzz + Tick	-1.44	2.86		.0063
Tick	1.85	5.46	3.3	

<i>O. fidicinium</i>	Silence	Buzz	Buzz + Tick	Tick
Silence		.373	.0023	.0054
Buzz	-1.87		.3647	.6492
Buzz + Tick	3.57	1.88		1
Tick	3.34	1.61	-.29	

<i>O. nigripes</i>	Silence	Buzz	Buzz + Tick	Tick
Silence		1	.0261	.452
Buzz	-1.13		.0005	1
Buzz + Tick	2.87	4.00		<.0001
Tick	1.78	.67	4.61	

Chapter 2

IDENTIFYING CANDIDATE TRAITS IN THE CALLS OF MULTI-SPECIES ASSEMBLIES OF MEADOW KATYDIDS

Introduction

Katydids generally have simple, stereotyped calls (Walker 1974, Alexander 1972, Walker 2010). These calls are made by moving a scraper located on one wing across a file located on the other, causing a mirror unit located on the wing to vibrate and radiate sound (Greenfield 1997). Each wing movement produces a single 'pulse' of sound (Gerhardt and Huber 2002). Katydid species typically have a stereotyped pulse period, and they string these pulses together into either a continuous call or rhythmically repeated chirps (discontinuous calls, Walker 2010).

Meadow Katydids, katydids in the genera *Orchelimum* and *Conocephalus*, have calls with a complex structure, compared to many other Tettigoniids (Walker 1976). They have two phrases, ticking and buzzing. The buzzing phrase is comprised of a string of pulses that come at consistent and predictable rates. The ticking phrase is made up of pulses that come at much slower and often irregular rates (Thomas and Alexander 1962). These two phrases are organized in a highly diverse and variable way. Some species consistently make a set number of ticks immediately prior to buzzing. Other species arrange ticks variably: everywhere from producing bouts that are impressively irregular and

long, to not ticking at all (Alexander 1956, Morris and Walker 1976).

As in most katydids, *Orchelimum* and *Conocephalus* calls are produced by males and function in large part to attract females for mating (Alexander 1956). The parameters implicated in female call recognition in katydids vary. We know that in just one genus, *Neoconocephalus*, females base mating decisions on a variety of traits, including pulse rate, pulse pattern, spectrum, second order call structure, or some combination of these (Schul et al., 2014). This group exemplifies the notion that mate-recognition signals often differ qualitatively, across several traits within a group. However, there are also several examples of arthropods in which closely related species differ mainly along a single continuous trait parameter (Shaw 2000, Greenfield 1983, Alexander 1956, Walker 1964).

Acoustic signals carry an array of information that encodes species identity, individual quality, individual identity, location, and more (Review in Gerhardt and Huber 2002). This information is carried in a variety of traits. Predicting where the information pertinent to premating-isolation and species-identity will lie is challenging. It is made more difficult in Meadow Katydid by the production of two phrases, which add another set of potentially synergistic call traits for male-female and male-male communication.

We can, however, leverage what we know about community composition to make predictions. Within a community, a call trait functioning in reproductive

isolation is expected to rarely overlap with those of other species. Such an overlap could cause interference and inhibit mate recognition (Schwartz and Wells 1983, Romer et al., 1989, Greenfield 1998, Paez et al., 1993). The distribution of male call traits also reflects the selective pressure (or lack thereof) put on them by female call preferences. Traits that establish diversity are typically under stabilizing selection (Helversen and Helversen 1994) and we can find signatures of that selective force by looking at the relative amount of variation in call traits (Gerhardt 1991, Gerhardt and Huber 2002).

Therefore, we hypothesize that a quantitative analysis of call parameters in Meadow Katydid will reveal traits with little within-species variability and little overlap within communities. These two characteristics will reveal candidate-traits that are likely to be involved in the mate-recognition system of female Meadow Katydid.

Here we investigate the call structure of fourteen species of Meadow Katydid, representing four communities, at various temporal levels. We measured each species' trait variation at the level of the buzzing phrase structure, the ticking pulse period, the buzzing pulse period, and the impulse period within a pulse. See figure 1 for terms. These parameters were put into the context of the communities these species occupy to identify traits of interest.

Methods

Animals

We collected males of fourteen species of Meadow Katydids (Tettigoniidae: Conocephalinae) as nymphs, or adults early in their breeding season.

Orchelimum vulgare Harris 1841, *O. nigripes* Scudder 1875, *O. silvaticum* McNeill 1891, *Conocephalus brevipennis* (Scudder 1862), *C. fasciatus* (De Geer 1773), *C. strictus* (Scudder 1875), and *C. nemoralis* (Scudder 1875), were all collected in Boone County, Missouri, USA. *Orchelimum gladiator* Bruner 1891, were collected in Adair County, Missouri. *Orchelimum agile* (De Geer 1773) were collected in Somerset County, New Jersey, USA. *Orchelimum erythrocephalum* Davis 1905, *O. pulchellum* Davis 1909, *O. concinnum* Scudder 1862 were collected in Franklin County, Florida. Individuals of an unidentified *Conocephalus* species were also caught in Franklin County. These individuals had identical calls and morphological features and we assumed that they belong to the same species, but were unable to identify them using existing keys or databases (including Eades et al., 2015, Walker 2010, and Rhen and Hebard 1915 a,b). Here we refer to them generically as *Conocephalus* sp. *Conocephalus cinereus* (Thunburg 1815) were collected from a population living in the Sachs Butterfly House in St. Louis, Missouri, USA. This population was likely introduced from Florida. Individuals were identified after Rhen and Hebard (1915 a,b) and Walker (2010). Insects were kept in plexiglass/screen cages (40x40x60cm) in an

environmental chamber at 22-26^o C, 40% relative humidity at a 14:10 h light:dark cycle. They were kept on apples, Fluker's high-calcium cricket feed, and Purina Pro Plan Adult Cat Food. Collection took place June-September in 2013-2016.

Call Recordings

Males were allowed at least 72 hours to acclimate to the light conditions in the environmental chamber. They were recorded in an anechoic chamber at an ambient temperature of 25±1°C; each male was recorded within 90 minutes of the start of the dark period. The specimens were placed in small screen cages 15cm in diameter. The microphone was placed 20cm dorsal of the calling male. Calls were recorded with a 1/4in (6.3mm) free field microphone (GRAS 40 BF; Holte, Denmark), amplified (GRAS 26 AC and 12 AA), high-pass filtered (1000Hz, Krohn Hite 3202; Brokton, MA, USA), and digitized using a custom-made A/D converter system (16 bit resolution, 250kHz sampling rate). This setup provided a flat (±1dB) frequency response in the range from 2kHz to 70kHz.

Analysis

For each male, we measured the buzzing-pulse period, interval, and duration; the ticking-pulse period, interval, and duration; and the buzzing phrase period, interval and duration. Within a buzzing-pulse we were often able to identify 'impulses' of sound, produced by the file and scraper impacting. For species with

calls where this was resolvable, we also measured the impulse period, interval, and duration (Figure 1). We measured a minimum of 200 buzzing pulses, 200 impulses, 50 ticking pulses, and 30 buzz phrases per individual, and a minimum of ten individuals per species. We used only high quality recordings where we could identify the beginning and ends of each trait with high accuracy. We analyzed the timing of the call parameters at a resolution of 1ms using custom software developed in the Schul lab. Data processing was conducted using Microsoft Excel 2013, and the R/RStudio software suite (R core Team 2014) For each trait, we calculated the grand mean and the standard deviation within each species. We plotted the range of these trait values (Fig 2), including the first and second standard deviations, as candlestick plots using DeltaGraph (SPSS inc). We then calculated the *Coefficient of Variation (CV)* of each trait within a species using these values and plotted them as histograms (Fig 3). We sorted each species into four communities in which they are found. Within these communities, we looked for overlap (or lack-of overlap) in the range of values for each trait.

Results

First we compared the variability among the 12 call traits across all species studied (Fig 2). From impulse period to buzz duration, the ranges over which these traits are expressed varied. Pooled together, there were no discernable patterns.

Histograms of the within-species *Coefficient of Variation*'s (CV's) for a given trait revealed distributions with a low mode in two call traits (Fig 3). For buzzing-pulse-period, nine species had CV's under 2.5% and five species above 9%. For ticking-pulse-period, seven species had CV's 10.6% or under, and nine had CV's 19% and above. Two other traits had individual species with remarkably low CV's. For buzz duration *O. concinnum* had a CV of 3.6% and *O. agile* had a CV of 7.8%, this for a trait dominated by CV's well above 20%. For impulse period *C. nemoralis* had a CV of 1.8%, the next lowest was 19.2%. Here we have labeled these three species as having low CV's.

To elucidate which traits could be reliable indicators of species, we grouped communities together (fig 4) and examined both trait variance (low CV traits colored to correspond to fig 3, high CV traits remain black), and trait overlap. In the Eagle Bluffs community (fig 4a), we found that buzzing-pulse-period had no overlap in range even over two standard deviations. One of these species, *O. gladiator*, was temporally separated from the rest as they mature into adulthood months before the other species in both the Eagle Bluffs and Tucker Prairie communities. *O. nigripes* did not overlap in buzzing-pulse-period with syntopic species but had a high CV. An analysis of other traits shows that *O. nigripes* had a low CV for ticking-pulse-period and only overlapped with the ticking-pulse-period of *O. vulgare*.

Species in Tucker Prairie (fig 4b) also had buzzing-pulse-periods that showed minimal overlap. *C. strictus* had a buzzing-pulse-period CV that fell into the high mode, precluding us from categorizing it as a candidate trait for this species. However, *C. strictus* had a low CV ticking-pulse-period with minimal overlap.

Within the community found at Stephens Lake (fig 4c), buzzing-pulse-period again had little to no temporal overlap between species. Two of the species had CV's that were high; *O. nigripes*, which had a low CV for ticking-pulse-period, and *C. nemoralis*, which had a low CV impulse period. *O. nigripes* ticking-pulse-period overlapped with the highly variable ticks of *O. vulgare*. *C. nemoralis* impulse periods overlapped minimally with the highly variable impulse period of *O. nigripes*.

Species in the community at Carrabelle (fig 4d) are at least minimally separated by habitat type. buzzing-pulse-period again showed no overlap. *O. pulchellum* had a high CV buzzing-pulse-period, and was the only species analyzed here for which we found no low CV traits. Two species, *C. cinereus* and *O. erythrocephalum*, had low CV ticking-pulse-periods with *C. cinereus* being free from trait-overlap. *O. concinnum* had a low CV buzz duration that minimally overlapped the high variability buzz durations other species.

Discussion

We examined twelve traits across fourteen species. Four of these traits (buzzing-pulse-period, ticking-pulse-period, buzz duration, and impulse period) had low amounts of relative variation. When put into the context of community (examining overlap of trait values), buzzing-pulse-period emerged as a candidate trait for nine species, ticking-pulse-period for three. Other traits and interaction effects are likely important as well.

Among closely related arthropods, signal diversity follows three basic patterns. First, diversity can occur along a continuously varying quantitative trait, e.g. pulse rate (Greenfield 2002). Walker (1964) provides several examples of cryptic cricket species which seem to maintain their diversity only by differences in the pulse rate of advertisement calls. Similarly, in Hawaiian *Laupala* crickets calls differ primarily in pulse rate among sympatric species and the diversification of this trait likely played an important role in their rapid speciation (Mendelson and Shaw 2005). Other traits that follow this pattern are, for example, the ratios of olfaction chemicals (Greenfield 1983), and peak frequency in the call spectrum (Westcott and Kroon 2002).

Second, diversification can manifest in the presence or absence of discrete call traits. For example, in *Neoconocephalus* katydids some species have calls with a distinct chirp structure, while most species have continuous calls. Other examples of such call diversity include the presence or absence of alternating

pulse periods (Deily and Schul 2004) or certain spectral components (Deily and Schul 2006). Females are selective for these differences, often requiring the presence of these traits (Schul et al., 2014, Deily and Schul 2004, Deily and Schul 2006). Quantitative diversity, of course, can occur within each of these qualitatively different traits: for example, after the evolution of a chirp structure, calls can diversify quantitatively in their chirp period. In turn, quantitative differences in one trait (e.g. pulse period) can be as large or larger than qualitative differences. In *Neconocephalus*, for example, pulse periods vary from around 5 ms (the likely ancestral state, Frederick et al. 2016) up to over 120 ms and thus can be on the order of magnitude of chirp periods.

Third, the available space for interspecific variation can be doubled through the evolution of complex calls with two (or more) phrases (e.g. Tarbrush Grasshoppers (Minckley et al., 2005), *Chorthippus dorsatus* (Stumpner and Helversen 1994), Coqui (Narins and Capranica 1978) and Tungara frogs (Ryan and Rand 1990)). The presence of this additional phrase adds avenues for diversification, including the potential for interaction effects of the two phrases (Ryan and Rand 1990). Meadow Katydid have this conspicuous form of call diversity with their buzzes and ticks. Here, we might find both previous examples of diversification (along a continuous trait and across distinct traits) in one, or both, of the call phrases. These trait parameters might also be important comparatively across more than one phrase (e.g. the period of pulses within

ticking *and* buzzing phrases) making synergistic interaction effects potentially meaningful.

For signal-diversity to be useful in reproductive isolation it must be both detectable and meaningful to females. Double pulsed calls in *Neoconocephalus* are a reminder that it is critical to understand not only call structure and female perception, but also female preference. The *Neoconocephalus* ancestral state call includes fast pulse rates on the order of 200Hz (Frederick et al. 2016). This rate is beyond the limit of temporal resolution for females, and female preference is for continuous, unmodulated signals (Greenfield 1993, Deily and Schul 2004, 2009, Bush and Schul 2010). Five species have an alternating pulse period that results in pulse pairs, or double pulses. These double pulses halve the effective pulse rate, bringing it into a range detectable by females. In three of the five species, females have a preference for this double-pulse rate. In the other two species, however, females have no preference for the species specific double pulse pattern over the ancestral single pulse calls (Review in Schul et al., 2014). This highlights that the presence of conspicuous call traits (or trait values) does not necessarily indicate behavioral relevance, even if this 'species-defining-trait' is derived and plays a role in other species. The analysis of male calls alone can only identify traits that have the potential to be behaviorally relevant (i.e. candidate traits). Preference tests with females are necessary to confirm their role.

The pulse period (or rate) is the fundamental parameter of Ensiferan calls, reflecting the most basic motor pattern of their calls (Gerhardt and Huber 2002). In many groups it is important for reproductive isolation (Laupala (Shaw and Herlihy 2000), *Oecanthus*- (Walker 1969), *Teleogryllus* (Bentley and Hoy 1972), Eneopteridae (Robillard and Desutter-Grandcolas 2004), Phaneropterine (Spooner 1968), Pterophylla (Shaw 1968), Pseudophyllinae (Morris et al., 1988)). Pulse rates in Meadow Katydids range from ~20-90Hz. While this is considerably faster than in many other groups, these rates are likely resolvable by their sensory system. The sensory system of Locusts can resolve ~170Hz at 23°C. At higher temperatures (31°C), such as might be found in the habitat of diurnally calling Meadow Katydids, pulse rates above 200Hz were resolved by the hearing system (Prinz and Ronacher, 2002). *Neoconocephalus* katydids can behaviorally detect double-pulse rates >100Hz at 25°C. Thus, the diversity of pulse rates has certainly the potential to be important here.

The buzzing-pulse-period has emerged as a trait likely to be meaningful in this group. Buzzing-pulse-period CV's separate into two modes that we have classified as being high and low variability. Nine species fell into the low variability category. When examined in the context of four communities, little if any overlap occurred among the pulse rate ranges of sympatric species. In the few cases where species' ranges overlapped, the tight distribution of a low variation species fell into the tail end of a species with high variability. Thus, even during simultaneous-calling, misleading calls would occur infrequently and rarely

for extended periods of time. So, in all nine of these species (*O. vulgare*, *C. faciatus*, *C. brevipennis*, *C. sp.*, *O. erythrocephalum*, *C. cinereus*, *O. agile*, *O. concinnum*, *O. silvaticum*) buzzing-pulse-period met both of our criteria for candidate traits. However, with such little overlap in the ranges of this trait (for every species we tested, in every community we examined) the species falling into the higher CV category might still be able to reliably utilize it.

Ticking-pulse-period is the other trait that had a low mode of CV's. Seven species fell into the low-variation category. Three of these species did not overlap with sympatric congeners. So, the criteria for candidate traits is met for ticking-pulse-period in these species (*C. cinereus*, *C. brevipennis*, *C. strictus*). The other four species with low variation ticking-pulse-period had overlapping ranges, which precludes it from being categorized as a candidate trait for them. Despite this overlap these traits should not be overlooked when considering what parameters might be relevant.

Interaction effects could result in ticking-pulse-periods with low CV's that, on their own, might overlap too much to be effective in reproductive isolation. The issues with species-discrimination that arise from overlapping signals might be overcome by these synergistic effects (Pfennig 1998). For example, female swordtail fish assess two species-specific traits (vertical bars and chemical cues) in a situation where one trait (vertical bars) is insufficient to prevent heterospecific mating (Hankison and Morris 2003). Within Meadow Katydid, we

know *O. nigripes* females preferentially walk to calls with both the ticking and buzzing phrases present (Miller 2010), despite male *O. nigripes* ticking-pulse-period overlapping with sympatric congeners and buzzing-pulse-period falling into the high variation category. This synergistic, interaction effect could be present in many of the species here. In the four species we examined that had low CV but overlapping ticking-pulse-periods, like *O. nigripes*, we might expect ticking-pulse-period + another trait, like buzzing-pulse-period, could be important together (synergistically).

Meadow Katydid calls are complex and here we find many traits that could be meaningful. Twelve of the fourteen species we examined had low relative variation in ticking-pulse-period and/or buzzing-pulse-period. These two traits are essentially the same parameter but in the two different phrases. We know at least one species uses these two phrases together for mating. The patterns we see in the buzzing-pulse-period show that variation along a continuum of rates is likely to be meaningful. We also find evidence that a distinct call trait, impulse period, might be implicated in reproductive isolation. In half of the species we examined, we could not resolve impulses from 250kHz recordings. In those that we could, one had a CV (*O. nemoralis*) that was very low (the lowest we tested for any trait). This mirrors the distinct presence/absence call trait patterns seen in groups like *Neoconocephalus*. So, here we find that all three patterns of diversification discussed above might be found in this one group.

Every acoustically communicating animal has a huge amount of potential information within their calls. Meadow Katydid are no exception. Here, the two-phrases make this problem even greater. In addition to the many call parameters that can potentially carry information within each of the phrases, the two phrases themselves can be meaningful synergistically, e.g. the ratios of the phrases, the pulse rate within the buzz coupled with the tick rate, or many other trait combinations. Further, the important traits in one species might not be meaningful in another. Indeed, we see that species arrange the two call phrases (and the parameters within the phrases) in diverse and variable ways. It would be difficult, maybe unlikely, to isolate these traits by trial/error in preference tests alone. While some might be revealed in this fashion, other meaningful traits could go untested and unnoticed. Here we've identified candidate-traits to create better supported, finer-resolution hypotheses about trait diversification and female preference. Whatever the results of future studies, these data can provide insight into how complex signals evolve, how preferences and signals shape one another, and on the constraints of signal production.

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Figures

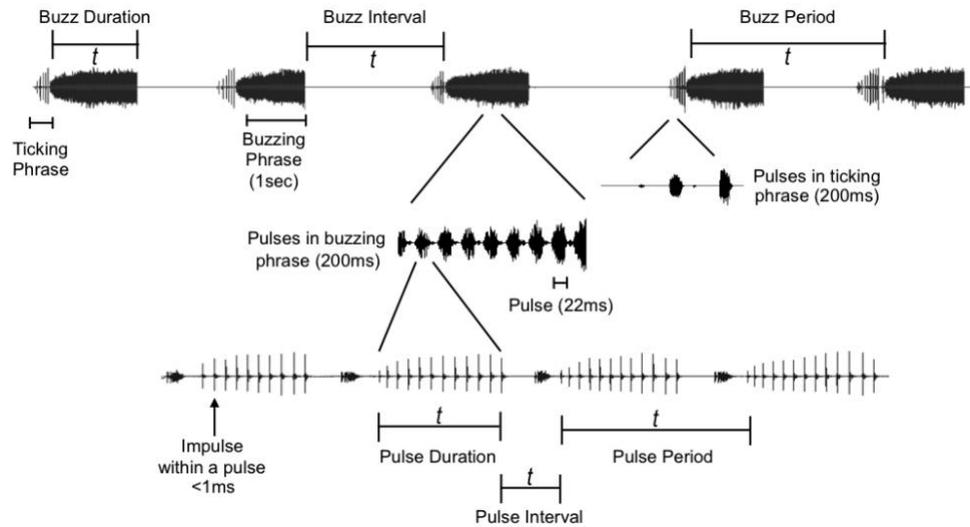


Figure 1

Meadow Katydid call terminology

Oscillogram of the male call of a Meadow Katydid with call structures labeled. At the largest time scale the two phrases, ticking and buzzing, are labeled. 200ms portions of each phrase are shown, containing pulses of sound produced from closing wing movements. Within each pulse are impulses, produced from the scraper contacting the file as the wing closes. Measurement terminology is also shown (duration, intervals, and periods).

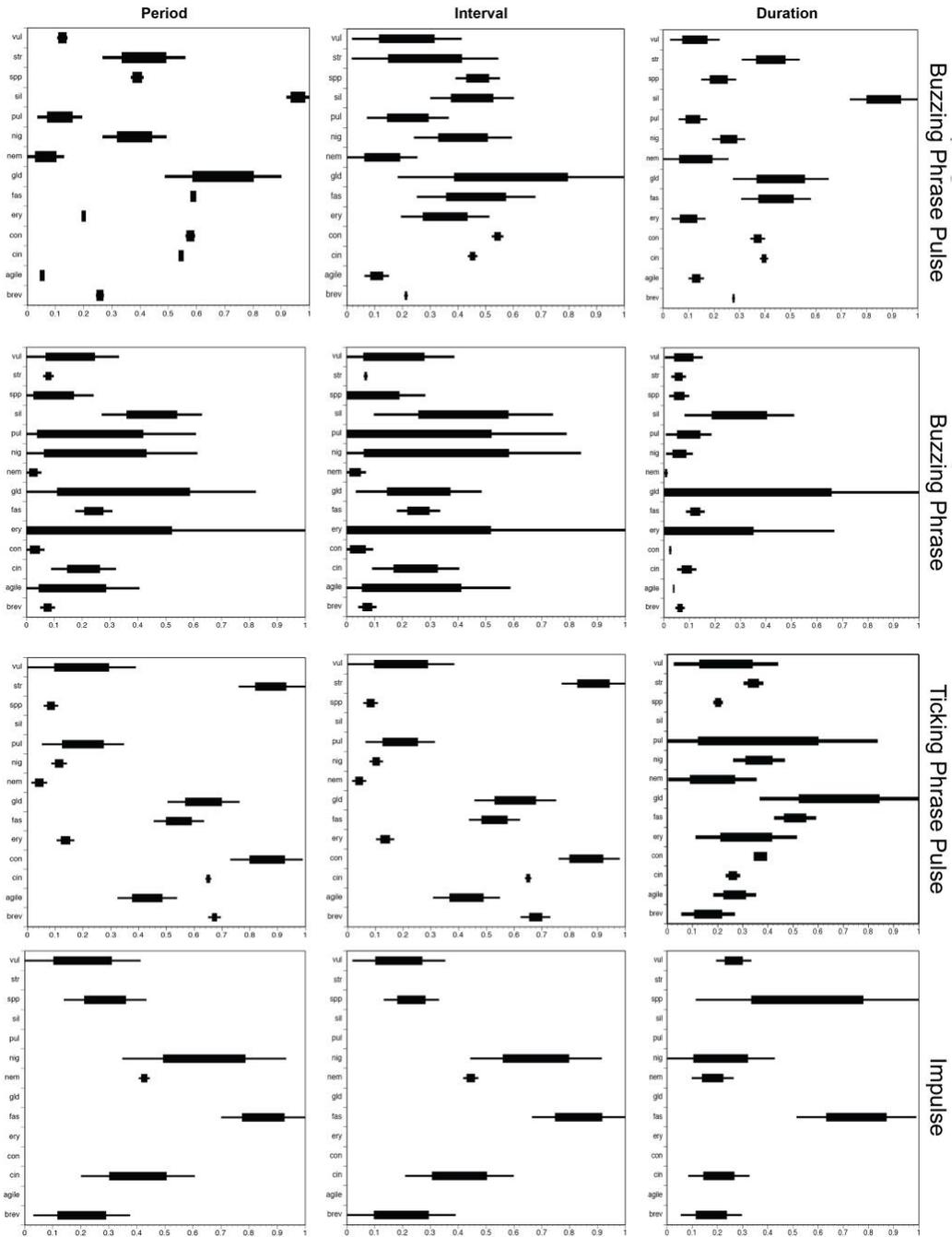


Figure 2

Range of all traits for all species

Candle stick plots of the first and second standard deviations for the 12 traits measured, across all 14 species. Values come from the grand mean and are normalized to be within the range of 0 to 1.

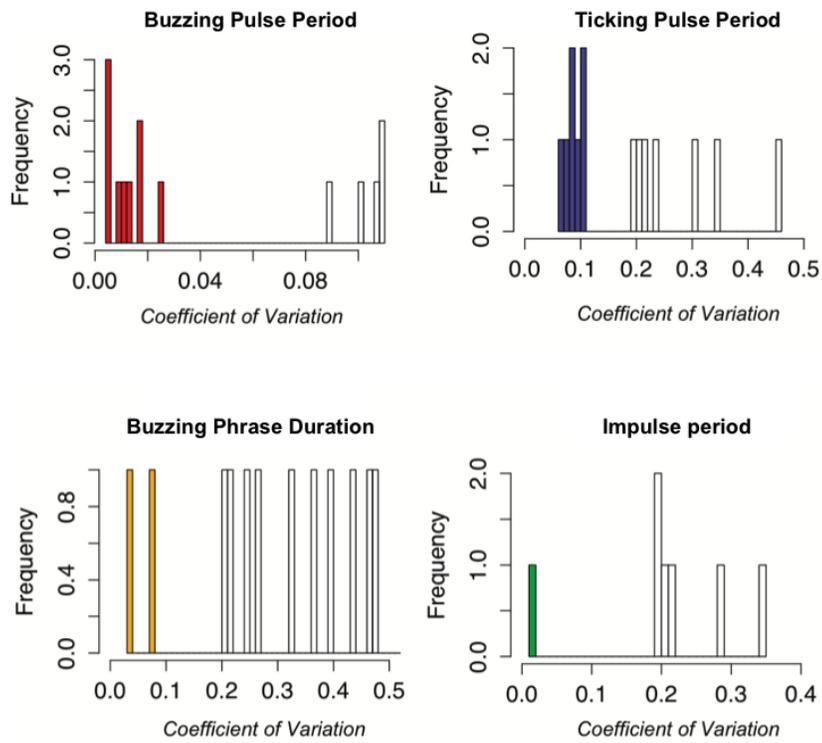


Figure 3

Distribution of trait CV's

Histograms of the coefficient of variation for traits that have a low mode.

Colored bars indicate species in the low mode, and categorized as having a

low relative amount of variation (i.e. CV). Colors correspond to species in Figure 4.

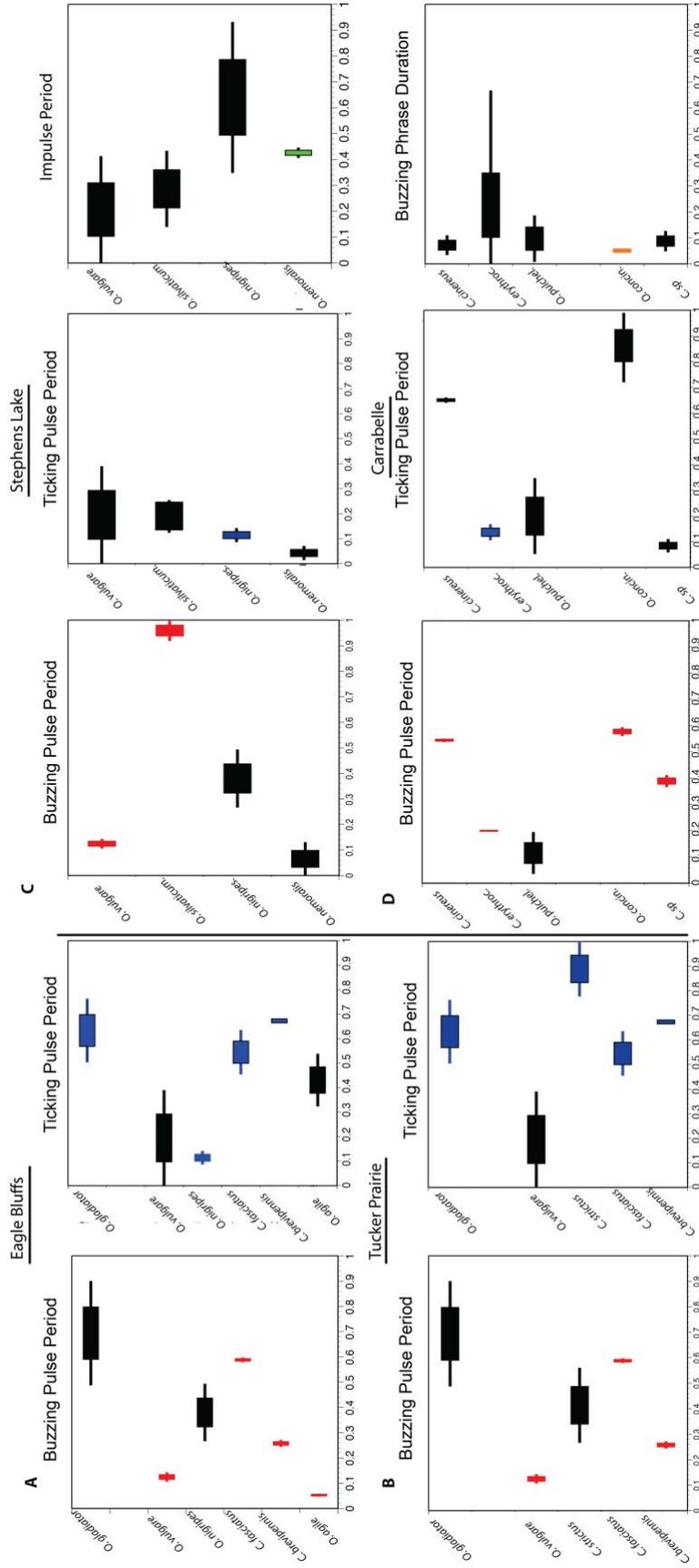


Figure 4

Ranges of temporal call values arranged by species assemblies

Temporal ranges of traits from four species assemblies (i.e. communities) from Eagle Bluffs Conservation Area, Boone County, MO; Tucker Prairie, Boone County, MO; Stephens Lake, Boone County, MO; and Carrabelle, Franklin County, FL. Trait values are normalized to the range of 0 to 1 and shown in a candlestick plot with one and two standard deviations. Traits with CV's are colored to correspond to figure 3. *O. gladiator* is offset in Eagle Bluffs and Tucker Prairie as this species is separated temporally (they mature many weeks earlier) from local congeners.

Chapter 3

PHYLOGENETIC SIGNAL IN THE COMPLEX CALLS OF MEADOW KATYDIDS

Introduction

Meadow Katydid, katydids in the genera *Orchelimum* and *Conocephalus*, provide a unique opportunity to examine the evolution of complexity in communication systems. Their complex calls consist of two-phrases, buzzing and ticking, which are organized in a diverse and variable way (Morris and Walker 1976, Thomas and Alexander 1962). Species vary in the stereotypy of call structure (i.e. how buzzing and ticking are arranged), and in values of temporal parameters, e.g. buzz duration, buzz period, tick number, etc. (Chapter 2, Walker 2012). Meadow Katydid are speciose and wide ranging with more than a hundred species worldwide (Walker 2012, Eades et al., 2015). Many of these species are found in communities with several other Meadow Katydid calling at the same time and place (Eades et al., 2015). This system provides an opportunity to examine how species interactions, habitat types, phylogenetic signal, geography and more relates to the evolution of complexity in a communication system.

Recently, the phylogenetic relationships within many groups of Tettigoniidae have been resolved (Kim et al., 2016, Zhou et al., 2010, Snyder et al., 2009, Shapiro et al., 2006, Hemp et al., 2016, Kaya et al., 2015). Historical

phylogenetic hypotheses based on morphological characteristics often changed when modern molecular tools were used (Nickle and Naskrecki 1997).

Mungleston et al., (2013) examined twelve subfamilies within Tettigoniidae and found that five of them were paraphyletic. The difficulty in using morphological characteristics alone in taxonomic descriptions is particularly evident in Meadow Katydid. The two genera are classified as *Orchelimum* vs *Conocephalus* based on how they fit into a complex of morphological characteristics (Naskrecki 2000, Walker 2012), rather than specific, discrete, diagnostic traits.

Interpreting call traits, (e.g. pulse rates, or the call structure of ticking and buzzing) in the context of phylogenetic relationships allows us to make inferences about the evolutionary history of Meadow Katydid. For example, comparing call traits among sibling species may generate hypotheses about the relevance of those traits for diversification and species isolation. Traits that seem to follow Brownian motion evolution (i.e., their diversity resembles a pattern as expected by chance) are unlikely to be meaningful (Blomberg et al., 2003, Warren et al., 2008). Traits that are less similar than expected by chance are likely to be implicated in establishing and/or maintaining diversity in the group (Blomberg et al., 2003). Traits more similar than expected by chance are likely to be involved in niche conservatism (Losos 2008).

Phylogenetic analysis of the call structure of ticking and buzzing, in particular how stereotyped the patterns are, may inform the question of the evolutionary

origin of the two call phrases. Signals functioning in species isolation should be stereotyped, to transmit reliable information. Thus, the level of stereotypy of the buzzing/ticking structure might indicate their function. The ancestral state reconstruction of call traits thus facilitates the development of hypotheses about the evolution of complexity in this communication systems.

Here, we use three molecular markers to build a species-level phylogeny across two genera. For four quantitative temporal traits, we reconstruct continuous character states in the phylogeny and analyze their distribution and phylogenetic signal using Blomberg's K statistic. Finally, we build an ancestral state reconstruction of call structure.

Methods

Taxon and sampling

We sampled nineteen species of Meadow Katydid (Tettigoniidae: Conocephalinae) from the genera *Orchelimum* and *Conocephalus*. *Orchelimum vulgare* Harris 1841, *O. nigripes* Scudder 1875, *O. silvaticum* McNeill 1891, *O. campestre* Blatchley 1893, *Conocephalus brevipennis* (Scudder 1862), *C. fasciatus* (De Geer 1773), *C. strictus* (Scudder 1875), and *C. nemoralis* (Scudder 1875), were all collected in Boone County, Missouri, USA. *Orchelimum gladiator* Bruner 1891, was collected in Adair County, Missouri. *Orchelimum agile*

(De Geer 1773) was collected in Somerset County, New Jersey, USA. *Orchelimum fidicinium* Rehn and Hebard 1907 was collected in Cape May County, New Jersey, USA. *Conocephalus cinereus* (Thunburg 1815) was collected from a population living in the Sachs Butterfly House in St. Louis, Missouri, USA. This population was likely introduced from Florida. *Conocephalus hygrophilus* Rehn and Hebard 1915, *Orchelimum erythrocephalum* Davis 1905, *O. pulchellum* Davis 1909, and *O. concinnum* Scudder 1862 were collected in Franklin County, Florida, USA. Individuals of an unidentified *Conocephalus* species were also caught in Franklin County. These individuals had identical calls and morphological features to one another and we assumed that they belong to the same species, but were unable to identify them using existing keys or databases (including #OSF, Walker 2010, and Rhen and Hebard 1915 a,b). Here we refer to them generically as *Conocephalus* sp. For use as an outgroup, we collected *Odontoxiphidium apterum* Morse 1891, (a species of Conocephalinae not within the genera *Orchelimum* or *Conocephalus*) from Taylor County, Florida, USA. We also included two species of *Conocephalus* collected, identified, and provided to us by Claudia Hemp: *Conocephalus conocephalus* (Linnaeus 1767) and *C. iris* Serville 1838, both collected in Tanzania, East Africa. All North American individuals were unequivocally identified after Rhen and Hebard (1915 a,b) and Walker (2010).

Molecular Markers

We isolated Genomic DNA from 95% ethanol-preserved hind femurs using a DNeasy Blood + Tissue Kit (Qiagen Inc., Valencia, CA, USA). The concentration and quality of each DNA sample were determined by spectrophotometry (NanoDrop 1000, Thermo Scientific, Wilmington, DE, USA). For this study we sequenced three loci: Cytochrome Oxidase I (CO1, ~650bp), Histone 3 (H3, ~375bp) and Wingless (WG, ~450bp). The PCR protocol we used has been previously described for H3 (Colgan et al., 1998), WG (Wild and Maddison, 2008), and CO1 (Svenson and Whiting, 2004). These protocols were followed using Platinum taq DNA polymerase (Invitrogen, Carlsbad, CA). PCR amplification was performed on an Eppendorf Mastercycler gradient (Eppendorf-Brinkman Instruments Inc., Westbury NY, USA). Amplified PCR products were prepared for sequencing using an enzymatic clean-up (1:1 ratio of Exonuclease I and Antarctic Phosphatase, New England BioLabs Inc., Ipswich, MA, USA) in a 1:10 reaction of enzymes to PCR product and incubated at 37°C for 50 min. Sequencing was performed at the DNA Core Facility, University of Missouri, Columbia, MO on ABI 3730 DNA Analyzers, using standard Big Dye Terminator cycle sequencing chemistry (Applied Biosystems, Foster City, CA, USA). Resulting sequence data was edited and aligned in Geneious version 11.1 (<http://www.geneious.com>, Kearse et al., 2012).

Phylogenetic Analysis

We utilized the BEAST software suite (Drummond et al., 2012) to leverage Bayesian analysis to estimate a species tree. Within BEAST we used *BEAST (starBEAST) (Heled and Drummond, 2009) to infer a single maximum clade credibility tree from our multi-locus sequence data. Using this coestimation method provides a more accurate estimation of a species tree than the supermatrix method where multiple single-locus-trees are first constructed and subsequently concatenated (Heled and Drummond, 2010). This approach is widely considered the best, most promising method to estimate a species tree (Molloy and Warnow 2017, Leache and Rannala 2010, Bayzid and Warnow 2013, Liu 2008, Edwards et al., 2007). We used JModelTest (Posada, 2008) to determine HKY as the best fit model for sequence evolution for each gene. We used a strict clock model, a chain length of 30,000,000 and logged 10% (30,000 trees). We analyzed the output of BEAST using Tracer, and identified a single tree best representing the posterior distribution (the maximum clade credibility tree), after a 15% burnin, using TreeAnnotator (Rambaut and Drummond 2015).

Call Analysis

Male calls were recorded in an anechoic chamber at an ambient temperature of $25\pm 1^{\circ}\text{C}$. Males were allowed at least 72 hours to acclimate to the light conditions in the environmental chamber. They were recorded within 90 minutes of first dark in their light:dark cycle of 14/10 hours. The specimens were placed in small screen cages 15cm in diameter. The microphone was placed 20cm dorsal of the

calling male. Calls were recorded with a 1/4in (6.3mm) free field microphone (GRAS 40 BF; Holte, Denmark), amplified (GRAS 26 AC and 12 AA), high-pass filtered (1000Hz, Krohn Hite 3202; Brokton, MA, USA), and digitized using a custom-made A/D converter system (16 bit resolution, 250kHz sampling rate). This setup provided a flat (± 1 dB) frequency response in the range from 2kHz to 70kHz. We recorded and measured calls from at least eight animals of each species, except for *C. hygrophilus* and *C. campestre* where we were only able to measure recordings from two and six animals respectively.

For each individual we measured the period of a minimum of 200 pulses within the buzzing phrase; a minimum of 50 pulses within the ticking phrase; and the interval and duration of a minimum of 30 buzzing phrases. We analyzed the timing of the call parameters at a resolution of 1.25ms. Data were saved as text files for further analysis. Data processing and analysis were conducted using custom software developed in the Schul lab, Microsoft Excel 2013, and the R/RStudio software suite (R core Team 2014). Grand means are reported in Table 1. We categorized species into either variable or stereotyped call-types. We measured the stereotypy of calls by quantifying how often the buzzing phrase was preceded by the ticking phrase. Stereotyped-callers fell into one group, having more than 98% of buzzes preceded by ticks. Variable callers ranged from 15% to 85% of buzzes preceded by ticks.

Ancestral State Reconstruction

We used 10,000 post-burnin trees from the Bayesian total evidence phylogeny to reconstruct the ancestral character states using BEAST for discrete traits (variable/stereotyped calls) and Phytools R package (Revell 2012) in R-Studio (*RStudio Team (2015). RStudio: Integrated Development for R. RStudio, Inc., Boston, MA <http://www.rstudio.com/>*) for continuous traits (temporal call values).

Phylogenetic Signal

To measure phylogenetic signal (the 'tendency for related species to resemble each other more than they resemble species drawn at random', Bloomberg and Garland 2002, Diniz-Filho et al., 2012) we used Blomberg's K statistic. We used the R package Picante (Kembel et al., 2010) and input our species tree to calculate K for buzzing-pulse-period, buzzing phrase duration, buzzing phrase interval, and ticking-pulse-period. A K value of less than one implies species resemble each other less than expected under Brownian motion evolution. K values equal to one indicate a trait shows exactly the amount of phylogenetic signal expected under Brownian motion evolution. K values greater than one suggest more related species are more similar than predicted (Blomberg and Garland 2003).

Results

In the maximum-clade-credibility species-tree (Fig 1.), two African *species* (*C. conocephalus*, *C. iris*) were sister to all North American Meadow Katydid species sampled. The genus *Orchelimum* formed a paraphyletic group nested within the North American *Conocephalus* (Fig 1) that also included *Conocephalus nemoralis*. The posterior probability for all nodes was 99% or above except for one (the “*Orchelimum*” clade), at 91%.

We analyzed four continuous call traits and used the results (Table 1) to perform an ancestral state reconstruction (Fig 2). Reconstruction revealed buzzing-pulse-period values that appeared to contrast a large amount between sister species (e.g. *O. gladiator* and *O. erythrocephalum*, the clade with *C. nemoralis*, *O. silvaticum*, *O. vulgare*, and *O. agile*, etc.). Ancestral buzzing-pulse-period for the North American clade (the only clade included in this analysis) was ~17ms. There was not a noticeable evolutionary trend toward faster or slower buzzing-pulse-periods. Ticking-pulse-period is similar between closely related species. There was a general trend towards a faster tick rate from the ancestral ticking-pulse-period value (~1400ms) to the tips (extant values). *O. concinnum*, and *C. strictus* were exceptions to this, with slower tick rates than ancestors. *O. concinnum* is the sister species to *O. fidicinium* (see fig. 1). *O. fidicinium* has lost the ticking phrase.

Character state reconstruction of buzz duration and buzz interval largely mirror each other. Clades with intermediate values in one have intermediate values in the other. Ancestral state for buzz duration was ~950ms, for buzz interval ~2500ms. There was not a noticeable trend towards longer or shorter values for either of the two traits.

We examined phylogenetic signal in four continuous traits. Only buzzing-pulse-period had a significant Blomberg's K statistic ($P=0.02$, $K=0.0021$, Table 2); the value of K was close to zero, which indicated that trait values between closely related species contrasted more than expected by chance. For ticking-pulse-period, the K-statistic approached significance ($p=0.09$); K was larger than 1, suggesting that ticking-pulse-period is more similar between closely related species than expected. For the other two call traits, this analysis was not significant.

In six species, buzzing phrases were preceded by ticking phrases more than 98% of the time; we classified these species as having a stereotyped call structure (Table 1, Fig 3 inset). In the remaining species, the call structure was variable with buzzes being preceded by ticks less than 85% of the time. The character state reconstruction of this trait revealed that the ancestral calling state for Meadow Katydid call structure was 'variable'. Stereotyped-call-structure evolved twice independently. In one clade, there was a reversal back to the ancestral, variable-call-structure.

Discussion

The phylogeny of Meadow Katydidids revealed no clear separation of its two genera. Character state reconstruction indicated that variable call-structure was ancestral. The buzzing-pulse period contrasted significantly more than expected by chance, while ticking-pulse periods had a trend to diverge less than expected by chance, but results did not reach statistical significance.

The species-tree (Fig 1) failed to support the two genera *Orchelimum* and *Conocephalus* as distinct taxonomic groups, as neither genus reconstructed as a monophyletic clade. For at least the past twenty years (Naskrecki 2000) the formal taxonomic distinction between them has been considered tenuous, as it has been based on muddy morphological categorizations that fit only “mostly” to one genus vs the other (Walker 2012). They continue to be separated by tradition (Naskrecki 2000). The molecular evidence here offers no support for two separate genera; if our finding would be confirmed with a larger set of species, including more taxa from Eurasia and Africa, *Orchelimum* species should be included in the genus *Conocephalus*. For interpreting data on the evolution of the communication system, these two genera can certainly be considered as one group.

The phylogenetic signal of the pulse period within the buzzing phrase was significantly less than expected by chance (Table 2, $K=0.0021$, $p=0.02$). This

means that they contrasted among closely related species more than would be expected if they evolved by drift alone (Arnegard et al., 2010). This often occurs in adaptive radiations (Blomberg and Garland 2003) such as with *Anolis* lizards (Pinto et al., 2008), Squamata (Burbrink et al., 2012), and African cichlids (Muschick et al., 2014). This pattern is found in traits that are evolutionarily labile (a trait with “extraordinary capacity for change” over generations, Emlen et al., 2005, Blomberg et al., 2003) and/or have outpaced ecological selection (Losos 2008). This suggests the pulse period within the buzzing phrase has been involved in premating isolation as this group evolved (Andersson 1994). It could have either driven species divergence, or reinforced it (Albert and Schluter 2004). Pulse rate and traits associated with it (pulse duration, interval) play a significant role in species recognition in numerous katydids, other orthopterans and anurans (Greenfield 2002, Gerhardt and Huber 2002).

The Meadow Katydid buzzing phrase resembles the echemes/verses in katydids with simple (one phrase) calls (Walker 2010). In the few species of Meadow Katydid that have only one phrase, it resembles buzzes (e.g. *O. fidicinium*, Chapter 1, *C. discolor*, Heller 1988). This suggests that buzzes are homolog to the simple calls of 'typical' katydids. We follow this assumption here for the sake of argument. This raises the question of how the ticking phrase has evolved.

Evolution of complex calls

Communication systems consist of two subunits, sender and receiver, which need to be reciprocally matched for the system to function. In this light it is difficult to understand how communication systems can diversify: Given that novel signal traits or receiver preferences lack matched receivers or signals, respectively, how do new signal traits evolve? Changes of signals involved in reproductive isolation (Gerhardt 1991) should not be tolerated by the stabilizing selection acting on them (Greenfield 2002). Nevertheless, communication signals are highly diverse and usually differ markedly between closely related species (Henry *et al.* 1999). In Meadow Katydid, this “problem” is remarkably evident. Their calls are not just quantitatively different (e.g. faster or slower pulse rates) from closely related groups, but qualitatively with the addition of a new phrase. Additionally, the structure of these two phrases is expressed in a diverse and varying way across many species. This makes Meadow Katydid a particularly good system to study the origin of call complexity in a comparative, phylogenetic context.

Character state reconstruction of call traits may suggest the likely ancestral functions of the different call components. In Meadow Katydid, the ancestral state of "call structure" was variable (Fig. 2), i.e, buzzing and ticking phrases were not arranged in a stereotyped pattern during each calling bout. For a signal

trait to contribute to reproductive isolation it needs to be stereotyped, with a low amount of relative within and among male variation (Gerhardt and Huber 2002, Helversen and Helversen 1994). In species with variable call structure, it is thus unlikely that both phrases, and in particular the specific pattern of the two phrases (i.e. buzzing coupled with ticking) play a role in species isolation. This suggests that the ancestral function was for one of the phrases to be used by females in reproductive isolation, and the other evolved in a separate context.

Our results, taken together, hint at the evolutionary history of complex calls in this group. The ancestral reconstruction suggests one of the two phrases is involved in reproductive isolation. The pulse period within the buzz is implicated in reproductive isolation (Table 2 and Chapter 2). Female preference data (Miller 2010) further supports this, with the buzzing phrase being necessary and sufficient for phonotaxis. In Chapter 1 we present evidence that ticking functions in male/male interactions. Ticking is not always involved in reproductive isolation (Miller 2010). This suggests that ticking has likely evolved in the context of male-male communication, and has been co-opted in some species (e.g. *O. nigripes*) for female preference. More extensive behavioral experiments, examined in a phylogenetic context, are ultimately needed to better resolve these questions.

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Figures

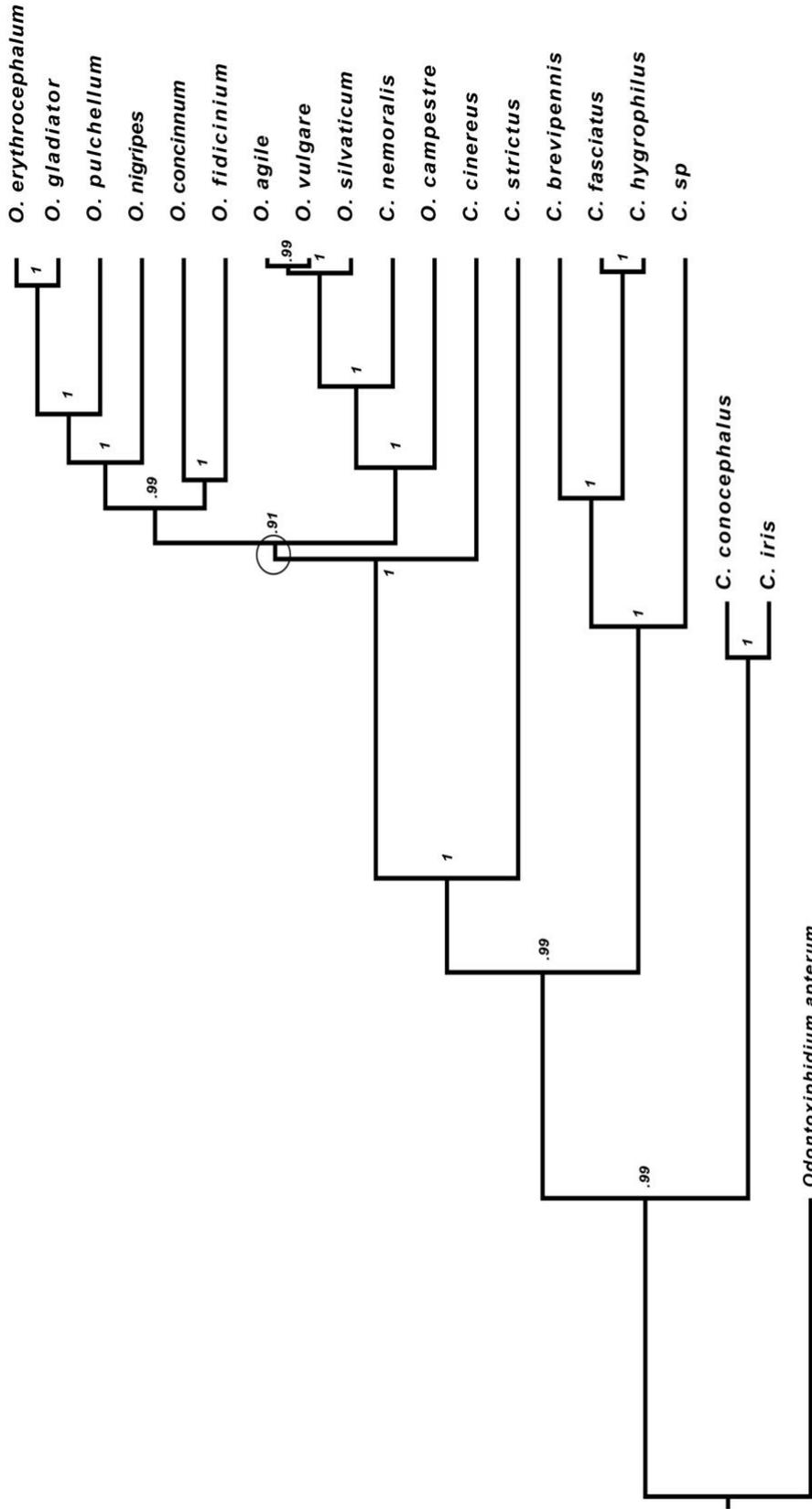


Figure 1

Multi-locus species tree of Meadow Katydid

A coestimated consensus species-tree built in *BEAST from COI, H3 and WG genes. Posterior probabilities are shown with two significant figures. The circle denotes the “*Orchelimum*” clade.

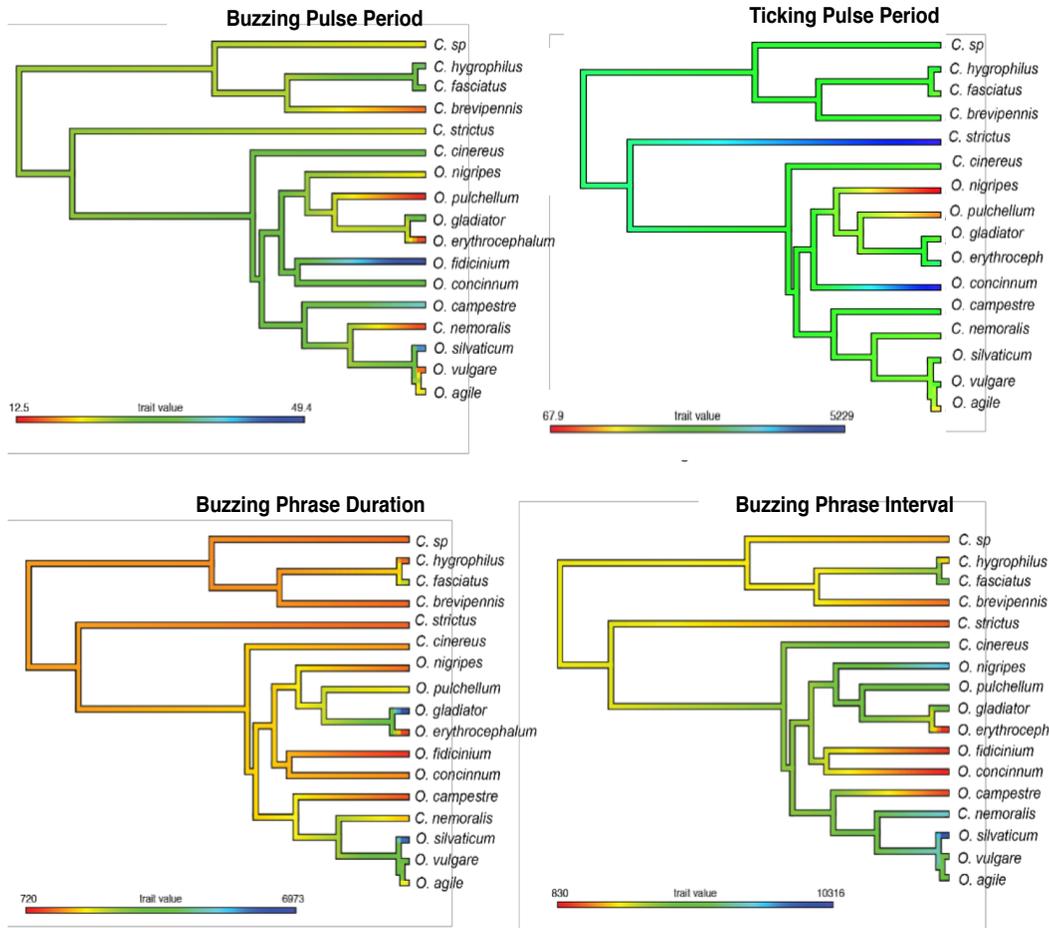


Figure 2

Character states of continuous call traits

Ancestral state reconstruction for Buzzing-pulse period, Buzz Duration, Buzz Interval, and Ticking-pulse period. Trait values are given in milliseconds. Values plotted on a log scale.

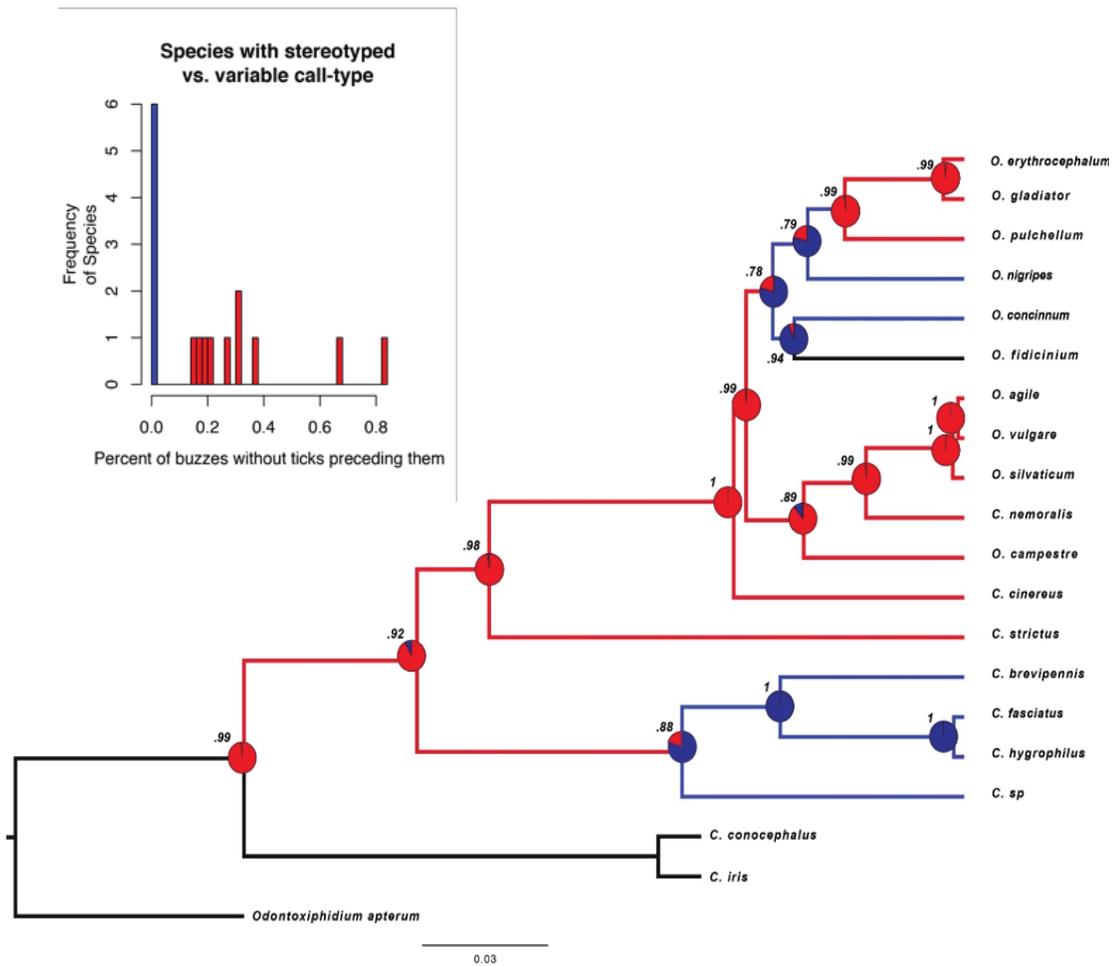


Figure 3

Character state reconstruction of 'call-structure'

The ancestral-state reconstruction of stereotyped/variable call structure.

Reconstructed states are represented by branch colors (variable calls indicated in red, stereotyped in blue). Values at nodes represent posterior probability of character states. Color at the tips is the current-state (table 1). Black branches indicate species with values that were not given for the analysis. *Inset*: Six species (in blue, *O. nigripes*, *O. concinnum*, *C. brevipennis*, *C. fasciatus*, *C.*

hygrophilus, *C. sp*) alternated the two call phrases more than 98% of the time, and were categorized as stereotyped-callers. The remaining species (in red) produced buzzing phrases without ticks between them 15% to 85% of the time, and we categorized them as variable-callers. We did not classify *O. fidicinium* as it produces only one of the two phrases.

Table 1

Description of Meadow Katydid Calls

Call traits for Meadow Katydid calls included in ancestral state reconstructions, and tests for phylogenetic signal (Blomberg’s K). All numerical values are given in milliseconds, and come from our own measurements. % of Buzz with tick preceding is followed by a V or S to denote if the calls are classified as variable or stereotyped, respectively.

Species	% of Buzz with Tick preceding	Buzzing-pulse-period (MS)	Buzzing Phrase Duration (MS)	Buzzing Phrase Interval (MS)	Ticking-pulse-period (MS)
<i>O. erythrocephalum</i>	33% V	17	720	830	902
<i>O. gladiator</i>	68% V	36	6973	6369	380
<i>O. pulchellum</i>	74% V	17	2165	6128	126
<i>O. nigripes</i>	100% S	24	1360	7924	67.9
<i>O. concinnum</i>	99% S	32	1551	987	5114
<i>O. fidicinium</i>	-	49	964	1244	-
<i>O. agile</i>	80% V	24	1237	5732	128
<i>O. vulgare</i>	83% V	12	4219	4169	552
<i>O. silvaticum</i>	64% V	46	6601	10316	433
<i>C. nemoralis</i>	18% V	18	1876	7468	349
<i>O. campestre</i>	69% V	39	1211	1399	518
<i>C. cinereus</i>	85% V	32	1605	6097	390
<i>C. strictus</i>	79% V	25	1287	1680	5229
<i>C. brevipennis</i>	99% S	19	1321	1829	456
<i>C. fasciatus</i>	100% S	32	2748	6348	328
<i>C. hygrophilus</i>	100% S	36	1100	2201	671
<i>C. sp</i>	98% S	24	1341	2328	596

Table 2

Blomberg's K among call traits

Results of an analysis of the phylogenetic signal in four call traits. Buzzing-pulse-period was the only trait with significant results, and was in the direction of $K < 1$.

<i>TRAIT</i>	<i>BLOOMBERG'S K</i>	<i>P</i>
BUZZING-PULSE-PERIOD	0.0021	0.02
TICKING-PULSE-PERIOD	1.227	0.09
BUZZING PHRASE INTERVAL	.6991	0.29
BUZZING PHRASE DURATION	1.083	0.11

Chapter 4

MEADOW KATYDIDS AS A TOOL FOR TEACHING BIODIVERSITY AND ENGAGING STUDENTS

Introduction

One way to help combat student apathy towards what many view as a complicated and intimidating subject is by engaging students in a gateway-discipline of biology, like animal behavior. Animal behavior has wide appeal to the general population, from which our students come. Student interest is one of the main motivational variables tied to success (Harackiewicz 2000), and we can leverage animal behavior to spark a greater interest in biology in general.

By introducing students to a topic and letting them form new hypotheses, and run experiments we are showing them what it is like to be a biologist, not just learn about biology. Such activities help students both understand the process as well as spike an interest in science (Crawford 2000).

Here, we introduce a system well suited for use as a tool to meet many Next Generation Science Standard Performance Expectations, but especially MS-LS1-4: *Use argument based on empirical evidence and scientific reasoning to support an explanation for how characteristic animal behaviors and specialized plant structures affect the probability of successful reproduction of animals and plants respectively.*

In this system male insects use characteristic, audible calls to attract mates

and interact with rival males. These calls are not only associated with reproduction, but are the very means by which individuals obtain matings. Below, we describe a hands-on lesson using this system that we have tested at both the high school and college level.

Background: Meadow Katydids

Meadow Katydids are a group of small to medium sized insects in the genera *Orchelimum* and *Conocephalus*. They have an unusually complex call among closely related katydids (Walker 2010). These calls are made by males by rubbing their wings together and include two distinct parts, a buzzing phrase and a ticking phrase. The function of these two phrases is not yet well understood. Some simple experiments designed by students are likely to reveal knowledge of the behavior of these insects that is entirely new to science.

They are particularly well suited to classroom experiments. They are among the few diurnal katydids, and are found in great abundance in most areas of North America, especially east of the Rocky Mountains (Eades et al., 2015). They are longer lived than most katydids and can be readily found from the early months of the summer until around the time of a hard frost or prolonged cold spell. Meadow Katydids don't require any special equipment to capture, and usually this is done just by hand. Caring for them in captivity is easily accomplished, even for prolonged periods of time, by using simple aquariums or plastic shoe boxes and the addition of green grasses and apple slices.

These complex calls make identifying this group somewhat trivial. Calls are loud and conspicuous, and most species will be well within the audible frequency range of teachers, and certainly students. Few other insects will be calling in grassy areas during the day. Once they are located by call, identifying them as katydids is simple. They have both large hind jumping legs and antennae longer than their bodies (these long antennae differentiate them from grasshoppers, which are also a commonly found diurnal orthopteran). Figure 1 illustrates these traits. While identifying the specific species is not required, it can be done by referencing the Singing Insects of North America website (<http://entnemdept.ufl.edu/Walker/buzz/>). Some species identifying characteristics can be complicated, out of the scope of traditional classrooms, but many of these species have conspicuous morphological characteristics that can quickly identify them (e.g. Black legged meadow katydid, stripe faced meadow katydid, red faced meadow katydid, etc.).

Materials List

- Aquarium with lid or large insect keeper to house insects. Homemade alternatives (e.g. 2L bottles with the tops cut off and replaced with plastic screen, see fig 3c) can be used with success
- Apples, solid water, and/or dry oatmeal
- Cups to hold cut green grasses for structure and food in the insect keepers.
- Classroom tablets with built in or external microphones, or laptops with

external microphones. Microphones built into headphones work well.

-Small bottles, such as repurposed water bottles with air holes, to hold captured insects in-the-field.

-Computers with the free and simple sound tool Audacity installed to analyze calls (http://download.cnet.com/Audacity/3000-2170_4-10058117.html)

-Small mesh containers for housing calling individuals during experiments (figure 2).

-Various equipment depending on the experiments students come up with (i.e. Thermometers, heat lamps, paper for making shades, etc.)

Procedure

DAY 1

1. Discuss animal behavior with the class, and the ways scientists study behavior. A few popular examples are the waggle dance in bees (Von Frisch, 1967)(https://www.youtube.com/watch?v=LU_KD1enR3Q), and a parasitized cricket population in Hawaii losing their acoustic sexual signal (Zuk et al. 2006) (<https://www.youtube.com/watch?v=qF2bJyCt5i0>).

2. The Hawaiian flat wing cricket story is a good opportunity to steer the discussion to acoustic communication in animals. This is a broad field that has advanced our understanding of sensory and motor systems, speciation, and evolution (Gerhardt and Huber 2002). Students are often familiar with the

sounds of nighttime calling animals and so this is a good opportunity to relate to their experiences.

3. Play a few insect calls for students. There are countless examples on places like YouTube, though one excellent resource is a sound compilation on Piotr Nascrecki's website (<https://thesmallermajority.com/2013/10/11/music-in-my-head/>). Before introducing the details of the calls, ask students what they think they are hearing, why they think different insects sound differently, and what those sounds might be used for. Guide them in a discussion on how much of the insect calls they hear are males advertising to potential female mating partners. Different species having different call functions to isolate them, preventing breeding mistakes and playing a role in speciation. Calls might also be used to communicate important information to other males such as spacing or information about territory defense.

4. Introduce students to the meadow katydids they will be working with. These are insects you hear calling from grassy areas during the day and at night.

Figure 1 has images of common meadow katydids and a description of how to differentiate them from grasshoppers you might also find in grassy areas during the day.

5. Go outside to a nearby grassy area; you can also check in landscaped gardens with knee high or taller plants, hedges, or forest edges. The ticking/buzzing call gives their presence unmistakably away. Usually any place that hasn't been recently mowed and has clumps of taller grass will be home to meadow katydids. This includes the tall grass around telephone poles, in/near

drainage ditches, or the edges of wooded areas. Have students first listen for calls. Have them write in their notebook what the various calls sound like.

6. Have students catch calling, adult male meadow katydids (reference figure 1). In daylight, this can require a little patience and practice, but generally they are easily caught by hand. Keep in mind that these insects can inflict a mild bite akin to being pinched. Most rarely do, and gardening gloves can eliminate the risk. Small green aquarium nets can be used if available, but they are not necessary.

7. As a group, begin separating the males into larger cages by apparent species. This can be done generally just by putting like-looking males caught near to one another together. Many species have conspicuous distinguishing morphological features such as red faces or black legs. Work at also collecting a few females, which won't be calling but can be spotted when looking for males.

8. Debrief the students on what they found. This is a good time to talk about biodiversity and ecological niches. If you have wet areas, such as ditches, students will find different meadow katydids there than in dry grassy areas.

Meadow katydids can be kept in ten gallon aquariums (with lids), plastic insect keepers, or even two liter bottles with the tops cut off and mesh fitted for ventilation (figure 3). It is best to keep the density of the cages to around 8 individuals per 2L bottle. A 10-gallon aquarium will hold 45 adults comfortably. No matter the container, they should be given cups of green grasses cut from the general areas the katydids were collected. Water in the bottom of the cup will

keep the grass green for several days. This will act both as a source of food and as substrate. Pieces of apple should be put in the bottom of the cages if they are to be kept beyond a couple of days. For longer term care (i.e. over ten days), providing these omnivores with a small amount of canned wet cat food will help keep them in good condition. If the humidity in the area they are kept is low, misting once or twice a day is recommended.

DAY 2

1. Before class starts put insects in individual small mesh cages (fig 3a). If they are to be in the cages for long it is a good idea to put a small piece of apple in with them.

2. Ask students about the katydids' call. They should notice that there are two parts, a Tick and a Buzz phrase (figure 1). Different species organize these ticks and buzzes differently, so depending on the species you've caught the calls will sound somewhat different.

3. For the initial experiment we will see if the tick/buzz composition changes in the presence of other calling males. Arrange different trials with varying numbers of males near to each other. The first trial should be a lone male. The next should be two males together, and so forth. Males of the same trial should be arranged in a circle with a diameter of about 45cm (figure 3b). The different trials should be as far from one another as possible. Each group will handle one

of the trials (i.e. Group 1 will do the solo male, group 2 will record a focal male that is in the presence of two other calling males, etc.)

4. Have students pick one focal male at random from each trial. The microphone will be placed pointed towards this male and as best as can be configured away from the other males. Use the recording application on the iPad or other recording device to start recording a few minutes after the males have been arranged.

Note: Several factors such as time of day, temperature, and how loud students are will affect how quickly the animals settle and start to call. It is a good idea to have some readings or worksheets relevant to the unit on hand. Usually animals will start calling within 10minutes, especially with other males around.

5. Students should record a calling male for about five minutes. These calls can then be emailed to the teacher or to themselves so they can access them on a laptop.

6. Open the calls in Audacity and have students see if they can identify what is a tick and what is a buzz in the waveform. This should ultimately be straightforward once they become familiar with the program (figure 2).

7. Have them count the number of ticks and the number of buzzes in a five-minute period. They should put their data on the board.

8. As a class analyze all the groups' data. Usually building a bar graph will reveal trends. You can expect that as the number of individuals in the trial goes up, the ratio of ticks to buzzes will also rise. An example of this is found in the bottom four trials in figure 4.

9. Ask the class to discuss what this result might mean. Guide them toward thinking about possible functions of the tick (possibly used for male/male interaction such as establishing territory). This aligns with the NGSS science and engineering practice, *engaging in argument from evidence*

<http://www.nextgenscience.org/sites/default/files/Appendix%20F%20%20Science%20and%20Engineering%20Practices%20in%20the%20NGSS%20-%20FINAL%20060513.pdf>

10. Have groups brainstorm other experiments they could do with these animals. Build in some time to come together so that groups can get input from the class. Examples of experiments include examining how calls change in the presence of females, or males of a different species, or in light/dark, or warmer/colder temperatures. Usually these experiments can be completed and the data analyzed during a single class period. This aligns with the NGSS science and engineering practice, *planning and carrying out experiments*

<http://www.nextgenscience.org/sites/default/files/Appendix%20F%20%20Science%20and%20Engineering%20Practices%20in%20the%20NGSS%20-%20FINAL%20060513.pdf>

DAY 3

1. Carry out the experiments designed at the end of day two.
2. Analyze the data. How this is done will vary with experimental design, but most students will be measuring numbers of ticks and buzzes in some duration of call (i.e. average number of ticks or buzzes per minute over ten

minutes). Alternatively, some students might be interested in looking at other call parameters, such as the length of the buzz or the number of ticks between buzzes. There is a lot of potential information in these calls that students can analyze. An example graph with example trials can be seen in figure 4.

PRESENTATION DAY

Arguably the most important part of a scientist's job is communicating their results. This might be via a peer-reviewed article in a scientific journal, a formal presentation to colleagues at a scientific conference, or a less formal presentation to the public. Depending on the class, each of these could be useful models of products to use as a final assessment. A paper turned in with a brief formal classroom presentation is likely to be a popular option. More difficult, and more interesting, are presentations designed to teach a general audience in an engaging, interactive, and entertaining way. This aligns with the NGSS science and engineering practice, *communicating results*

<http://www.nextgenscience.org/sites/default/files/Appendix%20F%20%20Science%20and%20Engineering%20Practices%20in%20the%20NGSS%20-%20FINAL%20060513.pdf>

Discussion

There are all sorts of challenges faced by scientists over the course of designing, carrying out, analyzing, and publishing their experiments. Students (and

teachers!) will find this is no different when doing these types of behavioral experiments in the classroom.

When carrying out this activity with secondary students, locating the spots that meadow katydids were calling from in a grassland was never a problem. Some students, however, had trouble finding individual males and capturing them.

Telling students that they should expect this and that they need to be focused and patient, helped most succeed. Groups of two looking together for one calling male tended to be more effective than individual, or large groups of students.

These are living animals with minds of their own, and sometimes they choose not to call. There are several ways to handle this. If there are enough captive katydids, students can set up two identical experiments, doubling their chances of getting males to call. Or, if other groups have successfully used some individuals in an experiment try and reuse those. Just moving away from the insects, to reduce distractions, typically results in calling within a few minutes. Once they start calling, it is often a good idea to let them call for a few minutes to stabilize their calling.

The power of this activity lies in its accessibility and simplicity. Students doing the simple experiments outlined here, or modifications of them, are engaging as scientists. In doing this they are also learning about scientific content, aligning with much of the emphasis the Next Generation Science Standards and Performance Expectations.

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Figures

Meadow Katydid

Male *O. nigripes*
Black Legged Meadow Katydid



Large jumping legs and antennae that are longer than their bodies are good identifying characteristics of Meadow Katydid. These physical characteristics plus their distinctive ticking and buzzing call make them easily identifiable.

Female, Juvenile *O. nigripes*
Black Legged Meadow Katydid



Female Meadow Katydid are readily distinguished from males by large sword-like ovipositors. Juveniles of either sex can be identified by the lack of fully developed wings. Only adult males call, so be sure to collect a variety of individuals.

Grasshopper



Katydid are commonly mistaken for grasshoppers. Grasshopper antennae are shorter than their bodies.



Meadow Katydid can be found in almost any tall grasses in North America. Unmowed ditches, the grass around telephone poles, and the tall grasses near ponds or streams are excellent places to hear and locate these insects. They are easily found even in urban areas. We found three species calling from the green bush in this photo.

This is the "waveform" of an insect call. It is what you will see in Audacity as you record katydids. The two phrases of these calls are readily identifiable. Ticks are slow, individual pulses of sound made from one wing closing. Buzzes are many of these pulses of sound strung very close together. You can easily measure things like the number of ticks or buzzes per minute, the ratio of these two phrases, or other changes of the call composition in different conditions.

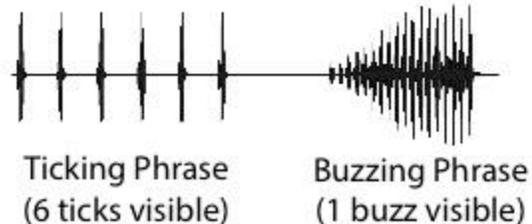


Figure 1

Handout for class discussion

A handout to familiarize students and teachers with Meadow Katydid, their habitat, and their calls. Photographs of: an adult male Meadow Katydid, a juvenile female Meadow Katydid, and a grasshopper. The osciligram shows the two phrases of Meadow Katydid calls.

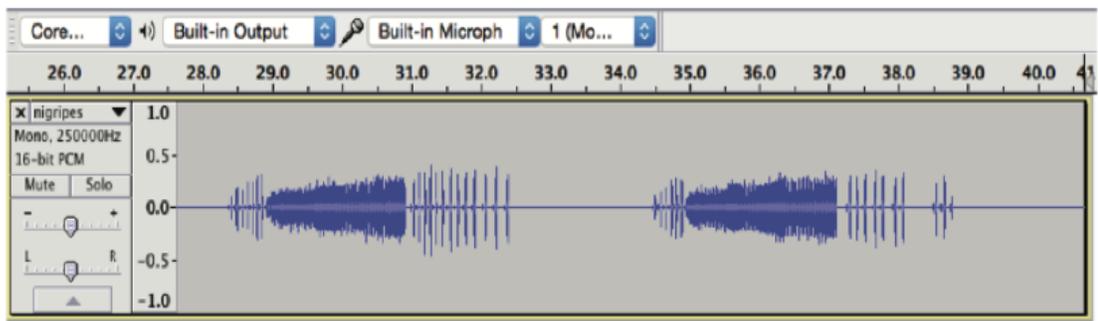


Figure 2

Example audacity recording

This is about twelve seconds of calling. Ticks and buzzes are easy to identify, ticks being discrete pulses of sound, buzzes being much more dense continuous sounds. In this recording two buzzes are clearly visible, sandwiched between dozens of ticks.

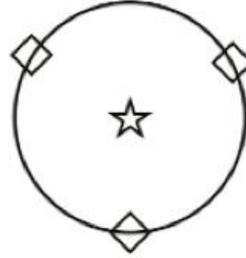


Figure 3a Example of mesh cage for housing a single katydid

These can be constructed of fiberglass screen sold at hardware stores for repairing screen-doors. Simply staple two ~10cm squares together on all sides but one. That side can be folded over and closed with a clip once the animal is in place. **3b Example layout for male/male density experiment.** The male to be recorded (focal male) is in a mesh cage at the location indicated by the star. Other males, indicated here by squares, are spaced evenly in a circle around the focal male, each ~45cm away. **3c Example of a 2 liter bottle cage.** A 2 liter bottle is washed, and the narrow top portion removed. Mesh screen commonly found in hardware stores is tied or banded to the top. Insects can be kept on grasses in these bottles for several days.

Number of Call Phrases Per Minute in 10min of calling

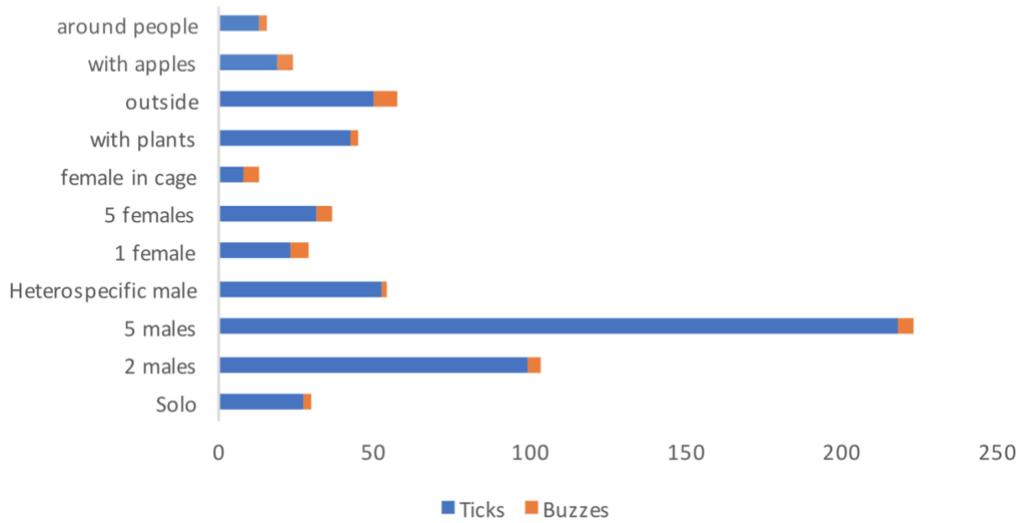


Figure 4

Classroom data of call phrase ratios

Data collected from a high school classroom. A single focal male was recorded in the presence of various treatments (described by the y-axis). Student groups ran treatments, and the data was combined.

VITA

Nathan Harness was born in St. Joseph, Missouri on September 16th, 1984. He graduated from Benton High School in St. Joseph in May 2003, having also taken course work and done research at Missouri Western State College with Dr. Todd Eckdahl. From there, he attended Truman State University and graduated with a B.S. in Biology in May 2008. He did research in Animal Behavior with Taro Narahashi in Meadow Valley, California and with Dr. Don Kangas in northeastern Missouri. Upon graduation he worked as a high school biology and chemistry teacher from 2008 to 2012, first at Bunceton High School in Bunceton, Missouri and then at Rock Bridge High School in Columbia, Missouri. Nathan subsequently matriculated at the University of Missouri, working under the advisement of Dr. Johannes Schul. He earned his Ph.D. in biological sciences in July 2018. Nathan has accepted a position as an assistant professor in biology at Francis Marion University in Florence, South Carolina.