SIGNALS, CUES, AND INFORMATION USE IN FORAGING DECISIONS OF HERBIVOROUS INSECTS AND A LEAF-FOLDING PLANT

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by

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The undersigned, appointed by the dean of the Graduate School,

have examined the dissertation entitled:

SIGNALS, CUES, AND INFORMATION USE IN FORAGING DECISIONS OF HERBIVOROUS INSECTS AND A LEAF-FOLDING PLANT

presented by Sabrina C.J. Michael, a candidate for the degree Doctor of Philosophy, and hereby certify that, in their opinion, it is worthy of acceptance.

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Professor Reginald Cocroft

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Professor Debbie Finke

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Professor Manuel Leal

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Professor Johannes Schul

DEDICATION

I dedicate this work to:

The curious little girl who once spent hours in the backyard, captivated by the world of ants, and whose relentless curiosity never ceased—you did it!

My dear parents, Gary Michael, Darla Williams, and Terri Michael who have provided the rock-solid foundation in life, enveloping me in their enduring love and support.

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Jesse Cocroft and Lydia Cocroft whose radiant presence has brought boundless light and joy into my life.

My friends who have shared in my triumphs and tribulations, your camaraderie has been a source of strength and laughter that I cherish deeply.

And to the world of Jiu-Jitsu, which has taught me the value of discipline, perseverance, and the indomitable human spirit.

“The important thing is not to stop questioning. Curiosity has its own reason for existing.” Albert Einstein

“Somewhere, something incredible is waiting to be known.” Carl Sagan

In loving memory of Michael Ray Jones and Bradley Lewis.

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ABSTRACT

Group living organisms rely heavily on cues and signals from other group members to make decisions. I studied how foraging decisions are influenced by communication in two species of plant-feeding insects that differ in ecology and life history. I also studied the cues produced when leaves of sensitive plants temporarily stop foraging for light. In treehoppers, I hypothesized that recruitment signals would guide the choice of feeding sites by group-living immatures. Both species engage in a food-for-protection mutualism with honeydew harvesting ants, and I found that ant bodyguards are more likely to be present when individuals are feeding in larger groups. I first characterized the context in which nymphs produced plant-borne vibrational signals, then experimentally tested the influence of those signals on the foraging decisions of other nymphs. I found that communication underlies cooperative foraging in both species but using different signals and signaling dynamics. In one species, searchers initiate back-and-forth exchanges with individuals already at a feeding site, while in the other, settled individuals produce series of collective signals that act as a beacon. In sensitive plants, I found that leaf-folding produces distinctive vibrational cues, which have the potential to influence the light-foraging decisions of neighboring leaves and the movement decisions of other organisms on the plant. The discovery of vibrational signals that underlie cooperative foraging in treehoppers, and of incidental vibrational cues that accompany leaf-folding in sensitive plants, sheds light on an overlooked aspect of information exchange among organisms.

CHAPTER 1

Bioacoustics signals, cues, and cooperation

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Overview

My research interest involves how individuals make decisions within social groups, and especially how personal information from environmental cues is integrated with information about the decisions of others. Social information acquisition can occur through the perception of incidental cues such as patterns of movement or feeding, as well as through communication signals.

I am also very interested in bioacoustics signals and cues, specifically those that fall beyond the range of human auditory perception. A substantial portion of acoustic signals and cues that are produced are predominantly by insects through vibrational mechanisms.

In my previous research I concentrated on movement decisions of schooling fish that were based on incidental cues produced by neighboring fish. My dissertation research focused on decisions based on both cues and social signals. My study organisms are insects and plants, two taxa where socially informed decisions are prevalent and well-documented (e.g., Karban 2015, Bradbury and Vehrencamp 2011).

There has been a significant amount of research on how social signals influence decision-making in insects, especially in the eusocial insects (e.g., von Frisch and Seeley 1993, Beckers 1990, Dussutour et al. 2009). I aim to contribute to our understanding of the influence of social signaling on decisions in non-eusocial insects. Currently, we are trying to understand how social signaling helped shape behavior such as maternal care, where nymphal signals and maternal responses have been conserved in closely related taxa (Figure 1.1). I am interested in how social signaling has also shaped other behaviors, such as foraging, and what the responses are in nymph-to-nymph communication.

Background

The study of collective animal behavior, the coordinated behavior of groups based on interactions between individuals, is important for understanding group-living species (Sumpter 2010). Group living is widespread in animals and is beneficial in many species because it can increase survivorship at the individual level (Hamilton 1971). There are major benefits of group-living such as avoiding predators, perhaps by encounter-risk dilution and/or being alerted when predators are near (e.g., Foster and Treherne 1981), or conserving energy, as when birds alternate flying in the back of a v-formation (Weimerskirch et al. 2001). There are also costs of group living, especially an increased exposure to infectious diseases (Loehle 1995, Altizer et al. 2003), as well as greater competition for resources such as food or mates (Krause and Ruxton 2002, Ward et al. 2006). Costs and benefits of grouping are species and context dependent, e.g. being in a group could decrease the likelihood of some individuals being targeted by a predator, while increasing the likelihood of other individuals in the same group being targeted

(Bednekoff and Lima 1998, Curley, Rowley, and Speed 2015), and is expected to be maintained if the benefits outweigh the costs (Alexander 1974). Lastly, one additional benefit of grouping is that it increases the availability of non-private information (see below), which may decrease uncertainty (Clark and Mangel 1984, Gil et al. 2017).

Individuals in groups can gain information about the decisions of other individuals by attending to signals and cues (Duboscq et al. 2016). The definition of information, cue, and signal is often debated; however I am using definitions commonly cited in the literature in my focus areas (e.g. social signaling and foraging decisions). Here, information is something that causes a "change in a receiver's estimated probabilities that a given condition is true" (Bradbury & Vehrencamp 2011). Individuals can gain information about the environment through sampling (i.e. private information) as well as through the social environment (i.e. non-private information), where individuals gain information about the decisions of neighbors through incidental cues arising from activity, or through perception of social signals, or communication (Dall and Johnstone 2002; Danchin et al 2004; Fig 1.1).

Communication involves a sender (the individual producing the signal), a message (stimuli or sources of information), and a receiver (the individual perceiving and responding to the stimulus) and if the message influences the receiver and the response is beneficial to both sender and receiver on average, it is referred to as a signal (Bradbury and Vehrencamp 2011, Schaefer and Ruxton 2011, Dall et al. 2005).

Signals can be defined as some aspect or characteristic of the phenotype which evolved because it elicits or influences the receiver response and on average benefits both signaler and receiver (Laidre and Johnstone 2013, Horisk and Cocroft 2013, Dall et al.

2005, Bradbury and Vehrencamp 2011). Signals differ from cues in that signals have been selected for because they influence the response of the receiver and benefit the sender as well as the receiver (Dall et al. 2005). Cues can be defined as actions, structures or traces left in the environment that are incidentally produced by an individual's activity, without having been selected for to provide information and without yielding an average equal benefit to both the producer and the receiver (Stegmann 2011). It is important to note also that the efficacy of the signal is dependent upon receiver perception, which is dependent upon habitat conditions (Endler 1992). Signals can be reliable or deceptive; they are reliable if a property of the signal is "consistently correlated with some attribute of the signaler or its environment" and the attribute benefits the receiver; they are deceptive if the signal characteristics are not consistently correlated with the attributes (Searcy and Nowicki 2005).

Social signals are important in many contexts including mating, avoiding and defending against predators, and foraging. Social signaling may be an especially important component in group foraging. Group foraging, i.e., searching and obtaining food with other individuals in a group, is a widespread behavior in animals (Giraldeau and Caraco 2000). When individuals are foraging, either solitarily or in a group, they must decide where to forage and for how long, i.e. when to stay and when to leave (Stephens and Krebs 1986, Stephens 2008, Giraldeau and Dubois 2008). If an individual is foraging alone, the information it has about the environment to make foraging decisions is based on personal information from direct perception of the environment, whereas if an individual is foraging in a group, the individual is able to access and use

information from others through local social interactions (Danchin et al. 2004, Katsikopoulos and King 2010).

Cooperative foraging strategies, i.e. the active recruitment of others to a feeding site, is most beneficial to individuals when the food source is sparse, difficult to locate, rapidly decreasing in nutritional value, and/or ephemeral (Torney, Berdhal, and Couzin, 2011, Couzin et al. 2011, Sherman and Visscher 2002, Dornhaus and Chittka 2004, Egert-Berg et al. 2018). Cooperative foraging also allows for individuals to quickly exploit resources as less time is spent foraging than when alone (Visscher and Seeley 1982). A model created by Senior et al. (2016) supports the hypothesis that collective foraging is most beneficial in a more complex or patchy foraging environment, if the nutritional needs across individuals within a group are evenly distributed.

Animals use different types of signals to actively recruit conspecifics to a feeding site. Perhaps the best-understood recruitment signaling behavior is the dance language of honeybees (Seeley 2010). Some animals, such as ants (Holldobler and Wilson 1990, Beckers et al. 1990, Dussutour et al. 2009) and tent caterpillars (Peterson and Fitzgerald 1991) use chemical signals to recruit conspecifics. An understudied type of recruitment signaling in non-eusocial insects is the use of vibrational recruitment signals, which is predicted to be widespread in insects (Cocroft and Hamel 2010). Hograefe (1984) found that sawfly-larvae signals also function to indicate the quality of feeding sites by changing their signaling rate (increasing with higher quality sites and decreasing with lower quality sites) based on the condition of the leaves, i.e., damaged vs. undamaged. The immature stage of a membracid treehopper, *Calloconophora pinguis*, is the only treehopper species documented to actively recruit conspecifics to a feeding-site (Cocroft

2005), although recruitment is likely to be present in other treehopper species (Cocroft and Hamel 2005, Cocroft 2005).

Treehoppers (family: Membracidae, ~3,270 species) are sap-feeding insects related to cicadas. The plant is an important component of treehopper communication, as treehoppers live and feed on plants, as well as use plants to communicate, i.e., produce and perceive substrate-borne vibrations that travel as bending waves through the plant (Cocroft and Rodríguez 2005). Treehoppers use social signaling to influence decision-making in primarily three contexts: mating, foraging, and warning against predators (Cocroft 2005, Cocroft 1996, Cocroft 1999, Cocroft and Hamel 2005). These communication systems allow for individuals and groups to solve problems such as finding mates, avoiding predators, and locating resources (e.g., feeding sites) and obtaining benefits of being in a group by aggregating (Cocroft 2005). Some treehoppers have been documented to display maternal care (e.g., egg and nymph guarding) (Wood 1977, Cocroft 1999).

In the species in the tribe Hoplophorionini, once the eggs hatch, the female modifies the stem by using her ovipositor to create feeding slits (Cocroft 1999); each slit generates a pocket of callose tissue on which nymphs feed (Shugart 2005). The female continues to produce these slits during the nymphs' one-month development to adulthood, on the same stems where the eggs hatched. These nymphal groups are thus stationary foragers, remaining on one plant stem throughout their development to adulthood. Similar maternal modification of the stem occurs in other hoplophorionine species. In stationary-foraging nymphal aggregations, the main signaling context is during predator encounters, and it is unlikely that cooperative foraging exists among

nymphs in the hoplophorionines. Instead, most communication occurs during predator encounters, during which nymphs produce synchronous signals that alert the defending mother to the predator's presence and location (Cocroft 1999; Ramaswamy & Cocroft 2009). Although egg guarding is also observed in the Aconophorini, the females do not create feeding slits for nymphs and because of feeding locality preference (i.e., on the base of new leaves), the nymphs will likely need to periodically find new feeding sites (Cocroft 2005).

In most treehopper species, maternal care is either limited to egg-guarding, reduced nymph guarding (guarding only during the first instar) or is absent completely (Lin, Danforth, and Wood 2004). Plant quality declines or changes over time, causing individuals to move around the plant in search of a new feeding-site, either as individuals or groups. It is thus likely to observe group foraging in species where nymphs are found in aggregations, maternal care is absent, and in species where nymphs are likely to move around more frequently.

Nymphs of the treehopper *Calloconophora pinguis* develop to adulthood in sibling groups containing up to 50 individuals, with the mother remaining with nymphs for at least a few days after hatching. Groups feed on sap at the base of rapidly growing new leaves, and because leaves mature rapidly, groups must move periodically during the nymphal stage (Cocroft 2005). Once the food quality begins to decline, individuals of a group of *C. pinguis* nymphs will begin moving their feet while remaining stationary and some will walk into or over other individuals and this behavior spreads to other individuals (Cocroft 2005), this is hypothesized to be individuals making a consensus decision (Conradt and Roper 2005) about whether the feeding site remains acceptable.

Then, one or more individuals will leave the declining feeding site. Once that individual has found a new high-quality feeding site, the nymph will produce a 'broadcast' or advertisement signal to recruit nymphs to that location. Members from the group at the declining site will begin to move towards the advertisement signal and begin forming a new group. Once individuals join a group, they too begin producing the advertisement signal in synchrony. Playbacks of the synchronous signaling to groups of *C. pinguis* nymphs caused the nymphs to move towards and join the location of the playback stimulus, regardless of feeding site quality.

Dissertation Outline

The main objectives of this dissertation are to 1) test the hypothesis that cooperative foraging occurs in two species of treehoppers that differ in host use and life history; and 2) describe a previously overlooked vibrational cue generated by rapid plant movements.

In Chapters 2 and 3, I will focus on how social signals influence foraging decisions and grouping behavior. In Chapter 2 I focus on how the feeding-site choice of nymphs in a group-living treehopper (*Tylopelta gibbera)* is influenced by signals produced by both searching and settled individuals. *Tylopelta gibbera* specializes on herbaceous hostplants (*Desmodium* spp.) and has two generations during the summer. Nymphs of *T. gibbera* form aggregations of 2-10 individuals, often with several groups occurring within the same host plant. I assess the likelihood that ant mutualism favors grouping behavior and test the responses of nymphs to signals produced by both searching and settled individuals. I will note that the ecological setting in which *T.*

gibbera nymphs make foraging decisions includes other potential receivers, and future research will investigate the influence of nymphal signals on their ant mutualists, which harvest honeydew and provide protection against predators, and on predators, many of which can detect plant-borne vibrational signals.

In Chapter 3 I focus on signaling by nymphs in a species in the *Enchenopa binotata* species complex of treehoppers, *E. binotata* 'Juglans nigra.' This species has a single generation each year and its host plant is a large woody plant, the black walnut tree (*Juglans nigra*). I again characterize the relationship between the presence of ants and the number of individuals in a group and test the responses of nymphs to signals produced by settled groups.

In Chapter 4, I document vibration production in the sensitive plant, *Mimosa pudica*, and identify the source of the vibrations produced during leaf-closing movements.

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Figures

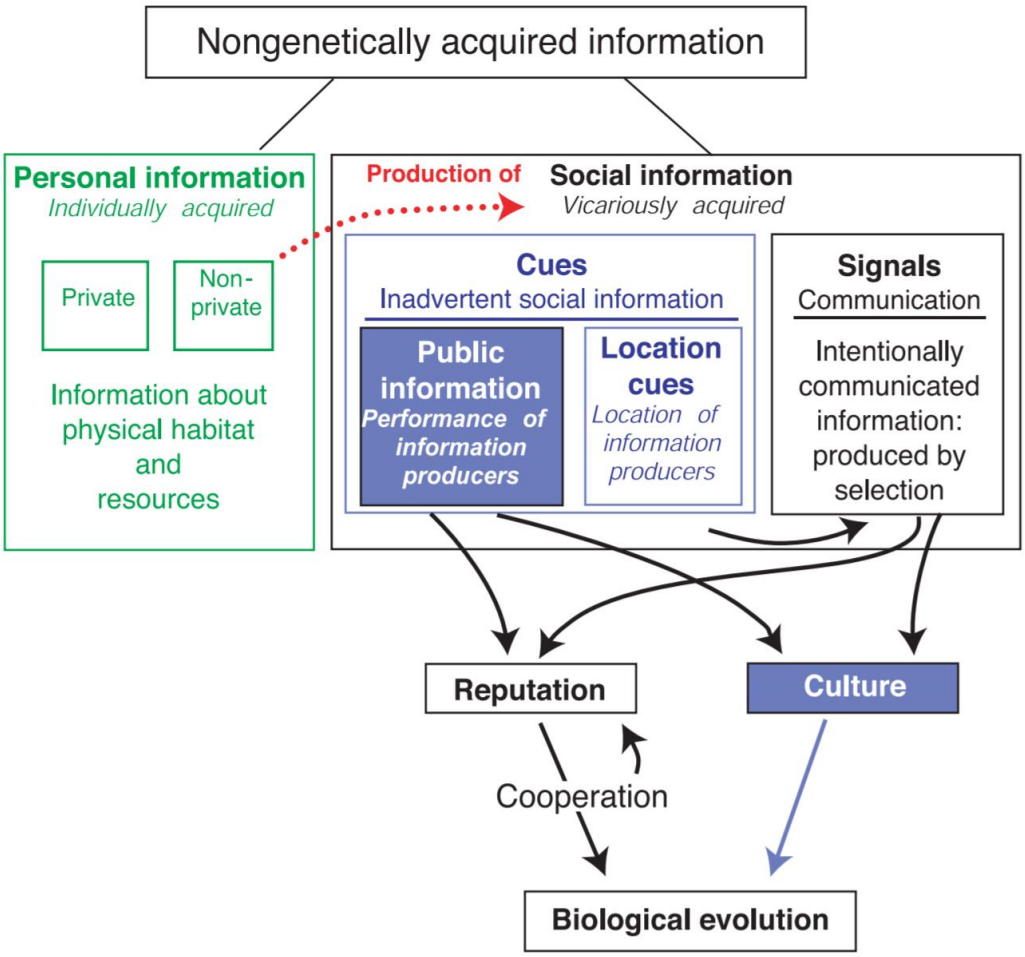

Figure 1.1: This figure from Danchin et al. (2004) illustrates the relationship between personal and social information, and between signals and cues.

CHAPTER 2

Dynamics of communication during cooperative foraging in a group-living treehopper

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Abstract

Communication signals that recruit other individuals to a feeding site occur in a wide range of species and are typically given in response to the discovery of the food resource. In contrast, signals that recruit separated individuals to a group are produced in response to signals from the isolated individual. I studied the communication underlying recruitment to a feeding site in the immatures of a sap-feeding insect, the treehopper *Tylopelta gibbera* (Hemiptera: Membracidae). Because groups in this species are stationary for days or weeks, I hypothesized that the benefits of continuous signaling by the group will be low and that, if there are benefits to being in a larger group, recruitment to the group will be initiated in response to cues from searching individuals, as with isolation signals. I first tested the hypothesis that nymphs benefit from being in a larger group by observing group sin the field, finding that larger groups are more likely to be attended by ants, which engage in a food-for-protection mutualism with *T. gibbera* nymphs. I then documented the production of two distinct signals by *T. gibbera* nymphs, a continuous signal (the purr) produced by moving individuals and a short signal (the

tick) produced by sedentary individuals. Recordings and playbacks experiments revealed that groups of nymphs at a feeding site produce tick signals at a low rate and increase their signaling rate six-fold in response to signals from searching individuals, with a peak of signaling immediately after the signal from the searcher. Furthermore, searching individuals oriented to and settled within a few cm of actuators playing back tick signals. Previous studies show that the presence of ant mutualists reduced predation on treehopper nymphs, so the finding that larger groups of *T. gibbera* nymphs are more likely to be ant attended suggests that natural selection will favor attraction of new individuals to a group. Cooperative foraging in *T. gibbera* shares some features of recruitment in other animal species, in that signals are produced spontaneously by individuals at a feeding site. However, recruitment also shares features of location-providing signals in other species, because individuals in a group produce spontaneous signals a very low rate, sharply increasing their signaling rate in response to signals from searchers, providing a beacon that attracts searchers to the signal source.

Introduction

Cooperative foraging, in the form of active recruitment of other individuals to a feeding site, occurs in many species (Bradbury and Vehrencamp 2011). Signaling to advertise a feeding site is hypothesized to be most beneficial to receivers when food resources are sparse, costly to locate, rapidly decreasing in nutritional value, and/or ephemeral (Tourney et al. 2011; Sherman and Visscher 2002; Dornhaus and Chittka 2004; Egert-Berg et al. 2018). Advertising a food resource benefits the signaler when the advantages of being in a larger group outweigh the costs of signaling, such as when

multiple individuals are more effective at exploiting the resource than a single individual, when there are other benefits of being in a group, when the receivers are closely related to the signaler, and when there is reciprocity (Tourney et al. 2011).

Food-associated signals are prevalent in the highly social insects (Seeley 1996; Holldobler and Wilson 2009), and are common in birds, primates and marine mammals (Clay et al 2012). Signals that recruit others to a feeding source are typically initiated in response to discovery of the resource, rather than by signals from searching individuals (e.g., Heinrich and Marzluff 1991; Seeley 1996) although signal production can be influenced by the presence of other individuals (Evans and Marler 1994). In contrast, location-providing signals are typically produced by individuals in groups in response to signals from an individual that is isolated from the group (e.g., Fletcher 2008, Mumm et al 2014). In cases where groups are largely sedentary and remain at the same food resource for long periods, as occurs in some phytophagous insects (Dixon 1998; Wood 1993), food recruitment signals might be expected to combine features of resource-discovery-triggered food signaling behavior and on-demand signaling like the response to separation calls. For example, if the arrival of searching individuals is infrequent, there may be little benefit to the continuous production of food-associated signals. However, if there are advantages to being in a larger group, it may benefit individuals at the feeding site to recruit searching individuals.

Membracid treehoppers are sap-feeding insects with a range of social behavior, including species that live in groups (Wood 1993). Many membracids form mutualisms with honeydew-harvesting ants and other Hymenoptera, especially during the immature stage (Wood 1993). Removal experiments demonstrate that ants increase treehopper

survival, primarily by defending the treehoppers from predators (Wood 1977; McEvoy 1979; Fritz 1982, Cushman and Whitham 1989; Morales 2000b; Billick et al 2001) but also increase survivorship even in the absence of predators, through mechanisms that are not well understood (Morales 2000a). Ant mutualism, in turn, selects for grouping behavior in treehoppers, because larger groups are more likely to be ant attended (McEvoy 1979; Wood 1982). Ant-attended groups can persist for weeks in the same location on a plant (e.g., Cocroft 2003).

Food recruitment signaling occurs in at least one membracid species (*Calloconophora pinguis*), in which immatures develop to adulthood in groups of siblings. In this species, signaling advertises the discovery of apical meristem tissue, a relatively scarce, ephemeral resource on the rapidly growing tropical plants on which *C. pinguis* occurs (Cocroft 2005; Coley and Barone 1998). Signaling occurs in the contexts where a current feeding site is declining in quality, and individuals that leave the old site and discover a feeding site begin signaling spontaneously, in the absence of signals or cues from other individuals (Cocroft 2005). Signaling ceases after all of the individuals in the group arrive at the new site, and groups at stable feeding sites were not observed to produce recruitment signals. Movements of these sibling groups are thus relatively synchronized, occurring over a period of a few hours.

In contrast to *C. pinguis*, many other membracid treehoppers develop in groups containing both related and unrelated individuals, and even multiple species (Wood 1993). Individuals in at least some such species have been observed to move individually between groups rather than *en masse* (RB Cocroft, pers. comm.). Given a different pattern of movement between feeding sites –asynchronous vs synchronous – we can

expect a different form of recruitment. Here I test the hypothesis that recruitment to a feeding site occurs in the treehopper *Tylopelta gibbera*. Immatures (nymphs) of this species receive no post-ovipositional maternal care and develop to adulthood in ant-attended groups. Based on several years of observation of *T. gibbera* in a greenhouse colony (Cocroft, pers. comm.), nymphs are sedentary, slow-moving and seldom change location. However, nymphs of *T. gibbera* produce a repertoire of vibrational signals (Cocroft, Michael and Lin, in prep.). I first characterized the natural history of grouping and ant attendance in *T. gibbera* and tested the hypothesis that ant attendance favors larger groups. I then used experimental manipulations to identify the contexts of signal production, and playback experiments to quantify the response of receivers to those signals. Likely receivers of signals produced by nymphs of *T. gibbera* include predators, mutualists and other nymphs, but here I focus only on other nymphs as receivers.

Materials, Methods, and Results

Methods I

Natural history: Does ant mutualism favor larger nymphal group sizes?

Tylopelta gibbera is a sap-feeding insect that specializes on host plants in the genus *Desmodium* (Fabaceae; Kopp and Yonke 1973, as *T. americana*). There are two annual generations in Missouri; one generation overwinters as adults that mate and oviposit in the spring, producing nymphs that develop during the early summer. The early summer nymphs mature in mid-summer, mate and produce offspring that mature in late summer and overwinter as unmated adults. I observed *T. gibbera* nymphs from the early

summer generation at the Columbia Cosmopolitan Recreation Area from June – August 2021. I found nymphs on *Desmodium canadense* (Fabaceae) in old fields and woodland edges.

To enable longitudinal tracking of nymphal aggregations I selected and marked 10 mature *D. canadense* plants that contained nymphs. I censused the nymphs occurring on those plants, along with their associated ants, on 1 June 2021 and thereafter at intervals of 3-11 days until the start of the late-summer generation. I recorded the location of groups (operationally defined as individuals occurring within 5 cm of each other), the stage of each nymph in the group, and the number and species identity of tending ants within the group.

After egg hatch, nymphs develop through five stadia until they eclose as adults. To assess whether group sizes changed during nymphal development, I used a mixed-model regression analysis of the effects of nymphal stage on group size. Because nymphs often occurred in groups with individuals of different instars, I calculated the average group size experienced by nymphs of a given instar. For example, if a group contained 8 first-instar nymphs and two second-instar nymphs, each of the individuals experienced a group of 10 individuals. I treated instar as an ordered categorial variable. Group size was not normally distributed, but the log of group size was normally distributed, as determined using quantile plots and an Anderson-Darling test (Anderson & Darling 1954). I used the *stats* library in R and included plant identity as a random effect to account for repeated observations of individuals on the same plants (model: glm(log(Group size) ~ nymphal_instar + (plantID), family = gaussian)).

To assess the effect of nymphal instar and group size on the probability that a group would be attended by ants, I used a mixed-model logistic regression with the occurrence of at least one ant (1/0) as the dependent variable and group size and nymphal instar as predictors, along with plant identity as a random effect (model: glm(ants present / absent ~ group size * average stage + (plantID), family = binomial (link=logit)). Because groups often contained individuals of more than one nymphal stage, I calculated an average nymphal stage value for each observation of a group using a weighted sum of the stages of the nymphs in the group. For example, a group containing three first instars and one second instar would have an average stage of (3x1 + 1x2)/4 = 1.25. I conducted data exploration and initial tests in Matlab 2022a and statistical analyses using the R software package v. 4.3.1 (R Core Team 2023).

In addition to tracking nymphal groups in the field, I collected individuals from non-tracked plants to conduct preliminary recordings of the vibrational signals produced by nymphs in the laboratory on cut stems of *D. canadense* using a Polytec PDV-100 laser vibrometer (Polytec, Inc, MA).

Results I

Natural history: Does ant mutualism favor larger nymphal group sizes?

On the initial census date there were 167 nymphs (100 first instars, 52 second instars, 8 third instars) on the set of 10 identified plants. The number of nymphs on the plants decreased through mortality and adult eclosion, until a census on August 2, 2021, at which 141 new first instars of the second summer generation were also present. I assumed that no nymphs switched plants, as the intervening grassland matrix contains

predators but no food or protection by ants. I observed only one ant species tending *T. gibbera* on the marked plants, tentatively identified as the Little Black Ant, *Monomorium minimum* (ID to be confirmed by Dr. James Trager, Shaw Nature Reserve using specimens collected from neighboring, non-tracked plants and preserved in 95% ETOH). Ant tending is easily identified in the field because tending ants frequently contact the nymph with their antennae in the vicinity of the anal tube to elicit the release of honeydew, and ants regularly walk over and around the nymphs (**Fig. 2.1**).

Nymphal stage was a significant predictor of group size, with individuals occurring in smaller groups as they matured (**Fig. 2.2, Table 2.1**). Both the number of individuals in a group and their average stage were significant predictors of the presence of at least one ant, with a significant interaction effect (**Table 2.1**): larger groups were more likely to be ant-attended regardless of nymphal stage, but older nymphs were more likely to be ant attended at smaller group sizes than younger nymphs (**Fig. 2.3**).

I recorded two types of signals from nymphs on cut stems in the laboratory (**Fig. 2.4**). I will refer to the signals using the descriptive terms 'purr' (a pulsed signal of variable length, from less than 2 sec to >60 sec) and 'tick' (a 20-50 msec burst of vibration). Purr signals were primarily produced by nymphs while walking. Indeed, the correlation between walking and purring was obvious even upon casual observation: nymphs typically began producing a purr signal as soon as they began walking and continued signaling continuously until they stopped walking. Stationary nymphs occasionally produced purr signals, and regularly produced tick signals.

Methods II

Playback experiment 1: Do signals from moving nymphs elicit replies from stationary nymphs?

In the context of social interactions among nymphs and the potential for cooperative foraging, I assumed that a walking nymph was searching for a new feeding site. I hypothesized that the purring signal produced during walking would elicit a response from nymphs already in an established group. I tested this hypothesis using vibrational playback experiments. To generate a library of playback signals, I first recorded purrs from a sample of nymphs by placing an individual nymph on a cut host plant stem and recording its signals using a laser vibrometer. To obtain individuals for recording I collected 90 *T. gibbera* nymphs (2^{nd}-4^{th} instar) from approximately 30 *D. canescens* host plants in July 2018 from Hart Creek, in Hartsburg, MO. To maintain field-collected nymphs I transferred them to clippings from unoccupied *D. canescens* stems placed in a water tube inside a Ziploc bag. I selected purr exemplars from recordings of over 100 *T. gibbera* nymphs, choosing a subset with the best signal-to-noise ratio.

For the playbacks I collected host plant stems that already contained one group of three nymphs between 3^{rd} and 5^{th} instar and used those stems as playback substrates without moving the nymphs. Nymphs in the field were often ant-attended, but ants abandoned the nymphs after the stem was collected, and there were no ants present on the playback stems.

I used methods described in Michael et al. (2019) to reproduce the signals, to ensure that the played-back signal had the frequency characteristics of the original recording and a realistic amplitude. In brief, this method uses a custom-written Matlab

script to correct for the frequency filter imposed by the playback equipment and host plant, then adjust the amplitude to the desired level. I generated playback tracks using Audacity software (company info) on a Lenovo laptop computer. I connected the laptop via a Tascam Celesonic 20x20 Audio Interface (Teac Corporation, CA, USA) to a Behringer PowerPlay HA8000 Headphone amplifier (MusicTribe, Manila, Philippines) which drove a linear resonant actuator ('LRA'; 10mm diameter, Samsung Inc.; see Nieri et al [2022] for a discussion of this and other playback methods) attached with wax to the plant stem at the desired playback location. I conducted the playbacks on a Newport (Fountain Valley, CA, USA) tabletop vibration isolation table in a temperature-controlled room at the University of Missouri (24.0 ± 0.7 °C air temperature).

The two playback treatments were purr signals and silence. For the purr treatment, I constructed playback tracks using filtered, amplitude-adjusted purrs separated by 30s of silence, repeated for 10 minutes. I placed an LRA at a similar location during the 'silent' treatment to ensure that the only difference between the treatments was the presence or absence of signals. I tested each group with two purr playbacks and one silent control. For each trial, I maintained the *Desmodium* stem with the settled nymphs in a water tube and placed the tube in a PanaVise to position the stem (**Fig. 2.5**). I conducted the playback compensation (which involves playing back noise through the stem and a brief segment of the playback stimulus) for each stem before the first test of the day for that group and allowed at least 15 minutes before starting a playback.

I constructed the playback files in Audacity, using the pre-filtered stimuli. For the 'purr signal' treatment I played back 10 minutes of silence to obtain a baseline level of signaling, followed by 10 minutes of purr signals separated by intervals of 30s, then

another 10 minutes of silence to capture any change in signaling behavior that persisted after the playback stimuli. Each plant-treehopper setup received two different purr exemplars, for a total of 20 exemplars. For the 'silent' treatment I played back 30 min of silence. To avoid introducing 'click' artifacts in my playback, I faded in and out for the searching signals in the playback track to ensure a smooth transition from silence to playback exemplar.

I recorded the signals produced by the stationary nymphs using a laser vibrometer focused on a small piece of reflective tape within 1 cm of the group of three nymphs. The output sent to a Tascam 20x20 Audio Interface with the input gain calibrated to 1x using a Tenma 72-2580 (Tenma Test Equipment, OH) oscilloscope. The average distance between the closest and farthest nymph and the location of laser recording was 3.8± 1.3 cm and 5.5 ± 2.3 cm, respectively. The average distance between the closest nymph and farthest nymph from the LRA was 6.5 ± 3.6 cm and 8.4 ± 2.4 cm, respectively, and the average distance between the LRA and laser recording site was 5.9 ± 0.9 cm.

Results II

Playback experiment 1: Do signals from moving nymphs elicit replies from stationary nymphs?

I conducted ten rounds of three trials. However, I discarded two rounds, one because the nymphs dispersed from their group and the other because of inadequate signal to noise ratio in the recordings. Accordingly, my final sample size was 8 groups of three nymphs with three trials each (total n = 24 nymphs). I analyzed the signal counts in three stages. I first compared the number of signals produced during the initial 10-min

baseline period between the purr signal and silent treatments, to reveal any differences in the baseline level of signaling that could affect the results. Second, I compared the total number of tick signals produced during the last 20 minutes of the playbacks between the purr and silence treatments. I combined the periods during and after the playback because these were the two periods that could potentially be influenced by the treatment.

To analyze the signal counts I first averaged the counts from the two purr treatments for each group. Because the data were not normally distributed, I compared the number of signals between the playback and silence treatments using a Wilcoxon matched pairs signed-rank test. I first confirmed that the baseline (first 10 min) periods were not different between the purr treatments and the silence treatment ($W=14$, $p=0.62$). I next compared the last 20 minutes (including the 10 min playback and 10 min post-playback period) between the playback and silence treatments. Nymphal groups increased their production of tick signals by 6x during and after the playback of purrs, compared to the silence treatments (**Fig. 2.6;** $W=0$, $p=0.008$). Finally, within the purr playbacks, I compared the number of signals produced before, during and after the playbacks using a Quade test, yielding a nonsignificant trend for the existence of differences among the before, during and after periods of the playback treatment (**Fig. 2.7;** $F=3.06$, $df = 2/14$, $p = 0.08$).

I also examined the post-stimulus delay times between the end of the purr playbacks and the start of the tick signals produced during the 30 seconds after each purr signal. Tick signals were produced throughout the 30s period, with an initial spike of ticks occurring in the first 1-2 seconds after the end of the purr playback (**Fig. 2.8A**, **B**).

The nymphal groups in this experiment sometimes produced purr signals, constituting 34 (±30) % of the signals produced. There was no difference in purr signaling rate between purr and silent playbacks in either the baseline number of purr signals produced ($W = 14$, $p = 0.11$) or in the number produced during and after the playback ($W=23.5$, $p = 0.48$).

Methods III

Playback experiment 1I: Do searching nymphs orient to signals from stationary nymphs?

Stationary nymphs that were already settled at a feeding site produced significantly more tick signals after playback of purr signals recorded from walking nymphs (Results II, above). I therefore tested the hypothesis that ticks produced after the purr playbacks were recruitment signals. Testing this hypothesis required playing back tick signals to walking nymphs newly introduced onto a stem.

Nymphs for this experiment (n=32) were collected from *D. canadense* at Columbia Cosmopolitan Recreation Area. Stems used for the playback were collected from *D. canadense* plants on a private property near Ashland, MO and maintained in water tubes in Ziploc bags until needed.

The signal playback methods are described in Methods II above. Because there was a peak in tick signal production in the first few seconds after the end of a purr signal, I used an interactive playback approach. After introducing a nymph onto a playback stem, I listened for purr signals and played back a tick signal immediately after the purr signal ended (the resulting playback times were within the natural range of variation, $x \pm SD = 0.9 \pm 0.45$ s). I used four exemplars containing between 1 and 4 tick signals each.

Playback calibration was done for each plant prior to placing the focal nymph on the plant.

There were two playback treatments: tick signals or silence. Nymphs in the field were typically positioned at the base of the leaves, often within the stipules at the base of the leaf. In the absence of a signal treatment, I would expect nymphs that were introduced onto a new stem to stop at the first junction with a leaf, so to test for a difference in the distance walked between silence and tick treatments I chose ~20 cm lengths of stem with two leaves, with each end placed in a water tube, resulting in a distance of 15-18 cm of stem available to the nymph (**Fig. 2.9**). Because leaves toward the apex of the stem are younger than those toward the base and might constitute preferred settling locations, I alternated whether the younger or older leaf was closer to the nymph's starting location. Finally, to control for the presence of an LRA at the leaf junction, I placed an LRA at both leaf junctions, and varied the location of the playback between individuals. I introduced nymphs immediately adjacent to the right-hand water tube, facing toward the opposite tube, with the design balanced to control for the location of the younger/older leaf and the signal/silent actuator.

I analyzed the results in two stages: first, I compared the total distance walked and the number of purr signals produced in the silence vs tick playback treatments. Second, for the tick playback treatments, I compared the final distance of the nymph to the LRA playing back a signal vs the silent LRA.

Results III

Playback experiment 1I: Do searching nymphs orient to signals from stationary nymphs?

Nymphs walked significantly farther from their starting location (**Fig. 2.10A**) and produced more purr signals (**Fig. 2.10B**) during the tick playback treatment than during the silence treatment (Mann-Whitney U test, distance: $Z = 2.895$, $p=0.0076$; purrs $Z=2.634$, $p=0.0084$; p-values adjusted for multiple tests using false discovery rate correction). Furthermore, within the tick playback treatment, 15 out of 16 nymphs settled closer to the LRA playing back ticks than to the silent LRA (**Fig. 2.11**; binomial test, $p<0.001$).

Discussion

Nymphs of the treehopper *T. gibbera* recruit other individuals to a feeding site using a back-and-forth exchange of signals. Searching individuals produce a continuous signal while moving. Searchers pause intermittently, and the recruiting individuals signal after the end of the movement-associated signal. Playback of the 'purr' signal produced by moving individuals elicits 'tick' signals from individuals already at a site, and playback of the tick signals to searching individuals cause them to orient to and approach the signal source. This communication system provides a mechanism by which individuals form groups on their host plants.

Food-associated signaling in *T. gibbera* nymphs, in which searchers produce signals that elicit 'beacon' signals from individuals already in a group, contains elements of both the typical food-associated signaling by animals and of isolation signaling by individuals that become separated from a group. Food-associated signals in animals are typically produced in response to the discovery of a food resource, rather than in response to cues from searching individuals (Clay et al 2012). In contrast, isolation signals are

initiated by the individual that becomes separated, and this elicits signals from the group that serve as a beacon that allows the separated individual to rejoin the group (Newman 2004). However, even without signals from a searching individual, *T. gibbera* nymphs at a feeding site signal at a low rate, and these signals may function as low-intensity recruitment signals. Because groups can persist at a site for days or weeks and movement between groups appears to be infrequent, there may be little benefit to producing more conspicuous recruitment signals in the absence of cues from searchers.

Grouping by *T. gibbera* nymphs appears to be favored at least in part because of its relationship with ant attendance. At any stage, *T. gibbera* nymphs in larger groups are more likely to be ant attended. Ant mutualism is hypothesized to be a major ecological driver of grouping in treehoppers, because in the species studied to date, larger nymphal groups are more likely to be attended by ants (Wood 1977; McEvoy 1979; Fritz 1982, Cushman and Whitham 1989; Morales 2000b; Billick et al 2001). Furthermore, in studies that have compared mortality in treehopper nymphs with and without ants, the presence of ants, some of which actively confront predators, results in higher survivorship (Wood 1982; Morales 2000a, 2000b).

In the related treehopper *Calloconophora pinguis*, the only other treehopper in which food recruitment has been studied, the dynamics of communication differ from those in *T. gibbera* (Cocroft 2005). Groups of *C. pinguis* typically move 2-3 times during their development to adulthood, in part because their preferred feeding sites on the petioles of rapidly expanding new leaves are an ephemeral resource that declines in quality as the leaf matures. The entire group moves in a process that can take several hours, as individuals eventually begin leaving the group and searching for other new

leaves. Discovery of a new site leads to ongoing and conspicuous signaling, and as other individuals are recruited, they form a growing chorus of signals that continue until the entire group has settled at the new site. It is likely that signaling is maintained by feedback from movement-related cues from searchers, which do not produce signals while orienting to the new site, but the initial signaling is produced in response to discovery of the resource. Because *C. pinguis* only leave the group when the group's current site has become less suitable, signaling at a new site will always occur in a context where there are 'needy' searchers nearby. Furthermore, there is no signaling by individuals in settled groups, but there are also unlikely to be searchers present.

Several aspects of communication in *T. gibbera* nymphs will be important to test in future studies. In this study we addressed the responses to signals of only one of the potential sets of receivers of the signals of *T. gibbera* nymphs. Other potential receivers of these plant-borne signals include mutualistic ants, predators, and the plant itself. With respect to predators, moving is risky for a sedentary animal (Ioannou & Krause 2009), and potential protective functions of purrs include masking of other cues of movement and advertising unpalatability. With respect to ant mutualists, it is possible that both the purr and tick signals attract ants to the source. With respect to the plant, although nearby plant tissues can likely detect the signals (Appel and Cocroft 2023), it is unclear how plant detection of the signals might benefit either the signaler or the receiver. Hypotheses about the effect of signals on predators and ant mutualists can be tested using playback experiments.

Two observations from this study also suggest further testing of the importance of signals in group decision making. One is that the purr signal, while most often produced

by walking nymphs, was also sometimes produced by stationary, settled nymphs. Given the frequent association of purrs with movement, I hypothesize that the purrs given by stationary individuals are a means of sharing individual assessments of declining site quality (these were cut stems, which even in water tubes will eventually decline in quality) and may make individuals in those groups more likely to move, as seen in some other animal groups (Stewart and Harcourt 1994; Bousquet et al 2011; Walker et al 2017; Mine et al 2022). The second observation is that settled nymphs produce ticks at a low rate in the absence of signals from searching individuals. Is the signal production rate related to food quality, as it is in many species with food-associated signals (Clay et al 2012)? And given the importance of ants in the biology of group-living treehoppers, and the evidence that signals in at least one species attract ant protection (Morales et al 2008), what is the effect of *Tylopelta* signaling behavior on mutualistic ants and *vice versa*?

Acknowledgments

I thank Dr. Debbie Finke, Dr. Manuel Leal, and Dr. Johannes Schul for comments on the manuscript. I thank Annette Marin, Alexia Pattman, Brandy Williams, Alexis Kollasch, Tessa Foti, and Abdul Rahman for help in collecting nymphs in the field. I acknowledge support from a Ridgel fellowship from the University of Missouri.

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Tables and Figures

Table 2.1. Effect of nymphal stage on log-transformed group size, using within-plant averages and a general linear mixed model with nymphal instar as an ordered categorial predictor and plant ID as a random effect. The effect of stage on group size was dominated by a negative linear relationship. * = $p<0.05$, *** = $p<0.001$.

Factor	**Estimate**	**Standard Error**	**T**	**P**
(Intercept)	1.17	0.081	14.41	***
Nymphal instar - LINEAR	-0.745	0.057	-13.04	***
Nymphal instar - QUADRATIC	0.13	0.057	2.33	*

Table 2.2. Effect of nymphal stage and group size on the probability of ant attendance, based on a mixed model logistic regression, including plant ID as a random effect. *** = $p<0.001$

Factor	**Estimate**	**Standard Error**	**T**	**P**
(Intercept)	-2.72	0.94	-2.90	***
Group Size	0.252	0.21	1.21	ns
Average Stage	-0.37	0.22	-1.70	ns
Group Size * Average Stage	0.334	0.10	3.42	***

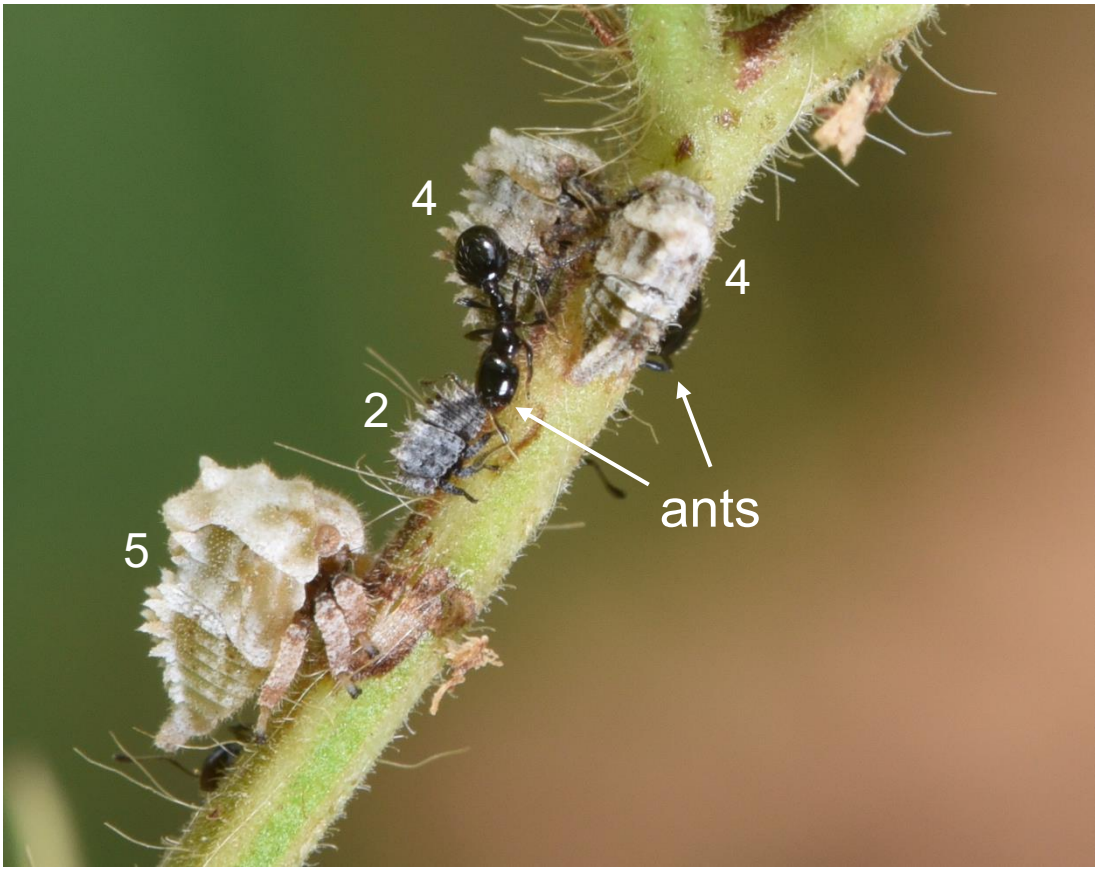

Figure 2.1. Aggregation of four *T. gibbera* nymphs (with stages indicated) being tended by two *M. minimum* ants on a *Desmodium canescens* stem. Photo: R. B. Cocroft.

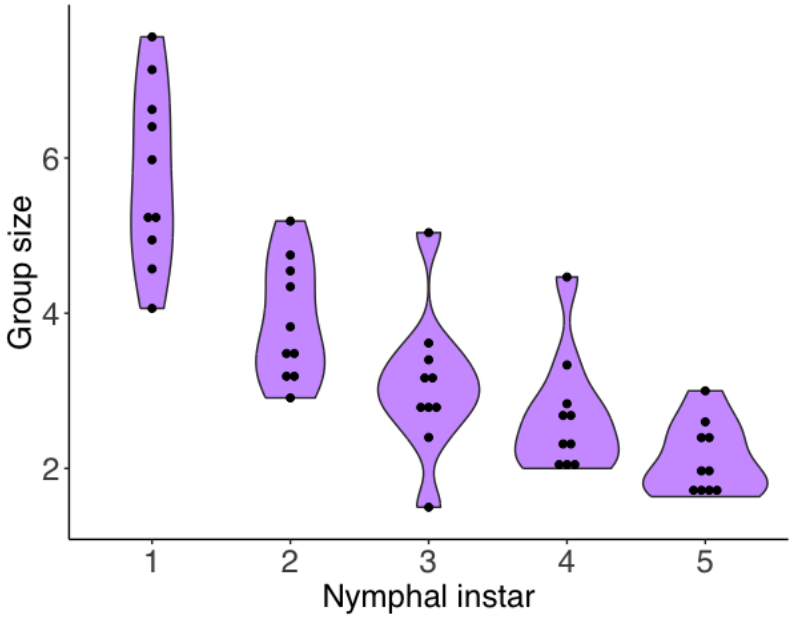

Figure 2.2. Later-instar nymphs occur in smaller groups (see Table 2.1). Sample size: n=50 (5 instars x 10 plants, averaged from n=203 group observations and 308 nymphs (groups were observed more than once).

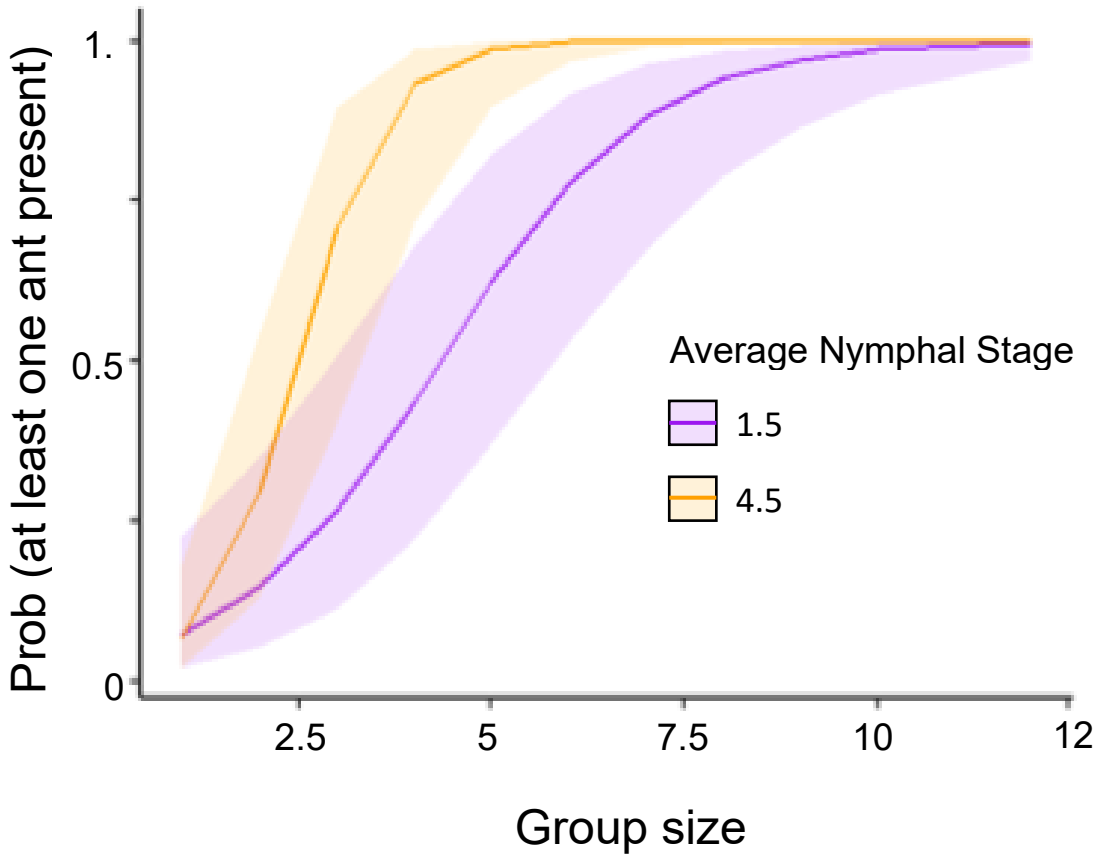

Figure 2.3. The relationship between group size and ant attendance is influenced by nymphal stage. At any stage, ants were more likely to be found with larger groups. However, for a given group size, older nymphs had a higher probability of being attended by ants than younger nymphs (predicted lines +/- SE; see Table 2.2).

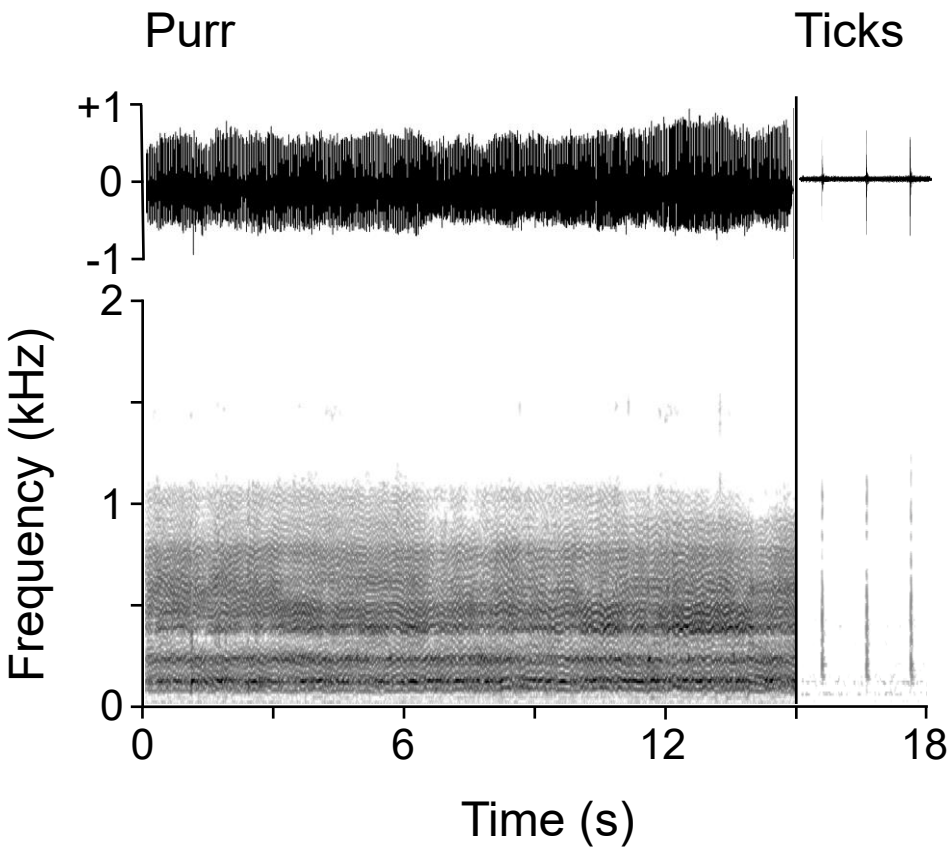

Figure 2.4. Waveform and spectrogram with examples of purr and tick signals.

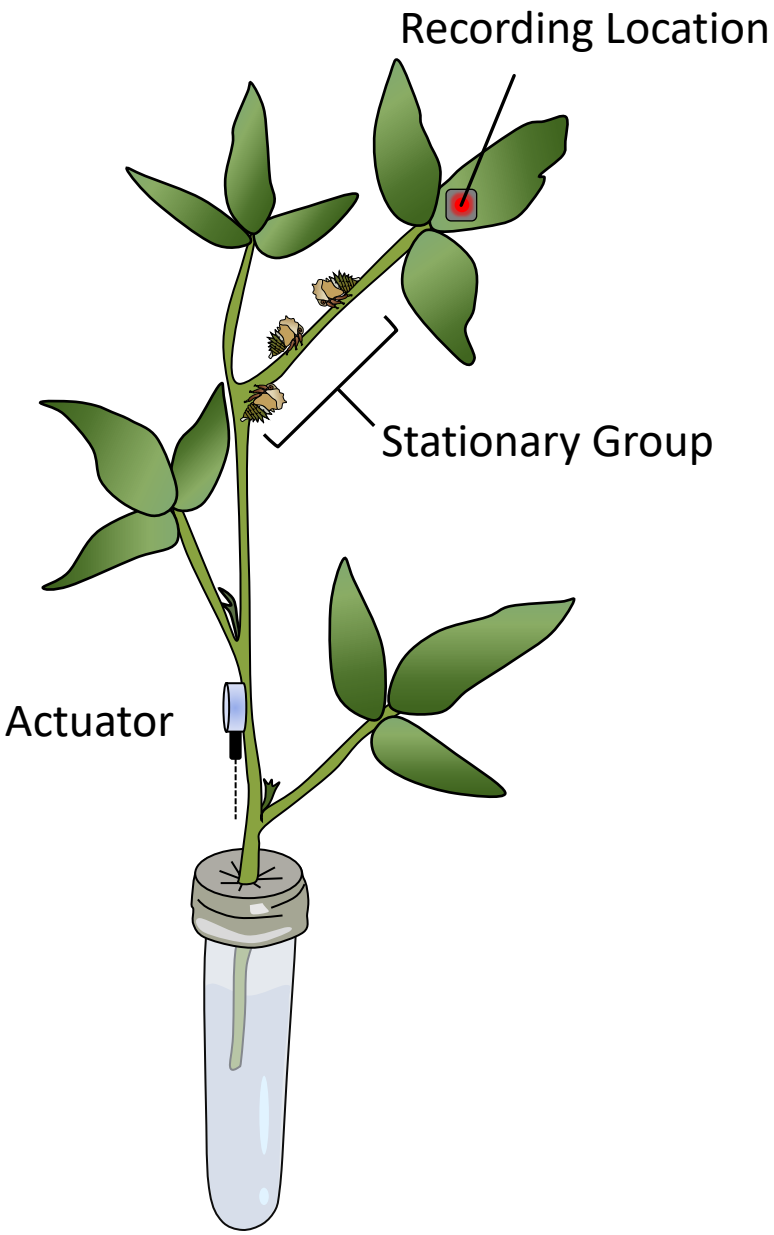

Figure 2.5. Illustration of the setup for the playback experiment in which purrs were played back to a group of stationary nymphs. The playback speaker was 10 cm below the group and the recording location was 5 cm above the group. The playback was calibrated <1 cm away from the actuator. Figure not to scale.

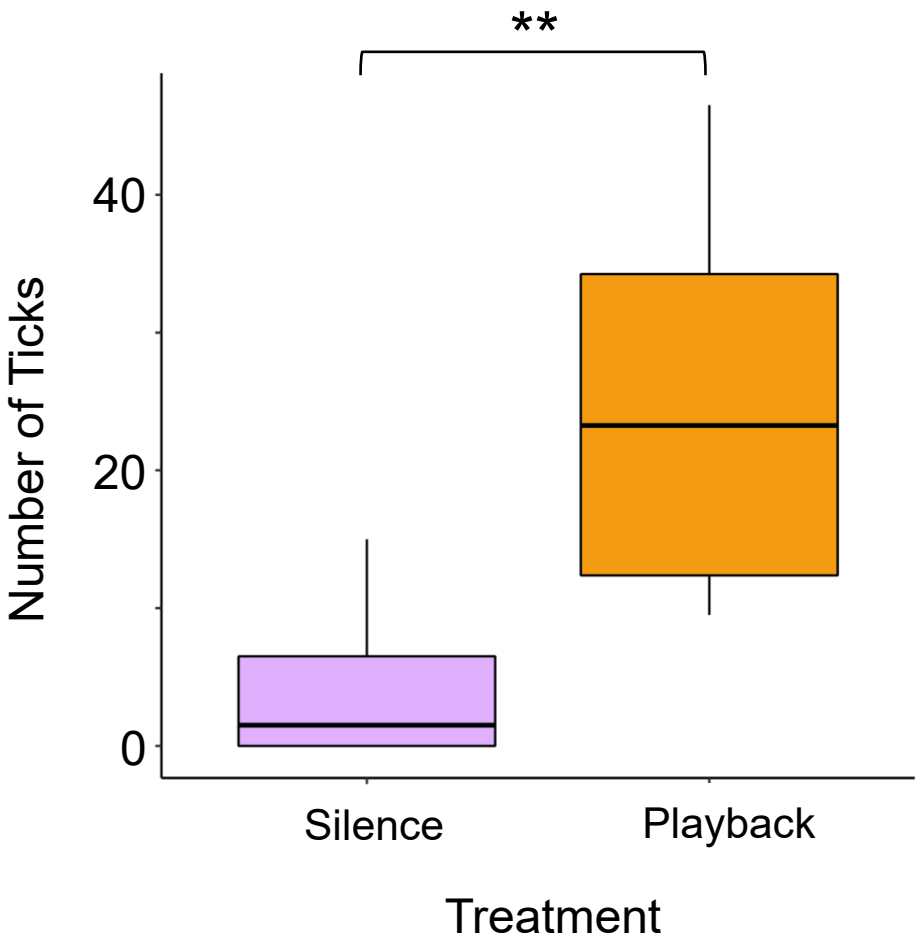

Figure 2.6. Nymphs in stationary groups increased their production of tick signals 6-fold, on average, in response to playback of purr signals. ** = $p<0.01$

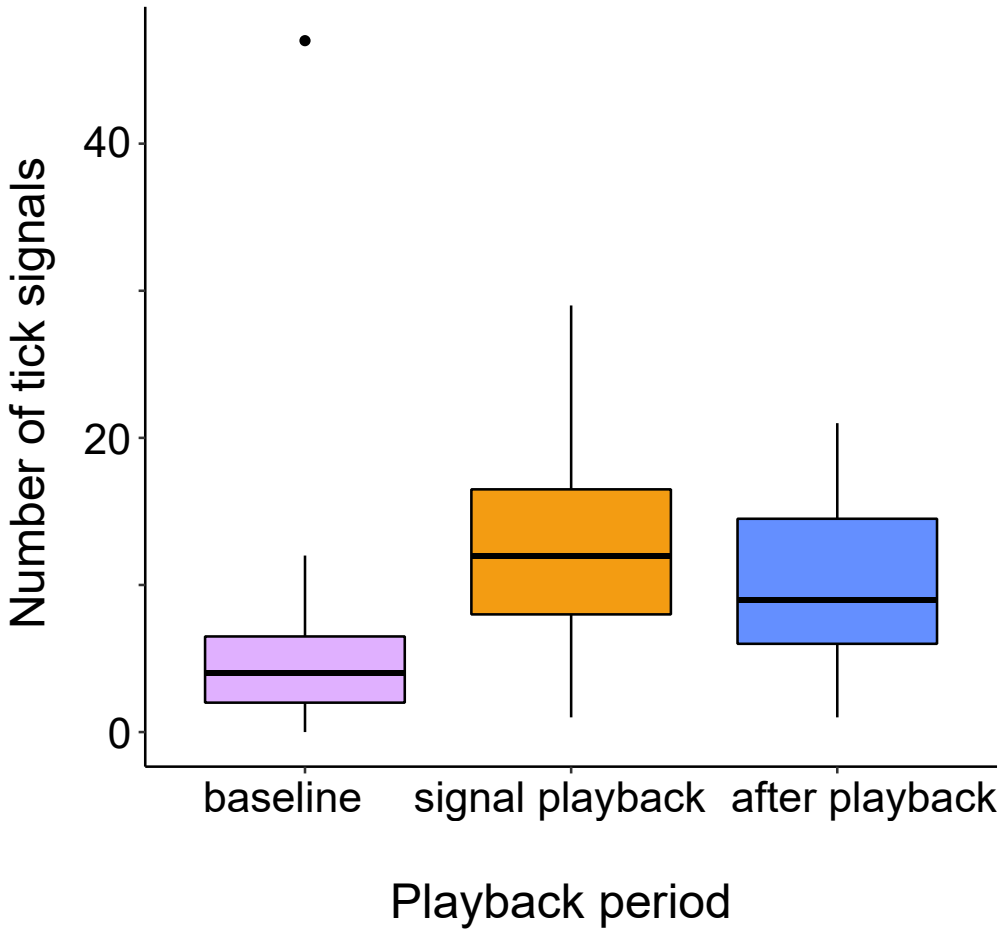

Figure 2.7. The number of tick signals produced did not differ significantly among baseline, playback and post-playback periods (p=0.08).

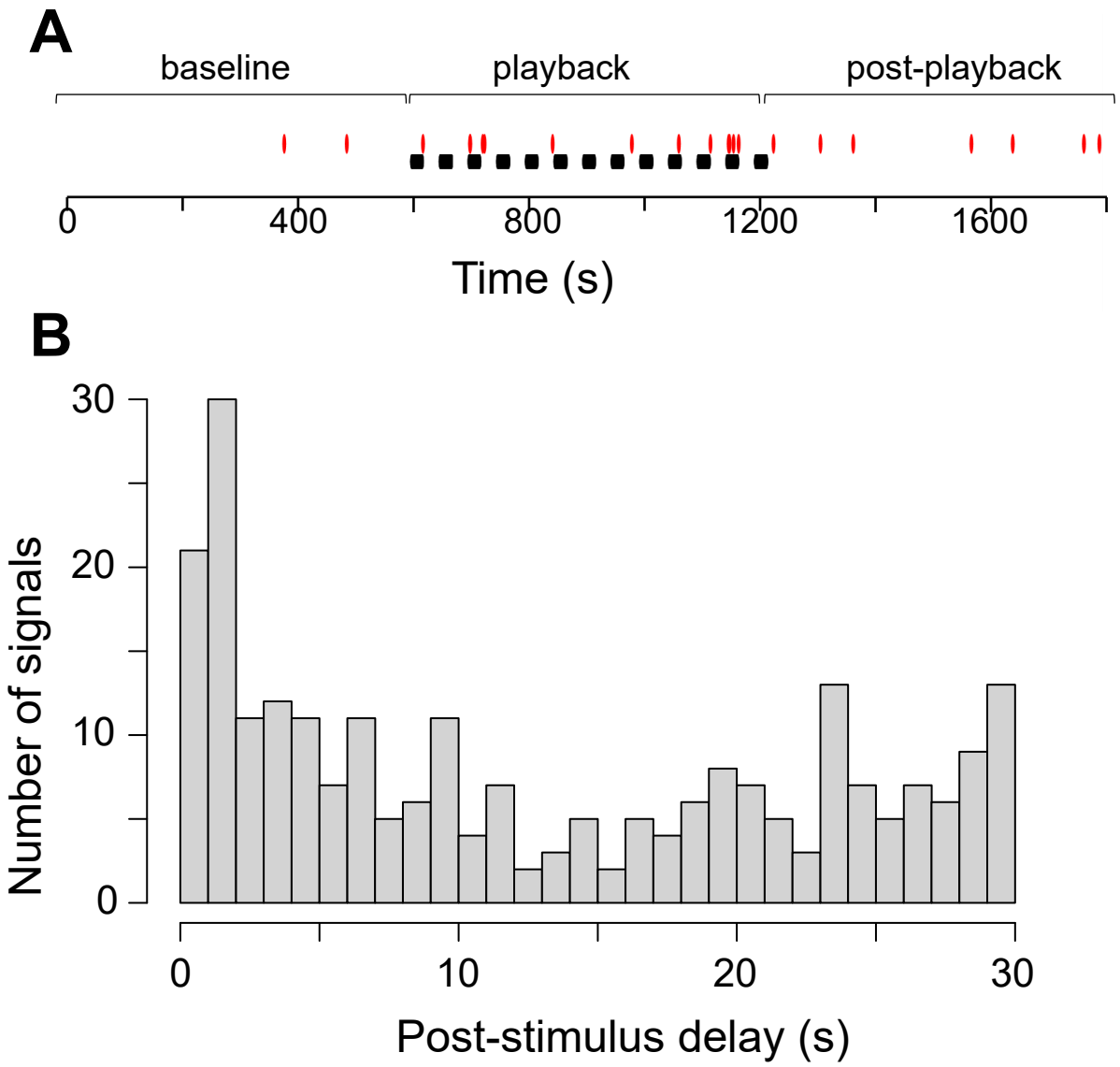

Figure 2.8. Timing of tick signals produced by stationary nymphs. **A**. Example of signal timing from one trial; black rectangles = purr playbacks, red lines = tick responses. **B**. Post-stimulus time histogram for the 30-sec periods following each playback signal. Note the peak in the first few seconds after the end of the purr signals. In this figure, tick timing is pooled across all individuals (n = 8 groups of 3 nymphs, 249 tick signals).

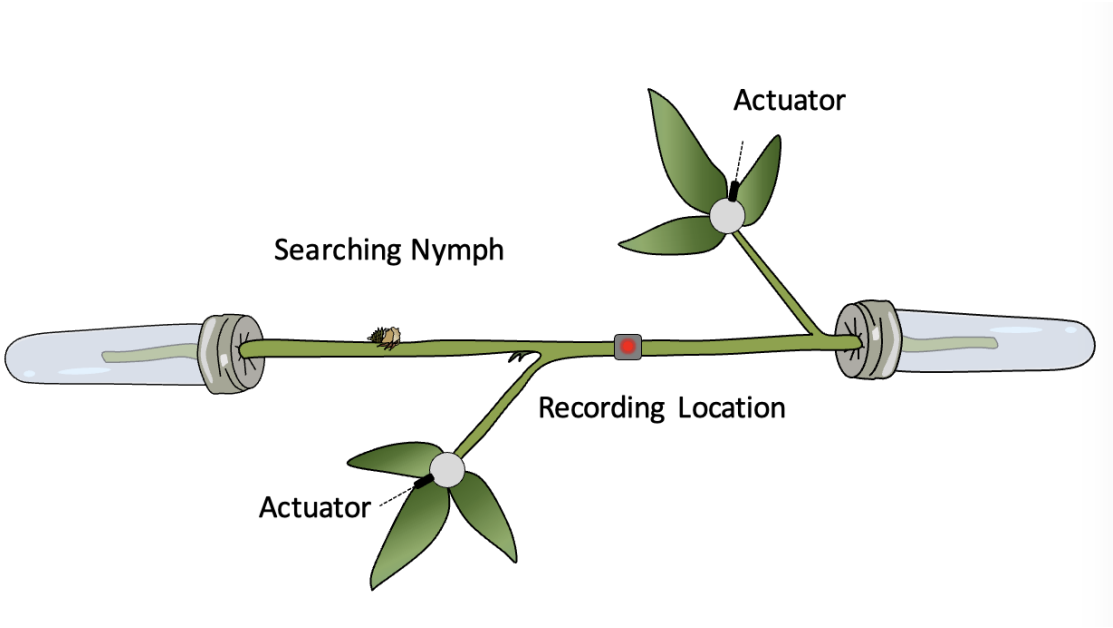

Figure 2.9.: Illustration of the setup for the playback experiment in which ticks were played back to newly introduced searching nymphs.

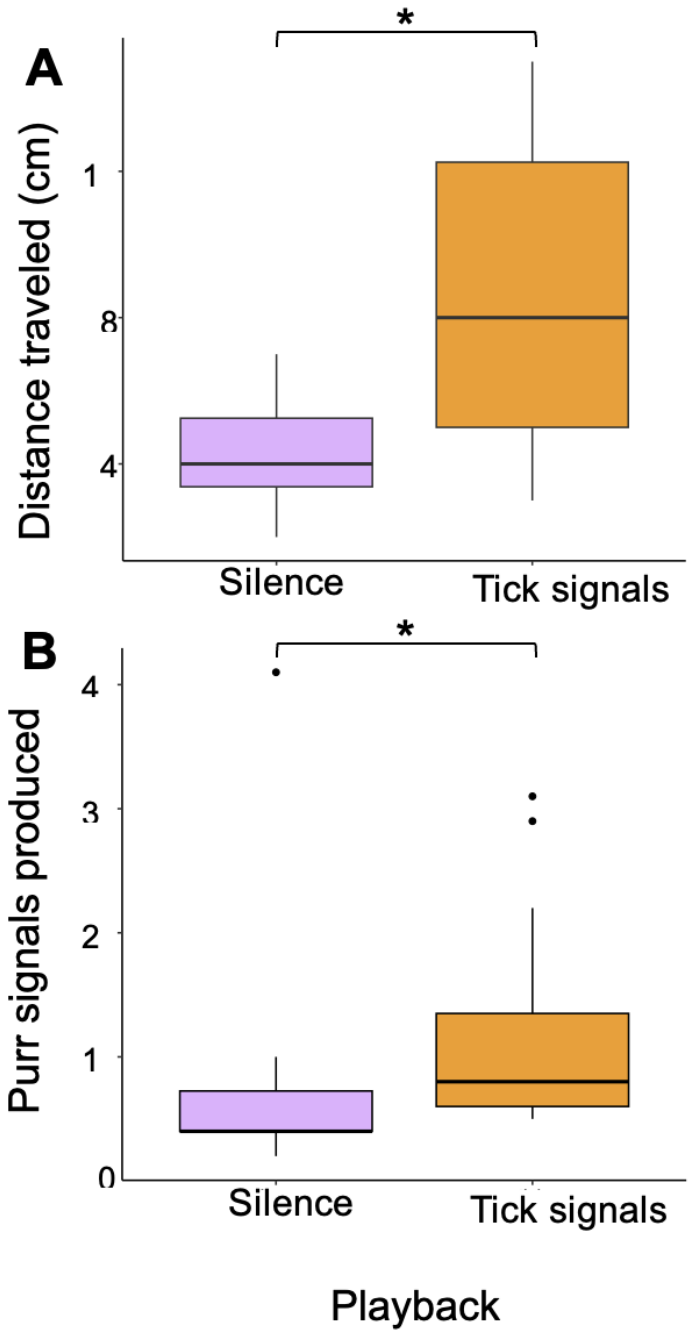

Figure 2.10. Searching nymphs moved farther from their starting position (**A**) and produced more purr signals (**B**) during tick playbacks than during silent controls. ** = $p<0.01$

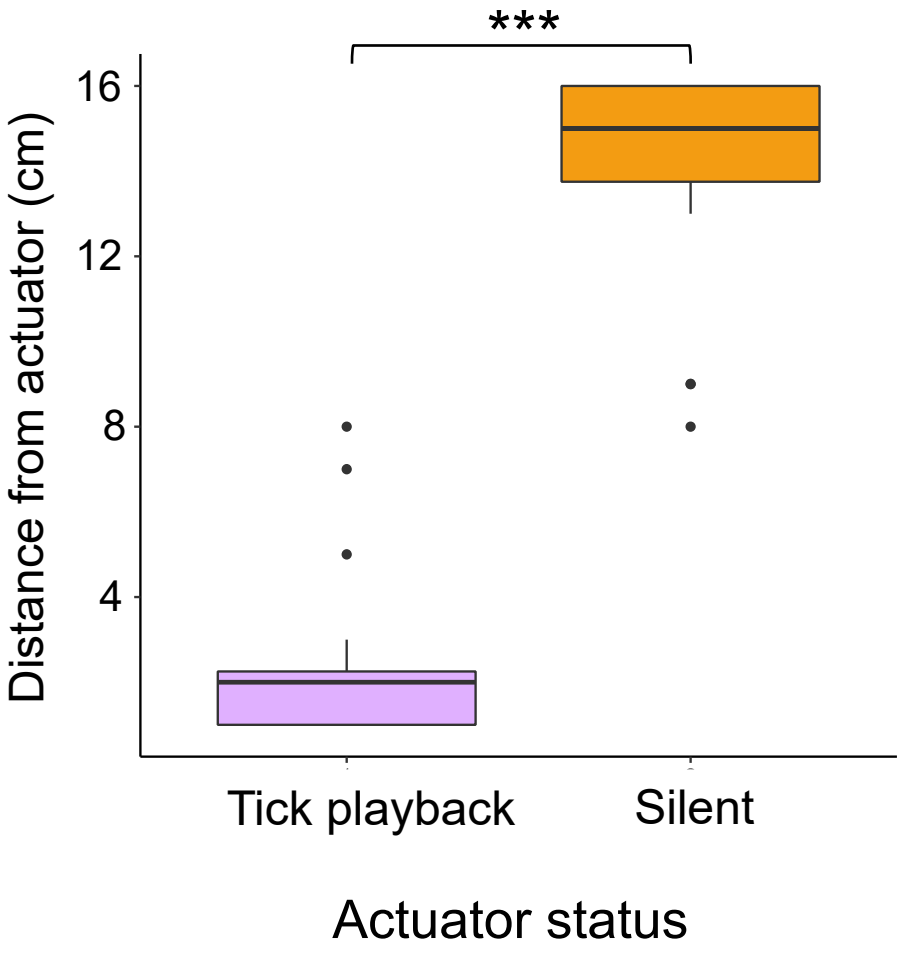

Figure 2.11. During the tick playback treatment, searching nymphs settled closer to the actuator playing back signals than to the silent actuator. *** = p<0.001

CHAPTER 3

Food recruitment signals in group-living immatures in the *Enchenopa binotata* species complex of treehoppers

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Abstract

Animal groups show great flexibility in adjusting their foraging and movement to the distribution of resources in their environment. Individuals in groups can rely not only on their own perception of environmental cues, but also on social information provided by other group members. I studied how individuals in a group-living herbivorous insect, the treehopper *Enchenopa binotata* 'Juglans nigra,' use social information to locate other group members already at feeding sites. I first tested the hypothesis that group living is advantageous, by observing individuals in the field and relating group size to the likelihood of attendance by their ant mutualists. I then surveyed the production of plant-borne vibrational signals in these insects. I then experimental tested the hypothesis that at least one of the signal types functioned in recruitment to feeding sites, by means of

vibrational playback experiments. Finally, I assessed the presence of these signals in close relatives using a library of recordings. The results suggest that group formation is favored because larger groups are more consistently attended by their ant mutualists, and that one signal type functions as a powerful recruitment signal. This recruitment signal is shared by each of the other five species surveyed in the *E. binotata* species complex and likely allows these insects to share information about the discovery of high-quality host plant resources.

Introduction

Behavioral flexibility allows individuals to adjust to temporal and spatial variability in their current environment and contributes to success in colonizing new environments (Sol 2003; Bush 2009; Wright et al 2010; Snell-Rood 2013). This ability of animals to adapt their behavior to changing environmental conditions is a hallmark of social insect colonies, which adjust their foraging effort in relation to changes in the distribution of resources in their environment (e.g., Gordon 1991; Seeley 1996). The ability of social insects to allocate the colony's efforts to current needs and opportunities arises from the interactions of individual colony members that differ in their experience of colony needs and external opportunities (Passino et al 2008; Gordon 2010). Many of the interactions among colony members rely in turn on the perception and production of cues and signals that can influence individual behavior in ways that lead to collective decisions (Holldobler and Wilson 2009). Similar forms of information sharing that allows

groups to respond to changing conditions occurs not only in the highly social insects, but also in animal groups in general (Sumpter 2010).

Plant-feeding insects are a hyper-diverse group of organisms (Hardy et al 2020), and many insect herbivores live in groups (Costa 2006). However, we know relatively little about how collective decisions in group-living insect herbivores allow them to exploit changing host resources (but see Peterson and Fitzgerald 1991; Fitzgerald and Costa 1999; Costa 2006; Cocroft and Hamel 2010). Studies of 'larval societies' in three insect orders, including Lepidoptera, Hemiptera and Hymenoptera, reveal collective decisions about when to move and where to feed (Fitzgerald and Costa 1999; Cocroft 2005; Michael et al 2019; Costa 2006; Fletcher 2008; McClellan and Montgomery 2023). The behavioral flexibility provided by collective decision-making allows groups of insect herbivores to exploit changing host resources (Cocroft 2005). Furthermore, diversification in phytophagous insects is associated with changes in host plant use (Funk et al 2002; Janz et al 2006; Nosil 2012; Hardy and Otto 2014), and the ability of groups to collectively monitor and share information about host resources may facilitate persistence on and colonization of novel hosts.

The *Enchenopa binotata* species complex of treehoppers (Hemiptera: Membracidae) is a model of ecological speciation via shifts to novel host plants (Wood and Guttman 1983; Nosil 2012; Hsu et al 2018). The 12+ host-associated species in this complex are morphologically similar but adults have diverged in their plant-borne vibrational mating songs (Cocroft et al 2010) and mate preferences (Rodriguez et al 2006). The immatures

(nymphs) of these treehoppers develop to adulthood in groups, which interact with ants in a food-for-protection mutualism (Wood 1982). The nymphs of different species feed on different locations on their respective host plants (Wood 1980), presumably reflecting host differences in the distribution of accessible resources. Nymph of different species also vary in their coloration, which matches the color of the plant part on which they feed, except for two aposematically colored species (Wood 1980). Finally, nymphs have a repertoire of vibrational signals (Cocroft et al in prep; this study; Desjonqueres et al 2019). The functions of those signals are currently unknown, but the signals likely play a role in collective decisions as do the signals of other treehopper nymphs (Cocroft 1996; Ramaswamy and Cocroft 2009; Cocroft and Hamel 2010; Hamel and Cocroft 2012; Michael et al 2019).

In this study I test the hypothesis that the vibrational signals of *E. binotata* nymphs play a role in recruitment to feeding sites. Changes in host resources over time can require membracid nymphs to change feeding locations multiple times during their development, and food recruitment is one of the key contexts for collective decisions in membracids (Cocroft 2005; Michael et al 2019). Recruitment of additional individuals to a feeding site is only likely to occur if there are benefits to increasing group size. Here I tested whether ant mutualism favors larger groups, as it does in other membracids (McEvoy 1979; Wood 1982; Morales 2000a; Michael et al 2019), by quantifying the relationship between group size and ant attendance in the field. I then experimentally tested the hypothesis that at least one signal in the repertoire functions in recruitment to a feeding site. Finally, this study focuses on one species in the complex (the species that occurs on

Juglans nigra [Juglandaceae], one of the two aposematically colored species), and I explored the possibility that potential recruitment signals occur widely in the *E. binotata* complex by surveying a library of recordings of membracid nymphal signals.

Methods

Ant mutualism and group living

To test the hypothesis that ant mutualism favors group living in nymphs of *E. binotata* 'Juglans nigra', I surveyed black walnut trees at four sites in Boone and Cooper counties, Missouri in May and June 2021 and June 2022. For each tree, I inspected branches <2m above the ground and recorded the number and stages of the nymphs per group and the number and identity (to genus) of tending ants. I operationally defined a nymph as belonging to a group if it was within 10 cm of other nymphs, and I considered an ant to be tending if it walked over and/or tended one or more nymphs (see **Figure 3.1**).

I first analyzed the relationship between nymphal developmental stage (nymphs develop through five instars before closing as adults) and the number of individuals in the group. Because groups often contained individuals at different stages, I calculated an average stage weighted by the number of individuals in each stage, as in Chapter 2. The log of group size was normally distributed, and the model in R included sampling location as a random effect: glm(log(group size) ~ average stage + (location), family = gaussian). Because sampling in the second year was restricted to nymphs of later instars, I only included data from the first year. I then assessed the relationship for each year separately

between group size, nymphal stage and the likelihood that at least one mutualist ant was present using a mixed model logistic regression, with location included as a random effect: glm(ant present ~ group size * average stage + (location), family = binomial (link = logit).

Experimental test of signal function

To obtain recordings of nymphal signals I collected groups of 2^{nd}-5^{th} instar nymphs from Stephens Lake Park, Columbia, MO in May and June 2019. I recorded nymphal signals from individuals on cut stems placed on a Vibraplane 5704 vibration isolation table (Kinetic Systems, Boston, MA) in a temperature-controlled room (24+1^{o} C) at the University of Missouri. I recorded signals using a Polytec PDV-100 laser vibrometer (Polytec Inc., Hudson, MA) focused on a small (5mm^2) piece of reflective tape attached to the leaf petiole within 5 cm of the nymphs (note that nymphs always occurred on the leaf petiole). The laser output was connected to a Tascam Celesonic 20x20 Audio Interface (Teac Corporation, CA, USA) with an input channel adjusted to have 1x gain using an oscilloscope to allow conversion to velocity units. The interface was connected to a Dell Latitude 5580 laptop. I recorded the signals using v. 3.3.3 of Audacity recording and editing software.

I obtained recordings of four signal types (**Figure 3.2**), and generated playback exemplars of the two most common types to use in a playback experiment. One signal type was produced by individuals in stationary groups for periods of a few seconds to several minutes, often with more than one individual producing the signal in synchrony. I

refer to this signal type as a ‘grouped signal’ because it consists of a group of syllables produced at a steady rate with several seconds between groups of syllables. The second common signal type was produced by stationary individuals, often preceding movement, and was sometimes produced by walking individuals. I refer to this signal type as a ‘pulsed signal’ in reference to the amplitude modulation within syllables. I selected four recordings of each signal type to generate exemplars, each from a different individual and with only one individual producing the signal in each exemplar.

To reproduce the signals, I attached a vibration actuator (a linear resonant actuator [LRA]; 10mm diameter, Samsung Inc.) with wax to the petiole of a leaf of a potted *J. nigra* plant ca. 50 cm tall (see **Figure 3.3** for an illustration of the setup). I played the signals back using Audacity v. 3.3.3 on a Dell Latitude laptop connected to a Tascam 20x20 interface and a Behringer PowerPlay HA8000 Headphone amplifier (MusicTribe, Manila, Philippines) that drove the LRA.

I used playback methods as described in Chapter 2; briefly, to ensure that the played-back signal matched the frequency spectrum of the original I used a custom-written Matlab script to compensate for the frequency response of the actuator and host plant stem (Michael et al 2019). I reproduced the signals at a peak amplitude of 0.06 mm/s measured 5 cm from the actuator, which is near the upper end of the amplitude distribution of both signal types when recorded within 5cm of the source on a host plant petiole. To improve the signal-to-noise ratio of the playbacks I used a bandpass filter with cutoff frequencies of 70 Hz and 5000 Hz, outside the frequency range of the signals.

The playback experiment included three treatments: silence, grouped signal, or pulsed signal. For each treatment, I attached the LRA to a leaf petiole of a potted *J. nigra* ~50 cm tall and for the playback treatments I calibrated the playback exemplar. Exemplars were 2-4 s long and were played back for 10 min using a signal timing that reflected the natural pattern of signaling. For grouped signals, the playback consisted of one group of 2-4 syllables plus 1s of silence, repeated continuously for the 10 min playback period. For pulsed signals, the playback consisted of 4-5s of signals plus 30s of silence, repeated for the 10 min playback period. I conducted the playback to n = 45 nymphs, systematically varying the playback treatments between individuals such that there were 15 nymphs for each of the three treatments. No nymph was used more than once. Within the signal treatments, I alternated the four exemplars between individuals. I used 18 potted *J. nigra* plants, employing each plant 2 – 3 times during the experiment. When re-using an individual plant, I waited at least 24 h after the previous use, and attached the LRA to a different leaf petiole. The playback plant was placed on a Vibraplane vibration isolation table.

For each replicate of the experiment, I used a soft-bristled watercolor brush to transfer a single nymph onto the base of the host plant stem, 5 cm above the soil and ca. 50 cm below the actuator. Individuals used in the experiment were 3^{rd}-5^{th} instar nymphs collected from Stephens Lake Park in Columbia, MO in May-June 2020. Nymphs were kept on cut stems in Ziploc bags prior to testing; after testing, the nymphs were

maintained until adulthood on a potted *J. nigra* tree and then released at the collection site.

I began the playback treatment immediately after introducing the nymph and continued playback for 10 min or until the nymph settled (i.e., stopped and remained stationary) whichever came first. I recorded the time the nymph spent searching, the number of times it stopped during the trial (where a stop typically lasted less than 2 s), and its final distance to the LRA. I measured all distances along the plant surface using a ruler.

For statistical analysis, because none of the response variables were normally distributed, I used a Kruskal-Wallis rank sum test, a non-parametric test analogous to a one-way ANOVA. I then compared pairwise differences between treatments using a Wilcoxon rank sum test.

Nymphal signal repertoire in the E. binotata *complex*

To assess the presence of similar vibrational signals in other species in the complex I surveyed a library of *E. binotata* nymph signal recordings made by RB Cocroft between 2000 and 2021. Because the recordings were made opportunistically, the number and length of the recordings varied among species. Accordingly, I considered the presence of a signal type as evidence that nymphs of a given species produced that signal, but I considered absence of that signal type as inconclusive, pending additional recordings.

Results

Group size and ant mutualism

In both years, larger groups were significantly more likely to be ant attended, though the relationship was less strong in year 2 (**Table 3.1, Figure 3.4**).

Experimental test of signal function

Nymphs oriented toward the grouped signal, with 73% (11/15) of nymphs settling within 1 cm of the playback actuator (**Table 3.2; Fig 3.5A**). Post-hoc comparisons revealed that the final distance between the nymph and the actuator in the grouped signal treatment was significantly lower than that in the other two treatments, but that the distance in the pulsed signal treatment was not significantly different from that in the silence treatment (**Table 3.2**). Nymphs also settled more quickly in response to playback of grouped signals (**Table 3.2; Fig 3.5B**). Nymphs made the fewest stops in response to playback of grouped signals, more in response to pulsed signals, and the most stops during the silent controls (**Table 3.2; Fig 3.5C**).

Nymphal signal repertoire in the E. binotata *complex*

Figure 3.6 shows the signal types recorded from seven members of the *E. binotata* species complex, including the species studied here (on *Juglans nigra*) and a species from Panama (on *Trema micrantha*). Grouped signals occurred in recordings of all seven species, and the other signal types occurred in recordings of 3-5 of the species.

Discussion

Nymphs of the treehopper *E. binotata* 'Juglans nigra' live in groups, and their vibrational signals provide one mechanism of group formation. Nymphs of *E. binotata* have a repertoire of at least four distinct types of vibrational signals. Among these, the 'grouped signal' is a powerful recruitment signal: searching individuals oriented strongly to this signal, approaching quickly with few stops and typically settling within 1 cm of the signal source. In contrast, the pulsed signals had little effect on nymphal searching behavior.

Why do nymphs of *E. binotata* 'Juglans nigra' live in groups? Larger groups were more likely to be ant-attended, and experimental ant-exclusion studies with membracids have shown that survivorship is higher for nymphs when ants are present (McEvoy 1979; Wood 1982; Buckley 1983; Morales 2000a; Del-Claro and Oliveira 2000; Fernandes et al 2005; Morales and Beal 2006; Reithel and Billick 2006; Fritz 2008; also see Buckley 1992; Reithel and Campbell 2008). The survival benefit of ant mutualism is sometimes found only when predators are present (Cushman and Whitham 1991) but can be present even when predators are excluded (Morales 2000b). Cushman and Whitham (1991a) found that larger groups of nymphs of the treehopper *Publilia modesta* benefited from ant-tending more than did individuals in smaller groups. Larger groups of *E. binotata* were also attended by more ants, although the relationship was less than 1:1, such that the per-capita number of ants was lower in larger groups. For *P. concava* treehoppers, which live in much larger aggregations, individuals in relatively smaller groups experienced more ants per capita and had higher survivorship (Morales 2000b). Furthermore, when Cushman and Whitham (1991b) experimentally reduced the number of tending ants, *P.*

modesta nymphs fed less and moved more. There is thus likely to be an optimal group size beyond which increasing the number of individuals in the group decreases average fitness. Groups of *E. binotata* nymphs in this study were an order of magnitude smaller than the groups of *Publilia* nymphs studied by Cushman and Whitham (1991a) and Morales (2000b), and it is unknown at what group size *E. binotata* nymphs may reach a point of diminishing returns. Overall, however, given that experimental studies show that excluding ants reduces treehopper survival, and this study and Wood (1982) both found that larger groups of *E. binotata* nymphs were more consistently attended by ants, recruitment of new individuals to groups likely provides fitness benefits to both signalers and receivers.

The grouped signals produced by *E. binotata* nymphs can be considered both as food recruitment signals and more generally as social recruitment signals (Townsend & Manser 2013). Given the survival benefits of ant attendance for nymphal treehoppers discussed above, and the relationship between ant attendance and grouping, solitary individuals that join groups will benefit from access to mutualists. Furthermore, nutrient quality varies among different growing shoots on the same plant, and treehoppers show strong preferences for higher-quality feeding and oviposition sites (Price and Carr 2000). Because aggregations of treehopper nymphs are most likely to occur at high-quality feeding sites, gaining access to mutualistic ants and to suitable feeding sites will often be joint, non-exclusive benefits to receivers of responding to recruitment signals. For the signaling individuals, there may be both direct and indirect benefits of recruiting additional individuals to the group. There is a potential for inclusive fitness benefits to signalers, to the extent that recruitment signaling benefits the receivers, because at least

some of the nymphs developing on the same branch will be full siblings: females in the *E. binotata* complex mate once (Wood and Guttman 1983; Sullivan-Beckers and Cocroft 2010) and deposit multiple eggs in a single clutch (x = 8.0+3.1 for *E. binotata* 'Juglans nigra; Wood [1980]). There are also potential direct benefits to increasing the number of individuals per group and thereby increasing the likelihood of ant attendance.

Recruitment signals like those of *E. binotata* allow animal groups to adjust their foraging in response to temporal and spatial variability in resources (Seeley 1996, 2010; Gordon 2010). Grouped signals are widespread among member of the *E. binotata* species complex, found in each of six additional species surveyed. Speciation in the *E. binotata* complex is initiated by shifts to new host plants (Wood 1980; Hsu et al 2018), and if nymphs also use these signals in a novel host environment, then the presence of recruitment signals would likely facilitate nymphal persistence when developing on new host plants. However, recruitment signals alone will be insufficient for allowing nymphal groups to make optimal use of host plant resources. In particular, when feeding site quality is declining – due to local plant conditions or location of the group by predators – other signal types will be needed to allow collective decisions about when to move. 'When to move' signals are widespread in animal groups (Bosquet et al 2011; Walker et al 2017; Demartsev et al 2022; Farrine 2022). In *E. binotata* nymphs, pulsed signals are a likely candidate because they are often produced by groups on cut stems whose quality is declining (Michael pers. obs.). In addition, given the importance of ants in the life history of *E. binotata* treehoppers, signals that influence ant behavior (as documented in another ant-attended treehopper; Morales et al. 2008) would not be unexpected. However, the

functional role of pulsed and other signals in the repertoire of *E. binotata* nymphs remain to be tested.

There is currently experimental evidence of food recruitment signaling in group-living immatures of three species of membracid treehoppers, and of recruitment to the site of a predator attack in at least two others (**Table 3.3**; this study; Michael et al 2019; Cocroft 1996, 2002, 2005; Ramaswamy and Cocroft 2009; Hamel and Cocroft 2019). Comparative evidence suggests that these signaling systems are widespread among immatures in membracids (this study, for the *E. binotata* complex; Cocroft and Hamel 2010; Cocroft et al. in prep), but some patterns emerge even among the five species that have been studied experimentally. Recruitment to signals is the common theme and occurs both during juvenile development and during mate choice, where receptive females respond to male signals to recruit sufficiently attractive males (McNett et al 2006; Gibson and Cocroft 2018; Virant-Doberlet et al 2023). Another common theme is that in four of the five species, signals are produced collectively by means of coordinated action among group members. However, the functional significance of coordination among signalers that produce a longer, higher-amplitude collective signal has only been studied for one species (Cocroft 1999). There are also striking differences among the three food-signaling systems, both in the signal features (harmonically structures signals in E. *binotata* and *C. pinguis*, broadband click in *T. gibbera*) and in whether the signals are produced collectively or singly (singly in *T. gibbera*, collectively in *C. pinguis*, and both in *E. binotata*). Further comparative study will be needed to understand how

differences in life history, host use, and social structure influence the evolution of communication systems in these group-living insects.

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Tables & Figures

Table 3.1. Effect of nymphal stage and group size on the probability of ant attendance, based on a mixed model logistic regression, including location as a random effect. Note that for year two, the sampling period was shorter and only later-instar nymphs were observed, so nymphal stage was omitted. * = $p<0.05$ ** = $p<0.01$, *** = $p<0.001$

Factor	**Estimate**	**Std Error**	**T**	**p**
Year 1				
(Intercept)	-6.35	1.75	-3.64	***
Group Size	1.07	0.36	3.02	**
Average Stage	0.67	0.41	-0.34	Ns
Group Size * Average Stage	0.002	0.09	0.10	Ns
Year 2				
(Intercept)	-0.10	0.46	-0.23	Ns
Group Size	0.11	0.05	2.23	*

Table 3.2. Response of *E. binotata* nymphs to playback of grouped signals (G), pulsed signals (P), and silent control (S), based on a Kruskal-Wallis rank sum test. P-values were adjusted across the three variables (and within each variable for post-hoc tests) using an FDR correction.

				Post-hoc tests		
Variable	**c^2**	**df**	**p**	**S v G**	**G v P**	**P v S**
Final distance from LRA	23.7	2	***	***	***	ns
Time to settle	29.4	2	***	***	***	ns
Number of stops	33.7	2	***	***	***	***

Table 3.3. Variation in recruitment signaling in group-living immatures of membracid treehoppers. In four of the five species, collective group signals are produced in response to an environmental trigger, while in *T. gibbera,* individual signals are produced in response to signals from searchers. Signals also vary in acoustic characteristics, as shown in the spectrograms. Signals of *C. pinguis*, *P. vittata* and *U. crassicornis* are drawn from Cocroft et al. (in prep.).

Recruitment to:	**Collective signals**	**Response to searcher**	**Spectrogram**
Feeding site			
Callonophora pinguis	X		
Enchenopa binotata	X		
Tylopelta gibbera		X	
Predator attack			
Platycotis vittata	X		
Umbonia crassicornis	X		

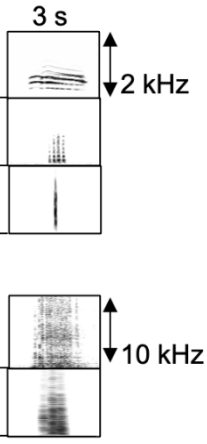

Figure 3.1. 4th-instar nymphs of *E. Binotata* 'Juglans nigra' and one of their ant mutualists (*Formica sp.*). Photo: R. B. Cocroft

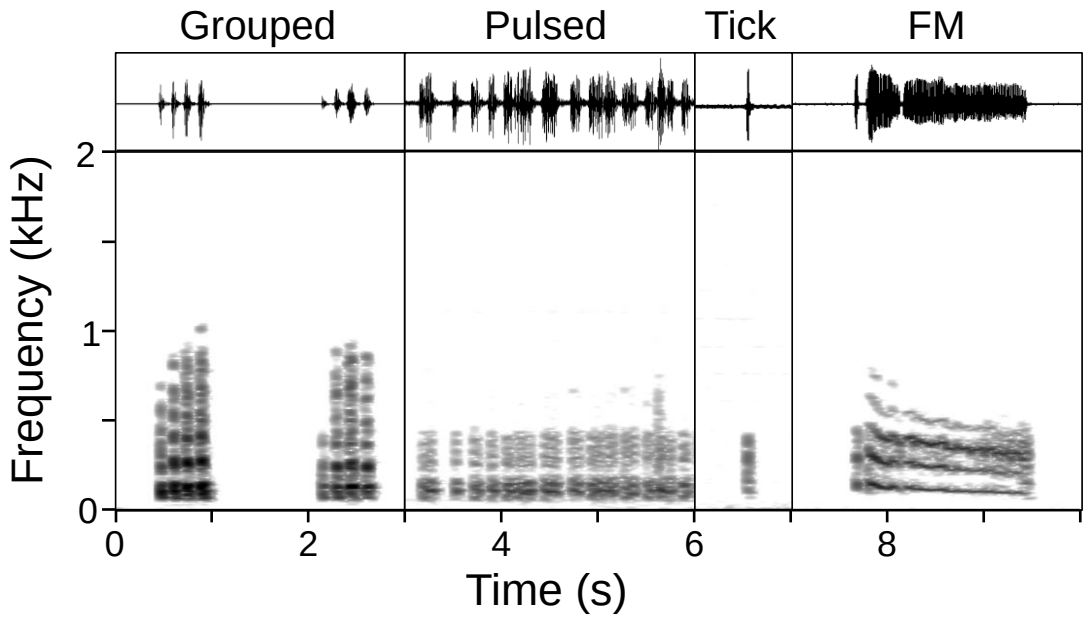

Figure 3.2. Signal types recorded from nymphs of *E. binotata* "Juglans nigra'.

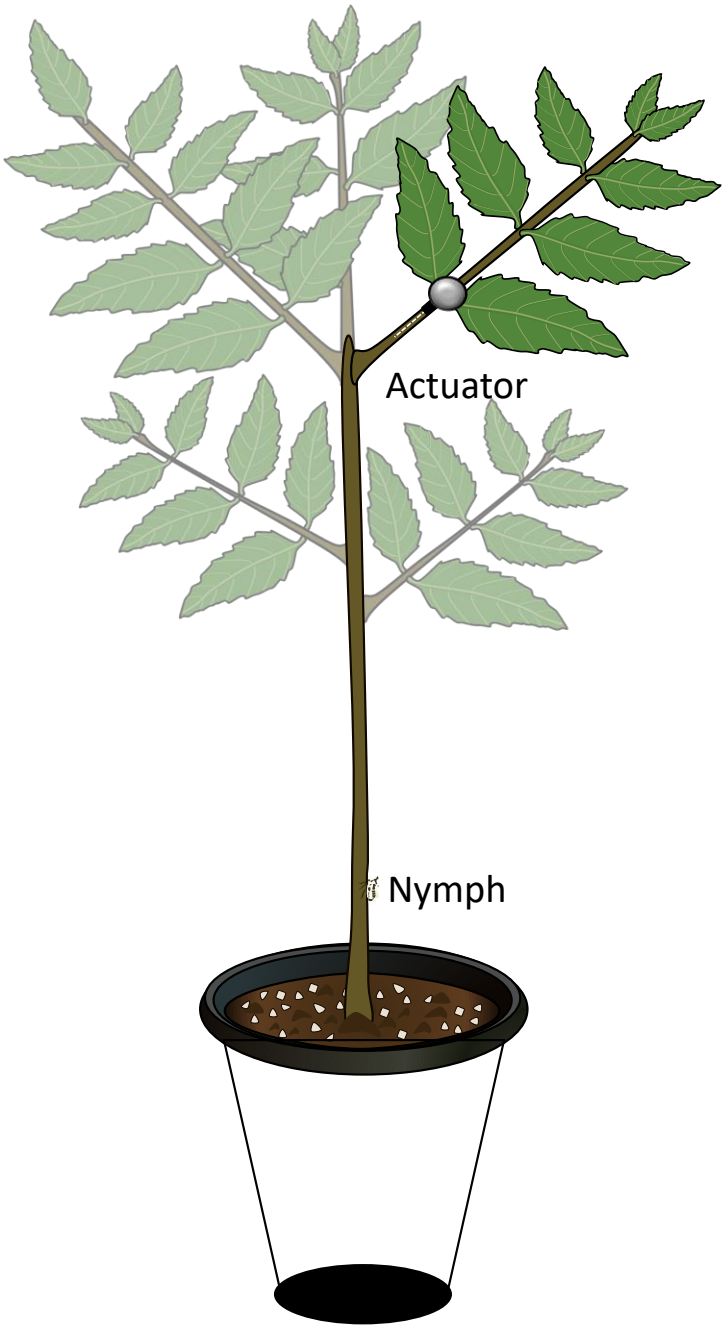

Figure 3.3. Illustration of the playback setup used for testing signal function.

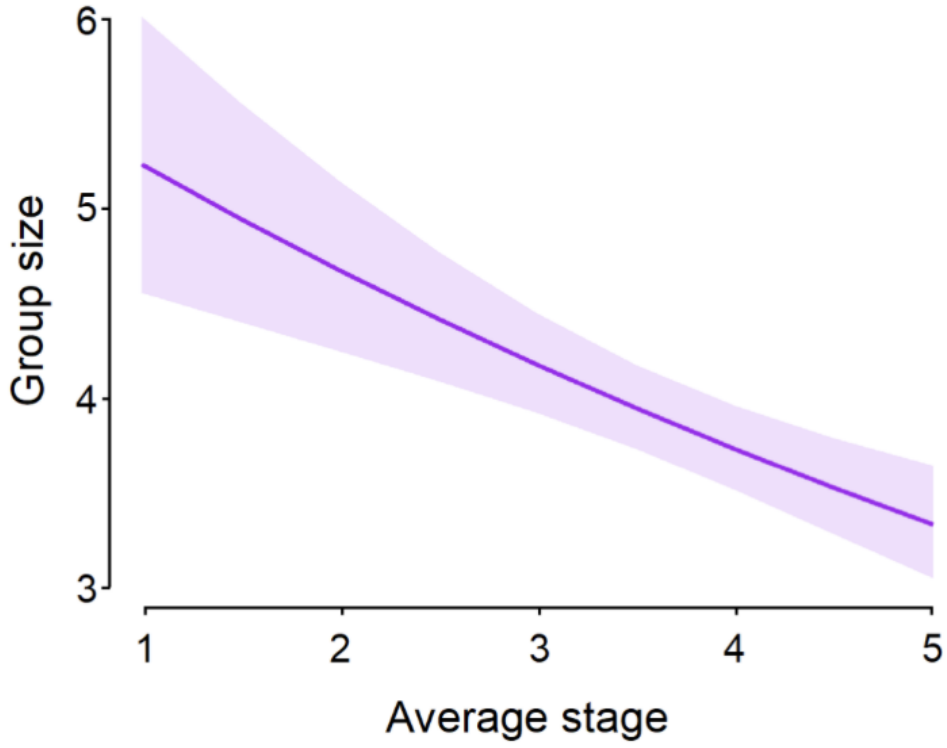

Figure 3.4. Group size decreases as nymphs mature (predicted line and SE from general linear model, year 1 only).

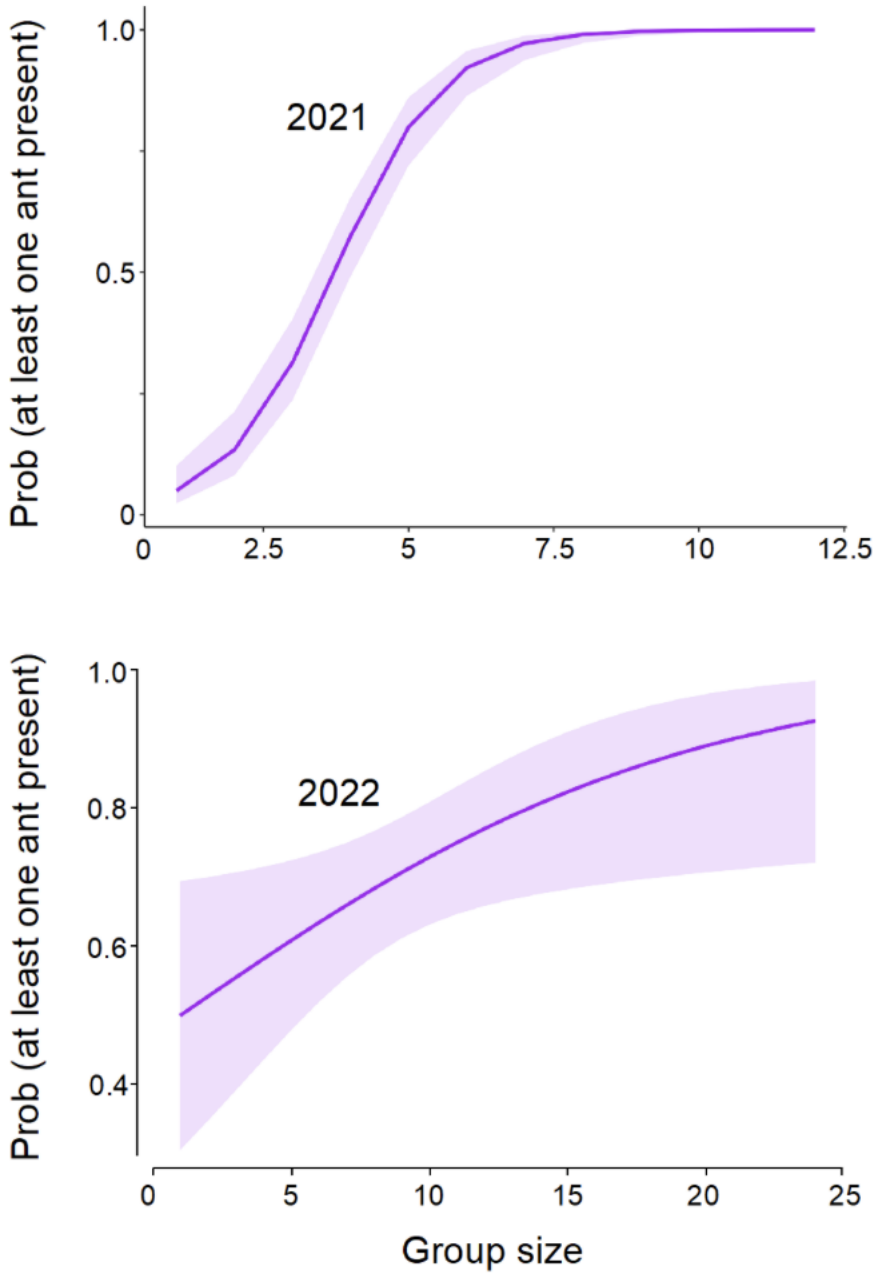

Figure 3.5. Larger groups of nymphs are more likely to be attended by mutualistic ants, although the slope of the relationship varied between years (predicted lines +/- SE; see Table 3.2).

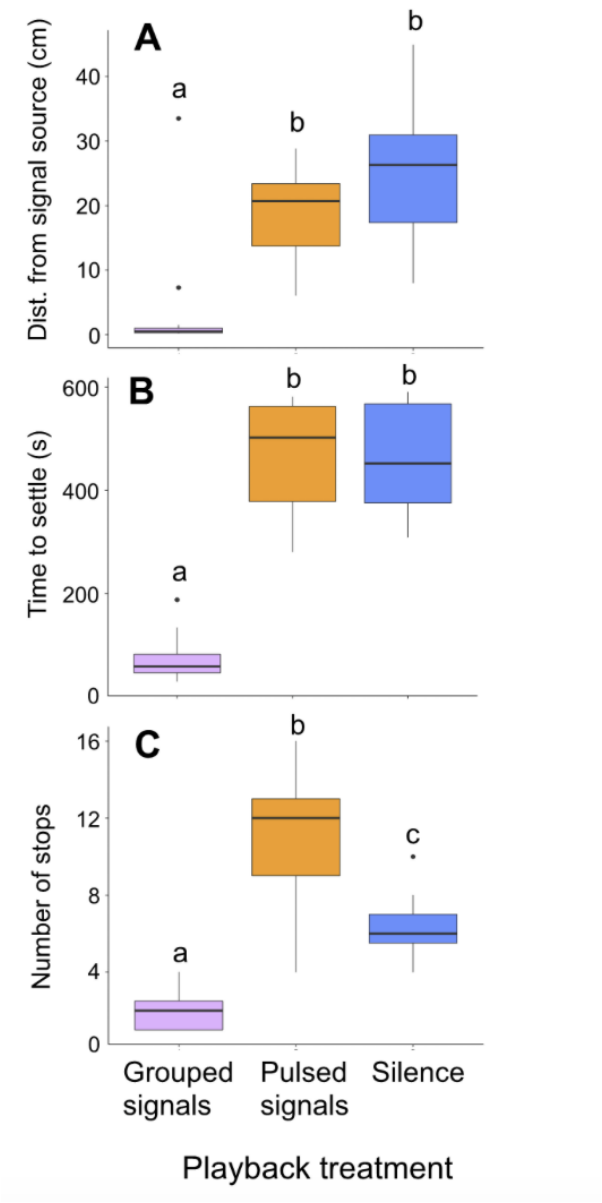

Figure 3.6. Responses of nymphs to playback of two signal types in their repertoire. During playback of grouped signals, nymphs **(A)** closely approached the playback source; **(B)** settled more quickly; and **(C)** made fewer stops before settling than in response to pulsed signals or silence. Within a panel, boxes with different letters are significantly different from each other.

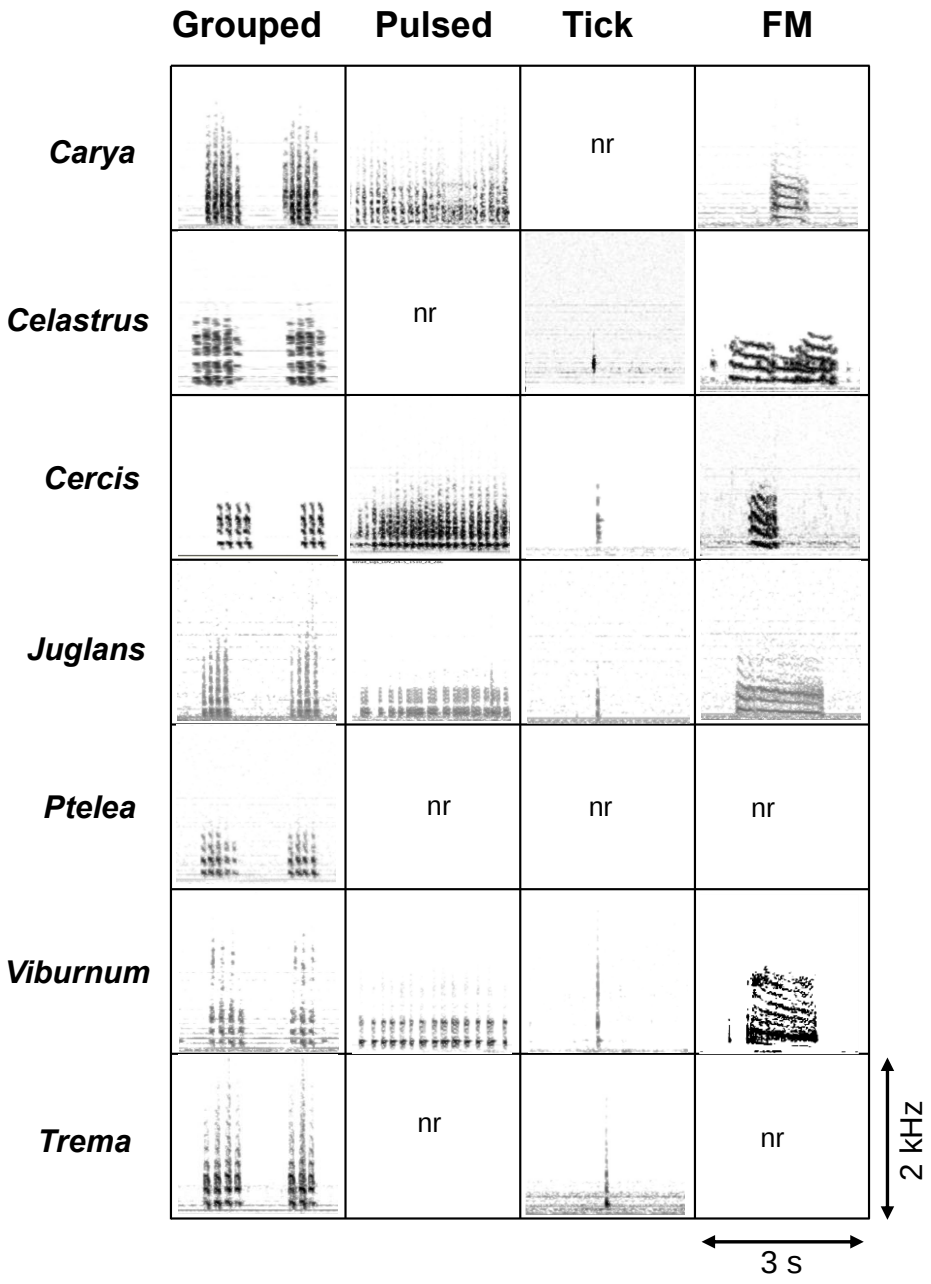

Figure 3.7. Nymphal signal repertoire recorded from seven host-associated species in the *E. binotata* complex, listed by host plant genus. Not all signal types were recorded from all species and 'nr' indicated that a given signal type was not recorded.

CHAPTER 4

Rapid plant movements provide an overlooked form of acoustic cue production in plants

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Abstract

There is great current interest in the field of plant acoustics, including the potential for plants to both perceive acoustic cues and produce them. Woody plants emit ultrasonic clicks under water stress as a result of cavitation, and such clicks have recently been documented in herbaceous plants where they correlate with both water stress and injury. Growing root tips of corn seedlings also produce clicks, in a lower frequency range. I investigated the possibility that rapid plant movements produce acoustic cues in the form of plant-borne vibration, focusing on the rapid leaf-folding movements of the sensitive plant, *Mimosa pudica*. I induced leaf-folding movements while recording vibrations from the leaf base using a laser vibrometer. Having recorded characteristic and relatively high-amplitude vibrations, I then assessed the source of the vibrations: the physiological motor that drives movement, or the contact between moving parts of the leaf. Vibrations were

produced by contact between pinnules and each other and/ or the rachis of the stem. The frequency range and amplitude of the vibrations are comparable to other vibrational cues that both plants and insects have been shown to perceive and respond to. In nature, leaf folding is induced by contact with potential herbivores, and in the laboratory, it has been shown to reduce herbivore damage. Potential receivers of this incidental cue of the presence of an herbivore include predatory insects as well as other leaves on the same or neighboring plants.

Introduction

Plants, like animals, continuously gather information about their environment (Karban 2015; Mescher and de Moraes 2015). Plants respond to various internal and external stimuli, including touch and pressure (Telewski 2006), gravity (Sack 1991), changes to the light environment (e.g., Jiao et al. 2007, Chory 2010), and plant-produced chemicals (Arimura et al. 2000, Baldwin et al. 2006, Heil and Bueno 2007, Heil 2009, Karban and Heil 2010, Karban et al. 2013). Plants also perceive and respond to acoustic and vibrational stimuli (reviewed in Chowdhury et al 2014; Hassanien et al 2014; Fernandez-Jaramillo et al 2018; Frongia and Forti 2020; Allievi et al 2021; Bhandawat & Jayaswall 2022; del Stabile et al 2022; Appel & Cocroft 2023). There is a large literature based on the use of computer-generated pure tones to influence plant traits in the service of agriculture (reviewed in Jung et al 2018). Recently, researchers have explored plant responses to ecologically relevant sources of acoustic stimuli in nature, including the feeding vibrations of caterpillars (Appel & Cocroft 2014; Body et al 2019a, b; Pinto et al 2019; Kollasch et al 2020), soil-borne sound (Gagliano et al 2017), and the vibrations induced by insect flight (Veits et al 2019).

Plants not only respond to acoustic stimuli; they also produce them. The best-known example of sound production occurs in drought-stressed plants, which produce ultrasonic clicks caused by the formation of cavitation bubbles (Ponomarenko et al 2014), and which can be detected at a distance from the plant and reflect the plant's level of hydric stress or injury (Khait et al 2023). Seedling corn plants grown in water produce clicks through an unknown mechanism (Gagliano et al, 2012). Other plant-produced sounds include the sometimes loud airborne sounds produced during explosive seed dispersal (e.g., Ribera et al 2020). Plants also produce sound and vibration when set into motion by wind (Barth 1988; McNett and Cocroft 2010). Although it is unclear whether there are plant processes or structures that have evolved in response to selection to produce sound, some plants produce 'passive' acoustic signals using a structure that reflects ultrasound and attracts bat pollinators (Simon et al 2011).

An overlooked source of potential acoustic production arises from plant-generated movement. Plant movements include the closing of leaflets in compound leaves at night (Rodrigues and Machado 2008), the movement of vine tendrils seeking a structure to attach to (Stolarz 2009), and the movement of floral and leaf structures in response to touch (Forterre 2013). Some of the most famous examples of this rapid movement in plants are the Venus fly trap (*Dionaea muscipula*) closing on an insect, *Codariocalyx motorius* with its rotating leaves thought to deter butterflies from laying eggs on its leaves (Lev-Yadun 2013), and the sensitive plant, *Mimosa pudica* L., folding its leaves in response to mechanical stimuli such as contact with an herbivore (Volkov et al 2010). None of these plant movements is associated with airborne sound. However, although the field of plant acoustics has focused largely on airborne sound, perhaps because our own

reliance on sound and poor sense of vibration makes airborne sounds more salient, plant movements will inevitably produce vibrations within the plant itself, even in the absence of airborne sound.

Mimosa pudica (Fabaceae: Mimosoideae) was named by Carl Linnaeus (1753). However, the leaf folding behavior was described even before that by Jean Jacques d'Ortous de Mairan (1729). Charles Darwin (1880) studied movement in *M. pudica* extensively in the lab, where he described the behavior of M. pudica throughout the course of its development. Bose also studied leaf folding in a laboratory setting (1926) and found that the leaves would fold in response to mechanical stimuli (wounding, wind, vibrations caused by shaking, extreme temperatures, light, and water). The mechanisms of leaf folding have also been well-described (Allen 1969, Coté 1995, Volkov 2010a, Volkov 2010b, Visnovitz et al. 2007). In contrast, the function of leaf folding is still not well understood. Current hypotheses for why *M. pudica* leaves fold in response to touch are to reduce predation by decreasing plant visibility (Braam 2005) or exposing thorns (Eisner 1981), and experimental immobilization of leaves increased tissue loss to herbivorous insects (Hagihara et al 2022).

Here I investigate the vibrations produced by *M. pudica* during leaf closing. Any vibrations produced are likely incidental, rather than an evolved feature of the leaf-closing process. However, incidental cues often play an important role in organismal interactions (Danchin et al 2004), and vibrations produced by folding leaves of M. *pudica* could, for example, provide cues to neighboring leaves or alert predatory insects on the same plant to the presence and location of herbivorous prey. I first characterized the vibrations produced during leaf closing, evoked by cutting the distal most pinnule, and

recorded vibrations on the petiole using a laser vibrometer. I then determined whether vibrations were produced by the 'motor' that drives movement or only by the contact of moving pinnules with each other or the leaf rachis.

Methods

I grew *Mimosa pudica* plants in the greenhouse (16L:8D) to a height of ~25 cm and a crown size of approximately [35] cm in diameter. To record vibrations associated with leaf folding I moved plants to a vibration isolation table (Vibraplane, Kinetic Systems, Boston, MA) in a temperature-controlled laboratory room (24 ± 1o C) and let them acclimate under a grow light (Sunblaze T540, 48", Intertek, OH) for 2h. Leaves folded during transport but re-opened after about 30 min.

I recorded leaf-folding vibrations using a Polytec PDV-100 laser vibrometer (Polytec, Inc, MA), acquiring the signal using a TASCAM 20 X 20 interface (Teac Corporation, CA, USA) and an iMac computer using Audacity v. 3.1 audio software. I calibrated the input using an oscilloscope (Tenma 72-2580, Tenma Test Equipment, OH) to achieve a 1:1 gain level, enabling conversion of the recorded laser output from mV to mm/s.

To standardize leaf choice, I selected the first mature leaf on the tallest stem. I recorded from base of the leaf, corresponding to the secondary pulvinus, after placing a small (5 mm^2) piece of reflective tape on the upper surface to increase reflectance of the laser signal. Leaves also folded during attachment of the reflective tape, so I waited an additional 30 min before recording.

Leaf folding in *M. pudica* involves two different structures: the pinnules, which close together around the central rachis; and the secondary pulvinus, which drops the entire leaf, changing the angle between the petiole and the main stem (**Figure 4.1**). Because dropping of the petiole under control of the secondary pulvinus would not allow the laser to remain focused on one spot on the leaf, before recording I stimulated the leaf to drop by touching the lower surface of the secondary pulvinus with a watercolor brush. I then elicited pinnule folding by cutting off one-third of one pinnule in the distal-most pair of one of the outer pinnae.

Identifying the vibration source

In principle, vibrations could be generated by the motor apparatus in the primary and secondary pulvini, or by contact of the pinnules with each other and the central rachis. Which of these is involved can be determined by the timing of the vibration relative to the pinnule motion: if vibration is produced during movement but before the pinnule has contacted another surface, this would support the hypothesis that vibration arises from operation of the motor units. In contrast, if the vibration is not produced while the pinnule is in motion but only after it has contacted the central rachis and/or other pinnules, this would support the hypothesis that vibration was caused by contact between different parts of the leaf.

Additional evidence to distinguish between the two potential sources of vibration comes from the direction of spread of the folding movements along the rachis. Pinnule folding proceeds in an orderly sequence from one pinnule to the next, regardless of whether the folding begins at the base or the apex of the rachis. However, the pattern of

contact between pinnules is very different in the two situations. When folding begins at the apex of the rachis, each pinnule moves freely until it contacts the rachis. In contrast, when folding begins at the base of the rachis, each pinnule contacts other, noy-yet-folded pinnules before it contacts the rachis. There is thus considerably more contact between pinnules when the folding begins at the base of the leaf than when it begins at the apex. Accordingly, if contact between pinnules or between pinnules and the rachis is responsible for the vibration, then vibration output should differ depending on the direction of spread of folding.

To test for a difference in vibration output depending on the direction of spread of folding, I chose leaves with only two pinnae. When a pinnule is cut at the apex of one rachis, pinnule folding spreads down that rachis, then up from the base of the neighboring rachis. Accordingly, the first pinnule closes beginning at the apex and the neighboring rachises fold beginning at the base. Choosing leaves with two rachises and cutting a pinnule at the apex of one rachis thus provides a recording of each member of the pair, but with the spread of folding in opposite directions. For each of the leaves I recorded (n=20 Plants, one leaf per plant), I cut a pinnule at the apex of one rachis; then, after the leaf re-opened, I cut a pinnule at the apex of the other rachis. The result was a recording of both rachises, each folding first in an apex-to-base direction and then in a base-to-apex direction.

I calculated the total energy of the vibrations produced during leaf-folding events as the sum of the squared samples; note that I used the total rather than the average energy because there was variable amount of silent time between the folding of neighboring rachises. Because both rachises from a single plant were recorded in both

folding directions, I first averaged the values from the two rachises within a plant, then assessed the normality of the distribution of energy values using an Anderson-Darling test. I then compared the amplitude of vibrations produced by the two directions of leaf folding using a general linear model (amplitude = intercept + plant identity (random) + folding direction). In addition, to facilitate comparison of the amplitude of leaf-folding vibrations to other vibrations reported in the literature, I also measured the peak amplitude of each recording after excluding the occasional impulses whose value was clipped.

Finally, I qualitatively compared the vibrations produced by experimental contact between pinnules with those produced by natural leaf-folding events. To simulate natural contact between pinnules, I lightly contacted unfolded pinnules with a pinnule on a rachis removed from another plant. This light contact did not cause the contacted pinnules to fold.

I characterized the frequency spectrum and amplitude of the recorded vibrations using custom-written scripts in Matlab v. 2022a (Mathworks, Inc., Natick, MA, USA).

Results

When the leaves of *M. pudica* closed in response to mechanical injury, they produced vibrations with a broad band of frequencies, mostly in the 100 - 5000 Hz range (**Figure 4.2**). At a finer temporal scale, bursts of vibration occurred as each pinnule made contact with the rachis or with neighboring pinnules. This pattern was easiest to observe when individual pinnule pairs folded freely, unimpeded by other pinnules, and contacted only the rachis (**Figures 4.3, 4.4**).

The distribution of energy values did not depart from a normal distribution (Anderson-Darling test, n=20, p = 0.30). The energy of the emitted vibrations was higher when folding was initiated at the base of the rachis than at the apex (**Table 4.1; Figure 4.5**). Furthermore, experimental contact between pinnules generated vibrations with a frequency spectrum equivalent to that of leaf-folding events (**Figure 4.6**). The average peak amplitude of the recordings was 3.8 ± 0.89 mm/s.

Discussion

The famous leaf-folding of the sensitive plant, *Mimosa pudica*, is accompanied by characteristic vibrations with a broad frequency spectrum and a relatively high amplitude. The leaves of *M. pudica* are doubly compound, with two or four rachises containing multiple pairs of pinnules that close in sequence as the electrical signal propagates along the rachis (Volkov et al. 2010). I showed that the vibrations originate not from the motor that drives the movement of the pinnules, but from contact between the pinnules and each other and the rachis. This conclusion is based on three lines of evidence: first, during the closing of an individual pair of pinnules, no vibration was detected until the end of the pinnule movement, when the pinnule contacts the rachis. Second, the vibrations were higher in amplitude when the first pinnule to fold is at the base of the rachis, rather than the tip; when closing begins at the tip of the rachis, the pinnules fold directly against the rachis, but when closing begins at the base of the rachis, the pinnules fold against other pinnules that have not yet begun to fold, a process that causes greater contact between pinnules. Third, the vibration spectrum was qualitatively replicated by experimental contact between pinnules.

The field of plant acoustics has long been in search of plant-generated acoustic signals. The best-known acoustic emissions of plants are the ultrasonic clicks produced by drying plants, through cavitation and other processes (Ponomarenko et al 2014). Production of these clicks has recently been shown to increase in drought-stressed or injured plants, and to be detectable as airborne sound near the plant (Khait et al 2023). In addition, seedling corn plants growing in water produce clicks from near the root tip, and these clicks have most of their energy in much lower frequencies, mostly under 1 kHz (Gagliano et al 2012).

The present study adds a previously source of acoustic production in plants: rapid plant movements. Although these movements do not generate audible sound, they do generate distinctive vibrations within the plant. These acoustic events have likely gone undetected because plant acoustic researchers have been focused on a search for airborne sounds. Although airborne sound is salient to us because of our hearing- and vision-centered sensory world, plants and many other organisms perceive airborne sound only indirectly, when it induces vibration of plant tissue and can be detected by mechanoreceptors. Instead, what plants and most plant-dwelling arthropods perceive is vibration of plant stems and leaves (see Appel and Cocroft 2023 for further discussion).

The outstanding issue for all studies of plant acoustic production to date, including this one, is whether any organisms (other than eavesdropping humans) make use of the acoustic information generated by the plant. It is possible that all plant-generated vibrations found to date are incidental cues, rather than signals that have evolved to influence the decisions of other organisms. In the case of *M. pudica* leaves, the incidental nature of the vibrations is obvious. Whether the clicks produced by growing

roots are likewise incidental is less clear because it is not known how they are produced. In any case, incidental cues produced by movement, feeding and other processes can be important in the interactions among animals (Danchin et al. 2004). Rather than treating incidental cues as if they were communication signals on the one hand or meaningless by-products on the other, we should ask what the potential receivers of those cues are, and what significance the perception of these cues might have for those receivers.

What are the potential receivers of the vibrations produced by *M. pudica* leaves, and what significance might these acoustic cues of leaf-folding have for those receivers? The first and most likely set of receivers are the other leave on the plant itself. But would other leaves be able to perceive these vibrations, and why would it matter? The answer to whether other leaves can perceive the vibrations is almost certainly yes, based on the similarity of leaf-folding vibrations to other vibrations perceived by plants. In particular, the frequency spectrum of leaf-folding vibrations is similar to that of the incidental vibrations produced in leaves by feeding caterpillars (**Figure 4.7**). Furthermore, the amplitude of leaf-folding vibrations is also comparable to that of a feeding caterpillar: the average peak velocity of leaf-folding vibrations was comparable to the average peak velocities of caterpillar feeding vibrations (leaf-folding: 3.8 mm/s; caterpillars: 1-3 mm/s; Kollasch et al 2020). For two plant species (*Arabidopsis thaliana* and *Nicotiana tabacum*), plants not only perceive caterpillar feeding vibrations, but also respond to them in an ecologically relevant way, by priming or directly increasing chemical anti-herbivore defenses (Appel & Cocroft 2014; Body et al 2019a, b; Kollasch et al 2020; Pinto et al 2019). Accordingly, if leaves can perceive caterpillar feeding vibrations, they should be able to perceive other vibrations of similar frequency and amplitude. But why would it

matter? Plants have many forms of within-plant signaling, from airborne volatiles to electrical signals to ROS waves to hormones to hydraulic waves (reviewed in Gilroy et al 2018). These within-plant signals are important because an event that occurs in part of a plant -- such as an herbivore attack -- can soon influence other parts of the same plant. Likewise, an event that causes one leaf of a sensitive plant to fold – such as contact by an herbivorous insect - may be relevant to other leaves on the same plant. This hypothesis can be tested by playing back leaf-folding vibrations to otherwise undisturbed leaves and asking whether those leaves fold, or fold more rapidly when they experience another stimulus.

Predatory insects on the same or a neighboring plant in contact are another likely set of receivers for leaf-folding vibrations because a plant behavior caused by contact with an herbivore provides a potential cue of the location of a prey item. Again, by comparison with caterpillar feeding vibrations, it is clear that predator insects such as pentatomid bugs can perceive similar vibrations, because at least one predator uses caterpillar feeding vibrations to locate and attack the feeding caterpillar (Pfannenstiel et al 1991). This idea can also be tested using vibrational playbacks.

The set of vibrations experienced by a living plant in a natural environment – its vibroscape – is arguably among the richest acoustic landscapes in its diversity of vibrational events (Sturm et al 2021). First, because plant leaves are good absorbers of airborne sound, the vibrations of a leaf contain a filtered but otherwise faithful representation of the local airborne soundscape (D'Allesandro et al 2015). Second, for the majority of terrestrial plants that are rooted in the ground, the plant also receives soil-borne sound that can cause above-ground tissues to vibrate (Sturm et al 2021). Third, the

rich arthropod community associated with plants generates plant-borne vibrations when individuals signal on, feed on, or move over the plant (Cocroft and Rodriguez 2005; Appel & Cocroft 2023). The result is a vibrational environment that constitutes a nexus of what are usually considered three distinct soundscapes, representing airborne sound, soil-borne sound, and arthropod-generated plant vibrations. In nature, leaves and the vibration-sensitive animals that live in or on plants experience one of the richest acoustic landscapes encountered by any organism. This study adds yet another acoustic event to this complex acoustic world, at least for plants such as *M. pudica* that are capable of rapid movements.

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Tables and Figures

Table 4.1. Results of general linear model for fixed effects (plant identity was included as a random factor to control for individual variation among plants).

Effect	**Estimate**	**SE**	**t**	**DF**	**p**
Intercept	55.89	1.10	50.81	18	<0.0001
Folding direction	-7.95	0.615	-12.93	18	<0.0001

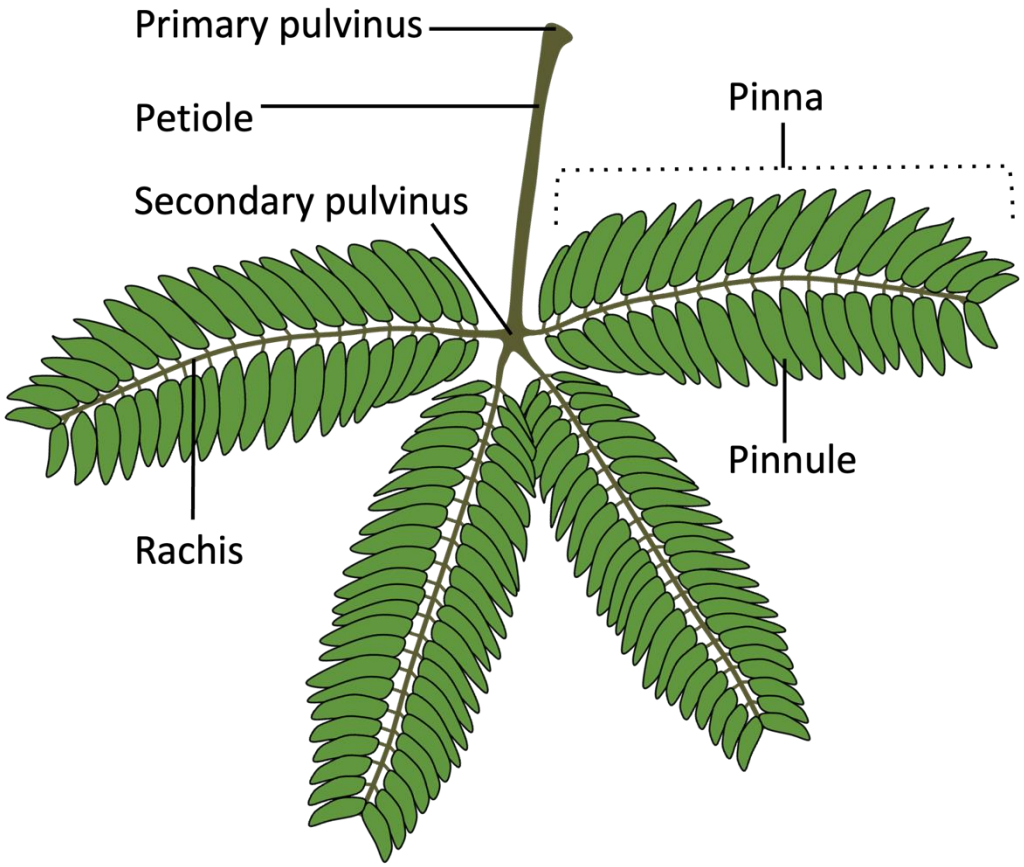

Figure 4.1. Illustration of a *M. pudica* leaf, showing the location where laser recordings were made (from the reflective tape attached to the secondary pulvinus).

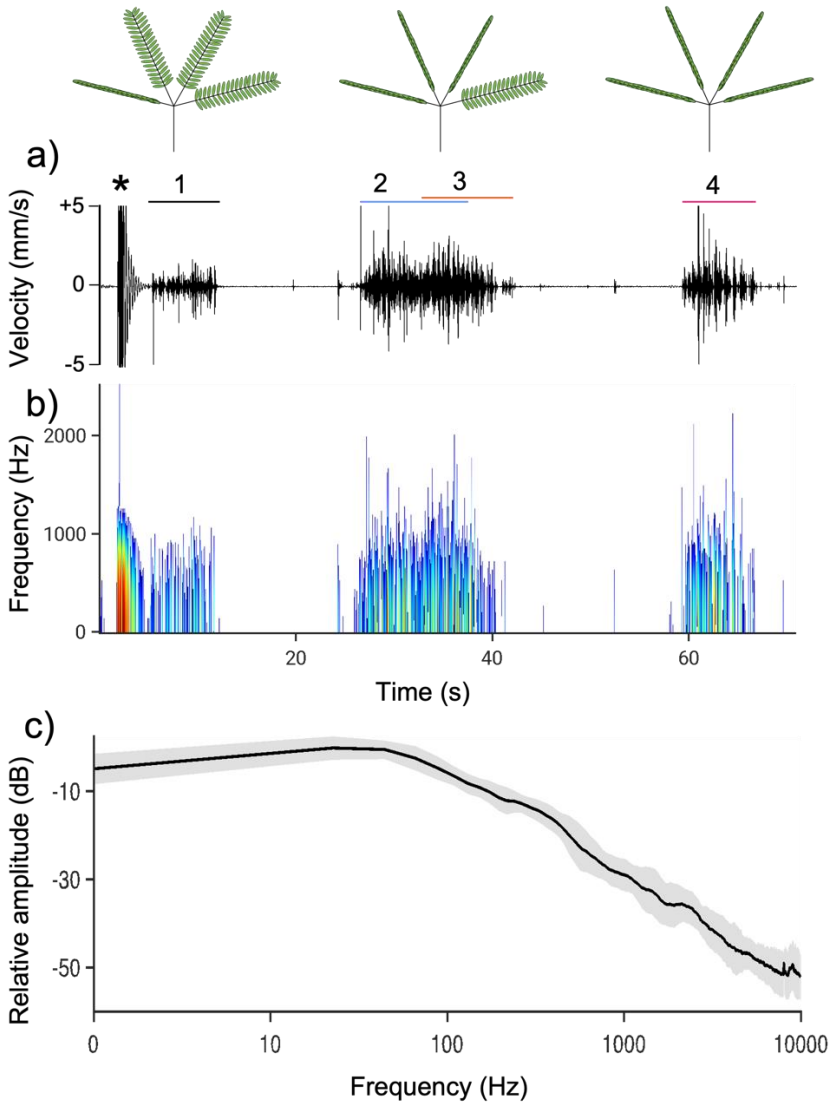

Figure 4.2. Vibrations produced by closing *M. pudica* leaves. **(a)** Example from one plant. The asterisk shows when one pinnule was cut, and the lines above the waveform indicate the time when pinnules on each rachis began and finished closing. **(b)** Spectrogram of the recording shown in (a). **(c)** Mean amplitude spectrum (± SD) of leaf-closing vibrations (N=20 plants, 1 leaf/plant).

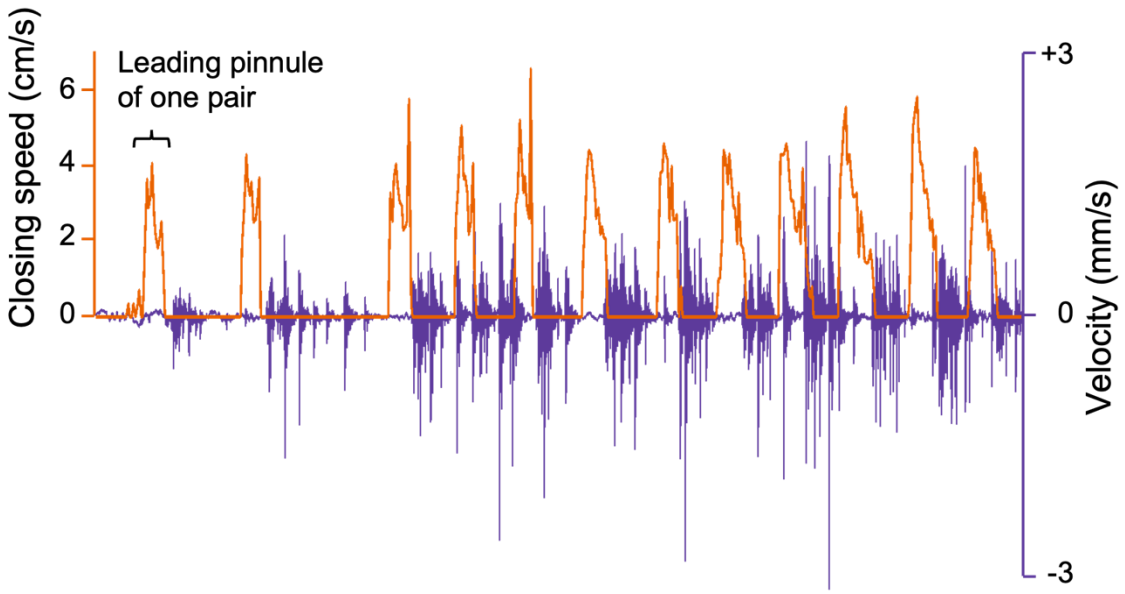

Figure 4.3. Temporal relationship between pinnule closing movements and the vibrations produced, in an example from one rachis with pinnule folding starting from the apex of the rachis. The orange line shows the speed of movement of the leading pinnule of each pair along the rachis.

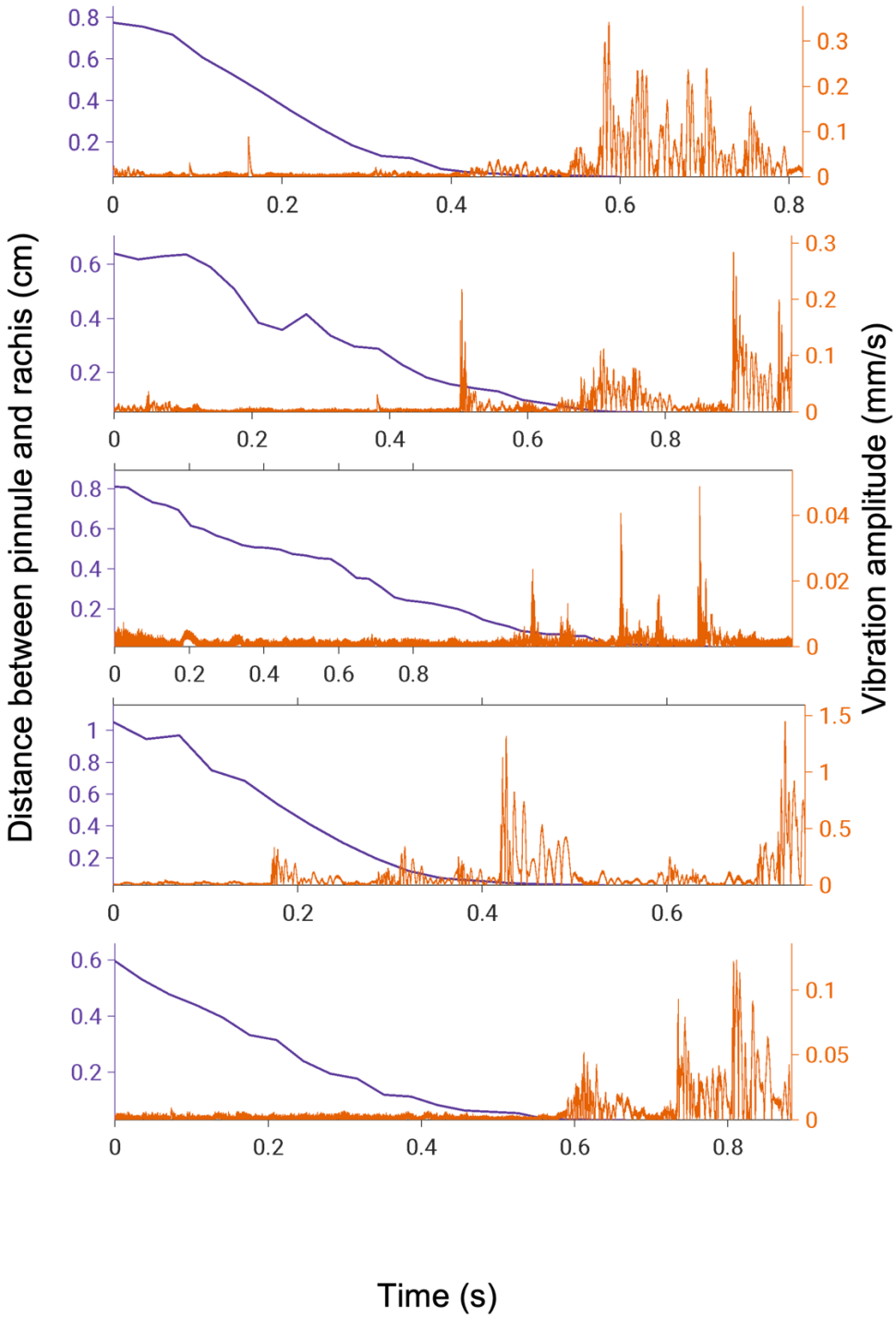

Figure 4.4. Vibrations are produced once the pinnule contacts the rachis, as shown here with examples of one pinnule pair from each of five plants.

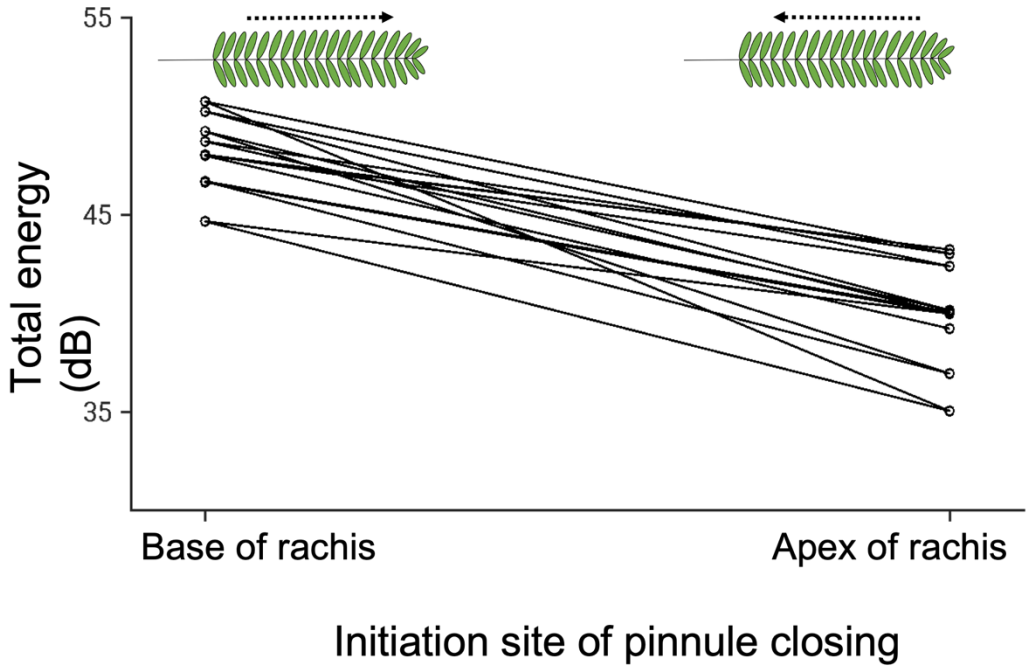

Figure 4.5. Leaf folding produces higher-amplitude vibrations when pinnule closing begins at the base of the rachis, rather than the apex (n=10 plants, $p<0.001$).

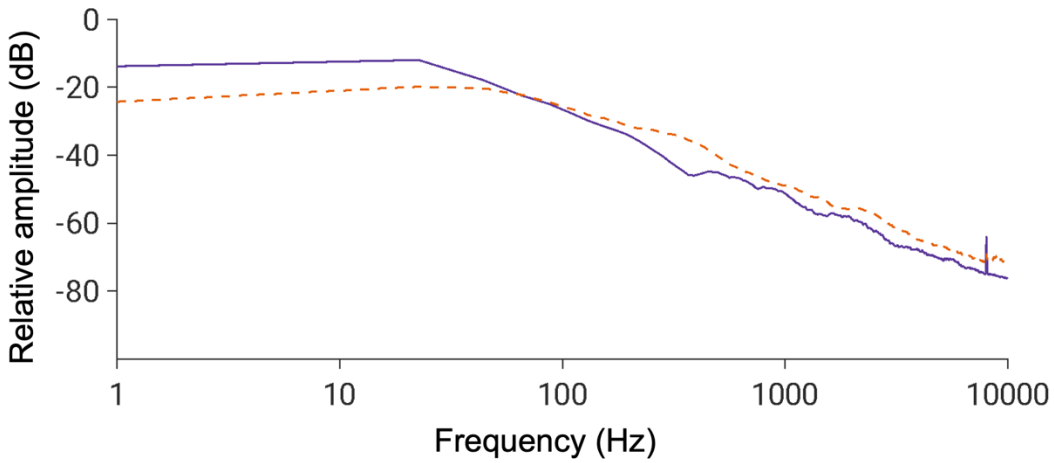

Figure 4.6. Experimental rachis pinnule contact (solid purple line ± SD) produces a frequency spectrum similar to that of natural leaf-folding events (dotted orange line ± SD, from Fig. 4.1C).

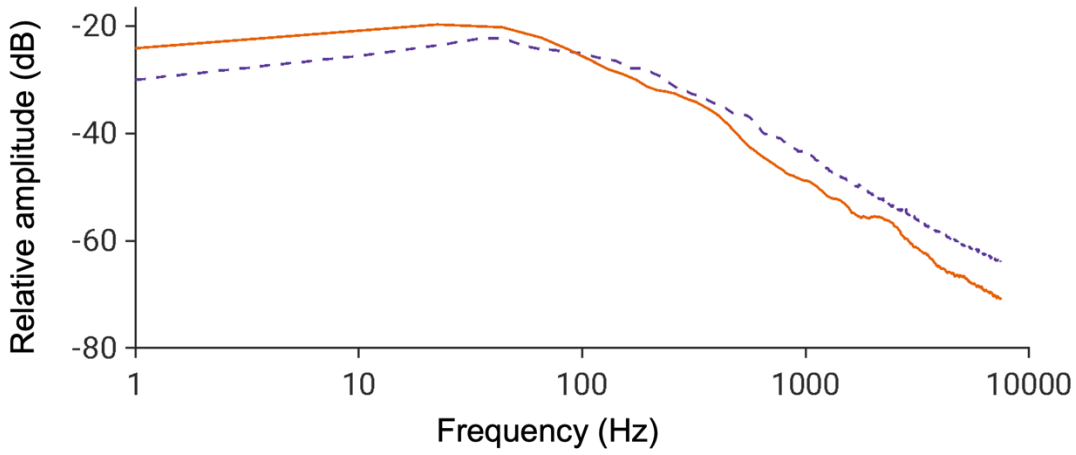

Figure 4.7. Leaf-folding vibrations of *M. pudica* (orange solid line) have a similar frequency spectrum to those of leaf-feeding caterpillars (purple dotted line = average spectrum derived from data in Kolasch et al 2020, Fig. 4.2a).

VITA

Sabrina Christine Jerlean Michael came into this world on a chilly winter day, January 24th, 1990, in the tranquil town of Carlsbad, New Mexico. Her parents, Gary Michael Sr., and Darla Williams, welcomed her into a bustling family of seven, making her the youngest of five siblings. She had two older brothers and two older sisters who, even at a young age, became her playmates, protectors, and teachers. Sabrina's arrival added a new chapter to their family's story.

Each sibling brought their own unique influence on Sabrina's early years. Michael, her older brother, ignited her taste buds for spicy food and chili dogs. Gary Dean, her other older brother, was the gateway to a different dimension of sound. He introduced Sabrina to the electrifying universe of rock music. Sarah, her older sister, embodied grace, style, and charm. Sabrina looked up to her with admiration. Monica, her other sister, connected with Sabrina through dance. Monica loved to make dances up (she was doing it before TikTok) and would often share her love of dance with Sabrina. They would create and dance to beloved boy bands like the Backstreet Boys and NSYNC, and of course Britney Spears.

In 1994, the family welcomed a new addition, Gary Daniel (Steel Toe), into their already chaotic household. Sabrina eagerly embraced her role as a big sister, nurturing a special bond with her youngest sibling as they embarked on adventures of their own. Their shared interests brought them even closer. They had a penchant for watching movies and TV shows together, and their favorites included "Blue's Clues," the zany antics of Ace Ventura, and the hilariously iconic Austin Powers movies. One of their

shared traditions was music time, where Sabrina would introduce her youngest brother to her favorite tunes. Britney Spears' infectious pop hits became a staple of these sessions. Sabrina would play Britney's CDs until her little brother could no longer stand it, sparking (mostly) playful arguments. The memories of these shared experiences continued to bring warmth and nostalgia to Sabrina's heart as she embarked on her own path.

Sabrina's parents, Gary Sr. and Darla, each played distinctive roles in her upbringing, leaving indelible marks on her character and values. Gary Sr., a hardworking man with deep roots in his community, imparted to Sabrina the values of responsibility and compassion from a young age. Working in the oil field, he had not attended college himself, but he possessed a profound belief in his daughter's potential. He encouraged Sabrina to reach for the stars and make her dreams a reality, emphasizing that she had the capability to transcend the boundaries of their small Carlsbad hometown. Gary Sr.'s faith in Sabrina was persistent, and he would even reward her financially for perfect attendance. This incentive motivated Sabrina to prioritize her education—so much so that she would try to attend while actively sick. As her dad taught her, don’t sweat the small stuff and after all, it is *all* small stuff.

Darla, Sabrina's mother, was the heart of their home. She had a culinary talent that was unparalleled, especially when it came to dishes like green chile stew, enchiladas, and all breakfast foods. Green chile was not just a delicious dish for Sabrina; it was her go-to comfort food during bouts of illness, plus it goes with most everything! Sabrina's fondest memories of her mother included Darla's passion for collecting miniatures, their shared moments watching "Maury," and her enthusiastic support for Sabrina's collection of

Britney Spears merchandise. Darla taught Sabrina to thoroughly enjoy life and to appreciate her own worth. "Treat yourself" was a mantra that resonated deeply with Sabrina, a philosophy she carried with her throughout her life.

Carlsbad, a small town nestled in the southeastern corner of New Mexico, was the perfect setting for Sabrina's early years. Its serene atmosphere served as the backdrop to her childhood adventures. Sabrina's early years in Carlsbad were filled with simple joys – riding bikes with her siblings, exploring nearby and savoring the flavors of New Mexican cuisine at family gatherings.

Sabrina's childhood was infused with a deep love for the outdoors. She would spend countless hours outside swinging, skipping rope, or running through the sprinklers on grass that was hardly ever green (she did grow up in the desert after all). One of her favorite pastimes was playing in her sandbox. Sabrina had a keen interest in the tiny inhabitants of the earth. She would watch ants for hours on end, observing their intricate trails and industrious work ethic. Her fascination with these little creatures was a testament to her curiosity and her ability to find wonder in the seemingly mundane.

Sabrina also loved being around water, which is hard to come by in Southern New Mexico. From the earliest days of her childhood, the sight of water alone brought her a sense of peace. Whether it was a meandering river, a tranquil pond, or even a swimming pool, water had an enchanting allure for her. Her affinity for water would later become a defining part of her life, shaping her choices, and leading her to become a scuba diving instructor and spending seven summers in the Caribbean.

Sabrina has loved learning and school from the very first day she set foot in a school. Her kindergarten classroom was where Sabrina's love for education ignited like a

flame, a passion that continues to burn brightly to this day. Her first day at school was a momentous occasion that left an inerasable mark on her young heart. The joy she experienced in the classroom on that day set the stage for a lifelong love affair with learning. Sabrina's passion and dedication to education was evident even beyond the school hours. At bedtime, she would immerse herself in the world of numbers, diligently repeating addition and multiplication problems out loud. Her bedroom became a sanctuary of intellectual exploration, a place where her love for mathematics found its voice. So fervent was her commitment that her parents would often have to gently remind her to lower her voice and let the night find its peace. There was one part of school that Sabrina struggled with—fitting in with the other kids. She always struggled to find a sense of belonging.

Her father, Gary Sr., eventually remarried to Terri Walker, who became a pivotal figure in Sabrina's life. Terri not only became Sabrina's stepmother but also played a crucial role in raising her into adulthood. One of the highlights of this new chapter in Sabrina's life was spending time with her stepmother and three new stepbrothers, Brad, David, and Cody. Together, they created a blended family. One of their favorite activities was tubing and jet-skiing on the Pecos River. In addition to their river outings, Sabrina and her newfound family also shared a love for rodeos, as all three of her stepbrothers were bull riders.

Sabrina's academic journey began at Carlsbad High School, where she proudly graduated in 2008. This achievement was a source of immense pride not only for Sabrina but also for her family, who had been rooting for her every step of the way. It was a significant milestone, marking a departure from the paths many of her family members

had taken. During her high school years, Sabrina took on a job at the local movie theater as a concessionist. Sabrina's time at the theater left her stained with butter and memories. It was here that she honed her work ethic and responsibility while juggling the demands of her studies. She would spend her time in between showings working on her Calculus homework.

The summer before her sophomore year of high school, Sabrina decided to take a science class, a decision that would change the course of Sabrina's life forever. This fateful choice would open doors to new possibilities and ignite a passion that would shape her academic and career trajectory in ways she could have never imagined.

Sabrina's unexpected journey into the world of science began with a class called SERP, the Summer Ecology Research Project. At the outset, her motivation was simple: she needed two science requirements to graduate, and she wasn't naturally inclined towards science. Her plan was to complete this class to avoid taking more science courses and make room for more theater classes, a passion that had always been close to her heart.

Under the guidance of their dedicated teacher, Michelle Perry, the SERP class embarked on an adventure that took them to the Gila Mountains. They camped, explored streams and ponds, and even delved into the depths of caves. The essence of their class was to collectively ask questions and use the scientific method to find answers.

Sabrina was drawn to this process of inquiry and curiosity. She began to observe subtle differences in cave crickets' morphology based on the type of cave they inhabited. Excited and intrigued, she approached Mrs. Perry with her observations. Instead of simply providing answers, Mrs. Perry encouraged Sabrina to think critically and said, "I

don't know the answer to that question, but let's talk about how we might find out." This response fostered Sabrina's natural curiosity and lit a spark within her.

Inspired by her newfound interest, Sabrina decided to pursue her own research project for the science fair, though it was not a requirement. She delved into the study of morphological and behavioral differences in cave cricket species between limestone caves and gypsum caves. To her astonishment, her research earned her first place at the regional science fair and later at the state level in New Mexico. This extraordinary achievement opened doors for her, allowing her to participate in the international science and engineering fair for three consecutive years in different cities, including Indianapolis, Indiana; Albuquerque, New Mexico; and Atlanta, Georgia.

The next chapter of Sabrina's life began in a beautiful sunny city, Las Cruces, New Mexico, where she began her academic journey at New Mexico State University. Here, she began working in a plant pathology and weed science research lab immersing herself in the fascinating study of plants and their diseases. Little did she know she would end up working with plants again later on in life. This marked Sabrina’s initial foray into collegiate research, which kindled her passion for scientific research.

During her time at university, Sabrina chose to participate in a study abroad program in Ronda, Spain. Her host family graciously made her feel at home with a homecooked meal as soon as she arrived. For six months, she called Spain home, submersing herself in of Spanish culture, tradition, and language. Sabrina was determined to learn Spanish and absorb every facet of the Spanish way of life. She forged meaningful connections with locals, dined on delectable tapas, and danced to the rhythm of Flamenco music.

Beyond the borders of Spain, Sabrina's adventurous spirit led her to explore neighboring countries. She traveled to the enchanting green landscapes of Ireland, the busy and loud streets of London, and the majestic views of Scotland. Each of these travels added another layer to her ever-expanding worldview.

Subsequently, Sabrina moved to Portales, New Mexico, to attend Eastern New Mexico University. Here, she successfully completed her Bachelor of Arts degree in Biology with a minor in Spanish. During her time at ENMU, Sabrina carried out a comprehensive study of a hermaphroditic species of algae, meticulously analyzing the variations in sex ratios under diverse environmental conditions. Her passion for research continued to flourish, greatly nurtured by her mentor, Dr. Marvin Lutnesky. Under his guidance and encouragement, Sabrina made the decision to pursue higher education, and thus, she remained at ENMU, ultimately achieving her Master of Science degree in biology.

While pursuing her master’s degree, Sabrina discovered her true research niche—collective behavior, which she would continue to explore during her PhD. She became captivated by the intricate dynamics of how groups of animals interacted and the myriad of factors that could influence these interactions. Her thesis focused on fish shoaling behavior. She studied intricate dynamics of how water quality influenced the collective behavior of fish shoals adding another layer to her burgeoning expertise in the field of biology.

Sabrina took a programming class to assist with a part of her thesis. She found herself among a mere three girls in a class of 50. This glaring gender imbalance struck a chord with her, as she once again felt out of place and like she did not belong there. In

response, she founded an organization aimed at creating a supportive network for women in STEM fields.

The programming course proved helpful as she acquired programming skills and, co-authored a Python software program. This program allowed her to meticulously track fish, providing her with the means to measure the precise distances between individual fish within their shoals.

While pursuing her master's degree, Sabrina encountered an unexpected twist in her academic journey—teaching. Initially, she had envisioned herself solely as a dedicated researcher, with little inclination towards the world of education. However, her perspective underwent a transformative shift on her very first day in the classroom. Assigned to instruct a biology class intended for non-biology majors, Sabrina entered the room with a preconceived notion that her students might not share her enthusiasm for the subject. To her delightful surprise, this assumption couldn't have been further from the truth. Her students, far from indifferent, displayed a genuine eagerness to learn. The truth is, she learned a great deal from her students—they helped her discover her profound passion for teaching. She had never felt more at home as she did while she stood in front of that classroom. This was what she had been missing in her life, a sense of belonging. She found that in teaching—she belongs in front of a class, teaching students.

Her passion for teaching extended beyond her own fulfillment; it had a tangible impact on her students. Two of them were so inspired by Sabrina's instruction that they made the life-altering decision to switch their majors to biology. This heartening affirmation of her influence cemented Sabrina's resolve to pursue a career as a college

professor, where she could continue to inspire, nurture, and learn alongside her students on a lifelong journey of discovery.

Sabrina's passion for teaching led to her appointment as an instructor for the Upward Bound program at ENMU. In this role, she took on the responsibility of educating middle school and high school students in both biology and English. The program was specifically designed to support first-generation, low-income students, making it a particularly impactful initiative. Teaching within the Upward Bound program presented Sabrina with a fresh set of challenges. She found herself at the helm of classrooms filled with approximately 30 high school students at a time, a significant departure from her previous teaching experiences. The new environment pushed her beyond her comfort zone, demanding adaptability and resilience.

However, as she embraced this role, Sabrina discovered a profound sense of fulfillment and purpose. Her dedication to her students and the opportunity to make a meaningful difference in their lives fueled her passion for teaching even further. Despite the initial challenges, she grew to cherish her time within the Upward Bound program, recognizing it as a valuable and enriching chapter in her teaching journey.

During her college and graduate school years, Sabrina longed desperately to explore the ocean. Her quest led her to the Seatrek program, a remarkable opportunity that would forever change her life. Her first Seatrek adventure was in the British Virgin Islands, where she spent three transformative weeks aboard a catamaran. Here, she immersed herself (pun intended) in the world of scuba diving, sailing, and dipped her toes into the vast field of marine biology.

Sabrina's connection to Seatrek deepened, leading her to return the following summer for an internship, where she assumed the role of a camp counselor. Over the course of five subsequent summers, she continued her involvement with Seatrek, eventually working up to earn her Scuba Diving Instructor Certification. She became a mentor, and instructor where she taught scuba diving and marine biology to middle school, high school, and college students.

Living on a boat in the ocean for the duration of her Seatrek experiences, Sabrina became intimately familiar with the rhythm of the sea. She adopted the ocean as her bath and dishwater, fully embracing a lifestyle intricately intertwined with the marine world. Her journeys took her beyond the British Virgin Islands, venturing to the Bahamas and even Hawaii. Along the way, she dove into the depths of the ocean, encountering majestic creatures such as massive manta rays, sharks, whale sharks, dolphins, sea turtles, and even pilot whales. However, Sabrina's appreciation extended to the smaller organisms that inhabited the ocean's vibrant ecosystems, from delicate flatworms and striking flamingo tongues to colorful coral reef fish, sponges, and, of course, the elusive and enchanting octopuses. Her fascination was primarily with group-living organisms. Sabrina would often hyperfocus on phenomena like the synchronized color changes of shoals of squid or the coordinated movements of surgeon fish. Even colonies of feather worms, each member contributing to the collective whole, held her attention. These observations continued her deep curiosity for the intricacies of collective behavior.

As Sabrina was finishing up her thesis, she eagerly applied to graduate programs. Among the mentors she conversed with, one individual stood out: Rex Cocroft. Their initial phone call, which stretched for over an hour, left a lasting impression on her.

Something about Rex and his work resonated with her deeply. Perhaps it was the aggregation of *Umbonia* treehoppers prominently featured on the banner of his website.

When the time came for her interview at the University of Missouri, she felt comforted by the graduate students, faculty, and staff. She had an inexplicable feeling of coming home as she drove, snow decorating the buildings of Columbia. After meeting Rex, she knew without a doubt that this was the place where her academic journey would continue to flourish.

The transition from a small, dusty town in New Mexico to Missouri took Sabrina by surprise. On the drive up to Missouri, she was taken back by the lush green landscapes that flashed by. Upon arriving to her new home, the awe-inspiring beauty of the surroundings left her in a state of sheer delight. As she stepped out of the moving truck, something caught her eye. In the grass and among the trees, tiny lights were twinkling in the darkness. Sabrina realized that those glistening lights were fireflies, which she had never seen before in her entire life. Overcome with emotion, tears of joy welled up in her eyes as she marveled at the enchanting spectacle of fireflies dancing in the night. It was a symbol of the new beginnings and endless possibilities that awaited her.

Sabrina's fascination with treehoppers became an all-consuming passion, one that ignited a deep love for these remarkable creatures from the very first encounter. She found herself spending countless hours observing them, listening to their unique sounds, and immersing herself in the world of treehoppers. Treehoppers were the perfect study organism as their gregarious behavior aligned perfectly with her profound interest in collective behavior. She discovered a newfound love for bioacoustics, particularly vibrational communication. She marveled at the fact that these acoustic signals often

went unnoticed by humans, yet they are all around us, hidden in plain sight. What truly captivated her was the realization that a vast majority of acoustic signals on Earth remained undocumented because they are not within the range of human hearing, too quiet for our ears to detect without sensors. The prospect of discovering these unheard signals, of being the first person to ever hear and comprehend a particular communication method, was an idea that sold her.

Sabrina would spend hours outside nestled in the grass, listening to blades of grass with a piezo-disc and amplifier trying to hear these subtle sounds in her backyard. Each moment held potential to hear something new. It was in these moments that Sabrina felt a deep connection to her inner child, the young girl that loved observing nature.

Sabrina acquired valuable skills during her PhD journey, including how to solder her own sensors for recording treehoppers, mastering Matlab, conducting playback experiments. Her most valuable skill she gained is trouble shooting. She even was able to have a month-long adventure in Bolivia to study treehoppers and collaborate with a group of researchers. She had the opportunity to help set up their lab to record treehoppers and conduct playback experiments. Sabrina conducted field work and was able to study tropical treehopper species.

During her second year of the PhD program, her oldest brother Michael, passed away unexpectedly at the age of 44, followed by the loss of her stepbrother, Brad, to cancer These devastating losses left her devastated, especially given her distance from her family. These experiences have instilled in her a deep empathy, knowing that life can take unexpected turns, and academia can be unforgiving during such times.

Sabrina has often grappled with feelings of not belonging due to her background and experiences. This has led her to prioritize ensuring that her students feel a sense of belonging and comfort in academia, particularly in STEM fields. Her aspiration as a professor is to create a welcoming environment where students always feel that they are at home when they are in her class. Sabrina envisions a career that blends her passions with teaching, recognizing that her greatest sense of belonging lies in guiding and inspiring the next generation.

Her commitment to blending her passions with teaching is a fundamental part of her identity. She is infinitely thankful to each person that has gotten her here today.

Sabrina dedicates her spare time doing things that make her happy. She trains jiu jitsu, often traveling to local tournaments. She also enjoys crocheting, drawing, and writing. Her most cherished pastime involves spending time with her niece and nephew, Lydia and Jesse. Together they love listening and dancing to Taylor Swift, swimming in the summer, sneaking treats, and playing Cheetah Race, Bumpy Road, and Jiu Jitsu among many others. Sabrina proudly holds the title of Auntie Brina to 14 nieces and nephews and eight great nieces and nephews. Her love extends to her four-legged companion, her two dogs, Morty and Charlie and her two cats Seymour and Tiger. Sabrina also finds solace in the simple things in life, savoring music, expressing herself through writing, eating sushi, and building Lego sets.

Every day is a chance to learn, a chance to teach, and a chance to make a difference. This is a testament to the ever-evolving story of Dr. Sabrina Michael.