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**AIRBORNE SOUND AND SUBSTRATE-BORNE VIBRATION  
IN ORTHOPTERAN COMMUNICATION**

A Thesis  
Submitted to the Faculty  
in partial fulfillment of the requirements for the  
degree of

Doctor of Philosophy

in

Ecology, Evolution, Environment & Society

by Ciara E. Kernan

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August 2023

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# Abstract

The challenge of attracting and locating potential mates has driven the evolution of diverse mate-finding signaling systems. These systems can be complex, with multiple signals or signal components spread across different sensory modalities. When multiple modalities are combined in one communication system, features of each can be important to individual fitness; over evolutionary time, these can shape investment in different parts of a signal repertoire. One group of animals that commonly uses multiple signal modalities is Orthoptera. Cricket and katydid species within this order often produce airborne sound calls to advertise to potential mates. Many also produce substrate-borne vibrational signals. I describe three investigations into the patterns of sound and vibration use by orthopteran insects with a combination of long focal recordings of individual callers and playback experiments to receivers. In the first chapter, I quantify the total diel sound and vibrational calling activity of ten species of katydid (Tettigoniidae: Pseudophyllinae) to investigate whether these signal types trade off with each other. I find that species that use more vibration tend to use less sound, and that other traits like sound call bandwidth may mediate this relationship. In the second chapter, I investigate intraspecific variation in one of these species (*Docidocercus gigliotosi*), recording the calling activity of recently mated vs. unmated males over several weeks. I find evidence for positive within-individual, but not among-individual, correlations between sound and vibrational signaling, with mating having large initial effects and more subtle, lingering ones on signal production. In the third chapter, I assess how duetting female crickets (*Lebinthus bitaeniatatus*, Gryllidae: Eneopterinae) respond to different components of male calls, finding that callers strike a balance between calling signal attractiveness and efficacy in duet timing. Increasing the length of a typically stereotyped sound call component results in vibrational replies that are higher amplitude and likely more perceptible—yet incorrectly timed. Together, these projects reveal patterns of differential investment in multiple signal modalities in orthopteran insects. Quantifying these differences is key to understanding how complex signaling systems function and how they might affect responses to environmental change.

## Preface

The work in this thesis would not have been possible without a constellation of people helping it, and me, along. Foremost among them is my primary advisor, Hannah ter Hofstede. I have often gone into meetings with her feeling totally daunted by a research problem, and left feeling much more capable of handling it. I have learned so much from her scientific clarity and willingness to engage with the details of experiments, statistics, and writing—while having complete freedom to pursue the research questions I’ve found most interesting. Thank you!

The rest of my advisory committee—which has included Ryan Calsbeek, Mark McPeck, Nate Dominy, Karin Pfennig, and Eileen Hebets—has provided essential guidance throughout my time in graduate school. Laurel Symes has been an incredibly supportive and thoughtful mentor, and I aspire to her levels of collaborative energy and insight. My other coauthors on each of these chapters—Tony Robillard, Jessica Jones, Stefan Schöneich, Jen Hamel, and Sharon Martinson—have all put so much time and care into helping shepherd each project along. Jess in particular made my initial time at Dartmouth very welcoming and I’m thankful to have had her help on the cricket project as it kept getting more and more complicated!

I’ve benefitted immensely by being part of the large and wonderful group of humans doing katydid work on BCI in recent years. This has included Hannah, Laurel, Jen, and Sharon, but also Christine Palmer, Estefania Velilla, Caitlyn Lee, Chase Novello, Alina Iwan, Matt Sears, Jean Ross, and Madi Gamble. Amber Litterer and McKenna Gray were the best assistants I could have asked for, and their cheerful willingness to accompany me on the oddest research sidequests (washing frog cages to acquire mealworms to feed to bats to learn about katydids) and most physically demanding parts of fieldwork (hiking up and down endless muddy hills carrying obnoxiously spiky bromeliads) made the 2019 and 2020 field seasons much more productive and enjoyable.

I must also thank a number of additional people who have shaped my thinking and supported me over the years. At STRI, Michael Caldwell and Rachel Page have helped me since

I was an undergrad just becoming interested in animal behavior research. Gabrielle Bueno, Zack Davis, Eva Arroyo, Pete Kerby-Miller, and many others have offered good company and good counsel. At Dartmouth, Matt Ayres, everyone in his Theories Models Data class (both times!), Craig Layne, Deb Goedert, Madi Gamble, Eran Amichai, Ridhi Chandarana, Pooja Panwar, and Miranda Zammarelli have all been similarly helpful sounding boards. Clare Doherty and Elliott Steele have been the best cohort-mates bar none—and Elliott the perfect co-TA during a Bio FSP field course filled with adventures, most good but some harrowing. I am so grateful to have shared a lab with Mia Phillips, whose friendship and shared Enthusiasm for Bugs has been vital. Any good scientific ideas I've had have emerged from interacting with this assortment of people and their delight in both big-picture theory and the smallest, strangest details of creatures and their natural history.

All of my thesis chapters involved a lot of data processing and animal care, and it would not have gotten done without Eva Legge, Stanley Rozentsvit, and Hannah Marr. Amy Layne, Anne Lavanway, Sherry Finnemore, and Bob Robertson provided essential logistical support at Dartmouth. Melissa Cano, Ariel Camaño, Jenniffer Saucedo, and Hilda Castañeda made everything on BCI work.

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There are a couple of people who I've never met but whose work has been so helpful, inspirational, or horizon-broadening to me that it would feel strange not to acknowledge them. Jacqueline Belwood's detailed foundational work and Heiner Römer's later studies on the Panamanian katydids so clearly illustrate why researchers have been fascinated by these animals for decades. Mary Jane West-Eberhard's thinking and writing on development, plasticity, and evolution has been clarifying at crucial moments. Marixa Lasso's and Megan Raby's books have given me a much deeper understanding of the complicated human history of the Canal Zone, Barro Colorado Island, and the enduring legacy of colonialism that continues to affect the science done there.

The broader EEES community has been wonderful, especially during the disruption and grief of the COVID-19 pandemic. So has the Graduate Organized Laborers of Dartmouth—I'm so happy to have been part of this newly formed union and witness the dedication of its members to improving the conditions of workers in our community and elsewhere. And I'm lucky to have had an outlet from thinking about insect music via making human music—in Panama, with Sharon, Jen, Yoel, Helene, Tammy, and Lee; and in the U.S., with the Upper Valley Chamber Orchestra. Each of these communities has buoyed me through different parts of this Ph.D. and I'm very grateful to all of them.

This section would be incomplete without acknowledging the love, support, and encouragement of my family—especially my parents, Karen and Maurice Kernan, who can pretty much be held directly responsible for my curiosity about the natural world and willingness to spend a very long time watching, thinking about and writing about insects. Thank you.

And to my partner Christopher Callahan, whose brilliance as a scientist is matched only by his sense of justice for others and whose contributions are too great to list: I love you! Your company has been the very best part of this whole thing.

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# Introduction

Attracting or locating a suitable mate is a fundamental challenge for almost all sexually reproducing species. The general nature of this challenge means that species have evolved a striking diversity of solutions to it (Emlen and Oring, 1977; Greenfield, 1997; Wells et al., 1998), an inevitable outcome of the specific pressures that each faces in an ecologically complex world (Endler, 1992; Patricelli and Hebets, 2016). In animals, many of these solutions involve the production of sexual advertisement signals that are broadcast to potential mates (Andersson, 1994). Signals can occur within and across different sensory modalities, and complex signaling systems in which signals in different modalities are combined are common (Partan and Marler, 1999; Higham and Hebets, 2013). When functionally related signals occur in different sensory modalities, the extent to which signalers rely on one or the other can reflect differences in the adaptive value of each. For example, complexity of various courtship signals is associated with different ecological and life-history traits among finch species: generalist species tend to have more complex songs; common species, larger dance repertoires; and gregarious species, more elaborate coloration (Gomes et al., 2017). Costs and benefits associated with a particular signal modality may be experienced differently by different species, leading to these types of species-level patterns. They may also be experienced differently by different individuals or groups of individuals within a species, or by a single individual at different times. In leafhoppers, for example, male vibrational signals incur greater risks from spider predators than do female vibrational signals (Virant-Doberlet et al., 2011). In eagle owls, acoustic calling behavior is elevated during full moon nights, potentially because lighting conditions during these times enhance the visual conspicuousness of a white plumage patch that is dynamically displayed while males are calling (Penteriani et al., 2010). Understanding how the risks and rewards of producing various types of signals are distributed across species, sexes, individuals, and lifespans—and investigating how these might shape patterns of signal expression across these scales—is a central focus of this thesis.

Many animals use airborne sound signals for long-range mate advertisement and short-

range courtship (Gerhardt and Huber, 2002; Marler and Slabbekoorn, 2004). Many more—including perhaps 92% of all arthropod species—use substrate-borne vibrations (Hill, 2008; Coccoft and Rodríguez, 2005). These two signal modalities are closely related: both involve the production of mechanical waves in different media; all receptors and organs used by animals for detecting sound are derived from mechanosensory receptors (Bradbury and Vehrencamp, 2011); and the production of airborne sound necessarily produces vibrations in the substrate upon which an organism is located (Caldwell, 2014). Given this shared evolutionary history and potential for cross-modal integration, it is perhaps not surprising that they are often combined in communication systems, either by being perceived cross-modally (Latimer and Schatral, 1983; Narins et al., 2018; Caldwell et al., 2022) or as largely separate signals (Ota et al., 2015). Similarities notwithstanding, there are also important differences between these modalities. For example, airborne sound attenuates predictably with distance, whereas substrate-borne vibrations are heavily influenced by the architecture and composition of the substrate (Michelsen et al., 1982; Coccoft, 2014). Airborne sound is likely to carry for further distances than substrate-borne vibrations (Coccoft and Rodríguez, 2005), though there are exceptionally long-range calls with vibrational components (e.g., elephant infrasound) and short-range sound calls (e.g., quiet song in birds) (O’Connell-Rodwell, 2007; Dabelsteen et al., 1998). The structures or mechanisms that different species use to produce signals can further influence signal features. Communication systems with distinct sound and vibrational signals present an opportunity to examine how the general and species-specific properties of each signal’s modality influence when, and under what conditions, they are produced.

In this thesis, I focus on the broad question of how airborne sound and substrate-borne vibration work together across different orthopteran species to aid in mate finding. In the first two chapters, I address this question in a Neotropical (Panamanian) community of katydids in the subfamily Pseudophyllinae (Orthoptera: Tettigoniidae). Males of pseudophylline species in this region produce two distinct mate advertisement signals. Airborne calls, produced via tegminal stridulation, are often extremely short, high-frequency, and longer-range signals. Conversely, substrate-borne calls produced via abdominal tremulation

are longer, low-frequency, and shorter-range signals. Despite these differences, the two types of signals indeed appear to functionally overlap. Males of many pseudophylline species will produce either type of signal spontaneously, without external cues from females (Belwood, 1988). Females of at least one species will exhibit elevated levels of searching behavior in response to either signal, and will respond with vibrational signals to either type of male call (Iwan et al., 2023, in prep.). Together, this evidence supports the idea that both sound and vibrational male calls play a role in long-range pair formation, with the female vibrational signals aiding in the final stages of mate location and leading into close-range courtship. These signals have been studied for several decades in the context of predation risk from eavesdropping, gleaning bats (Belwood and Morris, 1987; Heller, 1995; Römer et al., 2010; Falk et al., 2015; ter Hofstede et al., 2017; Geipel et al., 2020), which is a leading hypothesis for the evolutionary origin of the vibrational calls. Yet fundamental questions about the extent of inter- and intra-specific variation in each signaling modality remain unaddressed, hindering our understanding of how these distinct signals are integrated throughout the process of mate-finding—and whether any potential patterns of covariation they exhibit are consistent with the idea, often advanced in past literature, that one compensates for the other.

In Chapter 1, I investigate patterns of investment in sound and vibrational calling across several different pseudophylline katydid species from Barro Colorado Island, Panamá, in a comparative phylogenetic framework. Characterizing differences in total signal investment across time can shed light on the different factors that might have shaped multimodal signaling behavior, but requires long focal recordings. To examine patterns of sound and vibrational calling across species and test hypotheses about the drivers of signal use in each modality, my collaborators and I recorded focal individuals from ten pseudophylline species, each for 24 hours. We also collected data on demographic and morphological species characteristics, and features of calls such as bandwidth, peak frequency, and duration. Finally, we reconstructed the phylogenetic relationships of these species to examine the influence of phylogeny on trait distributions. Using these data, I assessed whether sound and vibration were positively correlated, negatively correlated, or uncorrelated across species. I also ex-

amined correlations between signaling effort and other species characteristics. I found that species that stridulated more tended to tremulate less, indicating that vibrational calling may compensate for reduced airborne signaling in this group. Sound call bandwidth and the proportion of males collected at lights, a proxy for male motility, also explained a significant amount of variation in sound calling across species, indicating that the overall relationship between the two types of calling signals may be mediated by the specific characteristics of the signals as well as other species traits.

In Chapter 2, I delve into the sound and vibrational calling behavior of a single one of these species, the blue-faced katydid *Docidocercus gigliotosi*. Among individuals in a species, relationships between the use of functionally overlapping signals can reveal possible behavioral syndromes (Dirienzo and Hedrick, 2014) or developmental trade-offs (Zambre et al., 2022). Alternatively, no relationship between the use of such signals may be the result of different selection pressures acting independently on each signal, despite their similar function. Distinguishing between these possibilities is important for understanding the evolutionary forces that shape signal repertoires. Testing for these relationships is complicated, however, by other factors influencing overall signaling variation, such as phenotypic plasticity over time. Signalers might flexibly alter their signaling effort for numerous reasons, such as their mating history, current motivation or condition, and sensitivity to predator or other environmental cues. To quantify potential among- and within-individual variation in sound and vibrational signaling, I recorded 24-hour patterns of signal production by male *D. gigliotosi* katydids. I repeatedly recorded males for up to 6 weeks and examined how factors including individual identity, body mass, and mating history affected the amount of signaling in each modality. Although individual males showed repeatability in their signaling behavior within each signal type, I find no evidence for among-individual correlations between sound and vibrational signals: most variation occurred within individuals. I further find that plasticity during a transient mating refractory period accounted for the majority of overall variation in signal production. Outside of this period, mating history also appeared to play a more persistent, long-term role: males that had mated less recently increased their signaling effort to a greater degree than males that had mated at the start

of the 6-week recording period. These findings underline the degree of plasticity that can be revealed by long-term, repeated measurements across different contexts. Strikingly, the within-individual correlations we observed between sound and vibrational signaling were in the opposite direction to the among-species correlation between these signals I describe in Chapter 1, indicating that the mechanisms influencing signaling behavior differ at these scales.

In orthopteran species, mate-finding interactions depend not only on the behavior of signaling males, but also of females, who attend to calls and search for males—or in some cases, produce their own signals in reply (Bailey, 2003; McCartney et al., 2012; Heller and Hemp, 2020). In these duetting species, the characteristics of both male and female signals can influence the outcome of mate-finding interactions. In the third chapter of my thesis, I examine one such duet in the cricket species *Lebinthus bitaeniatus* (Gryllidae: Eneopterinae), which is notable for two reasons. First, it is multimodal: males produce airborne sound calls which elicit a substrate-borne vibrational reply from females (ter Hofstede et al., 2015). Second, the male call has two distinct components: a series of longer syllables (ticks) followed by a rapid series of shorter syllables (a trill). The number of ticks per call is highly variable, whereas the number of pulses in the trill is stereotyped. One hypothesis for differences in signal component variability suggests that receivers provide stabilizing selection for components that are important for mate recognition, resulting in low variability, while also providing directional selection for signal components indicating mate quality, correlating with greater variability. Factors that affect signal efficacy, however, can also influence signal design, and might interact with receiver preferences to influence variability. In duets, which often depend on timing replies to fall within a species-specific latency after the signal, these efficacy constraints might be particularly important. To test how variation in each male call component influenced features of the female reply, and to make inferences about how replies might have constrained or allowed variation in each male call component, I recorded calling males and used measurements of these calls to develop a series of playback experiments with female crickets. Female response amplitude was not related to the number of (variable) ticks in the male call and it increased with (stereotyped)

trill duration, contrary to the stabilizing / directional selection hypothesis. The latency of the female vibrational reply, however, became erratic as the trill increased beyond the typical duration, indicating a reduced efficacy of the female reply for communication despite the females' greater amplitude response.

The properties of mate advertisement signals, including the sensory modality in which they are produced and how often they are repeated, can be critical to individual fitness. In general, signaling more often or with more conspicuous signals has been shown to enhance reproductive success (Hedrick, 1986; Ryan and Keddy-Hector, 1992), but energetic constraints (Taigen and Wells, 1985; Symes et al., 2015) and risks from eavesdropping predators and parasites (Endler, 1980; Zuk et al., 1995) can impose a high cost to increased signal production, while noise in a particular signaling channel can limit the efficacy of communication (Vasconcelos et al., 2007). Theory on complex, multimodal signaling systems like those I have studied in my Ph.D. research suggests that they may be more robust to these and other potential disruptions (Ay et al., 2007; Partan, 2017), which has implications not only for the success or failure of individual mate-finding interactions but also for the continued existence of populations and species (Wells et al., 1998; Anthony and Blumstein, 2000; Laiolo et al., 2008; Gascoigne et al., 2009). But our current knowledge of how complex signaling systems actually operate is inadequate, especially for tropical insects that are currently threatened by anthropogenic change on several fronts (Janzen and Hallwachs, 2019; Lamarre et al., 2020). I hope that the following chapters contribute to a more complete understanding of these animals—and the ways in which their communication signals will allow them to fare in our shared, and shifting, world.

---

## Chapter 1

---

Levels of airborne sound and substrate-borne vibration  
calling are negatively related across Neotropical false-leaf  
katydids

## Introduction

Locating potential mates is critical to individual fitness, and many species have evolved elaborate communication systems towards this end. Complex sexual advertisement signaling repertoires comprising multiple elements are widespread throughout animals, and are often produced in multiple sensory modalities (Andersson, 1994; Partan and Marler, 1999). Different elements (signals or signal components) within these repertoires can have different functions, perhaps conveying multiple messages (e.g., Narins and Capranica, 1976); a single pluripotent signal might also have multiple functions (e.g., Moskát and Hauber, 2019). A particularly interesting combination arises when structurally independent signals share an overlapping function. In systems biology terms, such signals are “degenerate” and may increase the robustness of a communication system by allowing it to function under different conditions in which one of the communication channels is disrupted (Ay et al., 2007; Kaczorowski et al., 2012). Degeneracy can also allow components to respond in different ways to selection (Hebets et al., 2016).

How complex signal structure and function interact has implications for examining signal diversity among species. Closely related species or populations may share some fundamental features of their signaling repertoire, but the extent to which species differentially express degenerate elements of this repertoire can reflect divergent evolutionary pressures. In birds, for instance, song and plumage coloration are each used by potential mates to assess male quality and in other conspecific interactions. Studies within different clades have variously shown positive (Shutler and Weatherhead, 1990; Ligon et al., 2018), negative (Badyaev et al., 2002), or no (Ornelas et al., 2009) correlations between the degree of elaboration of song and plumage, suggesting that taxon-specific differences mediate whether degenerate signals trade off with each other, are subject to correlated selection, or are subject to different selection pressures that cause their evolution to proceed independently.

Within a taxon, the responses of degenerate signals to selection may be influenced by attributes of the specific signals and the signaling environment, including potential receivers. Additionally, overall rates of signal production are likely to be very important across many

clades, because costs and benefits of different signal types can accrue over time due to differences in their specific properties. Directly comparing a single instance of two types of degenerate signals is not necessarily informative if they are produced at very different rates, or if one is expressed throughout the entire lifetime of an organism (coloration) while another is discretely expressed for shorter spans of time (acoustic calls). Thus, to compare signals, biologically relevant observation periods for each are necessary. So are considerations of additional factors that can mediate signal production, transmission, and reception, and the ultimate success of mate advertisement signaling. In *Monarcha* flycatchers, for instance, the physical signaling environment (degree of canopy openness) may interact with features of their visual and acoustic signals to differentially affect signal attenuation, resulting in a sequential assessment of signals that unfolds over the course of a single interaction and possibly has caused populations to diverge in song and plumage complexity (Uy and Safran, 2013). Additionally, predation risk, population density, or the costs of either producing or responding to advertisement signals could interact with signal features to influence the relationship between multiple types of signals. For example, repertoire size and degree of plumage dimorphism are positively related in sexually dimorphic wood warblers, but this relationship disappears when monomorphic birds are included—possibly because monomorphic birds are more likely to be groundnesting and thus at higher risk of predation, which may constrain plumage conspicuousness (Shutler and Weatherhead, 1990).

Degeneracy is ubiquitous within biological systems (Edelman and Gally, 2001), including animal communication systems (Hebets et al., 2016), but an unusually clear example is found in Neotropical katydids within the subfamily Pseudophyllinae. Calling signals to attract mates within this clade come in two distinct forms: airborne sound produced by wing stridulation, and substrate-borne vibrations produced by abdominal tremulations or (occasionally) drumming. Male katydids will produce either type of signal spontaneously, without a female being physically present (Belwood and Morris, 1987); females will respond to either by searching or by producing their own vibrational signals in reply (Iwan et al., 2023, in prep.). Airborne calling is widespread within Orthoptera, while spontaneous vibrational advertisement calling has been documented within pseudophylline and

copiphorine species in the Neotropics (Belwood and Morris, 1987; Belwood, 1988; Morris et al., 1988; Montealegre-Z. and Morris, 2004; de Souza et al., 2011; Sarria-S et al., 2016; Korsunovskaya and Zhantiev, 2022). This behavior has not been commonly reported in these or other clades in other regions despite the ubiquity of substrate-borne vibrational signals in arthropods generally (Cocroft and Rodríguez, 2005) and in the close-range interactions of many orthopterans (Stritih-Peljhan and Virant-Doberlet, 2021), including tettigoniids (e.g., Rajaraman et al., 2015). It is hypothesized that vibrational calling in these Neotropical species, as well as the short durations of airborne calls in the Pseudophyllinae, arose in response to high predation pressure from gleaning bats that eavesdrop on katydid airborne calls (Belwood and Morris 1987). Behavioral experiments (Belwood and Morris, 1987; Falk et al., 2015) and biogeographic comparisons (Heller, 1995) support this hypothesis.

If predation pressure has driven the evolution of calling signals in this group of katydids, we might expect investment in each signal type to trade off with the other: increased vibration, which is less conspicuous to gleaning bats (Geipel et al., 2020), could compensate for decreased sound production in species that faced especially strong eavesdropping predation pressure. Even absent the role of eavesdroppers, we might expect a trade off if vibration merely provides an additional channel for mate-finding in a dark and acoustically cluttered environment, as suggested by Korsunovskaya and Zhantiev (2022). However, it is not presently known how total vibration calling relates to total sound calling across different pseudophylline species. Additionally, because they are produced in different sensory modalities, these two types of signals have different properties that affect production, transmission, and reception by intended and unintended receivers. Therefore, although we would predict a negative relationship across species between total sound and total vibration, other species traits, including call features, morphology, and demographic characteristics, are likely to modulate this relationship. The combined influence of such traits could even result in a positive relationship, or none at all.

Examples of other traits might include those that affect the spatial relationships between signallers and their audience. For instance, because the active space of sound and vibration differs (Römer et al., 2010), abundance or density might play an important role. Males of

more common species, which are more likely to have a female receiver within range and are potentially at higher risk from predators due to density-dependent predator search dynamics than are males of less common species, might eschew conspicuous sound in favor of less conspicuous vibration. Alternatively, more common species might face greater intraspecific competition, and could tend to call more in both modalities.

Additionally, mate finding strategy might affect investment in either type of signal. Symes et al. (2021) found that the proportion of males caught at light traps, interpreted as a proxy of how much time males spend calling vs. searching for replying females, predicted sound calling investment in a subfamily of katydids that uses long-range sound duets for mate location. Pseudophylline species do not engage in airborne sound duets, but males of different species might differ in the proportion of time they spend calling from a single location vs. moving among different calling sites, and such differences in motility may be associated with relative investment in the two calling signals. For example, a katydid that frequently moves among calling sites might use more vibrational than airborne calls, because the male can assess presence of females at each new site according to whether he elicits a female duetting response with these shorter-range signals. A sit-and-call strategy, on the other hand, would rely on females being drawn in from as wide an area as possible, and would favor greater investment in longer-range sound calls.

Spectral or temporal features of individual sound or vibration signals might differ between species and could affect investment in either modality. For instance, katydid sound calls with lower peak frequencies and longer durations are more conspicuous to some eavesdropping bats (Falk et al., 2015), but these features could possibly also aid in localization by female katydids. Species with these or other conspicuous sound call characteristics might tend to call less often.

Finally, morphological or physiological features of the species might inform us about why a species might invest more in one or the other type of signal. Mass, for example, can affect the properties of a vibrational signal as it is propagated through the substrate (Cocroft, 2014), and it negatively correlates with inter-pulse-intervals of vibrational calls in meadow katydids (de Luca and Morris, 1998). It has also been argued that abdomen shape might

influence vibrational signal characteristics in pseudophylline species (de Souza et al., 2011). The size of an organism or its sound producing structures can affect various sound call characteristics within (Brown et al., 2006) and among (Montealegre-Z, 2009) stridulating orthopteran species. Finally, for at least one species of pseudophylline katydid, *Docidocercus gigliotosi*, sound and vibration calls appear to exert very different metabolic costs (Römer et al., 2010), and across species that differ in body size, differences in metabolic rate could affect relative investment into each type of signal.

To test the hypothesis that investment in airborne sound advertisement signaling trades off with investment in substrate-borne vibrational signaling in this group of katydids, we collected data from 10 species of pseudophyllines on Barro Colorado Island, Panama. We quantified the total number of calls produced over 24 hours in both signaling modalities for 3-6 individuals of each species. We compared sound and vibrational signaling among species using phylogenetic least squares models. To investigate additional relationships between species-specific characteristics and calling behavior, we considered additional factors including features of individual airborne calls such as duration, peak frequency, and bandwidth; abundance and the proportion of male captures of each species; and the mass of each species. These factors are neither exhaustive nor mutually exclusive, but understanding how they relate to diel levels of sound and vibration calling can shed light on signaling patterns across species and suggest avenues for further investigation.

## Methods

### Insect collection

We collected katydids at lights around the research station on Barro Colorado Island (BCI), Panama (9°09'17.9"N 79°50'50.4"W) on 248 days between December 2015 and March 2020. A standard set of building and other lights around the station was visited twice each collection day, once at approximately 04:30 and again at 23:00. All katydids that were present at these lights were captured in individual bags and brought back to the lab, where they were weighed and identified to sex and to species following Nickle (1992) and Cigliano et al.

(2020). Following capture, a subset of male katydids was retained for focal 24-hour recordings or other experiments, and 3–5 individuals of each species were preserved in 95% ethanol for phylogenetic reconstruction. Additionally, some katydids were retained as taxonomic vouchers at either the Muséum national d’Histoire naturelle (MNHM) in Paris, France or the Museo de Invertebrados G.B. Fairchild de la Universidad de Panamá (MIUP) in Panama City, Panama. The remaining individuals were released at sites several km distant to the lab clearing to minimize the chance that they would be recaptured at the lights. Pseudophylline katydids, which were less prevalent at lights relative to phaneropterine katydids, were also collected during nighttime walks through the forest on BCI or by searching day-roost locations like terrestrial bromeliads or curled leaves. These individuals were not included in the light-capture estimates of species abundance, but were used in recordings and for calculating average species mass.

Counts of each species’ light capture numbers were used as an estimate of abundance in this study, and roughly correspond to previous estimates of the relative rarity or commonness of species (Belwood, 1988). The genus *Idiarthron* contains several similar species that occur in Panama, and it is difficult to positively identify females of one species in this study, *Idiarthron majus*. Our estimates of *I. majus* abundance were therefore relatively low, and there were a larger number of *Idiarthron* katydids that were only able to be identified to genus level. To avoid underestimating abundance and the relative number of females of this species, we chose to assign some of these genus-level IDs to *I. majus* in proportion to positive identifications of *I. majus* males vs. other *Idiarthron* species in the light catch data.

## Phylogenetic tree

We reconstructed the phylogenetic relationships between the 10 focal species in this study using methods similar to those previously described in Symes et al. (2021). Briefly, we combined Genbank data from from Mugleston et al. (2018) and Symes et al. (2021) with new sequence data from an additional 29 species from our BCI collection efforts. In total our data matrix includes 301 species—186 more than in Symes et al. (2021)—including three

outgroup species from Mugleston et al. (2018) belonging to the families Gryllacrididae and Rhaphidophoridae. We used 6 DNA markers (mitochondrial: 12S rRNA and cytochrome c oxidase 2; nuclear: partial sequences of protein coding histone H3 gene, Wingless WG, and complete sequences of two non-protein corresponding to nuclear ribosomal subunits: 18S rRNA and 28S rRNA). DNA extraction, amplification, and sequencing was carried out at MNHN, with protocols as described in Dong et al. (2018). Phylogenetic analyses and divergence times estimations followed the following methods: Each gene sequence individually was aligned using the MAFFT algorithm under the default parameters found in Geneious R9.0.2. Then the aligned sequences of all six genes were concatenated in Geneious R9.0.2. The concatenated dataset was then analyzed using Bayesian inference (BI) and maximum likelihood (ML). For both BI and IQ we used PartitionFinder V2.1.1 (Lanfear et al., 2017) to determine best-fit partitioning schemes and the associated substitution models. Bayesian inference analyses were performed with MrBayes 3.2.6 (Ronquist et al., 2012) whereas ML analyses were performed with IQ-TREE 1.6.2 (Nguyen et al., 2015). All corresponding analyses were performed using the CIPRES Science Gateway 3.3 (Miller et al., 2012). For ML analyses best-scoring trees were obtained using heuristic searches relying on 100 random-addition replicates. Clade support was assessed using non-parametric bootstrap; for each analysis 1,000 bootstrap replicates were conducted. Nodes supported by bootstrap support values (BS) 70% were considered strongly supported following (Hillis and Bull, 1993). For BI analyses we conducted two independent runs with eight Markov chain Monte Carlo (MCMC): one cold and seven incrementally heated that ran for 50 million generations with trees sampled every 1000 generations. We used a conservative burn-in of 12.5 million generations per run after checking for stability on the log-likelihood curves and the split-frequencies of the runs in Tracer v.1.7 (Rambaut et al., 2018). Support of nodes for MrBayes analyses was provided by clade posterior probabilities (PP) as directly estimated from the majority-rule consensus topology. A clade with a PP value higher than 0.95 was considered as well supported. Divergence times were estimated using Bayesian relaxed clocks as implemented in BEAST 1.10.4. The partitions/clocks and substitution models were selected under PartitionFinder 2.1.1 following the settings presented above but with the ‘beast’ set

of models. BEAST analyses were performed on the CIPRES Science Gateway using BEAGLE to improve and speed up the likelihood calculation (Ayres et al., 2019; Miller et al., 2012). For each clock model/partitioning scheme an uncorrelated lognormal relaxed clock was implemented. The Tree Model was set to a birth–death speciation process (Gernhard, 2008) to better account for extinct and missing lineages. The ucl.d.mean prior of each clock model was set to an uninformative interval (0.0001–1.0) with a uniform prior distribution. Since our taxonomic sampling represented all extant families of infraorder Tettigoniidea, we referred to the calibration time from Wolfe et al. (2016), Song et al. (2015), and Mugleston et al. (2018). We thus used a secondary calibration point corresponding to the crown group of the katydids (infraorder Tettigoniidea) at the root of the phylogenetic tree, with the time range from 251 to 272 Ma, using a lognormal distributions centered on previously estimated median ages (Mean 261 – SD 6). BEAST analyses consisted of 400 million generations of MCMC with the parameters and trees sampled every 40,000 generations. A burn-in of 25% was applied after checking the log-likelihood curves. Trees obtained from distinct analyses were combined using LogCombiner v1.8.4 (<http://beast.bio.ed.ac.uk/>). The maximum credibility tree, median ages and their 95% highest posterior density (HPD) were generated with TreeAnnotator v1.8.4 (<https://github.com/beast-dev/beastmcmc/releases/tag/v1.8.4>).

## Signal recordings

### *Species call characteristics*

Each species’ stridulatory call has previously been described in ter Hofstede et al. (2020). For this study, we used the average values for bandwidth and peak frequency reported in that paper. To obtain a value for total call duration (summed sound excluding gaps between pulses), we took the mean duration of each pulse and the mean number of pulses and calculated the mean sum of sound per call among individuals of each species. Calls of several species can differ in the number of syllables they contain, so we weighted this sum of sound measure by the mean number of syllables observed by ter Hofstede et al. (2020).

### *Calling behavior*

To obtain the daily signaling behavior of each species, we counted the number of calls that individual males produced over a 24-hour period. Recordings took place in a metal greenhouse building that was exposed to ambient conditions near the forest edge. Each recording station consisted of a cylindrical fiberglass mesh cage (10 x 20 cm) that held a male katydid, a Plexiglas stand that positioned a recorder directly over the cage and also dampened some ambient noise and noise from other nearby recording stations, and a vibration-dampening platform of heavy floor tiles, foam mats and a bicycle inner tube. Males were recorded at up to 6 recording stations each day, but males of the same species were not recorded at the same time. Each recording was made with a Tascam DR-40 Linear PCM Recorder on the 4-channel setting, with the internal microphone pointed at the cage. To detect and record vibrational calls, a microaccelerometer (Knowles BU-21771, weight 0.28 g, sensitivity: 10.2 mV per m) was attached to the side of the cage with accelerometer wax, powered by a custom-built amplifier, and connected to the Tascam via the external recorder input. Recordings were made in 10-minute multichannel files with a 96 kHz sampling rate and 16 bit depth.

To identify airborne sound calls on the microphone tracks, we used an R script with functions from the 'seewave' (Sueur et al., 2008) and 'tuneR' (Ligges et al., 2023) packages to band filter recordings, then identify high-amplitude events in the frequency range of the focal species. To ensure that automated detections were accurate, we visually examined spectrograms of detections to eliminate false positives. Spectrograms of recording sessions with few detections were then also screened visually in Raven Pro (version 1.6) to ensure that calls were not passing below the amplitude detection threshold. Finally, to ensure that the automated detector was not repeatedly counting the same calling event, we plotted inter-call-intervals from each recording session and compared them to observed call repetition rates. One species, *Ischnomela gracilis*, has significant energy above 67 kHz with a peak frequency of 74 kHz, above the Nyquist sampling frequency of our Tascam recorders. For this species, we visually examined spectrograms of the airborne sound tracks for evi-

dence of the lower frequency component of the call (around 15 kHz). We confirmed these detections by looking at the vibrational tracks for the simultaneous vibrational signature of the stridulation call, which is a vibrational event containing low and high frequencies that was picked up by the accelerometer and was visually and acoustically distinct from the tremulation calls (Figure A.1).

To identify substrate-borne tremulation calls on the vibrational tracks, we visually examined recordings in Raven Pro. The vibrational calls are extremely low-frequency and resemble long purrs for all species in this study (unpublished data). The spectrogram view was zoomed to display 0–700 Hz in 20 s intervals, and each vibrational call was annotated using the selection table function.

### **Statistical analysis**

To test the hypothesis that there is a trade-off between airborne sound signaling and substrate-borne vibrational signaling across pseudophylline species, we constructed a series of phylogenetic least squares (PGLS) models in which the mean number of airborne sound calls per species (‘Number of sound calls’) was the response variable and the mean number of substrate-borne vibration calls per species (‘Number of vibrational calls’) was included as a fixed effect. We chose to use only the mean value rather than incorporating the intraspecific variance we recorded because individuals can fluctuate widely in calling activity due to many factors that operate at within- and among-individual levels (Chapter 2, this thesis), and for this study we were interested in the typical behavior of each species. To ensure that this mean value was representative, we only included species for which we had 24-hour recordings from at least three different individuals. In our initial model, we also included several other species traits that we considered to be potential drivers of variation in calling behavior as fixed effects: the mean sum of pulse durations in a single airborne call (‘Sum sound’); the bandwidth (‘Bandwidth sound’) and peak frequency (‘Peak frequency sound’) of species’ airborne calls; the number of each species caught at light traps over 5 years (‘Abundance’); the proportion of that catch that was male (‘Proportion male’); and the mean mass of each species (‘Mass’). Some variables were log-transformed to improve

the normality of residuals in the models (Table 1.1). For numbers of sound and vibrational calls, these transformations were first applied to the individual counts, prior to calculating the mean and standard deviation for a species, following Garamszegi (2014). Although all species produced both sound and vibration calls, we did not detect calls in one or the other modality for some individuals. To avoid issues with log transformation of these zero values, we used a transformation of  $\log(\text{number of calls} + 1)$ .

All analyses were conducted in R (version 4.2.0). We used the `drop.tip` function in the 'ape' package (Paradis and Schliep, 2019) to prune the complete tree to only the species for which we had complete data, and the `plgs` function in the 'caper' package (Orme et al., 2018) to run linear models that incorporated the phylogenetic relationships among species. We used a backwards selection approach to determine which of the initial variables best explained variation in the response variable by sequentially removing the fixed effect with the lowest  $t$  value and rerunning the model until only significant predictor variables remained. We checked the final model for multicollinearity using the variance inflation factor function from the 'car' package (Fox and Weisberg, 2019), and estimated the hierarchical partitioning between terms using the 'hier.part' package (Nally and Walsh, 2004) to determine the relative amount of variance in the model explained by each. We specified that the model should use maximum likelihood to estimate a value for Pagel's  $\lambda$  (hereafter  $\lambda$ , or phylogenetic signal). Near-zero  $\lambda$  values indicate low phylogenetic signal in the data. When estimates of  $\lambda$  were near zero we also ran a model with this value set at 1, its maximum, to see whether any observed patterns were robust to relatedness among species strongly affecting the distribution of trait values. Additionally, we used the `phylosig` function from the 'phytools' package (Revell, 2012) to estimate  $\lambda$  (with a log-likelihood function to test whether this estimate was distinguishable from 0) for each trait in isolation.

## Results

Between 2015 and 2020, we caught 6,539 katydids at station lights, of which 328 were in the subfamily Pseudophyllinae. The proportion of captures that were male was on average

60% for all pseudophyllines; captures of pseudophylline species in this study ranged from 50% to 88% percent male. The pruned phylogenetic tree, abundance, mass, and call trait characteristics are summarized in Figure 1.1 and Table 1.1.

The number of sound and vibrational calls varied substantially within and across species. There was a greater range of variation for numbers of sound calls across species (0–23,898) than for vibrational calls (0–1,378), although the greater duration of vibrational calls meant that the range in total time spent calling was similar for each modality (1,561 s range for vibrational calls vs. 1,433 s range for sound calls across all species). While several species did overlap, there were also clear differences between some species. For example, over 24-hours, *Thamnobates subfalcata* males stridulated between 7,300 and 12,392 times but only tremulated between 3 and 12 times; *Eubliastes pollonerae* males stridulated 12–406 times and tremulated 10–56 times; and *Ischnomela gracilis* males stridulated 0–4 times and tremulated 9–386 times (Table 1.1, Figure 1.2).

Across species, and in all but one of the PGLS models, the average number of sound calls was negatively related to the average number of vibrational calls (Table 1.2). In our final model, this relationship was mediated by sound call bandwidth and the proportion of light captures that were male. Of these three variables, the number of vibration calls explained a significant amount of variation in the number of sound calls on its own, while the  $t$  values for bandwidth and proportion male were very close to each other (-1.94 vs -1.91), indicating a similar effect on the model. We used likelihood ratio tests to compare a model with both terms to models excluding either term, and chose to keep the fuller model ( $R^2_{\text{adj}} = 0.75$ ,  $\lambda = 0$ ,  $P = 0.01$ ) because it fit significantly better than either of the reduced models (joint F-test:  $F_{2,6} = 7.45$ ,  $P = 0.024$ ; reduced model with bandwidth:  $R^2_{\text{adj}} = 0.65$ ,  $\lambda = 0$ ,  $P = 0.027$ ; reduced model with proportion male:  $R^2_{\text{adj}} = 0.65$ ,  $\lambda = 0$ ,  $\chi^2 = 4.76$ ,  $P = 0.029$ ), even though the individual terms for proportion male and bandwidth were not significant (Table 1.2). The number of vibrational calls explained most of the variation in sound signaling that was explained by the final model (55.3%; Figure 1.3 A), then bandwidth (30.6%; Figure 1.3 B), and lastly proportion male (14%; Figure 1.3 C). In this model,  $\lambda$  was estimated to be zero, indicating no detectable phylogenetic signal in

the residuals. All other models also had  $\lambda$  estimated at zero, though the upper confidence interval limits were large where it was possible to estimate these (Table 1.2).

None of the individual trait estimates for  $\lambda$  were significantly different from zero, indicating a general lack of phylogenetic signal in the trait variation among these ten species. Log likelihood estimates of  $\lambda$  for these traits were mostly near zero, except for the three sound call variables: number of calls, peak frequency of calls, and bandwidth of calls (Table 3, Figure 1.1).

## Discussion

In this study, we examined the relationship between investment in airborne sound advertisement signals and investment in separate, yet functionally overlapping, vibrational advertisement signals across a group of morphologically diverse katydid species. Specifically, we tested whether investment in sound trades off with, is positively correlated with, or is unrelated to investment in vibration. We found a negative relationship between the total number of sound signals and the total number of vibrational signals, supporting the trade-off hypothesis. The bandwidth of the sound signals and the proportion of males caught at lights for a species also appear to have a significant effect on investment in sound signaling. Overall, more sound calls tended to be produced by males of species that did not use many vibrational signals, had sound calls of narrower bandwidths, and for which males were not disproportionately captured at lights relative to females.

These relationships appear to be largely independent of phylogeny. In our models, estimates of phylogenetic signal were always near 0, and across the phylogenetic tree, more closely related species (e.g., *Thamnobates subfalcata* and *Eubliastes pollonerae*, Figure 5) were not more similar in traits than other species pairs. It should be noted that the two species least closely related to the remaining eight, *Scopiorinus fragilis* and *Ischnomela gracilis*, occupied the extremes of the sound signaling investment we observed, with *S. fragilis* producing 3,234–23,898 calls per day and *I. gracilis* producing at most 4 calls in our recordings. *Ischnomela gracilis* in particular has unusually high frequency and short duration

sound calls. Where there was evidence for a phylogenetic signal within the distribution single traits (in the number of sound calls, sound call bandwidth, and sound call peak frequency), this was possibly due to an outsized effect of the sound signals of this distantly related species being the least like the others. Excluding *I. gracilis* did not qualitatively change the model results. The other eight species are grouped within a single tribe (Pleminiini, Brunner von Wattenwyl, 1895) and, with the exception of one species (*T. subfalcata*) tended to overlap in the number of sound and vibration signals they produced (Figure 1.2). While our results are robust to the influence of phylogenetic relatedness, additional signaling data from species within and outside Pleminiini would be useful to know whether the trait relationships we observe are indeed strongest at the among-tribe level rather than within tribes.

That there was a relatively strong negative relationship between sound and vibration signaling is striking given the morphological and ecological differences among these species: they differ in body size (ter Hofstede et al., 2020), coloration, degree of crypsis, and other antipredator defenses (Robinson, 1969; Belwood, 1988; ter Hofstede et al., 2017), and possibly habitat and dietary preferences (Symes et al., 2022; Palmer et al., 2022). In this group of tettigoniids, sound and vibration calling signals are produced by separate mechanisms (tegminial stridulation vs. abdominal tremulation), with each subject to different costs and benefits to signalers and receivers. For instance, sound signals have a larger active space and are less energetically expensive to produce than vibrational signals (Römer et al., 2010), but vibrational signals are hidden from gleanings bats (Geipel et al., 2020) and are more likely to initiate close-range courtship duets if a female is within range of the signal (Iwan et al., 2023, in prep.). Thus, we might have expected no relationship between the two signal types, because selection pressures on each could differ quite drastically (Whitacre and Bender, 2010; Gomes et al., 2017).

Yet our data show that sound and vibration calls *are* negatively related across species, supporting the idea that vibrational signaling compensates for the much-reduced sound output (via low duty cycle, short duration, and high frequency airborne calls) of many of these pseudophylline species, as suggested by Belwood and Morris (1987). Previous

evidence for a gradient of sound signal investment has been largely descriptive: Belwood (1988), for instance, noted that a Panamanian species not present in our current study, *Ischnomela pulchripennis*, produced vibrational signals only rarely, usually when startled, and otherwise produced many conspicuous sound signals. Sound calls of this species are also detected very often on ambient soundscape recordings (Symes et al., 2022). In Ecuador, the pseudophylline species *Schedocentrus differens* is reported to produce no sound in mate advertisement contexts at all, and may rely solely on tremulation and drumming vibrational advertisement calls (Morris et al., 1994). Our findings provide quantitative context, demonstrating that there may in fact be a gradient between these extremes, with decreases in the average daily sound calling output of a species corresponding to increases in vibrational calling output over the same span of time.

We did not deeply investigate variation in vibrational signaling in these species, beyond that it correlates with sound investment. Species differ in not only how much vibration they use, however, but also in the spectral and temporal characteristics of their vibrational calls (Belwood, 1988). While these differences are less stark than for sound signaling—for instance, the dominant frequency of vibrational calls in a subset of these species ranges from 13–27 Hz, whereas the dominant frequency of the same species’ sound calls ranges from 14–74 kHz—they nevertheless raise questions about whether the vibrational calling signals can be used by receivers to reliably distinguish between suitable mates and heterospecifics. While there is evidence that pseudophyllines can distinguish between conspecific vibrations and those produced by a predatory katydid in a different subfamily (Iwan et al., 2023, in prep.), these two signals have very different pulse structures and peak frequencies, and it is not clear whether signals that show more overlap in their spectral and temporal characteristics, such as those of the ten species in this study, are readily distinguished by female receivers. Relatedly, there is a need for more investigation into differences in habitat preferences and signaling sites in these katydids. In other insects that use vibrational communication, features of signals such as frequency and bandwidth can interact with features of the solid substrate to affect propagation (McNett and Coccoft, 2008; Čokl et al., 2021). While some katydid species are known to tremulate on plants that transmit vibrations well

(Lang and Römer, 2008; Belwood, 1988), we do not know much about where many others tend to signal in nature. Structurally different substrates (e.g., tree trunks, lianas, or large palm leaves) could affect the transmission and filtering of these species’ vibrational calls, potentially blurring spectral or temporal differences between different species’ calls. Alternatively, associations with preferred host plants (for food, dayroosts, or both) could aid in aggregation and mate-finding for species that have low levels of signaling (Cocroft et al., 2010; Xu and Turlings, 2018). Questions also arise about how features of vibrations might interact with other species traits to influence the amount of vibrational signaling a species tends to use. In our dataset, for instance, intermediately abundant species like *D. gigliotosi*, *C. wheeleri*, and *I. gracilis* tended to produce the most vibrational calls, while very common (*T. subfalcata*) and very rare species (*P. tuberosus*) tended to produce the least. Additional signaling data for more species, and more robust methods of quantifying species abundance or density, will be useful for investigating this potential pattern.

In our study, bandwidth of sound signals helped explain a significant amount of sound signaling variation, with higher-bandwidth calls being produced by species that called less. This could be related to signal conspicuousness: even pseudophylline species with relatively narrowband calls have been shown to have broadband ultrasonic tuning of their auditory system because of features of their external pinnae (Woodrow and Montealegre-Z, 2023). Females might more easily perceive more broadband signals, which are more resistant to scattering or attenuation. Species with more broadband sound calls might therefore be able to “get away” with using fewer signals. However, at least one predatory bat species prefers lower bandwidth, more pure-tone calls (Falk et al., 2015), so it is not necessarily straightforward that higher sound bandwidths are more conspicuous to all possible receivers. Additionally, female meadow katydids have been found to prefer calls with more attenuation at higher frequencies, perhaps because these indicate a male’s ability to produce louder absolute call amplitudes (Harness and Campbell, 2021). Measurements of the absolute amplitudes of pseudophylline species’ sound calls and how these relate to call bandwidth may shed light on how detectable calls remain to conspecific and heterospecific receivers as they propagate through the forest.

Similarly to Symes et al. (2021), we found that the sex ratio of light captures explained a significant amount of variation in katydid sound signaling. Species that signaled with very few sound calls tended to have higher proportion male captures. That study focused on Panamanian katydids within the subfamily Phaneropterinae, in which many species engage in mate-finding duets using airborne sound calls. While the ten pseudophylline species in this study do not exhibit similar long-range airborne duetting behavior, they do engage in vibrational duets, with females producing vibrational replies to male vibrational calls (Belwood, 1988, CEK unpublished data). It is therefore likely that both sexes contribute some search effort, unlike in species where one sex (usually males) remains stationary and calls. Males of different species could differ in how much time they spend searching vs. signaling. In other words, the actual “mean crowding” (Lloyd, 1967) experienced by each individual male of a species might reflect not just the overall abundance or density of each species, but also the degree of searching by each sex. It would be worth examining differences in spermatophore size and content across these species to see if there is differential reproductive investment that could explain some of the differences in total signaling by males of different species, as suggested by Gwynne (2001).

Daily signaling rates can vary widely among individuals of the same species and within an individual over time. We chose to use a single number representing the mean signaling for 3-6 individuals of our ten focal species to examine differences among species without undue influence by individuals that might have signaling at unusually low or high levels due to, e.g., recent mating (Chapter 2, this thesis) or environmental factors like wind (Velilla et al., 2020). In addition to this intraspecific variation, we note that different species might have non-obvious differences in call function or other behaviors that could affect how much and when they signal. For instance, Belwood (1988) reports that one *S. fragilis* individual called for “several hours” one day after mating, which may indicate either a very short signaling refractory period or some sort of interference function for sound calls in this species. de Souza et al. (2011) reports that the vibrational signals of *Gnathoclitia sodalis* are “not designed to attract from a distance” and appear to be used primarily in close-range male–male contests. Although all ten species in our study readily

and spontaneously called in our recording setup, it is also possible that captivity and the artificial recording conditions affected them differently. For example, Symes et al. (2022) found a larger discrepancy between *D. gigliotosi* sound calling behavior in captivity and in passive acoustic canopy recordings than for several other species, and we recorded fewer *I. gracilis* calls in this study in comparison to previous studies where they were recorded under different conditions (e.g., ter Hofstede et al., 2010). Finally, almost nothing is known about how this group of katydids interacts with conspecific and heterospecific callers, but observations by Montealegre-Z (2012) and others indicate that the potential group signaling dynamics of Neotropical katydids should be further investigated.

Despite their ecological importance as prey items for many different taxa (Peres, 1992; Rosenberg, 1993; Belwood and Morris, 1987; Nickle and Castner, 1995) and their potential utility in understanding the evolution of complex communication systems that has been recognized by researchers for many years (Rentz, 1975; Belwood and Morris, 1987; Zuk and Kolluru, 1998; Lang et al., 2005), many aspects of Neotropical pseudophylline katydids' biology have remained enigmatic. Specifically, total levels of investment in the the sound and vibrational channels available to the species in this subfamily have not been quantified, nor examined in a comparative phylogenetic framework—essential pieces in understanding the relationship between these animals' degenerate calling signals. Here, we find that airborne sound calls are negatively related to substrate-borne vibrational calls, suggesting that species with especially reduced sound calls can compensate for this loss by broadcasting their availability to mate in the vibrational channel. Additional features of the sound calls (bandwidth) and measures related to searching behavior (sex ratios at light traps) also influence this relationship. While additional knowledge about the signaling and mate-searching behavior, ecology, and population dynamics of these and related species is still needed, our study reveals some of the selective pressures that may have worked in concert to shape the multimodal signaling repertoire of this group of insects.

## Tables and Figures

**Table 1.1: Focal species traits.** Signaling, mass, and demographic characteristics of the ten pseudophylline species in this study. Values in the three "Sound call measurements" columns are from ter Hofstede et al. (2020). All other values are from this study. Values in first two columns are mean  $\pm$  s.d. of sound and vibrational call counts. Starred columns were log-transformed in all statistical models.

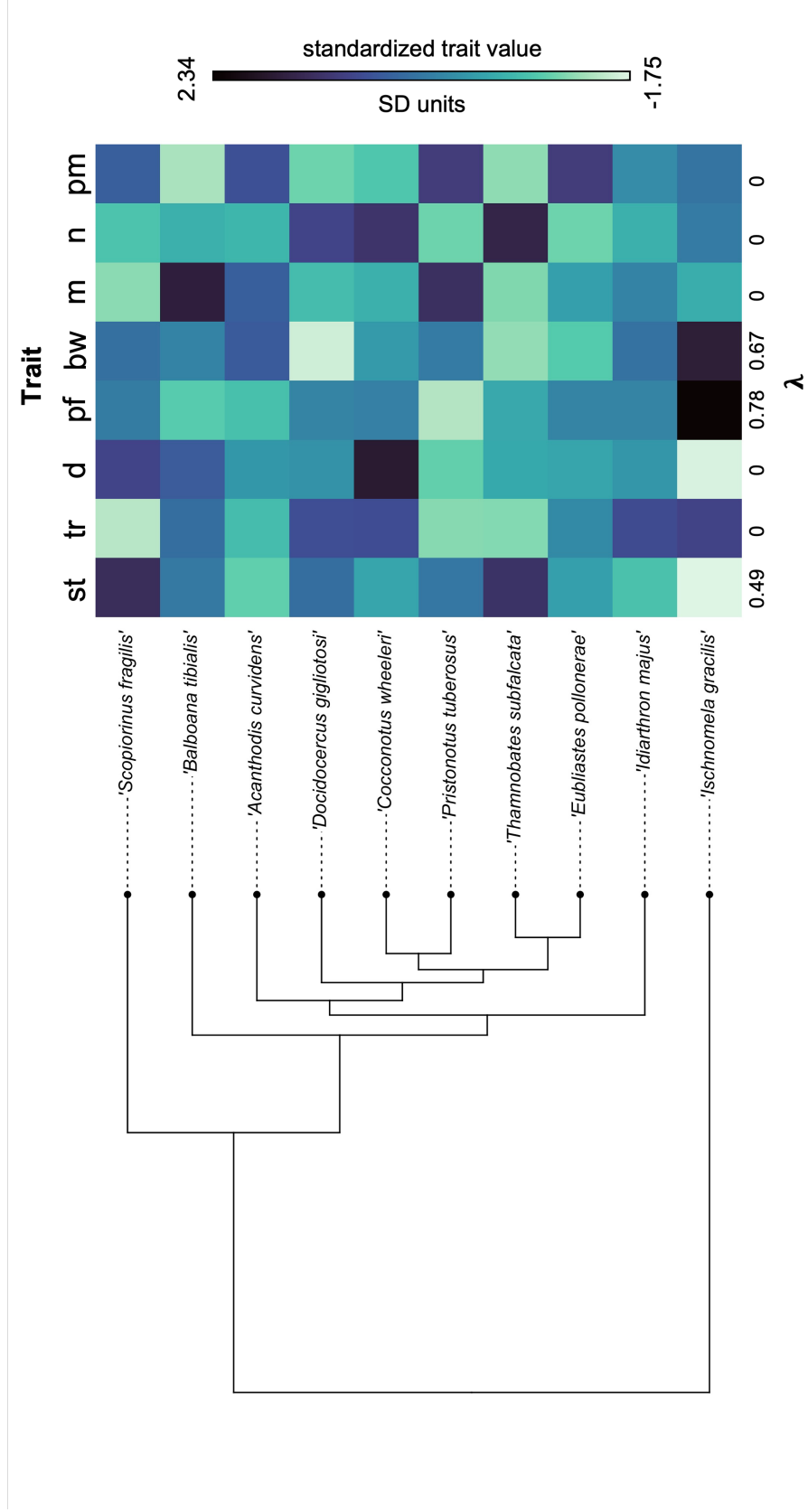
| Species                        | No. sound calls*      | No. vibrational calls* | Sound call measurements |                       |                  |          | Abundance* | Proportion male |
|--------------------------------|-----------------------|------------------------|-------------------------|-----------------------|------------------|----------|------------|-----------------|
|                                |                       |                        | Sum (ms)*               | Peak frequency (kHz)* | Bandwidth (kHz)* | Mass (g) |            |                 |
| <i>Acanthodis curvidens</i>    | 57.7 $\pm$ 100.2      | 16.7 $\pm$ 14.7        | 29.3                    | 15.6                  | 12.1             | 3.58     | 13         | 0.85            |
| <i>Balboana tibialis</i>       | 707.8 $\pm$ 372.6     | 75.8 $\pm$ 99.3        | 49.0                    | 14.4                  | 8.4              | 5.37     | 14         | 0.50            |
| <i>Cocconotus wheeleri</i>     | 224.8 $\pm$ 157.8     | 76.4 $\pm$ 33.0        | 97.8                    | 24.8                  | 6.7              | 1.68     | 61         | 0.59            |
| <i>Dacidocercus gigliotosi</i> | 1469.3 $\pm$ 766.2    | 318.2 $\pm$ 532.7      | 30.9                    | 24.4                  | 2.6              | 1.43     | 48         | 0.55            |
| <i>Eubliastes pollonerae</i>   | 212.3 $\pm$ 158.3     | 32.2 $\pm$ 16.9        | 25.8                    | 24.2                  | 4.3              | 2.10     | 9          | 0.89            |
| <i>Idiarthron majus</i>        | 69.7 $\pm$ 97.9       | 98.0 $\pm$ 74.6        | 29.4                    | 24.4                  | 9.8              | 2.76     | 14         | 0.71            |
| <i>Ischnomela gracilis</i>     | 2.3 $\pm$ 2.1         | 183.0 $\pm$ 188.0      | 11.0                    | 73.9                  | 24.1             | 1.75     | 26         | 0.77            |
| <i>Pristonotus tuberosus</i>   | 754.0 $\pm$ 457.9     | 6.8 $\pm$ 0.5          | 17.5                    | 10.9                  | 9.0              | 4.80     | 9          | 0.89            |
| <i>Scopiorinus fragilis</i>    | 15621.7 $\pm$ 10928.3 | 6.0 $\pm$ 5.0          | 60.0                    | 25.6                  | 10.0             | 0.56     | 11         | 0.82            |
| <i>Thamnobates subfalcata</i>  | 9202.8 $\pm$ 3656.8   | 7.5 $\pm$ 3.7          | 25.2                    | 18.8                  | 3.4              | 0.65     | 78         | 0.53            |

**Table 1.2: PLGS model comparisons.** Letters refer to traits that were included in the initial model, then selectively removed. Lowercase letters correspond to traits as in Table 3 and Figure 1 that were included as fixed effects. Numbers in square brackets in final column are the confidence interval estimates for Pagel’s  $\lambda$  (phylogenetic signal) by maximum likelihood, where these were able to be estimated. Model 4 could not be fit using maximum likelihood, so  $\lambda$  was set to 1. We also set  $\lambda = 1$  to check whether the the results of Model 5 were qualitatively similar in a model that assumed a strong phylogenetic signal. The bolded model (5) is the final model.

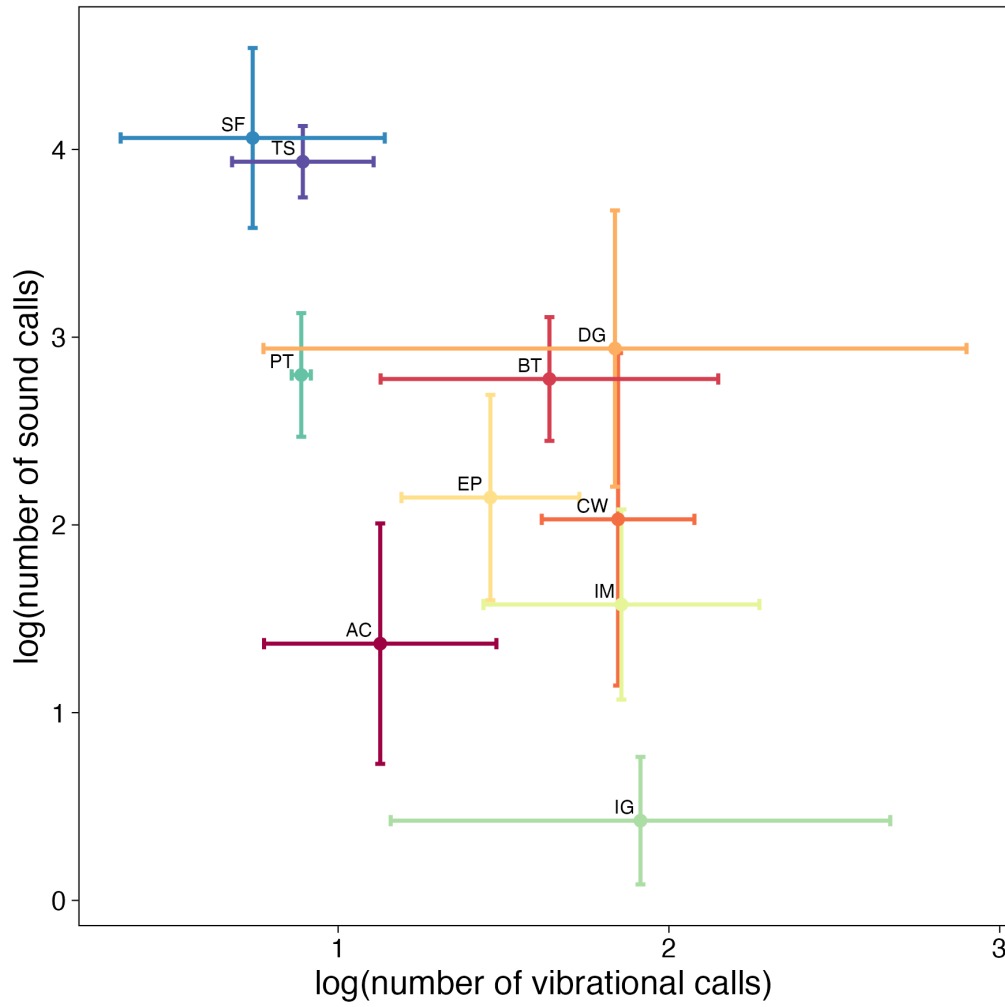
| PGLS model                                   | Significant predictors | P <sub>predictor</sub>               | P <sub>model</sub> | R <sup>2</sup> <sub>adj</sub> | Pagel’s $\lambda$ |
|--|------------------------|--------------------------------------|--------------------|-------------------------------|-------------------|
| (1) st ~ tr + d + pf<br>+ bw + m + n +<br>pm | None                   | NA                                   | 0.232              | 0.674                         | 0 [0, 0.814]      |
| (2) st ~ tr + d +<br>bw + m + n + pm         | tr                     | 0.028                                | 0.084              | 0.772                         | 0 [0, 0.930]      |
| (3) st ~ tr + bw +<br>m + n + pm             | tr                     | 0.0104                               | 0.027              | 0.815                         | 0 [NA, NA]        |
|  | pm                     | 0.039                                |                    |                               |                   |
| (4) st ~ tr + bw +<br>n + pm                 | tr                     | 0.004                                | 0.012              | 0.811                         | = 1               |
|  | pm                     | 0.049                                |                    |                               |                   |
| <b>(5) st ~ tr + bw<br/>+ pm</b>             | tr                     | 0.007                                | 0.010              | 0.747                         | 0 [NA,NA]         |
|  | bw, pm                 | 0.104,<br>0.100<br>(joint:<br>0.024) |                    |                               |                   |
|  | tr                     | 0.003                                | 0.005              | 0.801                         | =1                |
|  | bw                     | 0.054                                |                    |                               |                   |
|  | pm                     | 0.046                                |                    |                               |                   |
| (6) st ~ tr + pm                             | tr                     | 0.005                                | 0.011              | 0.650                         | 0 [NA,NA]         |
|  | pm                     | 0.024                                |                    |                               |                   |

**Table 1.3: Maximum likelihood estimates for phylogenetic signal in individual species traits.** Labels for each trait correspond to Figure 1.1 and Table 1.2. Pagel's  $\lambda$  is the phylogenetic signal estimate for each trait. The logL and logL0 columns provide values for the log-likelihood estimates for  $\lambda$  and  $\lambda = 0$ , respectively.

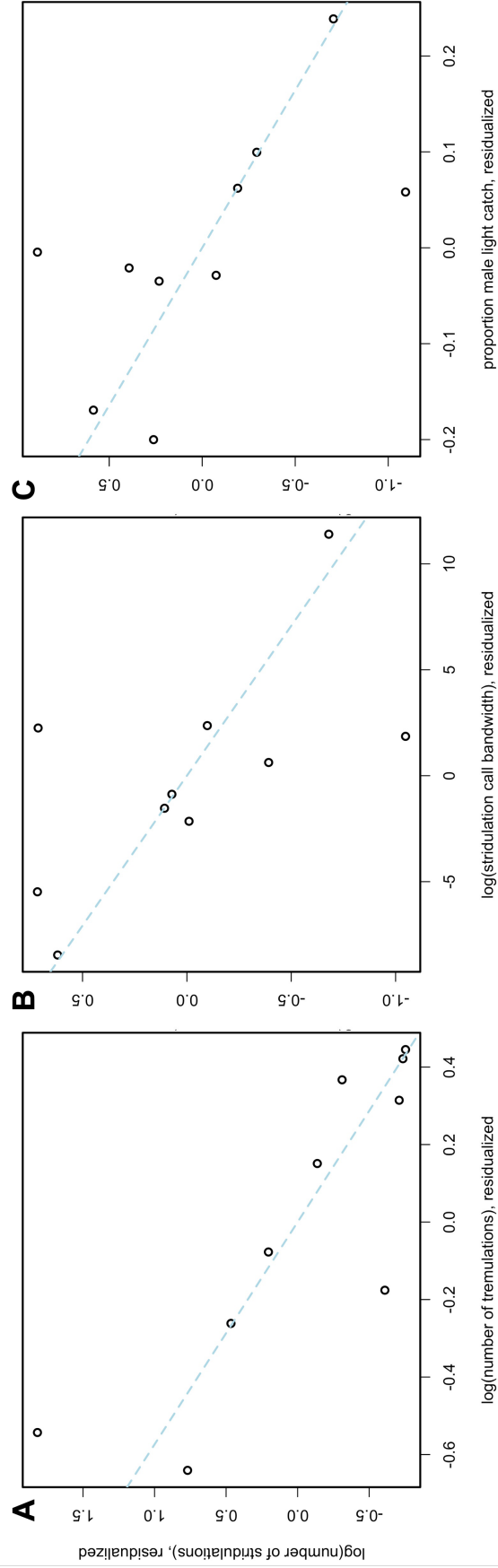
| Trait                           | Label | Pagel's $\lambda$ | logL    | logL0   | P     |
|---------------------------------|-------|-------------------|---------|---------|-------|
| Number of sound calls           | st    | 0.489             | -15.020 | -0.216  | 1     |
| Number of vibrational calls     | tr    | < 0.001           | -6.003  | -0.0004 | 1     |
| Sound call sum of sound (ms)    | d     | < 0.001           | -0.523  | -0.0002 | 1     |
| Sound call peak frequency (kHz) | pf    | 0.784             | 2.657   | 2.528   | 0.112 |
| Sound call bandwidth (kHz)      | bw    | 0.674             | -0.845  | 0.494   | 0.482 |
| Mass (g)                        | m     | < 0.001           | -18.665 | -0.0005 | 1     |
| Abundance                       | n     | < 0.001           | -3.292  | -0.0006 | 1     |
| Proportion of catch male        | pm    | < 0.001           | 5.013   | -0.0006 | 1     |



**Figure 1.1: Phylogenetic relationships and species traits** Left: Phylogenetic relationships among the 10 pseudophylline katydid species that were studied. Right: Heatmap showing standardized trait values for eight different species traits. Traits are labeled with lowercase letters as follows: st: Log-transformed mean number of sound (stridulation) calls recorded over 24 hours of 3–6 individuals per species. tr: Log-transformed mean number of vibrational (tremulation) calls recorded over 24 hours of 3–6 individuals per species. d: Log-transformed sum of sound per sound call, pf: Log transformed peak frequency of sound calls and bw: log-transformed bandwidth of sound calls, all from ter Hofstede et al. (2020). m: Mean species mass (including both males and females). n: Log-transformed number of individuals caught at light traps over a 5-year period on BCI. pm: Proportion of individuals caught at light traps that were male. Numbers at the bottom of each trait column are estimates of phylogenetic signal ( $\lambda$ ) in that trait. None of these estimates were significantly different from zero, but log-likelihood functions indicated that traits pf, bw, and potentially st were more influenced by phylogenetic relationships than any of the others (Table 1.2).



**Figure 1.2: Interspecific and intraspecific variation in multimodal advertisement calling.** Total sound and vibrational calls recorded over 24 hours for individuals of the ten pseudophylline species in this study. Call counts have been log transformed for each individual, then means and standard deviations (error bars) were calculated for each species. Letter codes indicate species. AC: *Acanthodis curvidens*, BT: *Balboana tibialis*, CW: *Cocconotus wheeleri*, DG: *Docidocercus gigliotosi*, EP: *Eubliastes pollonerae*, IM: *Idiarthron majus*, IG: *Ischnomela gracilis*, PT: *Pristonotus tuberosus*, SF: *Scopiorinus fragilis*, TS: *Thamnobates subfalcata*.



**Figure 1.3: Relationships between number of sound calls and number of vibrational calls, male motility, and sound call bandwidth.** Partial regression plots showing the effect of A. the number of vibration calls, B. the proportion of light captures of a species that were males, and C. sound call bandwidth on the number of sound calls. X-axis residuals are taken from a model regressing each predictor (e.g., number of tremulations) against the other two predictor variables (e.g., bandwidth and proportion male). Y-axis residuals are taken from a model regressing the number of sound calls against these two predictors while excluding the third, to isolate the variation that was not attributable to the other factors in the final model.

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## Chapter 2

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Transient and persistent effects of mating on individual multimodal signaling behavior in the katydid *Docidocercus gigliotosi* (Orthoptera: Tettigoniidae)

## Introduction

Animals can produce a variety of signals to attract potential mates, and these signals sometimes overlap in function (Andersson, 1994; Hebets and Papaj, 2005). Investment in signal production in terms of time and energy can be highly variable among individuals, and also within individuals over time. Variation in sexual advertisement signaling by animals has profound effects on their fitness, but untangling the factors that shape variation at different hierarchical levels is challenging, particularly for complex signal repertoires (Westneat et al., 2015; Patricelli et al., 2016). The components of such repertoires, which are often produced in different sensory modalities (Partan and Marler, 1999), can have different degrees of functional overlap: in the context of mate advertisement, they might convey independent information to receivers (“multiple messages,” Johnstone, 1996), interact to contribute to an emergent function (“inter-signal interactions,” Hebets and Papaj, 2005), or independently share a function (“degeneracy,” Hebets et al., 2016). Degeneracy, which is often referred to as “redundancy” (sensu Partan and Marler, 1999), has a number of theoretical benefits in communication. For example, it can allow signals to function as backups of each other, enhancing the robustness of a communication system in noisy conditions (Grafe et al., 2012). It might also broaden the functional range of a system by relaxing selection pressure on duplicate signal producing structures, and could be more likely to lead to novel signaling phenotypes (Hebets et al., 2016).

To overlap in function, signals should independently suffice to elicit a particular response in receivers—as with the ultrasonic clicks and pheromones of arctiid moths, either of which is sufficient to enhance the copulation success of males (Conner, 1987). However, such different signals are unlikely to be wholly equivalent: they might differ in terms of their cost to produce, their likelihood of reception by receivers, the strength of the response they elicit, and even their heritability. Such differences have implications for how and when particular signals are deployed, and can shape patterns of covariation in degenerate signals both within and among individuals. A lack of among-individual correlations might be predicted when degenerate advertisement signal elements are so structurally or physiologically

independent that they respond differently to selection, environmental pressures, or other forces despite their shared function (Hebets et al., 2016). Alternatively, degenerate signals could be correlated among or within individuals if animals benefit from different reproductive strategies or experience significant energetic trade-offs. If signaling traits are heritable, this scenario could result in concurrent changes across a population’s signaling repertoire, where selection on each signal element is not independent from selection on the others. For example, positive correlations between degenerate signals across individuals (i.e. frequent and infrequent signalers in a population) might reflect the presence of alternate reproductive tactics, which often involve multiple signals and behaviors that are expressed more strongly in only one tactic. “Type I” plainfin midshipman males, for example, maintain nests and frequently produce multiple types of acoustic signals; the smaller “Type II” males produce fewer, lower amplitude acoustic signals and rely on satellite behavior to reproduce (Brantley and Bass, 1994). Positive correlations that are more continuous in nature could reflect male quality, like the multiple correlated dimensions of visual and vibrational courtship signaling that predict mating success in peacock spiders (Girard et al., 2015). Conversely, negative correlations within or among individuals could reveal either transient or more permanent energetic or developmental trade-offs: individuals might be able to produce more of one signal element if they invest less in the other. For instance, male cabbage white butterflies develop either brighter wings or higher levels of pheromone expression, depending on their light environment as caterpillars (Zambre and Thaker, 2017).

Assessing how or why animals use multiple advertisement signals can be complicated by other potential contributors to overall variation in the signaling repertoire of a population or species. It has long been appreciated that observed phenotypic variation can arise both from persistent differences between individuals—for behavioral traits, “personality”—and because of plasticity within individuals (West-Eberhard, 2003; Westneat et al., 2015). While signaling behavior can vary between individuals due to genetic differences (Hedrick, 1988), it is also often extremely plastic at multiple time scales. Variation in expression levels of advertisement signals can occur over the course of an individual’s life (Bertram et al., 2021), seasonally (Griffith and Sheldon, 2001; Beckers and Schul, 2008), within the span

of a single night (Velilla et al., 2020), and from moment-to-moment (ter Hofstede et al., 2008). Predator cues, for instance, can curtail advertisement signal expression at short timescales, resulting in “flexible” alternative reproductive tactics between which a single individual can shift. Male guppies that move from conspicuous advertisement displays to sneaker tactics when exposed to model predators (Godin, 1995) and tree crickets that call on some nights and remain silent on others (Sadiq et al., 2023) provide examples of such within-individual signaling plasticity. Researchers can attempt to partition the amount of variation due to within-individual plasticity vs. persistent differences between individuals by measuring signaling over biologically relevant timescales and across different environmental or behavioral contexts (Dingemanse and Dochtermann, 2013).

We investigated variation at multiple hierarchical levels in the production of degenerate mate advertisement signals in *Docidocercus gigliotosi*, a katydid in the tettigoniid subfamily Pseudophyllinae. Male katydids in this subfamily spontaneously produce two structurally different signals with the same function of attracting females: airborne sound acoustic signals produced by tegminal stridulation, and substrate vibrations produced by abdominal tremulation (Figure 2.1). For the katydids, these alternate advertisement signals can differ not only in conspicuousness to eavesdropping bat predators (Belwood, 1988; Geipel et al., 2020) but also the range at which they can be detected by potential mates (i.e. the active space), their attractiveness to conspecific females, and their energetic cost (Table 2.1). The fitness consequences of producing each type of signal are likely to fluctuate over time for an individual male caller, depending on his life history, current condition, and surrounding audience. For instance, many orthopteran species that produce spermatophore nuptial gifts during mating (Figure 2.2) experience a signaling refractory period during which the energetic demands of mating and spermatophore production outweigh the benefits of immediately attempting to attract another mate (Vahed, 2007). There is also evidence that katydids may adjust their signaling behavior on full moon nights, when searching females are at lower risk from some types of predators (Römer et al., 2010).

In this study, we repeatedly quantified the number of sound and vibration signals produced by individual *D. gigliotosi* over multiple weeks. These data allowed us to assess both

within-individual and among-individual variability in signal production. We tested whether 1) the number of sound and vibration signals were correlated with each other, either within individuals (Figure 2.3 A), across individuals (Figure 2.3 B), or both (Figure 2.3 C); 2) individuals differed in their use of each signal type over time (i.e. showed plasticity in signaling behavior); 3) individuals showed consistent repeatable differences from each other in their use of each signal (i.e. showed “personality”); and 4) recent mating had any transient or more persistent effects on the production of either type of signal. We discuss the implications of our results for possible mechanisms influencing the use of degenerate signals.

## Methods

### Study sites and animals

The study was conducted on Barro Colorado Island, Panamá, from January through March 2020, during the dry season. We captured male and female *D. gigliotosi* during the day from three patches of *Aechmea magdalenae* bromeliads or from vegetation in the surrounding forest. Collections took place during the month of January, mating trials were conducted from 28 Jan–3 Feb, and recordings began the same week. Mating trials and recordings were conducted in metal mesh and shade cloth greenhouses that were exposed to ambient conditions. Males and females were initially housed in communal sex-specific cloth mesh cages (36 x 36 x 60 cm). After mating trials and recordings began, males were transferred to smaller individual cloth mesh cages (13 x 13 x 23 cm). When animals were not participating in 24-hour recording sessions, their cages were kept under shade cloth in an open-air greenhouse that was located ~330 m from the recording stations. All animals were fed ad libitum with cat food and apples and were provided with water.

### Matings

To assess whether recent mating history influenced signaling behavior in the wild-caught males ( $N = 26$ ), we allowed approximately half ( $n = 12$ ) to mate in the lab on the night preceding the first recording session. We introduced females to several (4–8) individual

males' cages each night. After each female was introduced, we monitored her interactions with the male under red light, noting whether copulation was attempted and whether spermatophore transfer occurred. Details of these mating trials are reported in the Appendix. Males and females were weighed before the female was introduced, and mated males were weighed immediately after trials concluded to obtain an estimate of spermatophore mass.

## **24-hour sound and vibration recordings**

On the night following each round of matings, the one or two males that had successfully mated began the first round of 24-hour recordings, along with two or three other males that had not mated. Males were weighed and then placed in one of four separate recording stations located in separate greenhouses. Each recording station was at least 14 m away from other recording stations. Each male was always recorded at the same station.

Recording stations consisted of a cylindrical fiberglass mesh cage (10 x 20 cm) that contained the male, a Plexiglas stand that held the recorder directly over the cage and acted as a baffle for some ambient noise, and a platform of heavy ceramic tiles and a bicycle inner tube that isolated the cage and recorder from some ambient vibrational noise. Males were provided with a tray of water with cotton balls and small apple pieces during the recordings. Recordings were made with a Tascam DR-40 Linear PCM Recorder on the 4-channel setting, with the internal microphone pointed at the cage and a microaccelerometer (Knowles BU-21771, weight 0.28 g, sensitivity: 10.2 mV per m) that was attached to the cage with petro wax feeding into the external recorder input. Recordings were made in 10-minute files with a 96 kHz sampling rate and a 16 bit depth. Recording sessions were started during the day and were allowed to run at least 30 minutes beyond the 24-hour mark on the next day. None of the recording sessions began later than 16:45 h, ensuring that males were undisturbed during their most active time. We recorded each male once every  $7 \pm 3$  days for up to 6 weeks. When individuals died, a male that had been unsuccessfully recorded on a previous night of the week at that station (because of card errors or electrical problems) was re-recorded instead.

## Acoustic data processing

To identify sound (stridulation) calls on the microphone tracks, we developed a custom R script using functions from the 'seewave' (Sueur et al., 2008) and 'tuneR' (Ligges et al., 2023) packages that combined a bandwidth filter and amplitude detector to find events with high amplitude and high dominant frequency (above 20 kHz). The amplitude threshold was determined by incrementally lowering it until few or no true positives were detected on a subset of the recordings. Spectrogram images of each call detection were examined visually to eliminate false positives. Similar methods have been described in Symes et al. (2021) and Symes et al. (2022).

In contrast to sound calls, which are high amplitude and high frequency, vibrational (tremulation) calls were not necessarily the highest amplitude events on the vibration tracks and have spectral properties that overlap with many other sources of noise, like walking or chewing vibrations (Figure B.1). To identify vibrational calls on the accelerometer tracks, we used the Python package 'koogu' (Madhusudhana, 2021) to train a DenseNet convolutional neural network model on 16% of the 24-hour recording sessions (20 out of 123 sessions). Tremulation events within these 20 recording sessions were first annotated by CEK in Raven Pro. The trained model was then tested on an additional four annotated recording sessions. Model training, testing, and application to the remaining 99 folders took place in a Google Colabs environment. Recording files were downsampled to 600 Hz to speed processing time. This resulted in spectra that were still well above the upper frequencies of the tremulations, which had a frequency range of approximately 10–80 Hz. Acoustic processing settings in koogu were adjusted until the performance of the model was satisfactory (97.5% recall of test annotations), and then the trained model was run on the remaining unannotated files with a confidence threshold of 0.57. The detections from the model were visually examined in Raven Pro to eliminate false positives (Figure B.2). Additional details of the koogu model performance are reported in the Appendix.

*Docidocercus gigliotosi* sound calls can comprise between 1 and 3 syllables, while vibrational calls are a single, sustained syllable (Figure 2.1). Because we were testing re-

relationships between the total call output in each modality, we treat each sound syllable independently in the statistical models. The finer-scale timing of calls, including the clustering and rhythm of syllables, is outside the scope of this study but should be investigated further.

### **Other variables**

Moonlight is the brightest source of natural light at night and can affect animal behavior (Kyba et al., 2017; Kronfeld-Schor et al., 2013), but moon phase does not take into account factors like differences in lunar disk brightness between waxing and waning phases or the angle of incidence of the moon over the course of a night (Śmielak, 2023b). To estimate moonlight intensity, we used the 'moonlit' R package (Śmielak, 2023a), which models moonlight as a function of disk brightness while correcting for factors like the distance and angle of incidence of the moon. We used the `calculateMoonlightStatistics` function to obtain the average light intensity value for BCI's latitudinal and longitudinal coordinates during the recording dates, with a sampling interval of 15 minutes.

To measure mass during the recording period of the experiment, we weighed each male at the beginning of each recording session.

### **Statistical analysis**

All analyses were conducted in R (version 4.2.0). We used a series of linear mixed models to investigate the roles of plasticity vs. individual differences in signaling behavior. In all models, the dependent variable was either the number of sound call syllables or the number of vibrational calls. Tremulation was log-transformed to improve the normality of residuals, and both response variables were divided by their standard deviations and centered to put them on a common scale with each other (Schielezeth, 2010). We included only one recording per week for each individual, and we excluded individuals if they had been successfully recorded fewer than three times.

To test for a refractory period in signaling after mating, and whether vibrational calling had a longer refractory period than sound calling, we fitted a linear mixed model using

the 'lme4' package (Bates et al., 2015) that included the week of recording (1-6), treatment (mated or unmated), and an interaction between week and treatment as fixed effects; individual as a random effect; and either the number of sound or vibrational calls as the dependent variable. We used the 'lmerTest' package (Kuznetsova et al., 2017) to obtain p-values for main effects using the Satterthwaite estimate for degrees of freedom. When there was a significant interaction between treatment and week, we used the 'emmeans' package (Lenth, 2023) to perform post hoc contrast tests to determine which weeks showed differences between treatments.

To calculate the individual repeatability of each type of signaling behavior, we used the 'rptr' package (Stoffel et al., 2017), which estimates the proportion of variance attributable to among-group means (i.e. differences between individuals) over the sum of group and residual variance. We included the number of vibrational or sound calls as the response variable and individual as a random effect. We considered these and all subsequent model estimates to be significant if the 95% confidence intervals did not cross zero.

Next, we investigated among- vs. within-individual variation and the covariance between vibrational and sound calling. We used a Bayesian model comparison approach to fit a series of bivariate mixed models using the 'brms' package (Bürkner, 2021). First, we fit a model that included all independent variables of hypothetical interest as fixed effects: male mass on the day of recording, moon intensity, week of recording, and mating treatment. In this model, we also included as fixed effects the recording station (1-4) and the amount of time between capture and the first recording (0.6-7.2 weeks), as well as interactions between treatment and week of recording, treatment and time in captivity, and treatment and mass. Finally, we included a random effect of individual. We fit a series of fourteen models by sequentially removing the terms with the lowest t statistics from the full model. For all models, we used a (default) uninformative prior and ran three Markov chains with 2000 iterations each, a warmup of 1000, and no thinning (interval = 1). We compared the resulting models using their "leave-one-out" (LOO) cross-validation values and the stacking model weighting approach described by Yao et al. (2018). We selected the model with the highest weight and used the posterior-predictive check function in the 'bayesplot' package

(Gabry et al., 2019) to examine the observed response variables relative to a randomly drawn sample of the simulated datasets. The ‘brms’ models estimate the covariance between group-level intercepts of each response variable, as well as the residual covariance between these that is not accounted for by the fixed or random effects in the model (O’Dea et al., 2022; Houslay and Wilson, 2017). We interpret these as the among- and within-individual covariance, respectively, between sound and vibrational signaling.

In these models, we noticed that the posterior fits were poor on the lower end of the observed y range, particularly for sound calls (Figure B.3). We surmised that this was largely due to the strong initial effect of mating, where the mated group produced very few signals before returning to about the same level as the unmated group. Our univariate models were able to detect this effect of mating treatment only during the first week. Therefore, we proceeded to fit a second series of bivariate models excluding the first week of recordings to examine more subtle or lasting differences in signaling behavior that were potentially swamped by the large change in signaling between weeks 1 and 2 in the initial models. We followed the same model fitting and comparison approach for this second series of bivariate models.

## Results

### Matings

For males that mated, there was a mean latency of  $97 \pm 55$  SD minutes between being placed in the cage with a female and successful spermatophore transfer, with copulation lasting for an average of  $2.3 \pm 0.5$  minutes. All mated males produced large tremulations post-copulation that were observably greater in amplitude than the pre-mating calling and courtship tremulations in this species. Females began consuming the spermatophore an average of  $12.7 \pm 6.8$  minutes after separating from the male and  $5.6 \pm 4.7$  minutes after the male stopped producing these large tremulations. The average difference in male mass before and after mating was  $0.35 \pm 0.04$  g, with spermatophores equaling about 23.5% (range: 17–29%) of pre-mating male body mass. There was a significant correlation between the

mass of the spermatophore and the number of minutes post-copulation that females began eating the spermatophore (Pearson’s product-moment correlation,  $r = 0.62$ ,  $N = 11$ ,  $t = 2.41$ ,  $df = 9$ ,  $P = 0.039$ , Figure B.4). There was no correlation between the starting mass of males and the mass of the spermatophore they produced (Pearson’s product-moment correlation,  $r = 0.03$ ,  $N = 11$ ,  $t = 0.09$ ,  $df = 9$ ,  $P = 0.93$ , Figure B.5).

### Signal trait patterns and correlations

For both signal types, there was a significant interaction between mating treatment and week of recording (sound:  $F_{5,84} = 6.1$ ,  $P < 0.001$ ; vibrational:  $F_{5,86} = 5.9$ ,  $P < 0.001$ ). Therefore, we used pairwise tests to assess significant differences between treatment and week combinations. For the mated group, the number of both sound and vibrational signals differed significantly between week 1 and all other weeks, which were not significantly different from each other (Figure 2.4). For the unmated group, the number of sound call syllables increased over time with significant differences spread across weeks, whereas the number of vibrational calls differed only between week 2 and weeks 4–6 (Figure 2.4). For both types of signals, the two mating treatment groups differed significantly from each other only during the first week. Thus, immediately after mating, males showed decreased signaling typical of a refractory period. However, this significant reduction in signaling was no longer present starting in week 2.

Individual male katydids varied in their sound and vibration signaling levels, both over time and from each other (Figure 2.5). Males produced an average of 89 tremulation calls each night, with some individuals producing as few as 1 or as many as 502 calls in any given night. Tremulation was highly and significantly repeatable when including and especially when excluding the post-mating refractory period of week 1 (Table 2.2). Males produced an average of 2,741 sound call syllables (grouped into an average of 1,658 one-, two-, or three-syllable calls) each night. The number of sound call syllables ranged from 54 to 5,548 in a night. Numbers of sound call syllables were not significantly repeatable when the refractory period was included (although the P-value was close to 0.05) and was significantly repeatable when it was excluded (Table 2.2).

Among individuals across both treatment groups, sound and vibrational calling were not significantly correlated, in either the full span of weeks ( $r = 0.44$ , 95% CI [-0.27, 0.87]) or when the first week was excluded ( $r = 0.29$ , 95% CI [-0.49, 0.80]). The residual correlation in both models, however, was positive and significant (week 1 included: estimate = 0.53, 95% CI [0.38, 0.67]; week 1 excluded: estimate = 0.44, 95% CI [0.23, 0.62]), indicating a within-individual relationship between signal types: on nights when males tended to stridulate more, they also tremulated more relative to their own behavior on other nights (Figure 2.5).

### **Effects of mass, time, and moonlight**

When week 1 recordings were included, male mass, week of recording, and weeks in captivity prior to recording each had a significant positive effect on the amount of sound signaling that males performed. Mass and week of recording, though not weeks in captivity prior to mating, had a significant positive effect on vibrational signaling (Figure 2.6, Table B.1).

When week 1 recordings were excluded, week of recording no longer had a significant effect on either sound or vibrational signaling: most of the previous variation was driven by differences in signaling between weeks 1 and 2 (Figure 2.4, Figure 2.6). For sound calls, however, there was a small but significant interaction effect between week and mating treatment: mated males tended not to increase sound signaling effort over weeks 2–6, whereas unmated males tended to increase sound signaling effort over the same time span (Figure 2.6, Figure B.6; Table B.2). The model estimate of the effect of interacting week and treatment was similar in direction and magnitude for vibrational calling, though not significant (Figure 2.6; Figure B.6; Table B.2). There was no significant overall effect of treatment for either signal. In this model, mass still had a strongly positive and significant effect on both sound and vibrational calling (Figure 2.6, Figure B.7), and males recorded at Station 3 continued to signal more than at Station 1 (Figure 2.6). Finally, there was a negative, though not significant, effect of moon intensity on vibrational calling: on brighter nights when the moon was fuller, males tended to tremulate less (Figure 2.6).

## Discussion

All of the male *D. gigliotosi* spontaneously produced both sound and vibrational calls, including those that mated at the start of the experiment. Signaling effort was significantly lower for both signal types one day after mating, suggesting that males experience a refractory period and do not attempt to immediately attract additional mates. There was no correlation between the nightly number of sound and vibrational signals among individual males. A lack of a positive correlation among individuals means that individuals did not fall along a continuum of consistently low-level to consistently high-level signalers, which we would have expected to see if the amount of signaling in both modalities was tightly correlated with male quality or if high and low signaling individuals represented two alternative (and inflexible) tactics of a behavioural syndrome. The lack of a negative correlation among individuals means that males did not fall along a continuum in which those that consistently produced many sound signals also consistently produced few vibrational signals (and vice versa). We would expect to see this pattern if there were energetic tradeoffs between the use of each signal and conditions that differed between individuals during development selected for the use of one signal type more than the other. *Within* individuals, there were positive correlations between the two signal types. This result indicates that individuals were plastic in their signaling effort, and that changes occurred in both signal types in the same way: a high acoustic signaling night would also be a high vibrational signaling night. In the unmated group, this plasticity manifested as an increase in signaling effort over several weeks, whereas the increase in signaling for the mated group only occurred between the first and second week after mating. Although individuals showed plasticity, they also demonstrated repeatable differences from each other, visible as most individuals occupying a unique area when their sound signaling effort is plotted against their vibrational signaling effort (Figure 2.5). In addition to mating and time, signaling effort was positively related to body mass.

Mating strongly depressed signaling levels in both modalities, and recent mating appears to be the cause of the greatest week-to-week change in signaling we observed during our

study (Figure 2.4). This refractory signaling period lasted no more than a week and did not differ in duration between sound and vibration signals. Because we did not observe males more frequently than once per week, this is possibly an overestimate: if one signal type returned to the level of the unmated group three days after mating, and the other recovered at five days after mating, it would not be apparent in our data. Beyond the strong transient effect of mating, we observed a small but significant persistent difference between the mated and unmated group: as time went on, the signaling effort of mated males tended to remain stable, while the signaling effort of unmated males was more likely to increase. While we do not know the mating history of any of the males prior to capture, nor how often males tend to mate in the wild, it is possible that males that have not mated or have mated less often are engaging in some sort of terminal investment strategy. In many species, it has been found that older animals males signal more (Nielsen and Holman, 2012), sometimes also exhibiting decreased responsiveness to predator cues (Lafaille et al., 2010; Kuriwada and Kasuya, 2011), possibly because the benefits of attracting additional matings begin to outweigh the risks or costs of producing advertisement signals (Kokko, 1997). Despite the predation risks and energetic costs of calling, older *D. gigliotosi* males might benefit from producing more calls, especially if they have not mated recently. Alternatively, we might have introduced bias in our mating trial methods: males that were less likely to quickly mate might have been younger, and as they aged they could have ramped up their signaling levels. Because we had a wide range of capture dates in both treatment groups, however, and because most males attempted courtship and copulation when placed with a female, we think that this potential source of bias is unlikely to totally explain the observed effect of treatment over time.

Males showed repeatability in their signaling levels within a modality, with some tending to produce relatively more sound or more vibration than other males over time. However, among-individual correlations between the degenerate sound and vibration signals were not observed. Thus, we did not find support for a behavioral syndrome or multimodal indicators of male quality, nor for a developmental trade-off between the two signal types. In other acoustically signaling animals where correlations between multiple signals have

been demonstrated, such as tree frogs whose advertisement and aggressive calls show similar acoustic properties, it has been suggested that among-individual correlations can result from constraint by shared mechanisms (e.g., vocal sacs) that produce the different signal types (Reichert, 2013): changes in one type of call are likely to affect the other type. In our system, the two different signals are produced by separate mechanisms, which may help explain this lack of among-individual correlation.

Males did show within-individual correlations between the two types of signals: on nights when they tended to produce more sound relative to their effort on other nights, they also tended to produce relatively more vibration—with some individuals spanning almost the entire range of observed calling behavior in either or both signal modalities across time (Figure 2.5). This finding reinforces the hypothesis that there is substantial functional overlap between the sound and vibration signals that males produce. It can be interpreted as a “plasticity syndrome” (O’Dea et al., 2022), where both signals respond to endogenous or exogenous cues in similar ways. What drives this correlated plasticity? Body mass showed a strong and significant effect across all our statistical models, and appears to not only be driven by on-average heavier males tending to signal more, but also by changes in an individual’s mass tending to positively correspond to signaling effort (compare numbers of positive vs. negative individual slopes in Figure B.7). It has been argued that phenotypic plasticity and flexibility in signal expression could be selected for because of associations between current condition and direct benefits to receivers, versus indirect genetic benefits that might lead to selection for static indicators of condition across different contexts (Bro-Jørgensen, 2010). Our current study finds high levels of signaling plasticity, and one possible prediction that emerges could thus be that males should flexibly signal more in both signaling modalities during times when they are able to produce a spermatophore nuptial gift that has greater direct benefits to females. However, the relationship between male condition and spermatophore investment is murky—there was no correlation between initial male mass and spermatophore mass in our mated group, for instance, and we did not measure the nutritional content of the spermatophores—nor is it known whether females are assessing male quality based on their long-range signals, versus merely using these to

locate signaling males and then electing whether to actually mate based on short-range courtship interactions. In the latter scenario, males might simply spontaneously signal more when they have the energetic reserves to do so because it would increase their chances of attracting the attention of a female. Further investigation into the relationships between *D. gigliotosi* male condition, spermatophore investment, and female behavior toward male calling and courtship signals at different distances is called for.

Exogenous environmental factors such as temperature and ambient noise have been found to cause flexible shifts in signaling effort in other acoustically signaling orthopterans (Walker, 1962; Costello and Symes, 2014). In this system, ambient light is one possible factor. Previous work by Römer et al. (2010) found a strong effect of moon phase on *D. gigliotosi* vibrational calling, which they interpreted as males increasing signaling effort during full moons when females are potentially at less risk from gleaning bat predators and are more likely to be searching for mates. While we found a nonsignificant effect of moon illumination, it was in the opposite direction: on brighter nights, the *D. gigliotosi* males produced fewer vibrational calls. Additionally, none of the males in our study tremulated nearly enough to cause the average sevenfold increase in vibrational signaling described by those authors. These differences could be explained by differences in methodology: Römer et al. (2010) put katydids in forest clearings in boxes, and each individual was recorded just once; we put them in cages in greenhouses, and individuals were recorded multiple times. However, they might also be due to “contamination” by unintentionally recorded male–female duets. We excluded several of our recordings from analysis due to apparent male–female duets between the caged male and a replying, tremulating insect. The timing of the replies indicates a male–female *D. gigliotosi* duet, with the female located either inside or outside the greenhouse, but outside the male’s cage. In addition to the extra replies by the second individual, males ramped up their calling rate when receiving replies. In the Römer study, females arriving at the males caged in plastic boxes could have caused a similar increase in both overall numbers of calls and male calling specifically. In our recordings, these duets ( $n = 3$ ) all occurred at or near full moons, consistent with the idea that females do actually search more at that time. We posit that the reported increase in

tremulation is not a spontaneous response on the part of the males and is instead effected by this change in female behavior causing similar problems in their experimental setup as with ours. If this interpretation is correct, the end result is the same as Römer et al. (2010) describe—in the wild, more duets and matings should happen around the full moon—but the mechanism by which these occur is different.

Understanding how multiple signals vary (and covary) within a species requires careful examination of patterns across individuals, but also within individuals over time and across different contexts. We have investigated such patterns within a communication system that contains two functionally overlapping, yet structurally distinct, degenerate advertisement signals. Our findings underline the degree of plasticity in multimodal signaling behavior that can be revealed by long-term, repeated measurements. They also demonstrate that the downstream effects of a metabolically costly mating event can be both transient and persistent. Lastly, our finding that within-individual correlations between total sound and vibrational signaling tend to be positive, in contrast to the among-species negative correlation between these signals (Chapter 1), indicates that different mechanisms have shaped signaling patterns at these different hierarchical scales.

## Tables and Figures

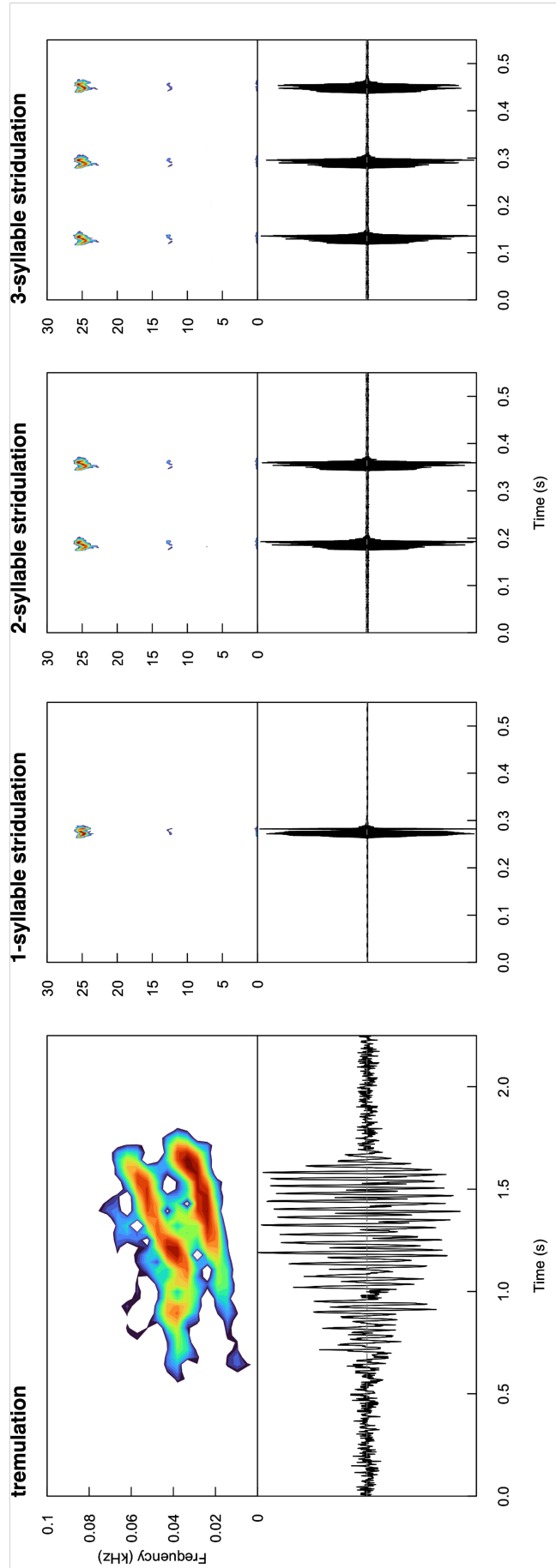
**Table 2.1: Characteristics of *D. gigliotosi* signaling repertoire.**

| Signal Feature                   | Sound call   | Vibrational call                                       |
|----------------------------------|--|--|
| <b>Mechanism of production</b>   | Tegminal stridulation                                  | Abdominal tremulation                                  |
| <b>Number of syllables</b>       | 1, 2, or 3 <sup>a</sup>                                | 1 <sup>b</sup>   |
| <b>Syllable duration</b>         | Shorter (20 ms) <sup>a,b</sup>                         | Longer (1000 ms) <sup>b</sup>                          |
| <b>Peak frequency</b>            | Higher (25 kHz) <sup>a,b</sup>                         | Lower (20 Hz) <sup>b</sup>                             |
| <b>Energetic cost per signal</b> | Smaller (0.73 $\mu$ l CO <sub>2</sub> ) <sup>c</sup>   | Larger (4.89 $\mu$ l CO <sub>2</sub> ) <sup>c</sup>    |
| <b>Active space</b>              | Larger (22–35 m) <sup>c</sup>                          | Smaller (up to 4 m) <sup>c</sup>                       |
| <b>Mate attraction</b>           | Less likely to establish close-range duet <sup>d</sup> | More likely to establish close-range duet <sup>d</sup> |
| <b>Predator attraction</b>       | Conspicuous to eavesdropping bats <sup>c,e,f,g</sup>   | Not conspicuous to eavesdropping bats <sup>h</sup>     |

References:<sup>a</sup>ter Hofstede et al. (2020); <sup>b</sup>This thesis, Chapter 1; <sup>c</sup>Römer et al. (2010); <sup>d</sup>Iwan et al. (2023, in prep.); <sup>e</sup>Belwood (1988); <sup>f</sup>Falk et al. (2015); <sup>g</sup>ter Hofstede et al. (2017); <sup>h</sup>Geipel et al. (2020).

**Table 2.2: Repeatability of sound and vibrational signaling behavior.** Individual repeatability (R), also known as the intraclass correlation coefficient or the proportion of phenotypic variation that can be attributed to within-subject variation, was calculated for each signal type across weeks. Week 1 (the day following mating for half of the individuals in the dataset) was excluded from some models and calculations. N is the sample size for each estimate, SE is the standard error, and 95% confidence intervals are shown in brackets.

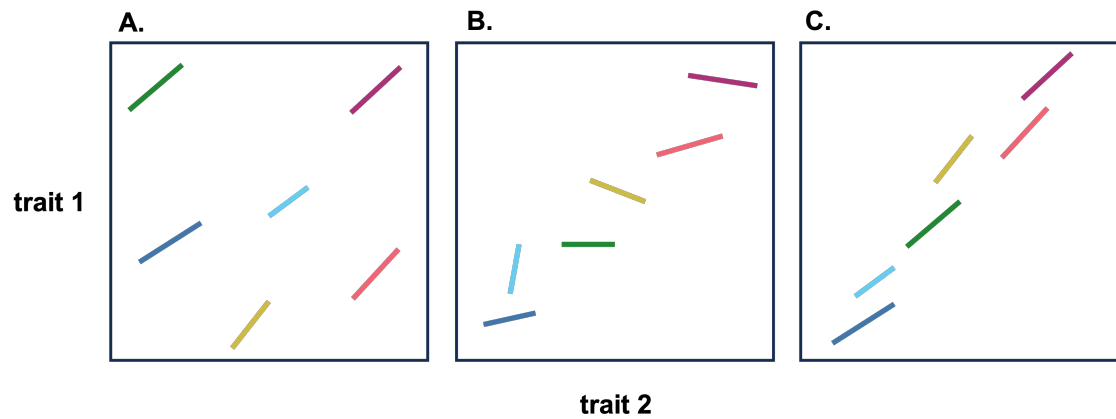
| Signal type  | Week 1 included? | N  | R     | SE    | 95% CI         | P      |
|--------------|------------------|----|-------|-------|----------------|--------|
| Stridulation | Yes              | 24 | 0.122 | 0.080 | [0, 0.291]     | 0.064  |
| Tremulation  | Yes              | 24 | 0.256 | 0.099 | [0.046, 0.434] | 0.001  |
| Stridulation | No               | 22 | 0.256 | 0.108 | [0.044, 0.467] | 0.002  |
| Tremulation  | No               | 22 | 0.444 | 0.115 | [0.196, 0.633] | <0.001 |



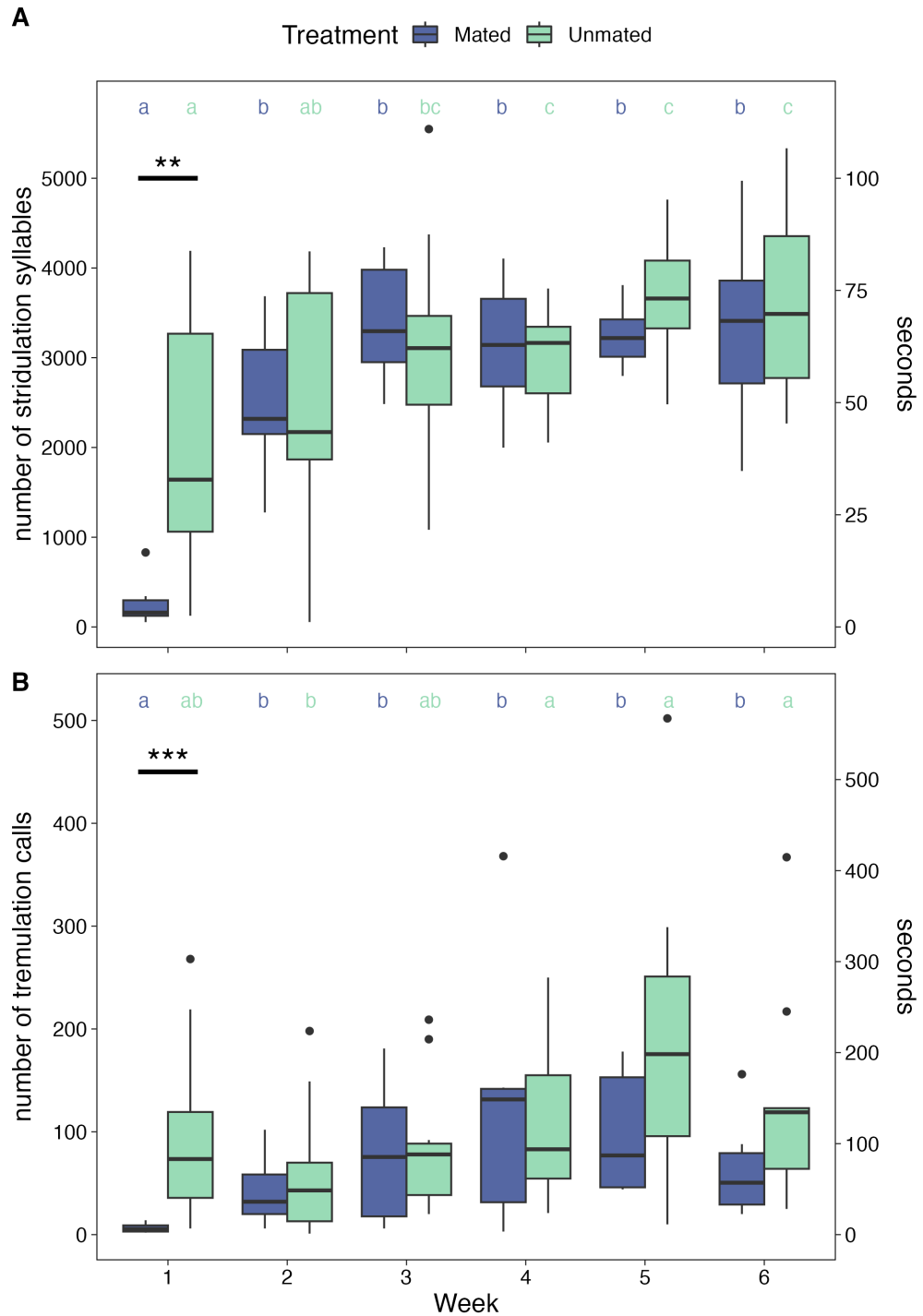
**Figure 2.1:** *Docidocercus gigliotosi* advertisement calls Vibrational (tremulation) and sound (stridulation) calls are shown as spectrograms (top row) and oscillograms (bottom row). Sound calls typically occur in 1, 2, or 3-syllable groups.



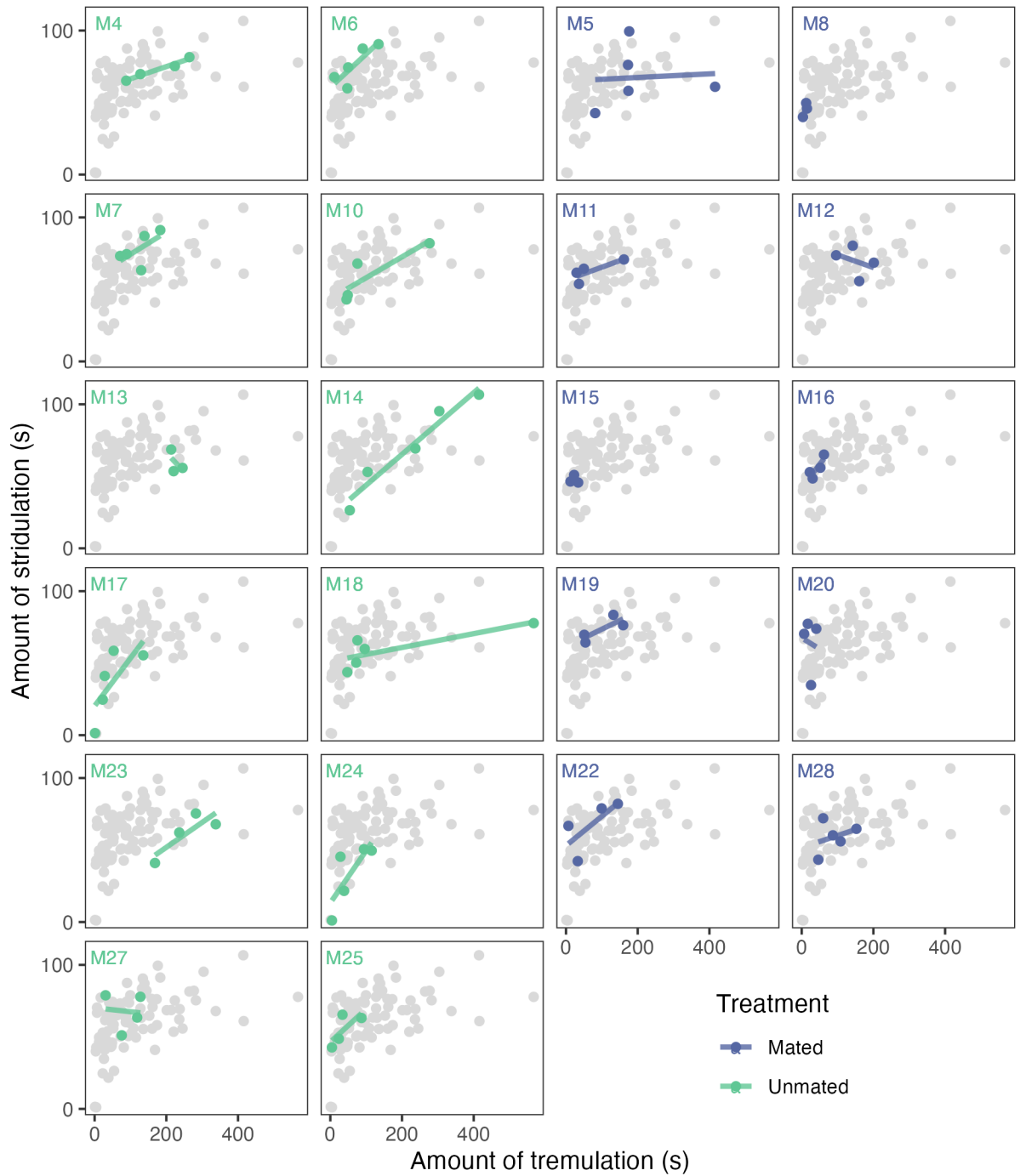
**Figure 2.2:** *Docidocercus gigliotosi*. Left: male. Right: mating pair observed on an *Aechmea magdalenae* terrestrial bromeliad. The white mass between the individuals is the large spermatophore and spermatophylax that the male transfers to the female.



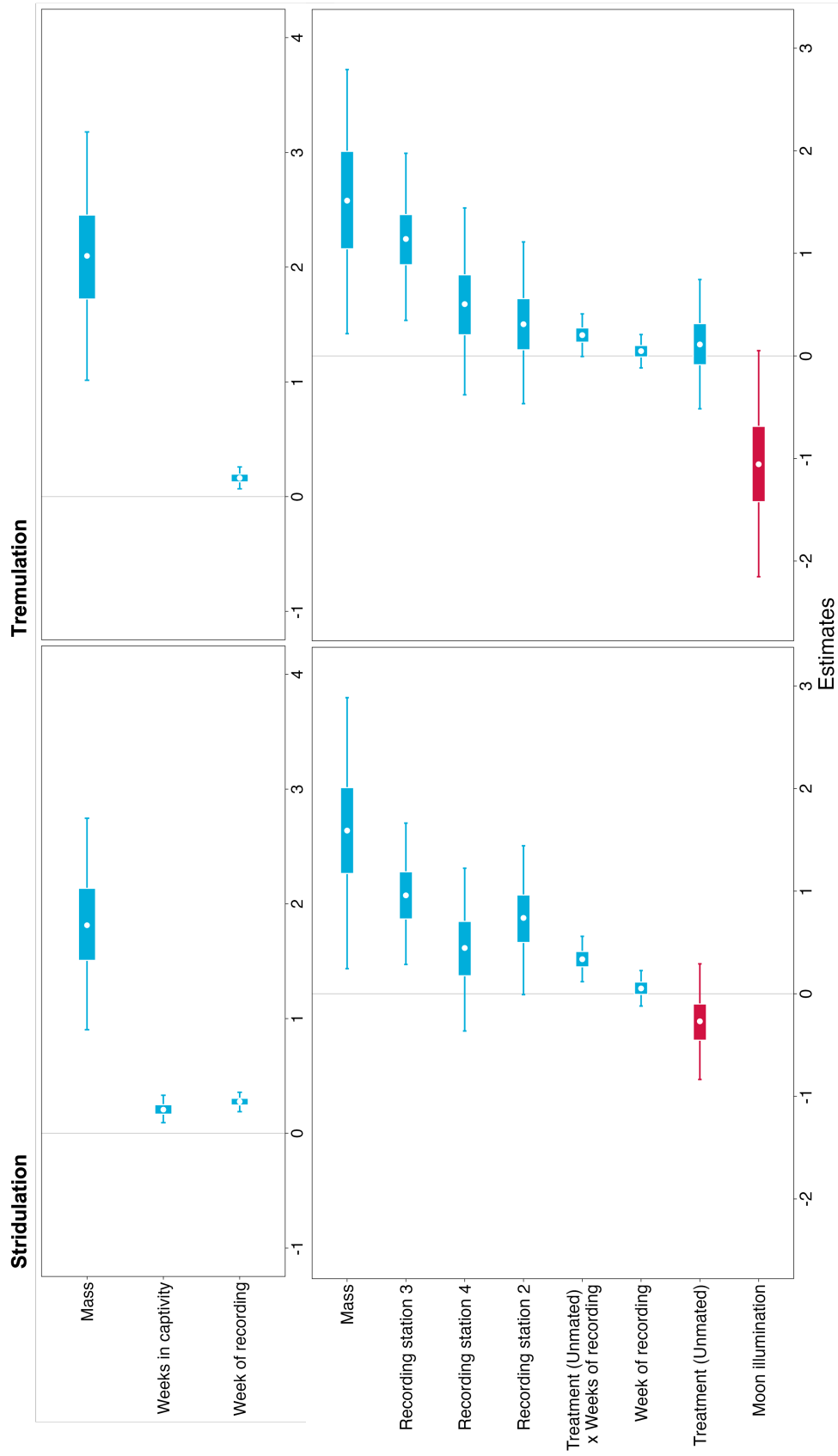
**Figure 2.3:** Theoretical within- and among-individual variation in signal production, with six individuals expressing two degenerate signals with high levels of individual repeatability for each signal. A: within-individual, but no among-individual, covariation between signal types. B: among-individual, but no within-individual, covariation between traits. C: both within- and among-individual covariation.



**Figure 2.4: Sound and vibrational calls over time.** Boxplots show each treatment group's number of calls (left axis) and corresponding amount of time (right axis) spent using sound (top) or vibrational (bottom) signals. Boxes represent the 25th and 75th percentiles (ends) and the median (center line); whiskers represent  $Q1 - 1.5 \times IQR$  and  $Q3 + 1.5 \times IQR$ ; and all outliers beyond these whiskers are shown. Starred black bars show significant differences between treatment groups, where present. Lowercase letters show differences across weeks within a treatment and modality.



**Figure 2.5: Individual covariation in sound and vibrational signaling.** Each box shows a specific individual's summed seconds of sound and vibration signaling from weeks 2–6, with each green (unmated treatment) or blue (mated treatment) dot representing one 24-hour recording session. Gray dots represent all other individuals over the same time span, to visualize where specific individuals fall relative to others.



**Figure 2.6: Effect estimates from Bayesian models.** Estimates from 'brms' models of independent variables' (left side of graphs) effects on the number of sound (left panel) and vibrational (right panel) calling signals. Top panels: Models with week 1 included. Bottom panels: Models with week 1 excluded. The final model did not include a term for moon illumination's effect on stridulation. White dots within bars indicate median estimates, boxes show 50% CIs, and whiskers show 95% CIs. Blue indicates a positive effect estimate, and red indicates a negative effect estimate.

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## Chapter 3

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### **Efficacy constraints on female directional preference stabilize a male call component in a multimodal cricket duet**

The following chapter was published in *Animal Behaviour* in 2022. I conceptualized the study, collected and analyzed the data, and wrote the manuscript with contributions to each stage by Jessica Jones, Tony Robillard, Stefan Schöneich, and Hannah ter Hofstede.

## Abstract

Mate advertisement signals can vary in their variability, with some signals or signal components being highly stereotyped and others highly variable. One hypothesis for differences in variability suggests that receivers provide stabilizing selection for signal components important for mate recognition, resulting in low variability, while also providing directional selection for signal components indicating mate quality, correlating with greater variability. Factors that affect signal efficacy, however, can also influence signal design and might interact with receiver preferences to influence variability. We investigated these hypotheses in the duetting cricket species *Lebinthus bitaeniatus* Stål. Like many other insects, these crickets use call-and-response signalling to find mates. Males produce an acoustic signal, females reply with a vibrational signal, and males use these vibrations to find females. Male calls have two components: a series of isolated syllables (ticks) followed by a rapid series of syllables (a trill). The number of ticks per call is highly variable, whereas the number of pulses in the trill is stereotyped. Male size was correlated positively with the number of trill pulses per call, but not with the number of ticks. Female response amplitude was not related to the number of ticks in the male call (the variable component) and it increased with trill duration (the stereotyped signal component), contrary to the stabilizing vs. directional selection hypothesis. We also found, however, that the latency of the female vibrational reply became erratic as the trill increased beyond the typical duration. Since males in duetting insect species rely on the species-specific latency of the female reply to recognize the signal, this reduces the efficacy of the female reply for communication despite the females' greater amplitude response. Given the prevalence of call-and-response signalling in insects, this relationship between female response amplitude and signal efficacy could be a common factor influencing signal variability across many species.

## Introduction

Most animals produce species-specific communication signals to attract mates and reproduce. This simple imperative has given rise to diverse and complex signals that comprise multiple components or can be perceived across multiple sensory modalities (Andersson, 1994). Various hypotheses have been proposed to explain the evolution of multimodal and multicomponent signals (Candolin, 2003; Hebets and Papaj, 2005; Johnstone, 1996). Across taxa, there is empirical support for both content-based hypotheses, where each signal component provides a different type of information (e.g., Nelson and Poesel, 2007), and efficacy-based hypotheses, which concern the recognition of a signal by its intended receiver despite environmental noise (e.g., Grafe et al., 2012). In addition to the presence of multiple components, a notable aspect of some complex signals is the variability of each component. Some signal components are highly variable within and between individuals, while others are more stereotyped. For mate advertisement signals, variation is both predicted and observed to correspond to open-ended receiver preferences, meaning that potential mates prefer more extreme signal properties; conversely, stereotypy corresponds to unimodal receiver preferences, meaning that potential mates prefer a narrow range of signal properties (Gerhardt 1991; Ritchie 1996; Shaw and Herlihy 2000; but see Millan et al. 2020). One common hypothesis for differing levels of variability in different advertisement signal components is that different information is being conveyed by each component (Gerhardt, 1991; Hebets and Papaj, 2005), potentially to different receivers (Zambre and Thaker, 2017). Information for mate recognition, where similarity to conspecifics is necessary, can then be transmitted in parallel—or at least in close proximity; see Gray (2022)—to information about individual quality, where distinction from conspecifics is important (Pfennig, 1998).

Studies on mate-finding behaviour have largely focused on unidirectional male signals towards which female searchers orient. Many animals across diverse taxa, however, use bidirectional, call-and-response duetting to locate mates (Bailey, 2003; Pika et al., 2018). In these dyadic interactions, both individuals produce signals, and males take on some or all of the searching role (Heller, 1992; Heller et al., 2021; Scherberich et al., 2017). To understand

mate-finding interactions in these species, it is therefore necessary to consider the signals produced by both animals. When these signals contain multiple components, each component has the potential to influence the behavioural response of the other animal (Kuhelj et al., 2016; Rodríguez and Barbosa, 2014). Even when signals are relatively simple, multiple signal features often influence successful pair formation because of the need for precise temporal coordination between the initiating caller and the replier. In many orthopteran duets, for example, calling males insert a ‘trigger’ component into their call to prompt correctly timed replies from answering females (Heller et al., 2015; Heller and von Helversen, 1986). Conversely, whether a calling male recognizes and responds to a replying female depends on two efficacy-related features of her reply: amplitude and timing. Amplitude affects whether the reply is above the hearing threshold of the male (van Staaden and Römer, 1997). Timing of the reply relative to the male call affects whether the reply falls within a species-specific window of recognition, outside of which the male will often not keep calling or perform phonotaxis (Forrest et al., 2006; Heller and von Helversen, 1986; Zimmermann et al., 1989). In at least some duetting katydid species, females adjust the timing and intensity of their reply based on cues within the male call (Villarreal and Gilbert, 2013) as well as their own motivational level (Bailey and Hammond, 2004). Duetting species therefore present an opportunity to examine how different features of two interacting signals shape each other. If female replies vary in intensity and latency, and particular call features of males predictably elicit greater reply amplitudes or less variable reply latencies, then males with those call features will be favoured by selection because they should locate replying females more easily or more often (Bailey and Hammond, 2003).

A cricket species with a particularly interesting communication system is *Lebinthus bitaeniatus* Stål (Gryllidae: Eneopterinae), a member of the tribe Lebinthini. Unusually for crickets, Lebinthini use duets, rather than unidirectional calling, to locate mates (ter Hofstede et al., 2015). Their communication system is one of the only examples of multi-modal acoustic–vibratory mate-finding duets currently known, with another described in a pseudophylline katydid (Rajaraman et al., 2015). These duets are distinct from exchanges of acoustic and vibrational signals by crickets during close-range courtship (e.g., Dambach

and Beck, 2010; deCarvalho and Shaw, 2010) in that they occur prior to antennal or other physical or even visual contact, with a specific temporal relationship between the initiating acoustic call and the vibrational reply. *Lebinthus bitaeniatatus* males produce high-frequency and temporally complex airborne calls made up of a series of longer syllables produced at long intervals (referred to here as individual ‘ticks’) followed by a series of shorter syllables produced at short intervals (referred to collectively as a ‘trill’) (Figure 3.1). The different components of the male call exhibit different levels of variability, with the number of ticks varying more than the number of trill syllables (Tan and Robillard, 2021, ; this study). Female crickets respond to this call with a short vibrational signal that travels through the plant substrate, and the male searches for her following these signals (ter Hofstede et al., 2015). We have also observed that the amplitude of the female reply can vary under some circumstances, whereas the latency of the reply remains relatively constant (H. M. ter Hofstede, personal observation). These observations raised the question: has selection by female crickets shaped male call structure in *L. bitaeniatatus*? Specifically, we asked the following questions. (1) Do the numbers of syllables in each call component vary with male size? (2) Does the shape of female preference functions differ for variable compared to stereotyped signal components?

To answer these questions, we first quantified variation in the tick and trill components of the male *L. bitaeniatatus* call and tested whether the average number of syllables in each call component was related to male size. Female crickets across diverse species typically prefer larger males (e.g., Forrest et al., 1991; Stoffer and Walker, 2012) and the songs of larger males (e.g., Gray, 1997; Ponce-Wainer and Cueva del Castillo, 2008). Then, by playing back synthetic male calls and recording female vibrational replies, we tested whether and how female crickets vary the presence, amplitude and timing of their replies in response to male calls with different acoustic properties. Having observed that the number of trill syllables was much less variable within and between males than was the number of ticks, we assessed whether the two call components provide different types of information to the female. Specifically, we tested the hypothesis that the less variable component (trill) functions in mate recognition, predicting that the trill would be both necessary and sufficient to

elicit a reply and that females would have a unimodal preference for the stereotypical trill, as measured by the intensity and latency of their replies. Concurrently, we tested the hypothesis that the more variable component (ticks) functions in mate discrimination. Because it is common for female orthopterans to prefer, or respond more strongly to, more frequent or longer-duration male calls (Gerhardt and Huber, 2002), we predicted that females would have an open-ended preference for more ticks in a call, producing greater amplitude replies to higher-tick calls. Finally, we considered an alternative hypothesis that the stereotyped duration of the trill is a compromise between a female preference for longer trills and limits on the efficacious timing of communication signals in a duet. In this scenario, the stereotyped trill would act to stabilize the latency of the female reply, improving the male's ability to recognize her signal, even if the number of ticks in male calls is highly variable.

## Methods

### Study animals

A colony of *L. bitaeniatus* crickets was established at Dartmouth College (Hanover, NH, U.S.A.) in 2013 from eggs laid by wild-caught females from the Philippines. Crickets were housed in plastic terraria (37 x 22 x 24 cm) and provided with water, food and egg carton shelters. Crickets that were used in experiments were isolated from the opposite sex as juveniles to ensure that all crickets were unmated at the time of initial testing. Newly emerged adults were housed in small containers with no more than two other individuals of the same sex. We recorded adult age as the number of days since the cricket's final moult.

### Male calling song

To test for correlations between the amount of calling and male size, we recorded individual *L. bitaeniatus* male crickets ( $N = 15$ ) for 24 h under a 12:12 h light:dark cycle. Crickets were placed in a box (50 x 33 x 45 inches, 127 x 84 x 114 cm) lined with acoustic foam and with a mesh top, and food and water were provided ad libitum.

Calls were recorded with a  $\frac{1}{4}$ -inch microphone (Type 4939-A-011; Brüel & Kjær, Nærum,

Denmark) with a flat frequency response ( $\pm 2$  dB from 4–100 kHz) and digitized with an Avisoft single-channel data acquisition board (UltraSoundGate 116H) and Avisoft Recorder software (Avisoft Bioacoustics, Glienicke, Germany). Each cricket was weighed before and after recording on a digital scale (Mettler Toledo XS204, 0.1 mg readability). Crickets were euthanized by freezing after the end of recording. We later measured pronotum width, anterior–posterior pronotum length and left and right hind femur lengths using a digital calliper (Ares 70019, 0.01 mm resolution). We counted the total number of calls, the number of ticks in each call and the number of trill syllables in a randomly selected subset of 20 calls per individual using the pulse train analysis function in Avisoft SASLab Pro (v.5.2.12). Calls were counted as separate events if they were separated by more than 1 s (i.e. almost three times the length of the average silent time between ticks).

To quantify acoustic parameters of the male call in more detail, we made additional measurements on 48 randomly selected calls from 10 individuals (6 individuals from the 24 h recordings described above and 4 from an additional set of males that was recorded in a cylindrical mesh cage (9 cm diameter x 17 cm height) in 2016. Using the Automatic Parameter Measurement tool in Avisoft SASLab Pro, we measured the following variables for each syllable in a call: duration (time from the start to the end of the syllable, ms), period (time from the start of one syllable to the start of the next, ms) and peak frequency (frequency with the most energy, kHz). We also measured the gap duration (silence) between the last tick and first trill syllable (Figure 3.1). The mean values calculated from these measurements are provided in Table 3.1.

## **Playback experiments to females**

### ***General approach***

Artificial call stimuli were synthesized using Avisoft SAS Lab Pro using the average call parameters from the male recordings as a guide (Table 3.1). For all playback stimuli, calls had a frequency of 20 kHz, a tick syllable duration of 45 ms, tick period of 400 ms, trill syllable duration of 23 ms, trill syllable period of 43 ms and a gap between the

ticks and the beginning of the trill of 200 ms. The number of ticks, the amplitude of the calls and the duration of the trill (i.e. the number of trill syllables) varied depending on the experiment (details below). Experiments were conducted in an attenuating and anechoic acoustic chamber under infrared light. This species calls both during the day and at night (Tan and Robillard, 2021), and we conducted experiments during the day but under dark conditions to avoid potential visual disturbance. Female crickets were placed inside a cylindrical fibreglass mesh cage (9 cm diameter x 17 cm height) and allowed 5 min to acclimate before playback of stimuli began. Stimuli were broadcast to crickets using an Avisoft ultrasonic speaker (Vifa model) and amplifier (USG216H) connected to a computer (HP Compaq Elite 8300), and the female vibrational reply was recorded using an accelerometer (Brüel & Kjær DeltaTron, Type 4517) or laser Doppler vibrometer (Polytec PDV-100, Waldbronn, Germany) positioned on the side of the cage. The cricket’s position was monitored using a video camera (ICD879 Type G, Ikegami, Tokyo, Japan) and external monitor outside of the sound chamber. Playbacks of stimuli were initiated only if the female was within 1 cm of the accelerometer or laser point; if she moved between treatments, she was re-positioned and allowed another 5 min acclimation period before continuing. For all experiments, treatments consisted of five repetitions of each stimulus (at periods specified below), and treatments were presented in random order, with 5 min of silence between each treatment. In experiment 1, female crickets were tested for responsiveness to average synthetic male calls (10 tick syllables plus a 40-syllable trill, other parameters as reported in Table 1) at 80 dB peak equivalent SPL (peSPL; Burkard, 2006) every 3–4 days after their final moult and participated in the playback experiment about 3 days after becoming responsive. The average age at the time of the experiment for the experiment 1 females was 19 days after the final moult (range 12–27 days). For experiments 2, 3 and 4, we tested responsiveness at about 20 days and performed experiments either on the same day or within 5 days. The average age of experiment 2 females was 25 days (range 22–28) and for experiments 3 and 4 (same crickets) was 23 days (range 21–32).

For the female playback experiments, we measured duration, latency and peak-to-peak amplitude of each vibrational reply (Figure 3.1). Amplitude and duration can each influ-

ence probability of signal detection, decision making and accuracy in locating the signal source in vibrationally communicating insects (Gibson and Cocroft, 2018; Polajnar et al., 2014). Latency can affect mate recognition in duetting orthopterans (Bailey and Hammond, 2003; Heller et al., 2018; Heller and von Helversen, 1986; Zimmermann et al., 1989) and other insects (Kuhelj et al., 2015; Rohde et al., 2013). We measured latency as the time between the end of the male call or playback stimulus and the beginning of the female reply. Duration and latency were measured directly from the oscillogram in Avisoft SASLab Pro, and amplitude was measured as the peak-to-peak difference of the signal in mV using the ‘time signal’ function. We did not calibrate these recordings, so they cannot be translated into absolute values in terms of displacement or velocity, but these values can be compared across all crickets in the same experiment that experienced the same recording conditions. In experiment 2, six stimulus presentations cut out during playback, and responses to these were excluded from analyses. For atypical replies that contained more than one distinct vibrational pulse, we measured the largest e usually the last e pulse in a series of premature replies occurring before the end of the playback. Atypical responses were uncommon in all experiments except experiment 3 and 4 (below), which mostly consisted of stimuli that were outside of the natural range of male call variation. For experiment 4, some individuals were excluded from statistical analyses because they produced atypical vibrational responses that could not be classified as discrete signals (see Appendix, Figure C.6, for examples).

### ***Experiment 1: Male call components required for female replies***

To test whether the presence of each component was necessary and sufficient to elicit a vibrational reply, we played the individual components of the male call separately and together to female crickets ( $N = 40$ ). The three treatments were: (1) 10 ticks with no trill; (2) a trill of average duration (1576 ms, 38 syllables) with no ticks; (3) the complete call (10 ticks followed by a trill of average duration and syllable number). Each treatment consisted of five repeated stimuli with a call period (time from the start of one call to the start of the next) of 15 s and an amplitude of 80 dB peSPL at the cricket. Treatments were presented in random order and female replies were recorded using the accelerometer.

The same females used in this experiment were subsequently used in a pilot experiment for varying the number of ticks in the call and for testing whether a greater number of ticks might lower the amplitude threshold of the female reply by playing calls with varying numbers of ticks. These females were then retested several weeks later after either being housed with a male or being isolated to address the possibility that age or mating status might affect responses to male signals (details in Appendix C: Age and mating effects on female responsiveness to male calls).

***Experiment 2: Effect of the number of ticks per call on female replies***

To test whether varying the number of ticks in the male call affected the female reply, we played a second group of female crickets ( $N = 17$ ) stimuli of 0, 10, 20, 30, 40 or 50 ticks plus a trill of average syllable number and duration. The treatment with 50 ticks represents the 95th percentile observed in our male recordings; the average number of ticks we observed was 14. Each treatment consisted of five repeated stimuli at 80 dB peSPL. The call period for all treatments was 40 s, which differs from the other playback experiments to avoid temporal overlap of calls. The remainder of the experimental set-up was the same as in experiment 1, except that the female vibrational replies were recorded with the laser Doppler vibrometer rather than the accelerometer.

***Experiment 3: Effect of the number of trill syllables per call on female replies***

To examine female cricket responses to variation in the trill component of the call, we played two additional series of stimuli to a third set of female crickets ( $N = 15$ ). Playback of stimuli were conducted using the same set-up as the experiments previously described; female vibrational replies were recorded with the laser Doppler vibrometer. All stimuli were played back at an amplitude of 80 dB peSPL with a period of 15 s and all consisted of 10 ticks followed by a trill. In the first series, we manipulated the number of syllables in the trill (and therefore the trill duration) but kept the call period and individual syllable duration constant. The treatments were 20, 30, 40, 50 and 60 trill syllables, with 40 being the average for male *L. bitaeniatus* crickets, 30 and 50 representing the 5th and 95th percentiles observed

in males, and 20 and 60 representing syllable numbers that we almost never observed in our male crickets.

#### ***Experiment 4: Effect of trill acoustic parameters on female replies***

Temporal properties of a call are not limited to the number of syllables it contains, and other factors like syllable period and duty cycle can be essential for mate recognition in crickets (Clemens et al., 2021; Schöneich et al., 2015; Schöneich, 2020). In a second playback experiment with the same group of crickets that were used in experiment 3 ( $N = 15$ ), we manipulated other parameters of the trill while keeping the tick component of the call constant (10 ticks). The treatments in this playback series manipulated syllable duration, syllable period and duty cycle. Eight of the crickets underwent experiment 3 first and the other seven underwent the experiment 4 first.

#### **Ethical Note**

All experiments with the crickets were noninvasive, consisting only of audio and vibrational recording and audio playback. For the male recordings, 16 crickets were euthanized by freezing. Crickets were maintained in a temperature-controlled room with a regular light/dark cycle, and they always had food and water ad libitum.

#### **Statistical Analysis**

All statistical analyses were conducted in R (v.4.0.5). Model assumptions for each statistical test were examined using residual quantile plots to test for normality of the data and the Levene test to test for homoscedasticity. In experiments 1 and 2, log transformation of the amplitude data achieved normal distributions of residuals. In some cases, latency data showed significant heteroscedasticity but could not be transformed to achieve homoscedasticity. Linear mixed models are highly robust to deviations from the assumptions of normally distributed data with equal variances across treatments (Schielzeth et al., 2020), so we report the results of these tests here with the acknowledgment that they did not meet the strict assumptions of the test.

To calculate the individual repeatability of the number of syllables of each call component, we used the function `rpt` from the package '`rptR`' (Stoffel et al., 2017). We calculated repeatability ( $R$  = ratio of the intergroup variance to the sum of the inter- and intragroup variance) (Nakagawa and Schielzeth, 2010) and used 1000 parametric bootstraps to obtain 95% confidence intervals. Likelihood ratio tests are used by `rpt` to obtain  $P$  values. We used a principal components (PC) analysis to obtain a multivariate measure of body size from the measurements of pre-recording mass, pronotum width and length, and left and right femur lengths. PC1 accounted for 78% of the variation in these measurements, with loadings for each original variable except pronotum width being approximately equal (20%). To test whether male calling behaviour is related to body size, we used linear regression models with the average number of ticks or trills produced by a male over 24 h as the response variable and PC1 (with lower values representing smaller males) as the predictor variable.

To test whether acoustic treatment influenced females' responsiveness to playback stimuli in each of the four experiments, we used generalized linear mixed models with a Poisson link function (`glmer` function from '`lme4`' package) (Bates et al., 2015), which is appropriate for count data. In each case, the number of female replies elicited in each playback treatment (out of the five repetitions within a treatment) was the response variable, acoustic treatment was a fixed effect and individual cricket was included as a random effect due to repeated measurements on the same individual across treatments. We used the mixed function from the '`afex`' package in R (Singmann et al., 2023) to calculate  $P$  values for generalized linear mixed models using the likelihood ratio test method. For experiments in which there was an increasing number of syllables across treatments (experiments 1–3), acoustic treatment was an ordinal variable, whereas it was an unordered categorical variable for experiment 4.

We also tested whether acoustic treatment affected the properties of the female reply, specifically amplitude and latency. Although we also measured duration, we found a significant correlation between the two variables measuring the intensity of the female reply, amplitude and duration (Appendix, Figure C.7). Reasoning that longer-duration female replies could necessarily result from higher-amplitude body movements during replies, but

not vice versa, we included only female reply amplitude and latency as response variables in our models. For experiments in which variation in the timing or intensity of the female reply was of interest (experiment 2, experiment 3), we used linear mixed models to test whether acoustic treatment influenced amplitude or latency. We used the mixed function from the 'afex' package to calculate  $P$  values for linear mixed models using the likelihood ratio test method. In our initial models, we included an interaction term between acoustic treatment and repetition; because the interaction term was not significant in any of the models, we dropped it from our final models. Final models included either female reply amplitude (mV output from the LDV or accelerometer) or latency (ms) as the response variable, acoustic treatment and repetition number as fixed effects and individual cricket as a random effect. Because we were interested in whether specific pairs of acoustic treatments elicited different intensities of female replies, we used the emmeans package (Lenth, 2023) to conduct post hoc tests (estimated marginal means, pairwise method) on models with significant main effects to see which treatments or repetitions differed from each other.

## Results

### Male calling song

We recorded 2643 calls from 15 males calling over 24 h. Males called  $176 \pm 147$  (mean  $\pm$  SD) times per 24 h, with a mean of  $21 \pm 11$  ticks and  $39 \pm 5$  trill syllables per call. The number of tick syllables per call was highly repeatable for individual males ( $R = 0.245$ ,  $CI = [0.111, 0.384]$ ,  $P = 0.001$ ), and repeatability was the same as a previously published estimate for a different population of the same species ( $R = 0.24$ , (Tan et al., 2021). Repeatability for the number of trill syllables per call was significant but low ( $R = 0.091$ ,  $CI = [0.007, 0.19]$ ,  $P = 0.001$ ). The low  $R$  value, however, was not due to an inconsistent number of trill syllables within individuals but rather to low variation both within and between individuals (Table 3.1; Nakagawa and Schielzeth, 2010). The relationship between male body size and the mean number of ticks per call was not significant, but the  $P$  value was marginal (Figure 3.2A). There was a significant positive relationship between body size and the mean number

of trill syllables per call (Figure 3.2B). The call parameters for a second set of males (see Methods, Male calling song) are summarized in Table 3.1. In general, the variation across males was greater for the tick component than for the trill for each measured parameter except syllable duration, more so for number of syllables and syllable period than for peak frequency.

### **Female responses to calls**

Across experiments, we found that females produced vibrational replies to almost all calls containing a typical trill, and that both acoustic treatment and the repetition number of the playback stimulus within a treatment influenced the amplitude and latency of these replies. Results for acoustic treatments in each experiment are summarized below. Repetition number influenced reply amplitude in all experiments in which it was measured (experiments 1–3), with the first repetition of the five eliciting lower-amplitude replies than subsequent repetitions. Repetition number affected the reply latency in experiments 1 and 2 but not in experiment 3. In experiment 1, the first repetition elicited shorter reply latencies (mean latency of replies to first repetition = 82 ms; mean latencies of subsequent repetitions = 141–160 ms). In experiment 2, the first repetition elicited slightly longer reply latencies (mean latency of replies to first repetition = 90 ms; mean latencies of subsequent repetitions = 80–85 ms). Additional details are provided in the Appendix.

#### ***Experiment 1: Male call components required for female replies***

No females responded to the tick component of the male call without a trill component, either immediately after the playback or at other points during the playback treatment (Figure 3.3). All but one of the females responded to at least one repetition of the trill component of the male call without the ticks. All females responded to at least three repetitions of the complete call, with no significant difference in the proportion of replies elicited by the trill alone and the complete call (Figure 3.3). The ticks-only treatment could not be included in statistical tests because no females replied to this treatment, preventing parameter estimates due to complete separation. Female replies had significantly higher

amplitude in response to the complete call than to the trill alone ( $\chi^2_1 = 41.87$ ,  $P < 0.001$ ). Replies to the trill alone occurred at significantly longer latencies ( $102 \pm 15$  ms) than to the complete call ( $99 \pm 17$  ms;  $\chi^2_1 = 7.61$ ,  $P = 0.006$ ).

### ***Experiment 2: Effect of the number of ticks per call on female replies***

Varying the number of ticks in the playback call did not affect whether a female replied to the acoustic treatment ( $\chi^2_5 = 0.04$ ,  $P > 0.999$ ; Figure 3.4g), but higher numbers of ticks did elicit higher amplitude replies ( $\chi^2_5 = 79.2$ ,  $P < 0.001$ ; Figure 3.4c). Tukey post hoc tests for the amplitude of the female reply showed that this effect was due to a significant difference between zero ticks (i.e. a trill alone) versus all other tick numbers for acoustic treatment, with no significant differences between any other pairs of treatments ( $P < 0.001$  for all contrasts with 0 tick treatment, see Appendix, Table C.4). Varying the number of ticks in the playback also influenced the latency of the females' replies ( $\chi^2_5 = 11.25$ ,  $P = 0.047$ ; Fig. 4e). However, Tukey post hoc tests of this model found no significant pairwise contrasts (Appendix, Table C.6). Five replies (out of 500 replies and 507 playbacks of stimuli) to 0, 10 and 30 tick treatments had negative latencies, occurring before the playback had ended.

### ***Experiment 3: Effect of the number of trill syllables per call on female replies***

Fewer female vibrational replies were elicited by the 20-syllable trill acoustic treatment compared to the treatments with more trill syllables, but this difference was not significant ( $\chi^2_4 = 5.52$ ,  $P = 0.238$ ; Figure 3.4h). Varying the number of trill syllables significantly and positively affected the amplitude of the female replies ( $\chi^2_4 = 197.8$ ,  $P < 0.001$ ; Figure 3.4d), and Tukey post hoc tests indicated that all pairs of playback treatments except for the 50- and 60-syllable acoustic treatments were significantly different from each other ( $P < 0.015$ ; see Appendix, Table C.8). Latency of the female reply was also strongly influenced by the number of trill syllables in the call ( $\chi^2_4 = 27.25$ ,  $P < 0.001$ ; Figure 3.4f). Tukey post hoc tests for pairs of acoustic treatments showed significant differences in latencies between the 20-syllable and 50-syllable treatments and between each treatment and the 60-syllable treatment (see Appendix, Table C.10), with no significant differences

between the other pairs. The spread of the latencies differed widely between playback treatments: the standard deviation for the 60-syllable treatment was 78, much greater than those of the 20-, 30-, 40 and 50-syllable treatments (with SDs of 9, 7, 9 and 25, respectively). For most individuals, within-individual variability also increased with longer trills (Fig. 5). Altogether we recorded 324 replies from 375 call playbacks during the experiments. Seventeen replies occurred before the playback call had ended, and all of these premature replies were in response to 50- and 60-syllable treatments.

#### ***Experiment 4: Effect of trill parameters on female replies***

Each of the manipulated trills in this playback series elicited at least one response, including the playback of 10 ticks plus a long tone without any pulse structure (treatment e; Fig. 6). The acoustic treatments significantly affected a female's propensity to respond (c26  $\frac{1}{4}$  161.31,  $P < 0.001$ ): all females responded to all of the control (average call) stimuli, and Tukey post hoc comparisons of each treatment to the control showed significant differences in the number of replies for all treatments except treatment b and treatment c (Figure 3.6; for details, see Appendix). Treatments b and c were the two in which the syllable period of the trill was the same as the control call (43 ms) but the syllable duration was either half the control duration (b) or 1.5 times the control duration (c). Treatment d had a normal syllable duration but twice the control period. The previous treatments changed one parameter and held the other constant, but this necessarily changes the duty cycle of the call (proportion of time with sound). To control for duty cycle, treatments e and g had syllable durations that were twice (e) or half (g) the control duration with syllable periods maintained at a duty cycle close to the control. Finally, to test whether syllable structure is needed at all, treatment f was a continuous tone the duration of the control trill. Changes in other call acoustic parameters did not appear to influence female responsiveness, but not all possible combinations were tested. This playback series also elicited more 'atypical' replies (39 out of 234 responses and 525 playbacks) than the other experiments. Examples of atypical replies, where the waveform did not closely resemble the female reply to actual (natural) male *L. bitaeniatus* calls, are provided in the Appendix. These atypical replies were not included in

the above analyses. Because this experiment was conducted with several playback stimuli that were unrealistic representations of male calls, we were primarily interested in the first-order question of whether females would respond at all, and so only report the responsiveness data.

## Discussion

As predicted, *L. bitaeniatus* females varied the amplitude and other parameters of their vibrational reply depending on specific parameters of the male call, and they responded differently to each component of the call. The two components of the male call have different functions: females never replied to tick syllables alone, and our results demonstrate that the trill of the male call is both necessary and sufficient to elicit a reply. Adding ticks to the call resulted in a significant increase in the vibration amplitude of the female reply, but we found no significant difference in either reply amplitude or latency to calls with few ticks (10) versus many ticks (50). Therefore, our results do not support the initial prediction of an open-ended preference for increasing syllable numbers in the more variable tick component of the call. This is surprising, as males can and often do produce upwards of 50 ticks when calling: one male produced 120 ticks in a single call. Our hypothesis that females would express a unimodal preference for the less variable component of the call was also not supported. We found that larger males produced longer trills (with greater numbers of syllables) and females produced higher-amplitude replies in response to longer trills. This pattern held even at the extreme range of the trills that males produce—i.e. an open-ended preference rather than a unimodal one. At these extremes, however, the average latency of the reply was shifted away from the typical reply latency, and replies sometimes occurred well before the end of the male call. This lends support to our alternative hypothesis that the trill functions at least in part to aid in the timing of the reply, similarly to ‘trigger’ pulses in some duetting katydid species (Bailey and Hammond, 2003; Stumpner and Meyer, 2001). A summary of these hypotheses, predictions and findings is provided in the Appendix (Table B.1). Our study shows that stereotyped communication

signals can result from an interaction between the efficacy of different signal parameters, such as those influencing detection distance versus signal recognition, rather than unimodal selection of signal parameters by receivers.

The function of the tick syllables remains unclear. Our experiments were not designed to examine possible interactions where response to changes in one component is altered or enhanced by response to changes in the other (Candolin, 2003; Hebets and Papaj, 2005). We did find, however, that the addition of ticks to a trill altered the amplitude and latency of the reply compared to trills with no preceding ticks (experiment 1). Alerting components perceived prior to a more informative or species-specific signal can hasten a response or increase the overall probability of signal detection (Grafe et al., 2012; McLennan, 2003). In this study, the addition of more ticks to a call did not significantly affect the latency (or the variation in latency) of the female reply, nor her propensity to reply. Other behaviours that increase the chances of successful mate finding are possible: for example, hearing ticks could prompt females to stop foraging or assume a better posture for producing a reply. We did not observe any postural changes in females during our playbacks, although these were not conducted under naturalistic conditions (females were perched within a mesh cylinder rather than on a plant stem or leaf). Furthermore, we found no evidence that adding ticks to a call decreases the threshold amplitude at which females will reply in a quiet laboratory setting (Appendix). It is possible, however, that calls with greater numbers of ticks would be more detectable to female crickets in acoustically noisy environments. Background noise can interfere with female responses to calls in *Gryllus bimaculatus* crickets (Schmidt et al., 2014) and can obliterate acoustically mediated mate preferences in crickets and other animals (Coss et al., 2021; Wollerman and Wiley, 2002). We note that an alerting function would not on its own account for the high variability in the number of ticks within and between *L. bitaeniatus* males recorded under consistent conditions.

The structure of the male call might be explained by the presence of multiple receivers (Hebets and Papaj, 2005; Zambre and Thaker, 2017), including receptive females of different ages (Tanner et al., 2019). The presence of ticks in a call appears to elicit replies more reliably from older females (Appendix), which could mean a larger pool of potential mates

for calling males if individuals of different ages are present at the same time in a habitat. From the present study, it is not clear whether *L. bitaeniatus* females became choosier as they aged, or whether the changes we observed were due to senescence. It is also possible that ticks are involved in mediating male–male interactions, rather than male–female duets. Several taxa are known to produce signals where male and female audiences either perceive the same call in different modalities (Hill and Shadley, 2001), or use different components of a call to make decisions (Narins and Capranica, 1976). As signallers, males may emphasize different call components while in the presence of males versus females (Krobath et al., 2017). Multiple components or signals that target different receivers are predicted to occur in situations where both types of receivers are likely to be encountered, such as in mate advertisement/competition. While there is little information available on male competition in *L. bitaeniatus*, there is some evidence that males of a related lebinthine species, *Ponca hebardii* Robillard, engage in competitive masking and female reply mimicry in response to the calls of other males (Benavides-Lopez et al., 2020).

The trill appears to be critical to successful mate recognition by females, as it reliably elicits replies regardless of the presence of ticks. From the set of playback stimuli in experiment 4, we can conclude that it is the (species-specific) syllable rate of the trill, and not some other parameter, that most strongly determines whether females respond. Interestingly, the stimulus in experiment 4 that elicited the fewest responses (g)—even fewer than the single long tone without pulse structure—was one that resembles the call of a congeneric species, *Lebinthus luae* Robillard & Tan. *Lebinthus luae* have shorter trill syllables that are produced at a faster rate (mean call parameters for *L. luae*: number of syllables = 25, trill syllable duration = 15 ms, trill syllable period = 25 ms, trill duty cycle = 0.52; cf. Figure 3.6; also see supplemental material in ter Hofstede et al. (2015)). These species overlap slightly in geographical distribution (Baroga-Barbecho et al., 2020), and it could be that the lack of female replies to this experimental treatment are explained by selection against hybridization with *L. luae*.

We did observe that longer trills elicited more intense replies in the playback experiments. Larger males produced calls with more trill pulses, so females might prefer calls that

reflect this measure of mate quality. It is possible that having a longer trill length helps females better sample the information in the trill and prepare their reply. From the occurrence of premature replies to elongated trills (Appendix, Figure C.4), we might conclude that the female reply is prompted by the number of pulses within the trill rather than the cessation of the trill. However, because the premature replies did not occur at a consistent time point (i.e. where the end of a natural-length trill would be; Figure 3.4f), and because several females produced a series of premature replies to long trills and then a reply at the typical latency (Appendix, Figure C.5), it seems more likely that both syllables within the trill and the cessation of the trill can affect reply timing. It is unlikely that females time their replies relative to the start of the trill since typical female reply latency was seen in response to unnaturally short trills (Figure 3.4f). Regardless of the mechanism by which the females time their replies, consistent trill lengths within and across males appear to help maintain the timing of the duet, despite the possible advantages that eliciting a greater amplitude reply might confer to males that produced longer trills, such as detection of females at greater distances or more accurate directional information (Gibson and Cocroft, 2018).

The unusual multimodal nature of this duet, however, raises questions about how males respond behaviourally to the vibrational reply. The precise female reply latency to naturalistic male calls suggests that timing is important in this interaction, but we do not yet know how tolerant lebinthine males are to deviation from the typical latency. Duetting katydids using only airborne sound can have strict latency windows, particularly when replies occur with extremely short latency (Bailey and Hammond, 2003). The latency for the female reply in *L. bitaeniatus* is short (ca. 100 ms) compared to many other insect species (Bailey and Hammond, 2003), suggesting that males might have a strict latency window for recognition. Unlike airborne sound, however, substrate-borne vibrations can travel at variable (and relatively slow) velocities (Michelsen et al., 1982; Virant-Doberlet et al., 2006). Therefore, the use of vibrational signals in this duetting system might require more relaxed latency windows (Kuhelj et al., 2015), since males would benefit from recognizing replies produced on a variety of plant substrates and architectures. In insects with mixed airborne and vibrational duets (*Onomarchus uninotatus* Serville; Rajaraman

et al., 2015) or purely vibrational duets (some Neuroptera, many Hemiptera; see Rodríguez and Barbosa (2014) for a review), reply latencies occur with characteristic timing and in some cases differ between closely related and co-occurring species (Rodríguez and Coccoft, 2006). The tolerance of the male recognition window, however, has only been tested in a few species (e.g., Kuhelj et al., 2015; Rohde et al., 2013). Furthermore, we do not know whether singing males are able to recognize vibrational replies that occur prior to the end of their call. We note that vibrational replies in *L. bitaeniatus* are typically repeated once per male call and are short in duration (122 ms in these experiments) compared to many other insect vibrational signals (Rodríguez and Barbosa, 2014). This, plus the observation that *Lebinthus* females do not appear to take on any of the searching role during duetting (ter Hofstede et al., 2015), could make the male’s search an especially challenging task. Males are observed to track down the source of vibrations while females remain stationary, but it remains ambiguous exactly how the males are using the information in the reply. It is possible that they perform vibrotaxis by gleaned directional information about the position of the female relative to them, as seen in some other vibrationally duetting insects (Gibson and Coccoft, 2018; Virant-Doberlet et al., 2006), or they might simply search or walk more when they have detected replies.

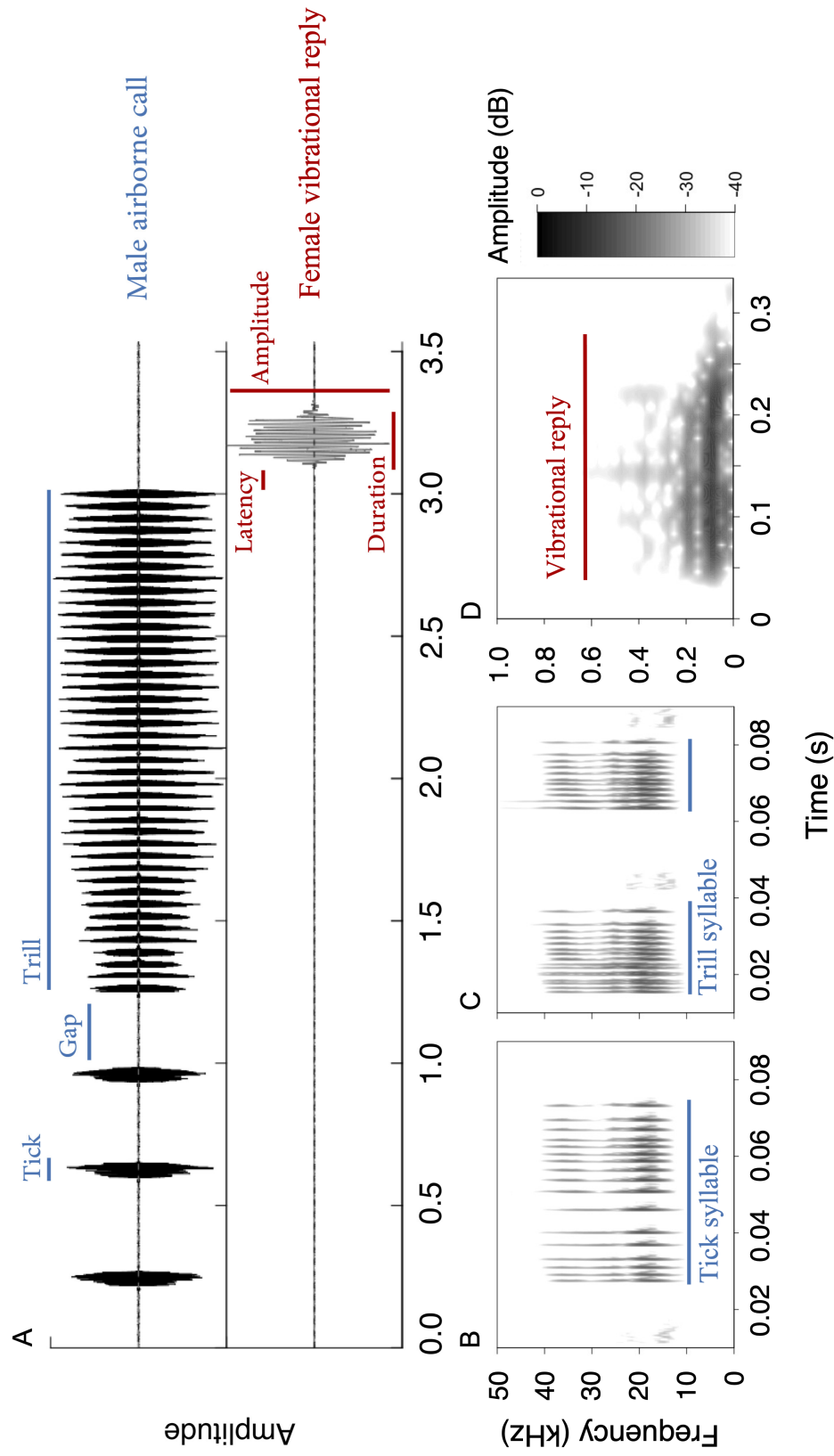
We conclude that the potential for the female vibrational reply to have shaped features of the male call via sexual selection exists in this communication system. The stereotyped number of trill syllables in the male call is not the straightforward result of stabilizing selection but likely reflects an interaction between a female preference for longer trills and the efficacy of the female reply. Trills with more syllables elicit replies at greater amplitudes, but the atypical timing of these replies could interfere with effective male mate recognition. In this mate-searching duet, the modality of each signal likely plays an important role, and there is a need for further studies in this and other multimodal duet systems to understand the differences between these unimodal acoustic duets and unimodal vibrational duets. Comparisons of reply timing, latency windows and searching behaviour across insect duets could reveal important constraints placed upon communication by the particular modality in which signals are produced—and whether such constraints affect close-range courtship

duets differently than those that function in longer-range mate searching. More broadly, our results suggest that interactions between mate preferences and signal efficacy can influence signal design. While variable signals are often the result of open-ended receiver preferences and stereotyped signals of unimodal receiver preferences, other aspects of communication systems (e.g. competitors, environmental transmission, receiver constraints) can potentially disrupt this relationship. Studies in animal communication will benefit from experiments that simultaneously consider both mate preferences and signal efficacy in addressing proximate and ultimate questions about signal design.

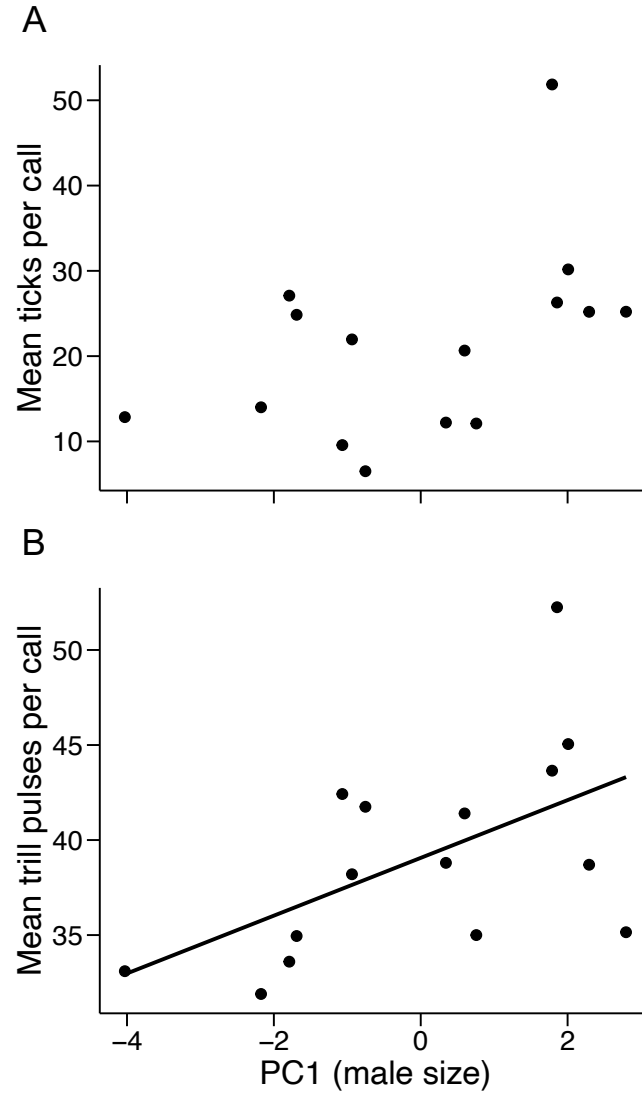
## Tables and Figures

**Table 3.1: Male call parameters for 10 individuals of the cricket species *L. bitaeniatus*.** Values are means  $\pm$  SD. Mean = grand mean ( $\pm$  SD) of the individual means for each call parameter; CV = among-male coefficient of variation for each call parameter.

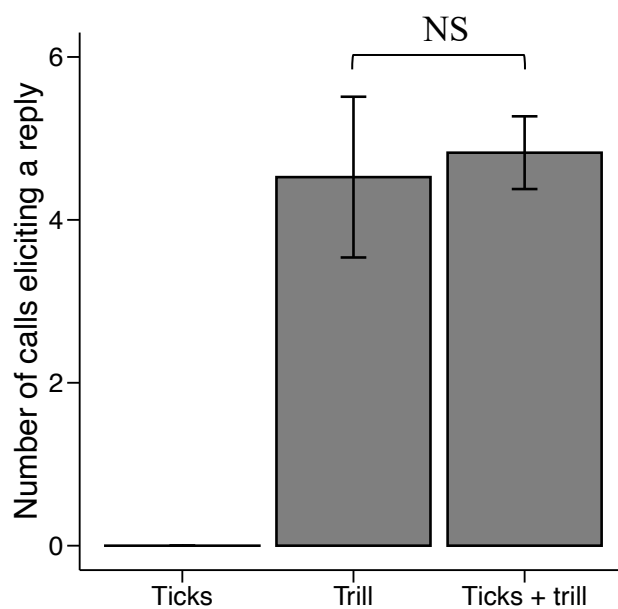
| Cricket | No. of calls measured | No. syllables   |                | Syllable dur. (ms) |                | Syllable period (ms) |                | Peak freq. (kHz) |                | Gap dur. (ms)     |
|---------|-----------------------|-----------------|----------------|--------------------|----------------|----------------------|----------------|------------------|----------------|-------------------|
|         |                       | Ticks           | Trill          | Ticks              | Trill          | Ticks                | Trill          | Ticks            | Trill          |                   |
| 1       | 5                     | 25.6 $\pm$ 15.6 | 37.0 $\pm$ 2.4 | 53.9 $\pm$ 3.1     | 23.7 $\pm$ 1.3 | 362.7 $\pm$ 19.5     | 43.8 $\pm$ 1.9 | 20.4 $\pm$ 0.7   | 20.5 $\pm$ 0.8 | 123.7 $\pm$ 68.6  |
| 2       | 5                     | 18.5 $\pm$ 26.6 | 35.2 $\pm$ 2.3 | 50.4 $\pm$ 11.5    | 20.9 $\pm$ 1.0 | 671.9 $\pm$ 385.1    | 41.5 $\pm$ 0.9 | 21.2 $\pm$ 1.0   | 20.9 $\pm$ 0.3 | 157.5 $\pm$ 37.2  |
| 3       | 5                     | 7.0 $\pm$ 8.0   | 43.2 $\pm$ 4.6 | 39.6 $\pm$ 8.4     | 17.9 $\pm$ 1.1 | 331.7 $\pm$ 28.2     | 38.8 $\pm$ 0.7 | 20.9 $\pm$ 0.9   | 20.3 $\pm$ 1.5 | 191.6 $\pm$ 28.1  |
| 4       | 5                     | 3.8 $\pm$ 0.5   | 41.6 $\pm$ 3.0 | 44.4 $\pm$ 8.5     | 19.4 $\pm$ 0.7 | 369.3 $\pm$ 70.7     | 38.3 $\pm$ 2.3 | 20.2 $\pm$ 0.5   | 20.1 $\pm$ 0.4 | 159.5 $\pm$ 103.0 |
| 5       | 5                     | 31.6 $\pm$ 25   | 40.8 $\pm$ 2.9 | 51.5 $\pm$ 2.5     | 22.4 $\pm$ 1.3 | 362.9 $\pm$ 23.3     | 39.1 $\pm$ 1.9 | 21.4 $\pm$ 1.6   | 20.4 $\pm$ 0.5 | 174.2 $\pm$ 56.9  |
| 6       | 5                     | 14.4 $\pm$ 5.2  | 40.6 $\pm$ 1.5 | 49.2 $\pm$ 2.6     | 23.0 $\pm$ 1.1 | 406.6 $\pm$ 22.5     | 45.9 $\pm$ 0.7 | 19.0 $\pm$ 0.3   | 18.7 $\pm$ 0.3 | 119.1 $\pm$ 63.7  |
| 7       | 5                     | 7.6 $\pm$ 2.6   | 32.0 $\pm$ 1.6 | 41.7 $\pm$ 2.1     | 23.2 $\pm$ 0.4 | 415.1 $\pm$ 24.2     | 44.0 $\pm$ 1.0 | 20.8 $\pm$ 0.3   | 20.7 $\pm$ 0.4 | 184.8 $\pm$ 76.2  |
| 8       | 3                     | 8.3 $\pm$ 2.5   | 41.0 $\pm$ 5.3 | 44.7 $\pm$ 4.1     | 22.3 $\pm$ 1.3 | 409.3 $\pm$ 87.3     | 44.0 $\pm$ 0.3 | 17.9 $\pm$ 0.2   | 17.4 $\pm$ 0.1 | 270.1 $\pm$ 2.3   |
| 9       | 5                     | 11.6 $\pm$ 2.3  | 38.4 $\pm$ 2.3 | 48.2 $\pm$ 1.5     | 21.2 $\pm$ 0.2 | 373.7 $\pm$ 23.7     | 39.3 $\pm$ 0.6 | 19.0 $\pm$ 0.2   | 19.0 $\pm$ 0.2 | 190.1 $\pm$ 35.9  |
| 10      | 5                     | 9.0 $\pm$ 6.2   | 38.4 $\pm$ 3.2 | 43.4 $\pm$ 5.4     | 24.2 $\pm$ 0.7 | 407.8 $\pm$ 30.6     | 44.9 $\pm$ 1.2 | 26.7 $\pm$ 2.0   | 22.8 $\pm$ 0.4 | 187.6 $\pm$ 55.3  |
| Mean    |                       | 13.7 $\pm$ 9.0  | 38.8 $\pm$ 3.4 | 47.7 $\pm$ 4.6     | 21.8 $\pm$ 2.0 | 411.1 $\pm$ 95.6     | 41.9 $\pm$ 2.9 | 20.7 $\pm$ 2.4   | 20.1 $\pm$ 1.5 | 173.8 $\pm$ 42.3  |
| CV      |                       | 1.04            | 0.11           | 0.15               | 0.10           | 0.25                 | 0.14           | 0.12             | 0.07           | 0.38              |



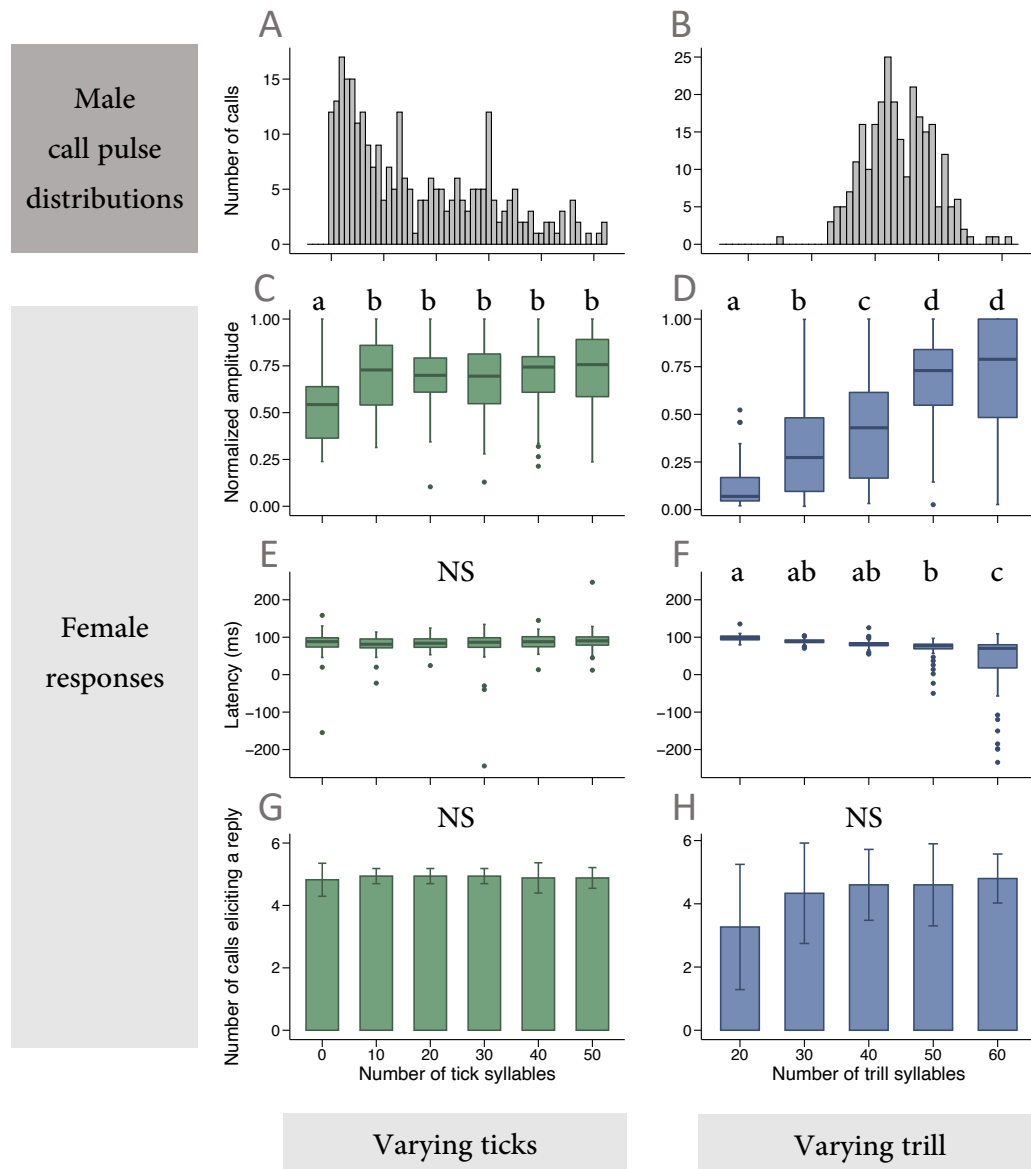
**Figure 3.1: Oscillogram and spectrograms of each component of the *L. bitaeniatus* duet.** This is a composite of a male call recorded in isolation and a reply from a female recorded during a playback experiment, placed at the appropriate (average) latency. Blue and red lines illustrate the measured acoustic and vibrational call parameters. (a) Oscillogram showing the last three ticks of a male call followed by a trill (top trace) and the female vibrational reply (bottom trace). Spectrograms on second row show a single tick syllable (b), two separate trill syllables on the same timescale as the tick (c), and the female reply (d).



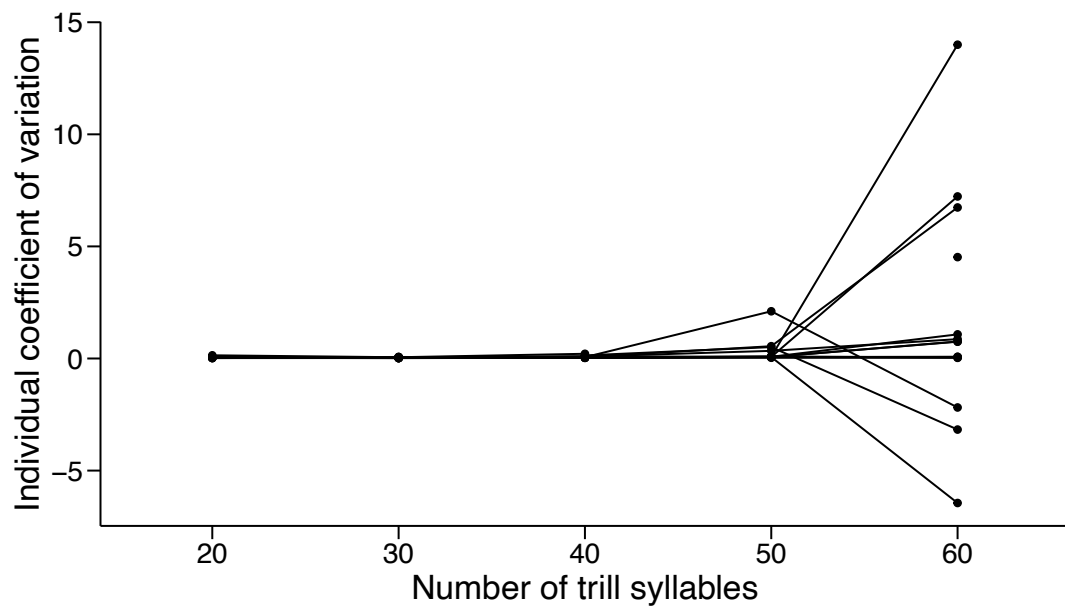
**Figure 3.2: Relationship between male size and number of syllables in call components.** (a) Number of ticks per call and PC1 ( $F_{1,13} = 3.83$ ,  $P = 0.072$ ) and (b) Number of trill syllables per call and male mass ( $F_{1,13} = 5.53$ ,  $P = 0.035$ ,  $R^2 = 0.30$ ) in *L. bitaeniatus*. Points show mean number of pulses in each component for all the calls produced by an individual over 24 h.



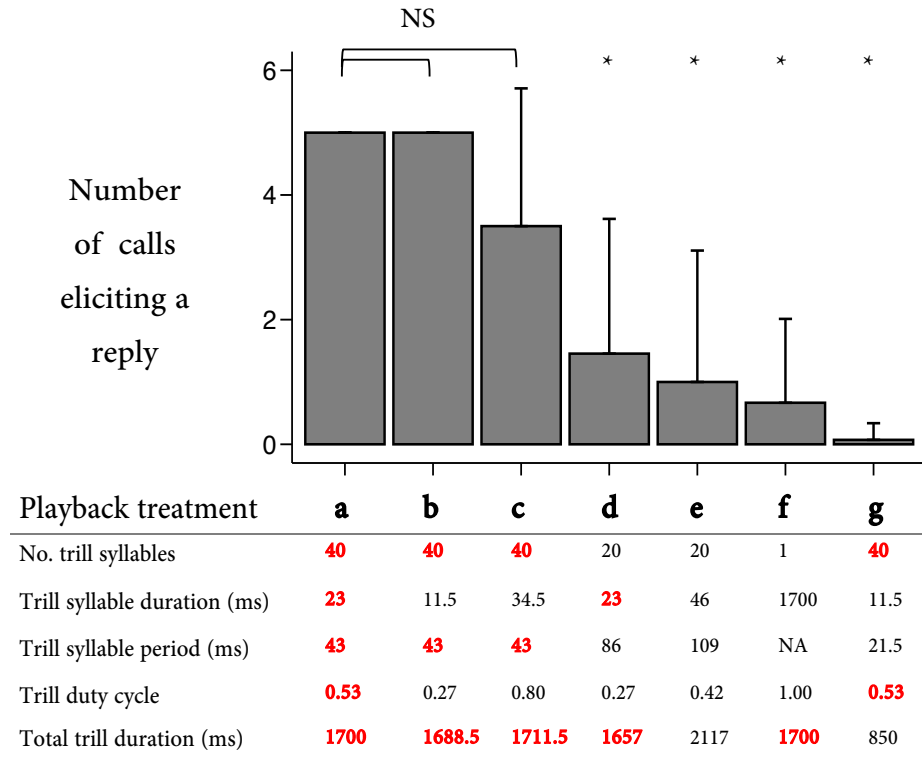
**Figure 3.3: Mean  $\pm$  SD number of male calls out of five replicates per acoustic treatment that elicited female vibrational replies.** Treatments contained one or both of the two main components of each male call: ticks and a trill. Treatments: a sequence of 10 ticks (Ticks), 10 ticks and a trill (Ticks + trill) and just the trill with no ticks (Trill).  $\chi^2_1 = 0.39$ ,  $P = 0.535$ .



**Figure 3.4: Distribution of syllable numbers in male calls and female responses to playbacks where the number of syllables in each component was altered.** (a, b) Histogram of syllable numbers for the tick component and the trill component, respectively, from the subset of 24 h calls that were scored for both the number of tick syllables and the number of trill syllables. (c, d) Amplitude of female vibrational replies (normalized by dividing the measured amplitude of each response by the maximum of each individual) for (c) varying ticks experiment and (d) varying trill experiment. (e, f) Latency of female response for (e) varying ticks experiment and (f) varying trill experiment. (g, h) Mean number of male calls out of five replicates per acoustic treatment that elicited female vibrational replies for (g) varying ticks experiment and (h) varying trill experiment. For box plots (c, e, f), boxes represent the 25th and 75th percentiles (ends) and the median (centre line); whiskers represent  $Q1 - 1.5 \times IQR$  and  $Q3 + 1.5 \times IQR$ ; and all outliers beyond these whiskers are shown. For bar plots (g, h), error bars show standard deviation. Lower case letters show significant differences between acoustic treatments determined by post hoc tests, with shared letters indicating no significant differences.



**Figure 3.5: Individual coefficients of variation for female replies to male calls containing different numbers of trill syllables.** Coefficients are the standard deviation divided by the mean reply latency of the five presentations of each treatment. Some coefficients are negative because the mean reply latency for that individual occurred before the end of the male call.



**Figure 3.6: Mean number of five replicates that elicited a female reply in experiment 4.** The treatments in this playback series were as follows: a = control with 40 trill syllables, similar to the average syllable duration, number and period of recorded males; b = 40 trill syllables with the average syllable period but half the average syllable duration; c = 40 trill syllables with the average syllable period but 50% longer syllable duration; d = 20 trill syllable with an average syllable duration but twice the average syllable period; e = 20 trill syllables with twice the average syllable duration; f = a single, long continuous tone rather than a series of syllables, with duration equivalent to total trill duration of control; g = 40 trill syllables with half the average syllable duration and period, but an equivalent duty cycle to the control. An asterisk denotes a significant difference between an acoustic treatment (beg) and the control (a).

## Synthesis and future directions

Patterns of signal expression can shed light on the proximate and ultimate causes that shape the evolution of communication signals and systems. In these manuscripts, I have presented data, analysis, and insights about the use of two signaling modalities, airborne sound and substrate-borne vibration, across a number of orthopteran species. These studies span multiple scales of biological organization, from species-level differences, to individual variation within a species, to single communicative exchanges between males and females. In Chapter 1, I showed that sound and vibrational advertisement calling signals appear to trade off across pseudophylline katydid species: heightened levels of vibrational signaling tended to occur in species that had lower levels of sound signaling. In Chapter 2, I found that within at least one of these species, the two signals are uncorrelated among individuals but positively correlated within individuals, with signaling males showing plasticity in expression of each type of signal as their time passed without successfully attracting a mate. In Chapter 3, I shifted my focus to a multimodal cricket duet where production of sound and vibrational signals is partitioned by sex, with males producing airborne sound calls to which females vibrationally respond. I found that characteristics of the male call can influence the amplitude and timing of the female reply, and identified the male call component that seems to be both necessary and sufficient for mate recognition by the females.

How can the contrasting directions of the correlations between sound and vibration signaling in Chapters 1 and 2 be interpreted? Here, the concept of phenotypic integration—how functionally related traits covary within organisms (Berg, 1960; Pigliucci, 2003)—is pertinent. Within this framework it has been argued that structural modularity, where display elements are linked genetically, developmentally, or physiologically, might both constrain signal evolution and enhance the correlated expression of signal traits in a complex display (Hebets et al., 2016). What about functionally modular, yet structurally distinct, signals and their components? The pseudophylline katydids, whose functionally overlapping sound and vibrational signals display negative relationships among species, positive relationships within individuals, and no relationship among individuals despite significant individual re-

peatability of each signal in isolation, potentially present one such case. The independent structures of these degenerate signals might free them to react differently to selection over evolutionary time, while the maintenance of at least some of their shared function may mean that they are likely to occur together in time during individual lifespans. In other words, the typical number of sound calls vs. vibrational calls that a species produces may be mediated by many traits that have diverged among species—including call conspicuousness, searching habits of each sex, or other traits (e.g., body shape) that were not measured in Chapter 1. Concurrently, for a single individual male within a single one of these species, the fact that conspecific females will respond similarly to each type of signal means that he might produce more sound and more vibrational calls on nights when he is highly motivated to mate. I will note that in this thesis, I have largely concentrated on the total number of signals or syllables produced in each modality—an important component of a signaling system, but one that is insufficient to fully address ideas about phenotypic integration, modularity, and evolutionary dynamics. Further work could investigate finer-scale temporal patterns in signal production (Are sound and vibrational calls produced randomly with respect to each other, or is there evidence for a shared pattern generator?), more comprehensive characterization of multivariate variation in each type of signal and corresponding functional implications for receivers (How do single, double, or triple pulse *Docidocercus* sound calls affect signal perception and attractiveness? Are females generally able to recognize conspecific vibrational calls in isolation because of subtle differences in frequency or amplitude modulation, or must they co-occur with sound calls? Is the substrate-borne component of the stridulation calls used by females or other receivers?), as well as the underlying genetic or developmental factors that potentially influence variation in signals and signaling behavior within and across species.

For the *Lebinthus bitaeniatus* crickets, there are many outstanding questions that arise from the specific features of their duetting system. The two-component male call, some form of which is common across diverse taxa, appears to convey multiple messages—but we do not know the function of the variable tick component, nor its intended receiver. Preliminary work on male–male interactions has revealed possibly signal interference behavior in

the timing of call components (CEK, unpublished data), which has been potentially documented (in a different form) in a related lebinthine species (Benavides-Lopez et al., 2020). And although I have demonstrated that changes in the stereotyped trill component of the male call can cause changes in the female reply that *could* alter the efficacy of the duet, we do not know exactly how male searching behavior would be affected. Can males recognize replies if they occur at atypical latencies? If they occur before the end of the male’s call? Does a female replying with higher vibrational amplitudes actually help the male locate her, given that the unpredictable propagation of substrate vibrations can pose issues for gleaned directional and distance information (Cocroft, 2014; Polajnar et al., 2023)? How do members of this species conduct their mate-finding duets in their natural environment, given that both the high-frequency airborne calls and short, simple, low-frequency vibrational replies seem potentially subject to scattering or disruption by architecturally complex vegetation? Finally, is the lack of responses to certain of our experimental stimuli (Figure 3.6, Stimulus ‘g’) driven by selection against hybridization with the closely related species *Lebinthus lae*? Clearly, there are many directions that future research in this system could take.

More generally, there are elements of each of the signaling systems described in this thesis that underscore the need to study not only mate advertisement signals themselves, but also the associated behaviors of their producers—and their intended audience. For the *Lebinthus* crickets, this need is obvious: males and females shift between being sender and receiver over the course of their mate-finding duets, and this system represents a reversal of the typical mate-searching paradigm in gryllid crickets, where females do the majority of searching for calling males (Weber and Thorson, 1990; ter Hofstede et al., 2015). In the pseudophylline katydids, females will also produce vibrational responses to male signals, though they appear to do so more reliably to vibrational male calls than to airborne ones and also contribute some to most of the search effort, performing phono- or vibrotaxis in response to male calls in both field and controlled experimental settings (Iwan et al., 2023, in prep.). Reproductive behavior in most animals is shaped by the balance of reproductive investment between the sexes, with gamete size differences at its core and a number of

possible outcomes—e.g., multiple matings, nuptial gifts, costly advertisement signals, reversal of the “choosy” sex, sexual cannibalism—that stem from it (Andersson, 1994). In orthopteran insects, who calls and who searches is one facet of this sexual conflict (Bailey, 2003). Much research has focused on the costs of signaling to advertising males, including from eavesdropping predators and parasites (Zuk and Kolluru, 1998; White et al., 2022). Mounting evidence, however, suggests that the process of searching for signaling males can be as dangerous—if not more so—than producing signals. Female field crickets are at more risk arriving to the burrows of calling males than the males (Sakaluk and Belwood, 1984); female birds with dull plumage are attacked at similar rates to brightly colored males (Cain et al., 2019); spiders have been shown to prey upon male and female tree crickets at similar rates in controlled mesocosm experiments (Torsekar et al., 2019); and (with particular relevance to this thesis) females of many pseudophylline species appear in the diets of gleaning bats in much greater numbers than males (Belwood, 1988; ter Hofstede et al., 2017). This last example may reflect pseudophylline males’ investment in large spermatophylax nuptial gifts, which can be upwards of 30% of male body mass (Chapter 2, this thesis; Belwood, 1988). In other katydids, spermatophore size is associated with mate-searching role reversal: non-duetting species of *Poecilimon*, where females search for males, have significantly larger spermatophores than duetting species, where males search for females (McCartney et al., 2012). In this same genus, females of a non-duetting species were shown to have drastically lower survival rates than those of a duetting species (Heller and von Helversen, 1993), a pattern that is also discernable in the sex ratios of iNaturalist observations (“captures”) for duetting vs. non-duetting katydid taxa (Kernan, 2023, in prep.). Despite this potential to influence signaling vs. searching strategy via the sex-specific costs of mate finding, differences in spermatophore investment remain an underexplored aspect of the Neotropical pseudophylline katydids’ biology (Gwynne, 2001). Similarly, and despite compelling hypotheses about sensory exploitation of females’ acoustic startle response (ter Hofstede et al., 2015), the pressures that led to the shift from unidirectional calling to multimodal duetting in the lebinthine crickets are not yet fully understood.

Multimodal, complex signals have been theorized to be especially robust to disruption,

allowing accurate information decoding when parts of a signal are lost (Ay et al., 2007). Duets, while subject to both intra- and inter-sexual conflict that can result in phenomena like signal jamming or deceptive mimicry of female signals, do also allow signalers more information about their audience than do unidirectional calls, permitting them to adjust their behavior in ways that maximize their chances of mating success. For instance, feedback from repliers can allow signalers to increase signaling rates only when a potential mate is present and motivated to respond. Each of the communication systems I have described in this thesis occurs in multiple sensory modalities, and also contains different components within a modality (e.g., the multiple components of the cricket calls, multiple syllables of many of the katydid airborne sound calls, or multiple frequencies present in both the sound and vibrational calls). At closer ranges, both the cricket and katydid systems become duets, involving the production of temporally stereotyped signals by both males and females trying to locate each other. In an increasingly noisy world (Barber et al., 2010) where animal population abundances and densities are sometimes much lower than they have been historically (Rosenberg et al., 2019; Outhwaite et al., 2022)—with a correspondingly lower likelihood of a receiver being within range of a signal (Gascoigne et al., 2009)—these sorts of complex mate-finding communications would seem to be a bulwark against communication disruptions. Signaling katydids, for instance, may have previously had better success when flexibly shifting from vibrational to sound signaling during times of higher wind noise (Velilla et al., 2020) and from sound to vibrational signaling when the cues of potential predators were detected (Belwood, 1988); this flexibility may aid signalers whether disruptions are natural or anthropogenic. To my knowledge, however, the joint implications of multimodality and duetting for communication system robustness have not been explored. Are multimodal duets less subject to disruption because of the principles of “robust overdesign” described by Ay et al. (2007) in regards to unidirectional communication systems? Or does the partitioning of modalities between different actors in the duet, together with the tight temporal relationship of call-and-reply that is common in these systems, mean that disruption in a single modality is enough to unravel the entire interaction? If the latter, does this concern specifically apply to these types of multimodal mate-finding duets, presently

known from a limited number of species (Rajaraman et al., 2015; ter Hofstede et al., 2015; Benavides-Lopez et al., 2020)—or to any mate-finding duet, which are widespread across many different taxa (Bailey, 2003; Pika et al., 2018)? Taking a longer view, have duets or unidirectional calling systems been more evolutionarily successful? Previously robust systems that have surprising fragility under novel circumstances may end up being a hallmark of the current period in our planet’s history (Scheffer et al., 2001; Liu et al., 2022). In order to more effectively anticipate and respond to this fragility, we should continue to examine complex systems—including those that govern the lives of “the little things that run the world”—to better understand why they originated, how they currently operate, and what potential disruptions they might face in the future.

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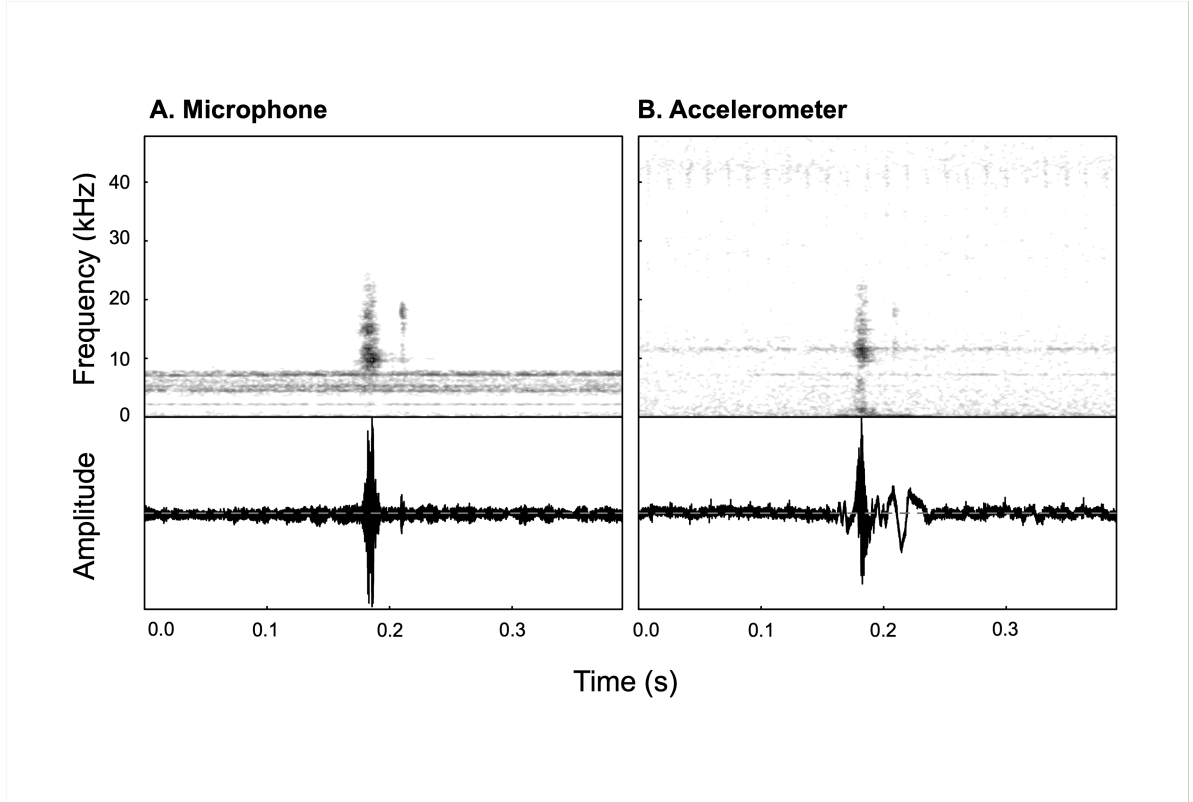
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## Appendices

### A Supplementary material for Chapter 1



**Figure A.1: Sound and vibrational components of an *Ischnomela gracilis* stridulation.** Sound production mechanisms like stridulation necessarily produce both airborne and substrate-borne signal components. For this species, which has a sound call with a dominant frequency at  $\sim 74$  kHz, above the available sampling frequency, I used the lower harmonic to identify calls and confirmed these were produced by the focal caged individual by looking for the substrate-borne component.

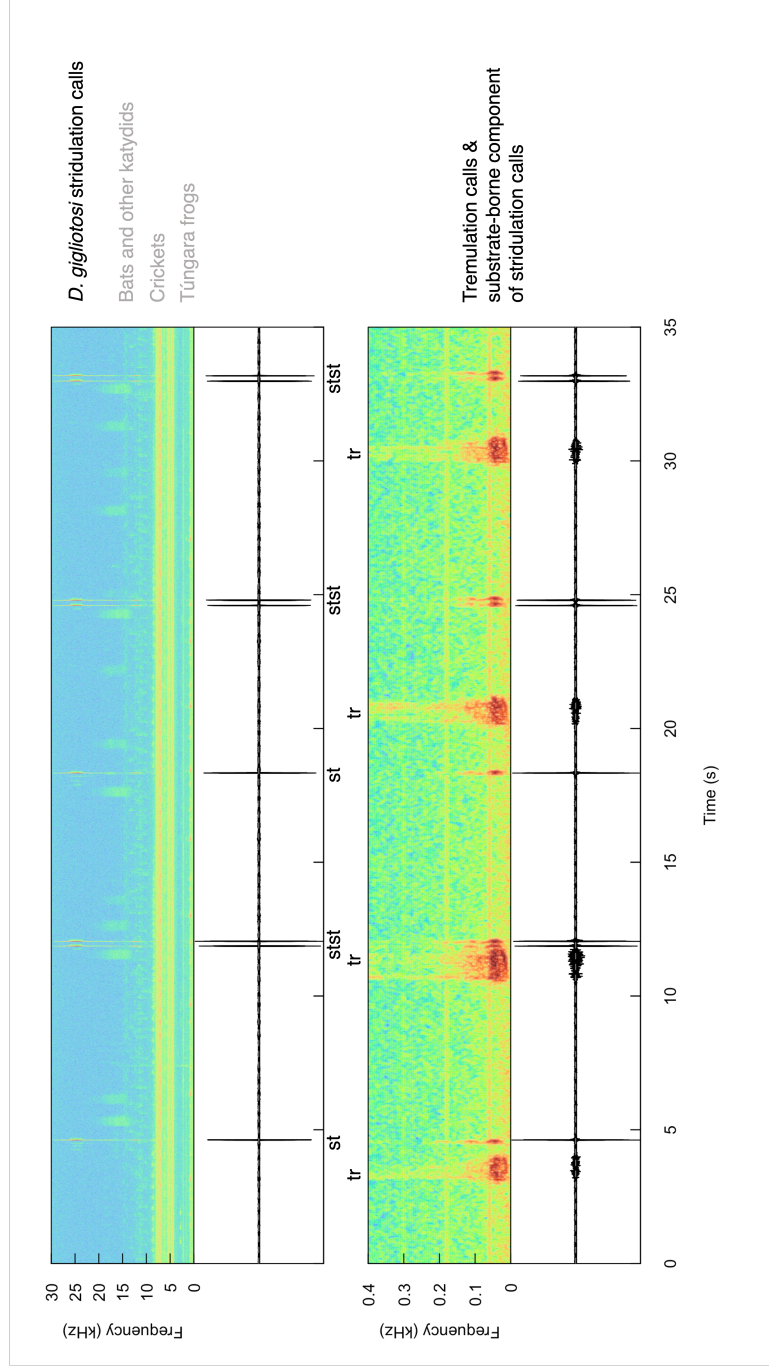
## B Supplementary material for Chapter 2

**Table B.1: Final 'brms' model effect estimates and 95% uncertainty intervals from model with Week 1 included.** Bolded CIs do not cross zero and were considered significant.

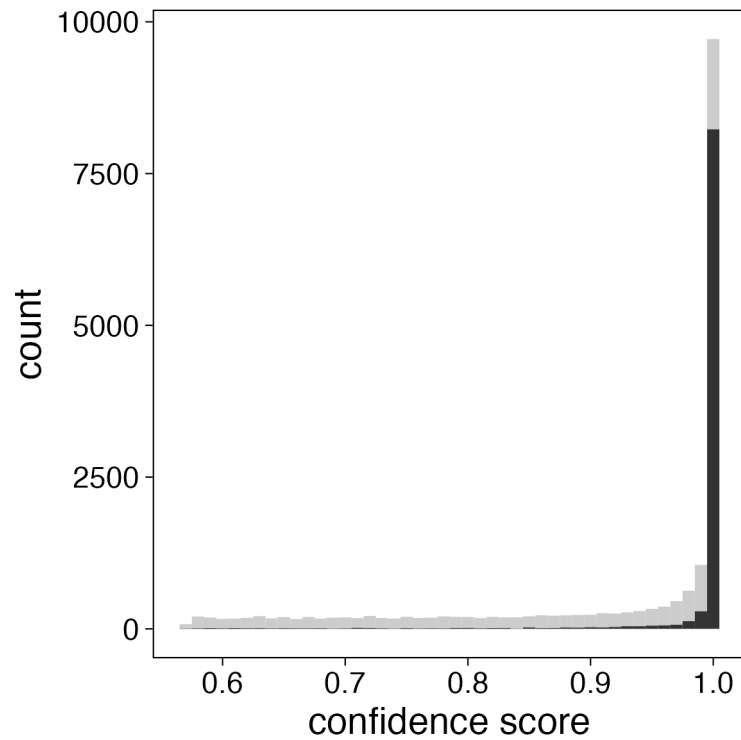
| Signal type  | Factor                                    | Estimate | SE    | 95% CI                 |
|--------------|---|----------|-------|------------------------|
| Stridulation | Mass                                      | 1.813    | 0.464 | <b>[0.903, 2.746]</b>  |
| Stridulation | Week of recording                         | 0.276    | 0.043 | <b>[0.189, 0.358]</b>  |
| Stridulation | Weeks between capture and first recording | 0.206    | 0.061 | <b>[0.0927, 0.332]</b> |
| Tremulation  | Mass                                      | 2.098    | 0.542 | <b>[1.014, 3.179]</b>  |
| Tremulation  | Week of recording                         | 0.162    | 0.050 | <b>[0.068, 0.259]</b>  |

**Table B.2: Final 'brms' model effect estimates and 95% uncertainty intervals from model with Week 1 excluded.** Bolded CIs do not cross zero and were considered significant.

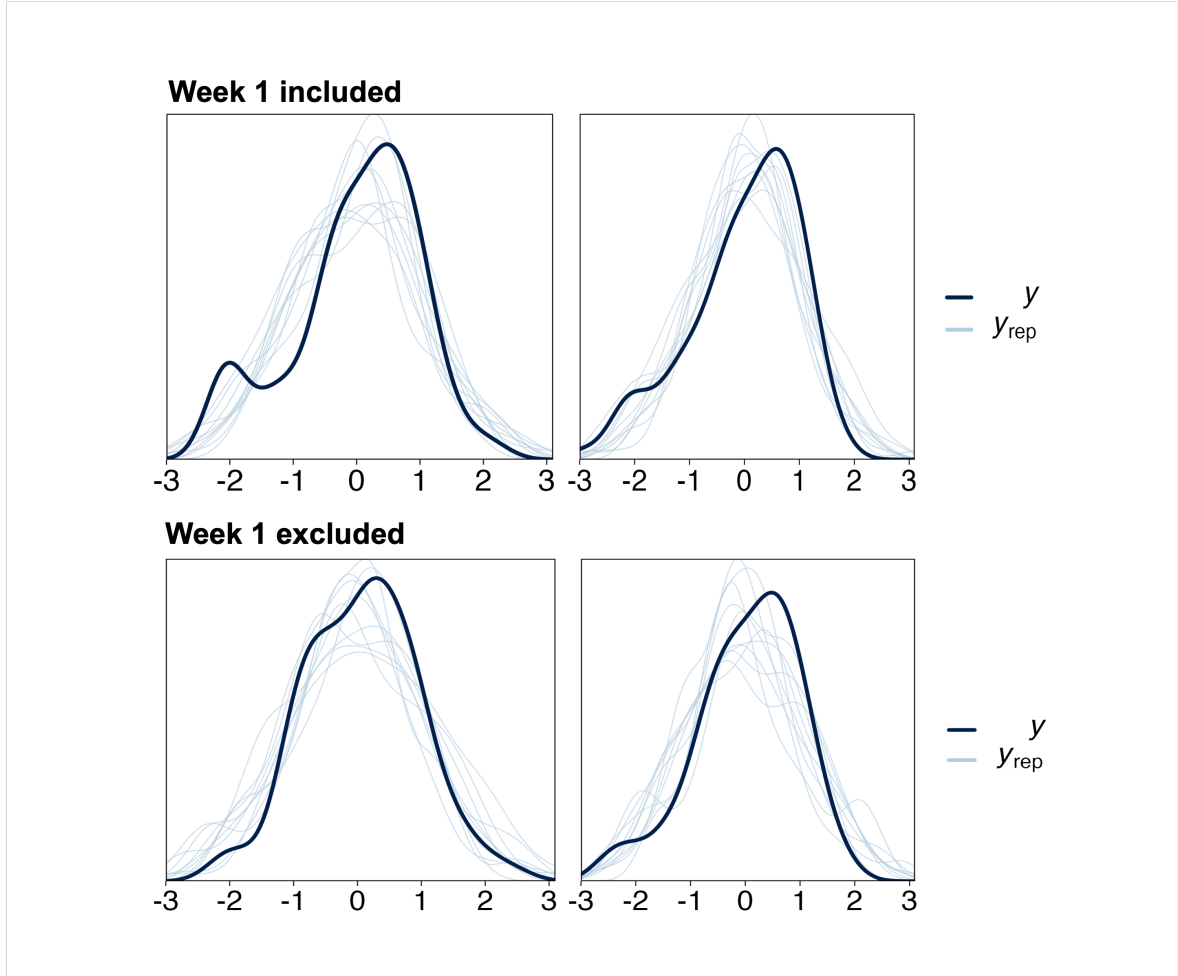
| Signal type  | Factor                                     | Estimate | SE    | 95% CI                 |
|--------------|--|----------|-------|------------------------|
| Stridulation | Mass                                       | 1.591    | 0.621 | [0.245, 2.886]         |
| Stridulation | Station (3)                                | 0.959    | 0.341 | <b>[0.286, 1.663]</b>  |
| Stridulation | Station (2)                                | 0.739    | 0.345 | <b>[-0.009, 1.442]</b> |
| Stridulation | Station (4)                                | 0.446    | 0.390 | [-0.363, 1.223]        |
| Stridulation | Treatment (Unmated)<br>x Week of recording | 0.337    | 0.111 | <b>[0.118, 0.560]</b>  |
| Stridulation | Week of recording                          | 0.052    | 0.089 | [-0.120, 0.226]        |
| Stridulation | Treatment (Unmated)                        | -0.269   | 0.258 | [-0.835, 0.292]        |
| Tremulation  | Mass                                       | 1.514    | 0.705 | <b>[0.217, 2.791]</b>  |
| Tremulation  | Station (3)                                | 1.140    | 0.361 | <b>[0.347, 1.975]</b>  |
| Tremulation  | Station (4)                                | 0.505    | 0.436 | [-0.379, 1.442]        |
| Tremulation  | Station (2)                                | 0.309    | 0.370 | [-0.465, 1.111]        |
| Tremulation  | Treatment (Unmated)<br>x Week of recording | 0.203    | 0.103 | [-0.006, 0.410]        |
| Tremulation  | Treatment (Unmated)                        | 0.112    | 0.295 | [-0.515, 0.744]        |
| Tremulation  | Week of recording                          | 0.047    | 0.083 | [-0.117, 0.208]        |
| Tremulation  | Moon illumination                          | -1.056   | 0.545 | [-2.152, 0.051]        |



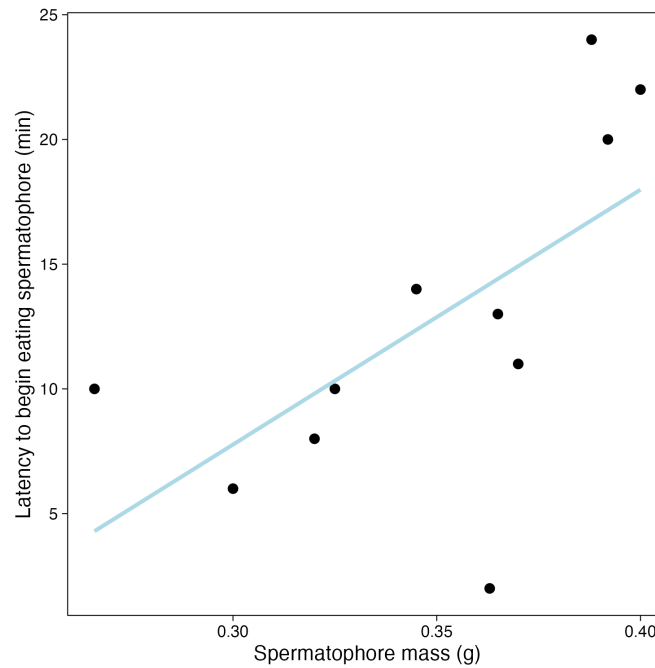
**Figure B.1: *D. gigliotosi* calling signals with ambient background noise.** Spectrograms and oscillograms of stridulation and tremulation signals from the 24-hour recording tracks, including the substrate-borne component of both call types. Top: airborne sound recorded by a Tascam microphone. Bottom: substrate-borne vibration recorded by a Knowles microaccelerometer. Tremulation events are denoted by 'tr'; stridulation events by 'st'. The general frequency ranges of other common sounds in the environment are labeled in light gray text.



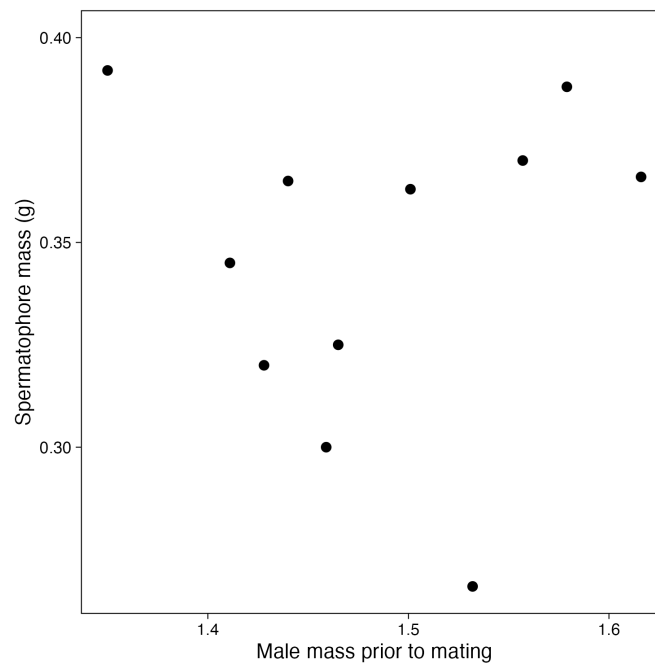
**Figure B.2: Machine learning call detection performance.** Black bars show the number of vibrational call detections that were true positive hits for each confidence level assigned by the ‘koogu’ model. Gray bars show false positives. For detections that were assigned a score of 0.99 or 1.0, the proportion of true positives over the proportion of true and false positives was 0.86. The model performed with 97% recall when the score threshold was set to 0.57 on a test subset of recordings.



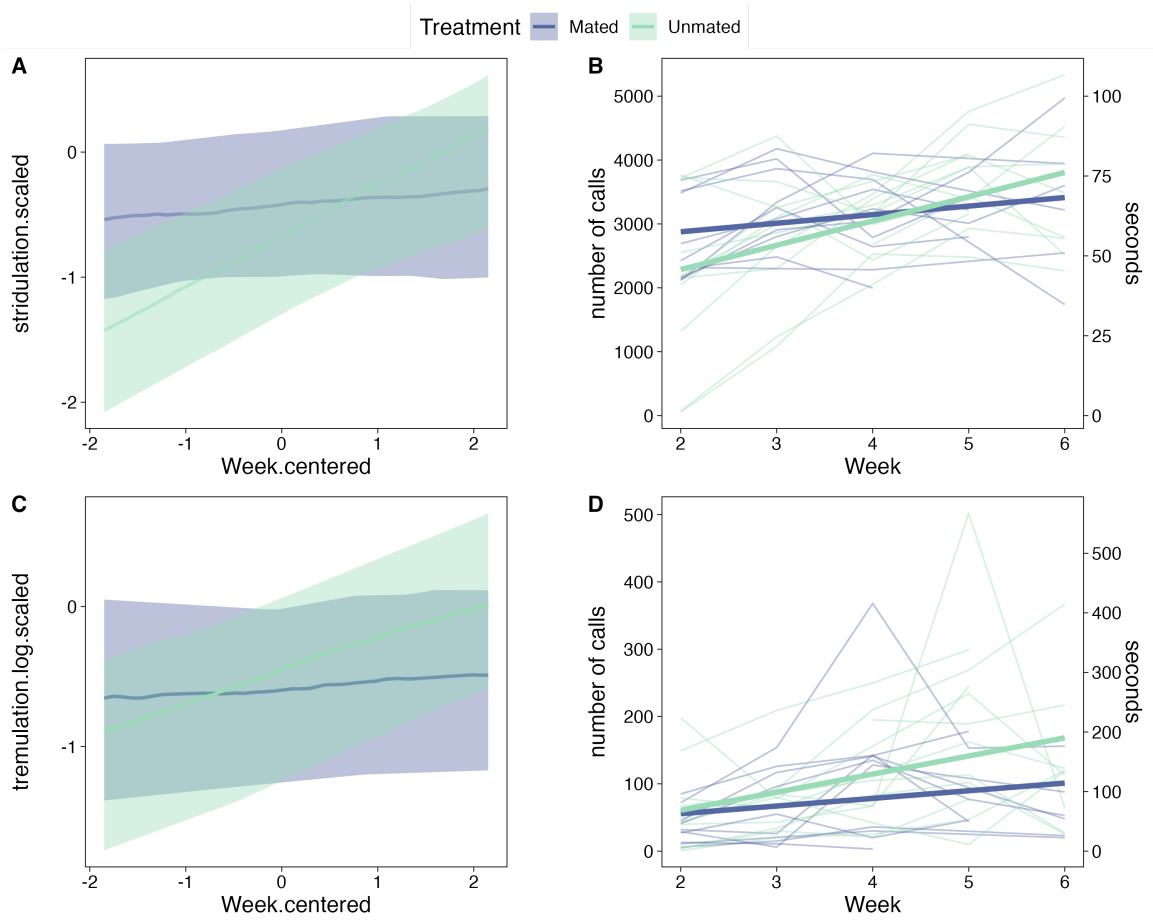
**Figure B.3: Posterior distribution prediction plots for brms models** Black lines show the observed distribution of stridulation (left) and tremulation (right) values (which have been scaled and centered). Gray lines show 10 random draws of model iteration fits.



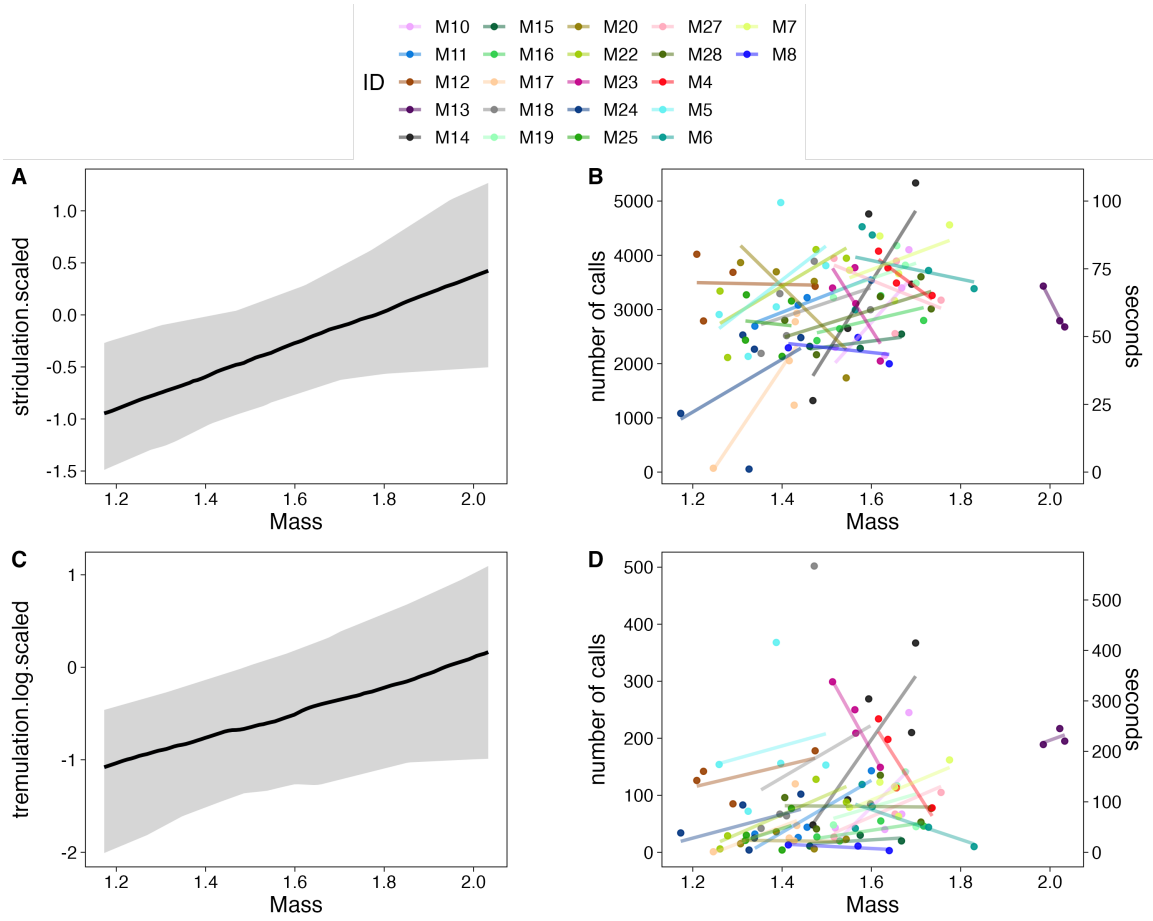
**Figure B.4: Spermatophore attachment duration and spermatophore mass.** There was a significant positive relationship between the mass of the spermatophore produced by a male and the amount of time post-copulation that a female waited to begin consuming it.



**Figure B.5: Spermatophore mass and male mass prior to mating.** There was no relationship between the mass of a male at the outset of a successful mating trial and the size of the spermatophore he produced.



**Figure B.6: Conditional effects of week and treatment on signaling.** Estimates of conditional effects of week and treatment from 'brms' models (left column: A,C) as well as the raw values (right column: B,D) for stridulation (top row: A,B) and tremulation (bottom row: C,D). On left, the median effect estimate is shown as a thick line with shaded 95% CIs. On right, thin lines show each individual's signaling trajectory across weeks, and thick lines show the mean amount of signaling for each treatment group across weeks.



**Figure B.7: Conditional effects of male mass on signaling.** Conditional effect estimates for week and treatment from 'brms' models (left column: A,C) as well as the raw values (right column: B,D) for stridulation (top row: A,B) and tremulation (bottom row: C,D). On left, median estimate and 95% CIs. On right, different colors represent each individual, with fitted color-matched lines showing signaling for each individual across weeks.

## C Supplementary material for Chapter 3

### *Effect of ticks on female reply threshold*

We tested the hypothesis that a greater number of ticks in a male call lowers the amplitude threshold at which a female will reply to the call, which would potentially allow males located farther away from females to elicit responses by adding ticks to their call. Using the same group of crickets that participated in experiment 1, we played calls with varying numbers of ticks (0, 5, 10, 15, 20) repeated five times, with each repetition increasing in amplitude (60–85 dB peSPL in 5 dB steps). We found the amplitude threshold of responses by noting the lowest-amplitude repetition in each treatment to which an individual responded, then tested for differences by comparing the mean response threshold of all the crickets for each treatment. As these data were not normally distributed, we used a Friedman rank test to examine potential differences in amplitude thresholds between acoustic treatments. To preserve equal blocking, 11 individuals who did not experience all five acoustic treatments were excluded from this analysis, resulting in a final sample size of 29 crickets. We found that thresholds were not significantly different between acoustic treatments ( $\chi^2_4 = 3.4764$ ,  $P = 0.4815$ ). Across all treatments, only one female responded at 60 dB peSPL, approximately half responded first to 65 dB peSPL, approximately half responded first to 70 dB peSPL, and a few in most treatments first responded to 75 dB peSPL (Figure C.1).

### *Age and mating effects on female responsiveness to male calls*

For the same group of 40 crickets that was used in experiment 1, we also investigated the effects of age and mating history on the likelihood and amplitude of the female reply. Following the first round of experiments, conducted when the crickets became responsive to calls 2–3 weeks after their adult moult, each cricket was assigned to either a mating treatment or an isolated treatment. Females were each placed in a separate small terrarium and those in the mating treatment were paired with a male cricket. After 3 weeks, we repeated the experiment 1 playback series with the females that remained responsive to male calls. Zero of 18 of the females in the mated treatment remained responsive to male calls at

older ages, with two females in the mated treatment dying before their responsiveness could be retested. Most (18 out of 20) females in the nonmated treatment remained responsive to playbacks of male calls after 2 weeks, with two becoming nonresponsive. As in initial experiment 1 playback experiments, females never responded to the ticks-only stimulus. At older ages, females were somewhat less likely to respond to all five repetitions of the full call and the trill-only stimulus than at younger ages (Figure C.2). A generalized linear model with a Poisson link function (R package 'lme4') (Bates et al., 2015) that included the number of replies out of five playbacks as the response, individual identity (ID) as a random effect, acoustic treatment and age (first or second test) as fixed effects, and an interaction term for age and acoustic treatment found no effect of age or acoustic treatment, nor any interaction between them (likelihood ratio test: age:  $\chi^2_1 = 1.65$ ,  $P = 0.199$ ; acoustic treatment:  $\chi^2_1 = 0.06$ ,  $P = 0.803$ ; age\*acoustic treatment:  $\chi^2_1 = 0.42$ ,  $P = 0.516$ ).

### ***Description of female vibrational replies***

Across experiments 1–4, we recorded female replies that fell into four categories: typical, premature, false-start and atypical. Most replies were ‘typical’ (Figure C.3) and occurred after the playback stimulus had ended. Some replies occurred before the playback had ended (Figure C.4) or contained multiple pulses, usually smaller than the final pulse (Figure C.5). In experiments 3 and 4, which contained stimuli that did not closely resemble natural male calls, we observed unusual responses, some of which did not contain recognizable replies at all (Figure C.6).

### ***Relationship between female reply amplitude and duration***

Initially, we measured both the duration and amplitude of the female vibrational reply for experiments 1–3. Duration and amplitude might affect signal propagation and receiver sensory systems in different ways. For example, higher-amplitude vibrational signals might travel further through the substrate, although propagation of vibrational signals through plant stems (the preferred substrate for *Lebinthus* crickets) is not straightforward. However, we found that for the female vibrational replies across all of our experiments, duration and

amplitude were highly correlated. Thus, we have included amplitude only in our statistical models, as we reason that longer durations are likely to be a by-product of higher-amplitude replies for this species, which has a simple, single pulse vibrational reply produced by a single jerk of the body. Female reply amplitude and reply duration were significantly correlated across experiments (Pearson’s product-moment correlation:  $r = 0.49$ ,  $t_{1195} = 19.6$ ,  $P < 0.001$ ). Amplitude and duration of replies were more strongly correlated for experiment 1 ( $r = 0.60$ ,  $t_{371} = 14.3$ ,  $P < 0.001$ ) and experiment 2 ( $r = 0.66$ ,  $t_{498} = 19.5$ ,  $P < 0.001$ ) than for experiment 3 ( $r = 0.45$ ,  $t_{322} = 9.1$ ,  $P < 0.001$ ). This difference could be due to differences in the playback stimuli of each experiment—unlike experiments 1 and 2, in which stimuli were all within a natural range for male calls, experiment 3 included stimuli with artificially high or low numbers of syllables. Experiments were conducted on different equipment with different gain settings, which might also explain differences in slope between the plots. We did not measure reply parameters besides responsiveness for experiment 4.

### ***Details of statistical tests for experiments 1–3***

Here, we report the statistical effects that stimulus repetition number had on female reply amplitude and latency. We also summarize the post hoc tests that were conducted to examine differences in reply amplitude, latency or responsiveness between pairs of acoustic treatments (Tables C.1, C.3, C.5, C.7, C.8, C.9) or between the repetition number of each stimulus within an acoustic treatment (Tables C.2, C.4, C.6). All stimuli were repeated five times within an acoustic treatment; see Methods in main text for details of timing. In general, the initial repetition of a stimulus elicited lower-amplitude replies than subsequent repetitions.

### ***Experiment 1: male call components required for female replies***

The amplitude of the replies was significantly and positively affected by the repetition number of the playback ( $\chi^2_4 = 185.66$ ,  $P < 0.001$ ; Table C.2). The latency of the replies was also significantly affected by the repetition number of the playback ( $\chi^2_4 = 129.79$ ,  $P < 0.001$ ; Table C.3).

*Experiment 2: effect of the number of ticks per call on female replies*

As in experiment 1, the repetition number of the playback significantly and positively affected the amplitude of the replies ( $\chi^2_4 = 66.60$ ,  $P < 0.001$ ). There were significant differences only between the first repetition of a playback and all subsequent repetitions (Table C.5). The repetition number of the playback stimulus also significantly affected the latency of the replies, with later repetitions eliciting shorter latencies ( $\chi^2_4 = 12.21$ ,  $P = 0.016$ ). Post hoc tests showed that the latency of the reply was significantly longer in the first than in the third and fifth repetitions (Table C.7).

*Experiment 3: effect of the number of trill syllables per call on female replies*

In this experiment, repetition number significantly affected the amplitude of female replies ( $\chi^2_4 = 10.82$ ,  $P = 0.029$ ). Tukey post hoc tests showed a difference only between the first and fifth repetition (t ratio = -2.939,  $P = 0.029$ , all other contrasts not significant; Table C.9). Latency of the replies was not affected by the repetition number of the playback ( $\chi^2_4 = 5.14$ ,  $P = 0.274$ ).

**Table C.1: Hypotheses, predictions, and results for experiments on relationships between male signal variability, male quality, female preference, and signal efficacy in the cricket *Lebinthus bitaeniatus*.**

| Hypothesis   |   | Prediction   | Supported by results?   |
|--|---|--|---|
| Highly variable male signal components correspond with male quality, whereas stereotyped signal components do not. |   | 1. The multicomponent signal has both highly variable and highly stereotyped components              | <b>Yes:</b> number of ticks in male calls is more variable than number of trill pulses (Table 3.1, Fig. 3.4A,B)   |
|  |   | 2. More variable signal component (ticks) correlates with male body size                             | <b>No:</b> larger males do not produce more ticks than smaller males (Fig. 3.2A).   |
|  |   | 3. Less variable component (trill pulses) does not correlate with male condition                     | <b>No:</b> larger males produce trills with more pulses than smaller males (Fig. 3.2B).   |
| Stereotyped signals are required for mate recognition  |   | 1. Trill component is necessary and sufficient to elicit female response                             | <b>Yes:</b> females only replied to calls with trills, and do not need ticks to reply.  |
| Highly variable male signal components correspond with open-ended female preferences                               |   | 1. Female responsiveness increases with number of ticks in male call                                 | <b>No:</b> no relationship between proportion of calls with female replies and number of ticks in male call (Fig. 3.3, 3.4G).                                 |
|  |   | 2. Female reply amplitude increases with number of ticks in male call                                | <b>No:</b> no relationship between amplitude of female replies and number of ticks in male call, only zero ticks different from calls with ticks (Fig. 3.4C). |
|  |   | 3. Female reply latency becomes more stereotyped with number of ticks in male call                   | <b>No:</b> no relationship between reply latency and number of ticks in male call (Fig. 3.4E).  |
| Stereotyped male signal components correspond with:  | H1: unimodal female preferences                                 | 1. Female responsiveness greatest for mean number of sound pulses in male trill                      | <b>No:</b> no relationship between proportion of calls with female replies and number of trill pulses in male call (Fig. 3.4H).                               |
|  |   | 2. Female reply amplitude greatest for mean number of sound pulses in male trill                     | <b>No:</b> female reply amplitude increases with number of trill pulses in male call (Fig. 3.4D).   |
|  |   | 3. Female reply latency most stereotyped for mean number of sound pulses in male trill               | <b>No:</b> variability of female reply latency increases with the number of trill pulses in male call (Fig. 3.4F).  |
|  | H2: opposing selection by female preference and signal efficacy | 1. Female responsiveness is greatest at trill lengths for which reply latency is least predictable.  | <b>No:</b> female responsiveness does not vary with trill length, but reply latency does (Fig. 3.4F,H).   |
|  |   | 2. Female reply amplitude is greatest at trill lengths for which reply latency is least predictable. | <b>Yes:</b> female reply amplitude increases with number of trill pulses but reply latency becomes more variable (Fig. 3.4D,F; Fig. 3.5).                     |
|  |   |  |   |

**Table C.2: Results of post hoc tests comparing amplitude of the female reply for different repetition numbers of playback stimuli in experiment 1** ‘Contrast’ refers to the specific repetition numbers being compared in each pair of contrasts. ‘Estimate’ shows the difference between treatment means. Results are averaged over the level of acoustic treatment. Note: contrasts are still on the  $\log_{10}$  scale. Degrees-of-freedom method: Kenward-Roger.  $P$  value adjustment: Tukey method for comparing a family of five estimates. Significant  $P$  values are shown in bold.

| Contrast | Estimate | SE   | t-ratio | $P$               |
|----------|----------|------|---------|-------------------|
| 1–2      | -0.21    | 0.02 | -8.734  | <b>&lt;0.0001</b> |
| 1–3      | -0.25    | 0.02 | -10.532 | <b>&lt;0.0001</b> |
| 1–4      | -0.33    | 0.02 | -13.526 | <b>&lt;0.0001</b> |
| 1–5      | -0.34    | 0.02 | -14.049 | <b>&lt;0.0001</b> |
| 2–3      | -0.04    | 0.02 | -1.809  | 0.370             |
| 2–4      | 0.11     | 0.02 | -4.941  | <b>&lt;0.0001</b> |
| 2–5      | -0.12    | 0.02 | -5.457  | <b>&lt;0.0001</b> |
| 3–4      | -0.07    | 0.02 | -3.182  | <b>&lt;0.0138</b> |
| 3–5      | -0.08    | 0.02 | -3.697  | <b>&lt;0.0024</b> |
| 4–5      | -0.01    | 0.02 | -0.507  | 0.987             |

**Table C.3: Results of post hoc tests comparing latency of the female reply for different repetition numbers of playback stimuli in experiment 1.** Details as given in Table C.2.

| Contrast | Estimate | SE   | t-ratio | $P$               |
|----------|----------|------|---------|-------------------|
| 1–2      | 11.68    | 1.79 | 6.540   | <b>&lt;0.0001</b> |
| 1–3      | 15.19    | 1.77 | 8.571   | <b>&lt;0.0001</b> |
| 1–4      | 18.58    | 1.77 | 10.487  | <b>&lt;0.0001</b> |
| 1–5      | 19.93    | 1.77 | 11.286  | <b>&lt;0.0001</b> |
| 2–3      | 3.50     | 1.69 | 2.068   | 0.237             |
| 2–4      | 6.90     | 1.69 | 4.072   | <b>&lt;0.0006</b> |
| 2–5      | 8.25     | 1.69 | 4.884   | <b>&lt;0.0001</b> |
| 3–4      | 3.39     | 1.67 | 2.036   | <b>&lt;0.251</b>  |
| 3–5      | 4.74     | 1.66 | 2.851   | <b>&lt;0.0371</b> |
| 4–5      | 1.35     | 1.66 | 0.810   | 0.928             |

**Table C.4: Results of post hoc tests comparing amplitude of female reply for different acoustic treatments in experiment 2.** ‘Contrast’ refers to the specific treatments (number of tick syllables) being compared in each pair of contrasts. ‘Estimate’ shows the difference between treatment means. Results are averaged over the level of repetition number. Note: contrasts are still on the  $\log_{10}$  scale. Degrees-of-freedom method: Kenward-Roger.  $P$  value adjustment: Tukey method for comparing a family of six estimates.

| Contrast | Estimate | SE   | t-ratio | $P$               |
|----------|----------|------|---------|-------------------|
| 0–10     | -0.13    | 0.02 | -7.16   | <b>&lt;0.0001</b> |
| 0–20     | -0.13    | 0.02 | -6.75   | <b>&lt;0.0001</b> |
| 0–30     | -0.11    | 0.02 | -5.92   | <b>&lt;0.0001</b> |
| 0–40     | -0.13    | 0.02 | -6.80   | <b>&lt;0.0001</b> |
| 0–50     | -0.15    | 0.02 | -8.04   | <b>&lt;0.0001</b> |
| 10–20    | 0.01     | 0.02 | 0.42    | 0.998             |
| 10–30    | 0.02     | 0.02 | 1.25    | 0.810             |
| 10–40    | 0.01     | 0.02 | 0.34    | 0.999             |
| 10–50    | -0.02    | 0.02 | -0.91   | 0.945             |
| 20–30    | 0.02     | 0.02 | 0.84    | 0.961             |
| 20–40    | -0.00    | 0.02 | -0.08   | 1.000             |
| 20–50    | -0.02    | 0.02 | -1.32   | 0.773             |
| 30–40    | -0.02    | 0.02 | -0.91   | 0.944             |
| 30–50    | -0.04    | 0.02 | -2.16   | 0.261             |
| 40–50    | -0.02    | 0.02 | -1.24   | 0.815             |

**Table C.5: Results of post hoc tests comparing amplitude of female reply for different repetition numbers of playback stimuli in experiment 2.** Details as given in Table C.2.

| Contrast | Estimate | SE   | t-ratio | $P$               |
|----------|----------|------|---------|-------------------|
| 1–2      | -0.09    | 0.02 | -5.08   | <b>&lt;0.0001</b> |
| 1–3      | -0.11    | 0.02 | -6.25   | <b>&lt;0.0001</b> |
| 1–4      | -0.12    | 0.02 | -7.23   | <b>&lt;0.0001</b> |
| 1–5      | -0.12    | 0.02 | -6.93   | <b>&lt;0.0001</b> |
| 2–3      | -0.02    | 0.02 | -1.17   | 0.767             |
| 2–4      | -0.04    | 0.02 | -2.13   | 0.210             |
| 2–5      | -0.03    | 0.02 | -1.87   | 0.337             |
| 3–4      | -0.02    | 0.02 | -0.95   | 0.877             |
| 3–5      | -0.01    | 0.02 | -0.70   | 0.957             |
| 4–5      | 0.00     | 0.02 | 0.25    | 0.999             |

**Table C.6: Results of post hoc tests comparing the latency of the female reply for different acoustic treatments in experiment 2.** Results are averaged over the level of acoustic treatment. All other details as given in Table C.4

| Contrast | Estimate | SE   | t-ratio | <i>P</i> |
|----------|----------|------|---------|----------|
| 0–10     | 2.79     | 3.66 | 0.76    | 0.974    |
| 0–20     | -0.27    | 3.66 | -0.08   | 1.000    |
| 0–30     | 3.62     | 3.66 | 0.99    | 0.921    |
| 0–40     | -3.34    | 3.67 | -0.91   | 0.944    |
| 0–50     | -6.71    | 3.67 | -1.83   | 0.450    |
| 10–20    | -3.06    | 3.64 | -0.84   | 0.960    |
| 10–30    | 0.83     | 3.64 | 0.23    | 0.999    |
| 10–40    | -6.13    | 3.65 | -1.68   | 0.547    |
| 10–50    | -9.50    | 3.65 | -2.60   | 0.099    |
| 20–30    | 3.90     | 3.64 | 1.07    | 0.893    |
| 20–40    | -3.07    | 3.65 | -0.84   | 0.960    |
| 20–50    | -6.43    | 3.65 | -1.76   | 0.491    |
| 30–40    | -6.96    | 3.65 | -1.91   | 0.399    |
| 30–50    | -10.33   | 3.65 | -2.83   | 0.055    |
| 40–50    | -3.37    | 3.66 | -0.92   | 0.941    |

**Table C.7: Results of post hoc tests comparing the latency of the female reply for different repetition numbers of playback stimuli in experiment 2.** Details as given in Table C.2.

| Contrast | Estimate | SE   | t-ratio | <i>P</i>     |
|----------|----------|------|---------|--------------|
| 1–2      | 4.58     | 3.34 | 1.37    | 0.647        |
| 1–3      | 9.89     | 3.34 | 2.96    | <b>0.027</b> |
| 1–4      | 4.42     | 3.33 | 1.33    | 0.673        |
| 1–5      | 9.58     | 3.35 | 2.86    | <b>0.036</b> |
| 2–3      | 5.31     | 3.33 | 1.59    | 0.503        |
| 2–4      | -0.16    | 3.32 | -0.05   | 1.000        |
| 2–5      | 5.00     | 3.34 | 1.50    | 0.567        |
| 3–4      | -5.47    | 3.32 | -1.65   | 0.468        |
| 3–5      | -0.31    | 3.34 | -0.09   | 1.000        |
| 4–5      | 5.16     | 3.33 | 1.55    | 0.530        |

**Table C.8: Results of post hoc tests comparing amplitude of female reply for different acoustic treatments in experiment 3.** ‘Contrast’ refers to the specific treatments (number of trill syllables) being compared in each pair of contrasts. ‘Estimate’ shows the difference between treatment means. Results are averaged over the level of repetition number. Degrees-of-freedom method: Kenward-Roger.  $P$  value adjustment: Tukey method for comparing a family of five estimates.

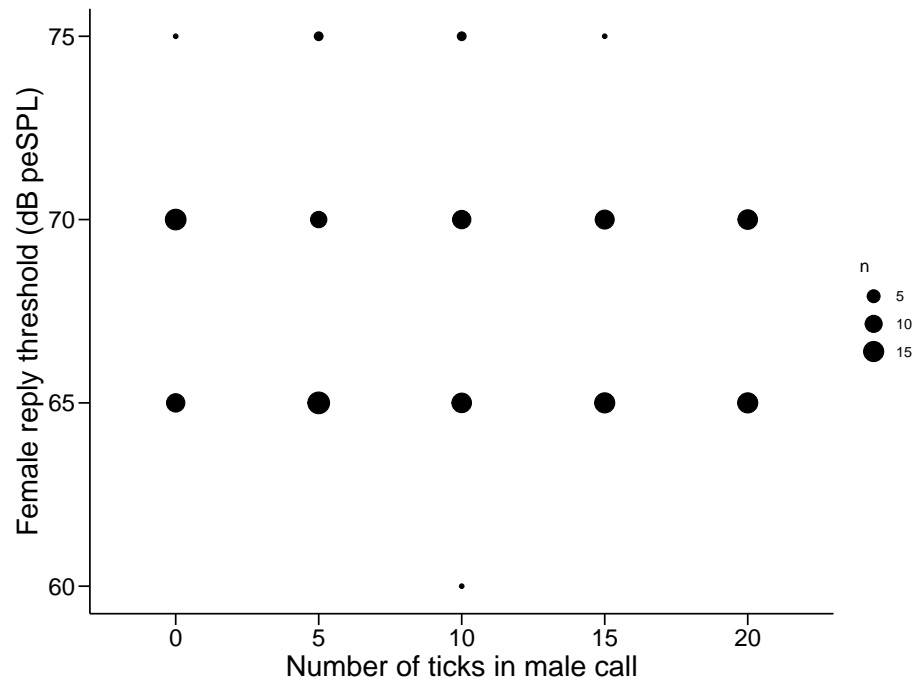
| Contrast | Estimate | SE    | t-ratio | $P$               |
|----------|----------|-------|---------|-------------------|
| 20–30    | -404.27  | 67.78 | -5.96   | <b>&lt;0.0001</b> |
| 20–40    | -600.92  | 68.00 | -8.84   | <b>&lt;0.0001</b> |
| 20–50    | -883.18  | 67.89 | -13.01  | <b>&lt;0.0001</b> |
| 20–60    | -974.98  | 66.71 | -14.62  | <b>&lt;0.0001</b> |
| 30–40    | -196.65  | 62.38 | -3.15   | <b>&lt;0.015</b>  |
| 30–50    | -478.91  | 62.28 | -7.69   | <b>&lt;0.0001</b> |
| 30–60    | -570.71  | 61.04 | -9.35   | <b>&lt;0.0001</b> |
| 40–50    | -282.26  | 60.77 | -4.65   | <b>&lt;0.0001</b> |
| 40–60    | -374.06  | 60.24 | -6.21   | <b>&lt;0.0001</b> |
| 50–60    | -91.80   | 60.22 | -1.52   | 0.547             |

**Table C.9: Results of post hoc tests comparing the amplitude of the female reply for different repetition numbers of playback stimuli in experiment 3.** Details as given in Table C.2.

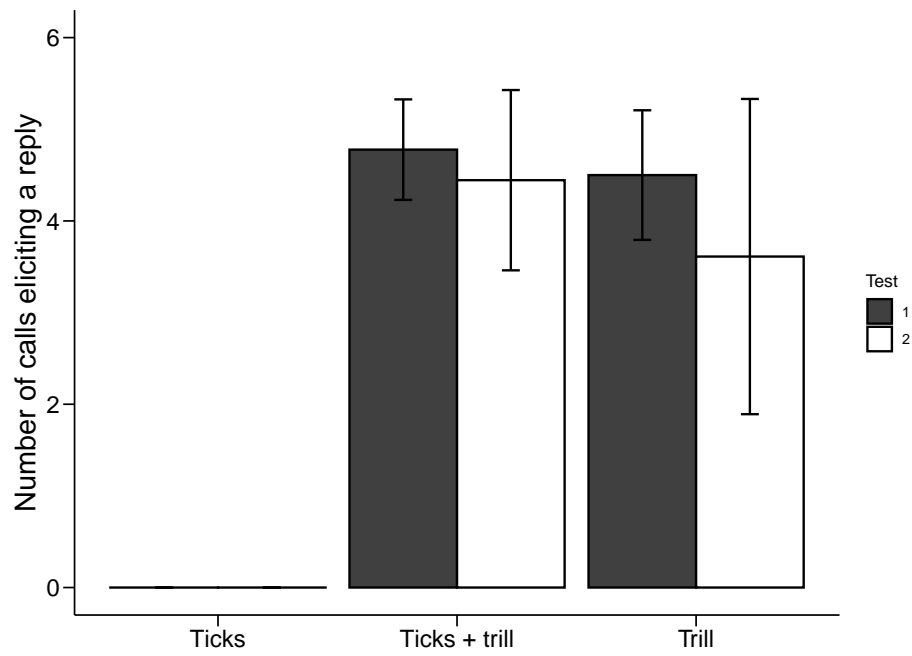
| Contrast | Estimate | SE    | t-ratio | $P$          |
|----------|----------|-------|---------|--------------|
| 1–2      | -79.00   | 62.63 | -1.26   | 0.715        |
| 1–3      | -116.76  | 62.59 | -1.87   | 0.338        |
| 1–4      | -160.42  | 62.88 | -2.55   | 0.082        |
| 1–5      | -183.41  | 62.39 | -2.94   | <b>0.029</b> |
| 2–3      | -37.76   | 62.35 | -0.61   | 0.974        |
| 2–4      | -81.42   | 62.60 | -1.30   | 0.691        |
| 2–5      | -104.41  | 62.12 | -1.68   | 0.447        |
| 3–4      | -43.66   | 62.62 | -0.70   | 0.957        |
| 3–5      | -66.65   | 62.08 | -1.07   | 0.820        |
| 4–5      | -22.99   | 62.41 | -0.37   | 0.996        |

**Table C.10: Results of post hoc tests comparing the latency of the female reply for different acoustic treatments in experiment 3.** Details as given in Table C.8

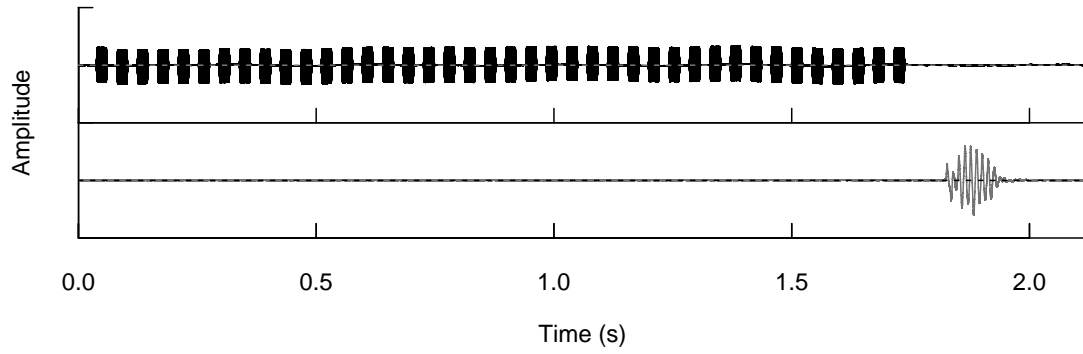
| Contrast | Estimate | SE   | t-ratio | <i>P</i>          |
|----------|----------|------|---------|-------------------|
| 20–30    | 10.98    | 7.10 | 1.548   | 0.532             |
| 20–40    | 15.25    | 7.09 | 2.151   | 0.201             |
| 20–50    | 27.13    | 7.08 | 3.833   | <b>0.001</b>      |
| 20–60    | 62.73    | 6.97 | 8.995   | <b>&lt;0.0001</b> |
| 30–40    | 4.26     | 6.51 | 0.655   | 0.966             |
| 30–50    | 16.15    | 6.50 | 2.483   | 0.097             |
| 30–60    | 51.75    | 6.39 | 8.094   | <b>&lt;0.0001</b> |
| 40–50    | 11.89    | 6.36 | 1.868   | 0.337             |
| 40–60    | 47.48    | 6.31 | 7.531   | <b>&lt;0.0001</b> |
| 50–60    | 35.60    | 6.30 | 5.647   | <b>&lt;0.0001</b> |



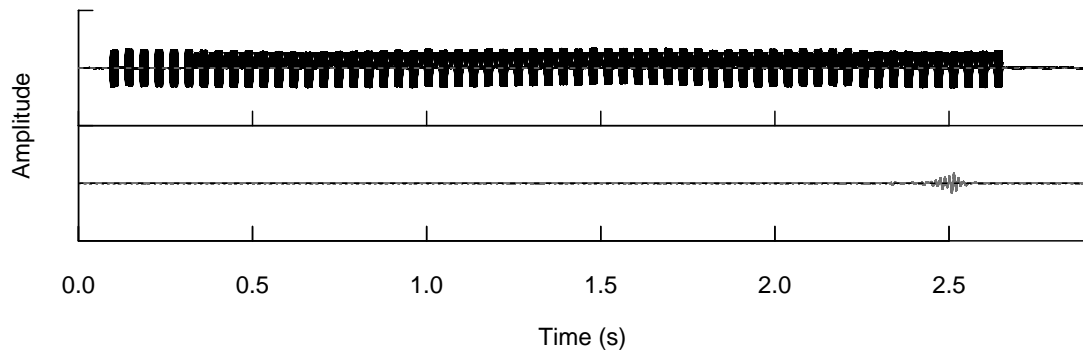
**Figure C.1: Relationship between the amplitude threshold at which a female cricket replies to a male call and the number of ticks in the call.** Black points showing the amplitude at which females first responded are scaled to the number of crickets at each amplitude.



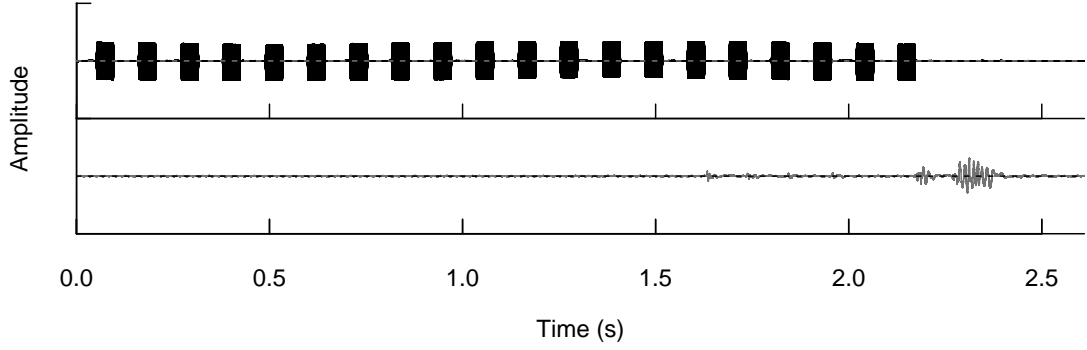
**Figure C.2: Mean  $\pm$  1 SD number of male calls eliciting female replies out of five repetitions to each individual.** Only crickets that were assigned to the unmated test and that remained responsive in the second round of playback tests are included here. Mean age of crickets: test 1 = 18.3 days after final moult; test 2 = 39.3 days after final moult.



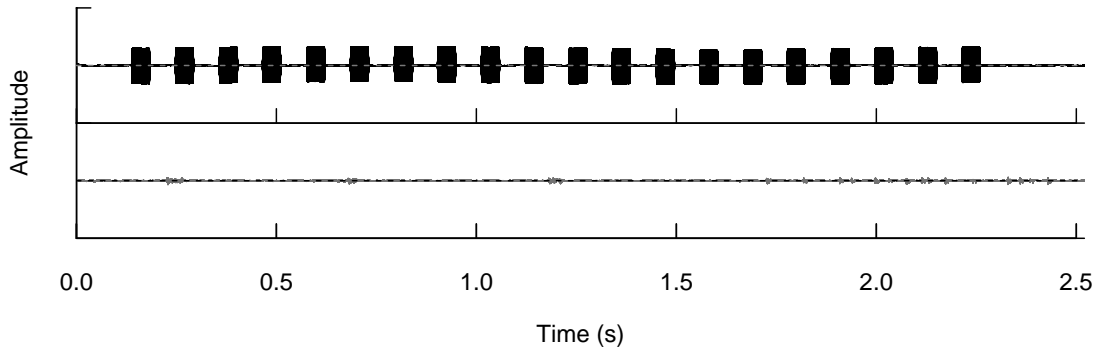
**Figure C.3: Oscillogram of a typical female vibrational reply, common across all experiments except experiments 3 and 4.** The end (trill) of the playback stimulus (here, control 10 ticks and 40-syllable trill call) is shown on the top trace, and the female reply is shown on the bottom trace.



**Figure C.4: Oscillogram of a premature female vibrational reply, occurring before the end of the playback stimulus.** The end (trill) of the playback stimulus (here, 10 ticks and 60-syllable trill call from experiment 3) is shown on the top trace, and the female reply is shown on the bottom trace.



**Figure C.5: Oscillogram of a false-start female vibrational reply, where one or several smaller pulses occurred before a ‘typical’ response.** This type of reply occurred throughout all playback experiments but was most common in experiments 3 and 4. For this type of call, parameter measurements (amplitude, duration, latency) were made on the largest pulse, which tended to occur last. The end (trill) of the playback stimulus (here, stimulus ‘e’ from experiment 4) is shown on the top trace, and the female reply is shown on the bottom trace.



**Figure C.6: Oscillogram of an atypical female vibrational reply, which was not scored as a response and was not measured.** This type of reply occurred mostly in experiment 4. The end (trill) of the playback stimulus (here, stimulus ‘e’ from experiment 4) is shown on the top trace, and the female reply is shown on the bottom trace. Atypical replies were generally of very low amplitude compared to typical, premature and false-start replies.

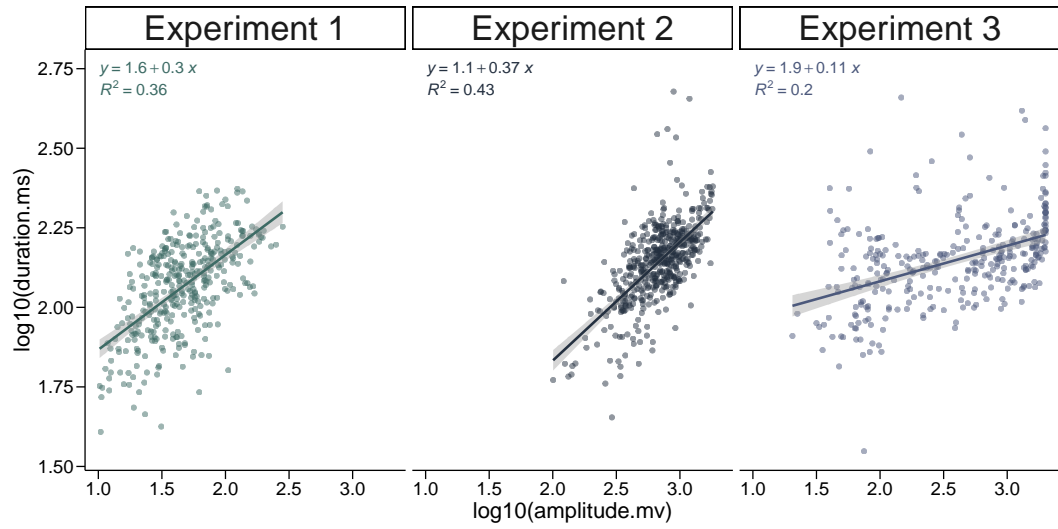


Figure C.7: Relationship between log-transformed amplitude and duration across the three experiments in which we measured reply parameters.

