NOISE AND SIGNAL TRANSMISSION PROPERTIES AS AGENTS OF SELECTION IN THE VIBRATIONAL COMMUNICATION ENVIRONMENT

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by

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

NOISE AND SIGNAL TRANSMISSION PROPERTIES AS AGENTS OF SELECTION IN THE VIBRATIONAL COMMUNICATION ENVIRONMENT

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a candidate for the degree of Doctor of Philosophy

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

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To Angela and my family, you have had a larger role in this than you know, and larger than I could ever convey in words. Your love, belief, and pride sustain me. For that and so much more, I love you.

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Well, so this is a Ph.D.? I must admit there were times I wondered how I would make it to this point. In some ways I imagined it differently, in other ways it is exactly as

it should be. I thought about quoting the Beatles here, since it has definitely been "a long and winding road," but that would be cheesy and not make any sense. Or there's the Grateful Dead, for "what a long strange trip it's been," but that is potentially worse. How about this: for the motley crowd in Tucker 205, I offer the immortal words uttered by Corey Hart back in 1985, when he shouted with 80's glory and gusto, "Never Surrender!" Yeah. That makes sense.

Gabe McNett

4 December, 2007

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Chapter 1

Introduction

Natural selection, communication, and the origin of species

For the first 100 years of speciation theory, geography was considered by many as the primary force in the evolution of reproductive isolation (Darwin, 1859; Dobzhansky, 1940; Mayr, 1947). Populations were isolated by macrogeographic (i.e., ecogeographic) differences, such as island versus mainland, forest versus prairie, and large scale barriers to movement, such as rivers and mountains. The role of natural selection in the evolution of reproductive isolation was secondary. A geographic emphasis is still witnessed in major textbooks with bold headings (e.g., "Geography of speciation") and prominent graphics of mountainous or oceanic barriers (e.g., Raven et al., 2008). Exceptions to this dominant view existed, as in the work of Walsh (1864) on phytophagous insects and Thorpe (1945) on habitat selection, which showed how variation in local habitats can drive the speciation process. Some exceptions can also be seen in the early work on adaptive radiation, during which different environments favor divergent phenotypes, and in some cases, lead to speciation (Mayr, 1963; Simpson, 1953). However, many of the classic examples of adaptive radiation also involve taxa that occur on different islands and thus, are also separated by substantial geographic barriers (Carr and Kyhos, 1981; Lack, 1947; Losos et al., 1997; Mayr, 1963; Simpson, 1953).

There have been two significant changes in recent decades with respect to the role of natural selection in speciation. First, the emphasis on geography and natural selection has switched, with natural selection having a primary role (Schluter, 2000). Proponents of "ecological speciation" have been at the forefront of this development. In ecological speciation natural selection is primarily responsible for the evolution of reproductive isolation (Rundle and Nosil, 2005; Schluter, 2000). Demonstrations of "parallel speciation" (Johannesson, 2001; Schluter and Nagel, 1995) show the repeated, independent evolution of similar morphologies in similar environments, independent of geography. Reproductive isolation evolves as a by-product: individuals from similar environments separated geographically readily breed in the lab, while those from different environments in close proximity do not (McKinnon et al., 2004). Recent examples can be found in fish (Allender et al., 2003; Boughman et al., 2005; Rundle et al., 2000), lizards (Richmond and Reeder, 2002), insects (Nosil et al., 2002; Stoks et al., 2005), and mollusks (Rolán-Alvarez et al., 2004). Another fundamental change in speciation research has been a shift in focus to a microgeographic scale. Researchers began to realize that parapatric or sympatric populations could differ considerably in their environments. With this development there has been increasing acceptance of the idea that population divergence can occur in sympatry (Berlocher and Feder, 2002; Bush and Butlin, 2004; Via, 2001). Well-supported examples of sympatric divergence are found in lake-dwelling fishes (Barluenga et al., 2006; Schliewen et al., 1994) and host-associated insects (Feder et al., 1988; Savolainen and Vepsäläinen, 2003; Wood and Guttman, 1983).

While the recent emphasis by proponents of ecological speciation has been on natural selection, there has been less consideration of communication-related traits, particularly mating signals and their perception. This omission is surprising since differences in communication signals follows logically from the microgeographic scale of speciation: individuals in close geographic proximity are much more likely to interact, in which case differences in communication systems may be the only source of reproductive isolation. Traits more commonly considered in studies of ecological speciation include body size or limb length, feeding structures (e.g., beaks, gill rakers), feeding performance, or life history. A limited interest on communication may partly reflect a sort of "historical inertia." Schluter (2000) stresses that "builders of the ecological theory of adaptive radiation attached little special significance to divergence of arbitrary secondary sexual characters." Exceptions can be found in early work on reinforcement and reproductive character displacement (see Discussion, Chapter 5, Blair, 1955; Dobzhansky, 1951).

The relationship between natural selection and communication systems is directly addressed by the sensory drive hypothesis. The sensory drive hypothesis predicts that, when populations occupy different environments, natural selection for efficient communication will cause their communication systems to diverge (Dawkins and Guilford, 1997; Endler, 1992; Schluter and Price, 1993). An important tenet of this hypothesis is that the transfer of information occurs within a set of specific environmental and ecological conditions, which constitute a "communication environment." Characteristics of the communication environment affect the efficiency of information transfer, with natural selection favoring maximum efficiency. Behaviorally, greater

efficiency assures that "correct" decisions are made, such as who to mate with, or whether to pursue or flee. However, while sensory drive focuses on natural selection on communication signals, it is not explicitly a speciation hypothesis. Sensory drive studies have exploded in the years since Endler's 1992 publication, but surprisingly few have focused on how reproductive isolation results from divergent selection on signals and sensory systems (but see Nosil et al., 2007; Patten et al., 2004; Rundle et al., 2005).

At the intersection of ecological speciation and sensory drive is a research program investigating the role of natural selection on mating signals in the evolution of reproductive isolation. Communication systems, arguably, are the most important traits with respect to speciation. They facilitate mate recognition and therefore, in cases where they differ, can also facilitate reproductive isolation. Rapid evolution of differences in signals and preferences can produce rapid reproductive isolation (Goldberg and Lande, 2006; Gray and Cade, 2000; Pfennig and Ryan, 2006; Uy and Borgia, 2000). A key link between ecological speciation and sensory drive is that the target of natural selection and the trait conferring reproductive isolation are the same. The importance of this association has been recognized by theorists in the form of "magic trait" models (Gavrilets, 2004). The "magic" of these traits is that no longer is it necessary to postulate separate traits (loci) for assortative mating and habitat-based selection, as earlier models had done (e.g. Felsenstein, 1981; Maynard Smith, 1966). This requirement was problematic for sympatric speciation theory, since genetic recombination will break down any association. This problem is resolved with magic traits since assortative mating becomes a direct consequence of divergent selection. Understanding the cause of

divergence in communication signals, therefore, may provide considerable insight into speciation.

My goal in this introduction has been to emphasize the importance of a greater union between ecological speciation and sensory drive. In my dissertation I focus on speciation in Chapter 4, sensory drive in Chapter 2, and methods for studying signal transmission through the environment in Chapter 3. With greater unification between ecological speciation and sensory drive we stand to gain additional insight into a range of topics. We may better understand the long range impact humans have on other populations as we alter the environments that animals inhabit (e.g.'s Foote et al., 2004; Slabbekoorn and Peet, 2003). We may also better understand that "question of questions" (Darwin, 1859): the origin of species.

Communication environments across modalities

Any taxon has one or more communication modalities it relies upon most—a "modality" being the dominant mode of information transfer (i.e., chemical, acoustical, vibrational, visual, or electrical; Bradbury and Vehrenkamp 1998). Common to all modalities are ecological factors and structural habitat components that attenuate, degrade, and distort communication signals. These factors define a communication environment. In the ensuing chapters I deal with two factors of the communication environment: background noise and properties of the transmission medium. Noise and transmission properties are two different agents of selection that both favor signals that transmit with the greatest efficiency.

Noise represents an unwanted source of energy that leads to inefficient communication by decreasing signal detectability. For visual signals that incorporate pattern-movement noise might be background movement patterns of wind-blown vegetation (Fleishman 1992); for chemical signals noise may take the form of pollutants (Fisher et al., 2005); or for acoustics, noise may come from wind (Aubin, 2004). Considerable recent attention has been given to anthropogenic noise (Fisher et al., 2005; Foote et al., 2004; Seehausen et al., 1997), particularly with respect to the effect of "urban noise" in acoustic signaling (Katti and Warren, 2004; Sun and Narins, 2005; Wood and Yezerinac, 2006).

Whether signals transmit information in the form of chemicals, color patterns, or pressure waves, they interact with a transmitting medium. Acoustic signals, for example, are transmitted through air and water and are affected strongly by variations in wind (or current), and obstacles in the transmission path. Wind attenuates signals, and obstacles reflect, absorb, or bend incoming sound waves (Bradbury and Vehrencamp, 1998; Forrest, 1994). An exception with wind can occur when atmospheric differences become arranged in stratified layers that run parallel with the ground, in which case signals may be "guided" by reflections off the ground and an atmospheric boundary, rather than attenuated and distorted (Wiley and Richards, 1978). Visual signals have been studied heavily over the last fifteen years, particularly color signals in birds, fish, and lizards (Allender et al., 2003; Endler and Thery, 1996; Heindl and Winkler, 2003; Leal and Fleishman, 2004; Macedonia et al., 2003; Seehausen and Schluter, 2004). The dependence of these signals on being seen means that efficient transmission depends on the unique characteristics of intervening obstructions and the amount and color of

ambient light (Endler, 1993). Chemical signal transmission depends on wind, current, and heat, which affect dispersion and bulk molecular flow (Atema, 1995), while electrical signals transmitted through water are affected by current and salinity, which affects conductivity (Brenowitz, 1986).

My dissertation focuses on transmission and noise in one modality: vibrational communication. Substrate-borne vibrations are used by many animals and are transmitted through a wide range of substrates, such as sand (Brownell, 1977), water (Wilcox, 1972), leaf litter (Elias et al., 2004; Scheffer et al., 1996), ground (Hill and Shadley, 1997; O'Connell-Rodwell et al., 2000), honeybee combs (Sandeman et al., 1996), and spider webs (Masters, 1984). My focus is on vibrations transmitted through plants by phytophagous insects. Plant-borne vibrations represent the most taxonomically widespread transmitting medium in vibrational communication (Cocroft and Rodríguez, 2005). In this transmission medium, noise comes primarily from incidental vibrations from rain and wind (Barth, 1988; Casas et al., 1998; Cocroft and Rodríguez, 2005).

The difficulties for communication created by variation in transmission properties and background noise can be mitigated with changes in signal design or changes in signaling behavior. I consider both of these topics with respect to noise and transmission in plant-borne vibrations by addressing two questions. First, how do signalers and receivers respond behaviorally to noise? I present the results of observational and experimental studies on changes in communication behavior by signalers and receivers in response to wind-induced noise vibrational noise (Chapter 2). Second, how are vibrational signals changed during transmission through the stems and leaves of plants? Accurately assessing the transmitting medium required the development of methods not

previously used in the study of vibrational communication. Previous research studied plant stem motion along only a single axis; however, because plant stems can vibrate in a complicated manner (Chapter 3), I detail a new method for measuring plant stem vibration (Chapter 3, McNett et al., 2006). I then apply this method to the question of whether variation in the transmission medium can affect the evolution of signal design. Specifically, I present data on how plant transmission properties can promote divergent selection on vibrational mating signals (Chapters 4). I address these two questions using members of a closely related group of herbivorous insects in the family Membracidae: the *Enchenopa binotata* species complex.

The Enchenopa binotata *species complex*

The *Enchenopa binotata* complex comprises eleven or more host specialist insect species (Cocroft et al. 2007; Wood 1993). Each *E. binotata* species has a single generation per year with females laying egg masses in the woody tissue of their hosts each fall, and eggs hatching in the spring. Life history timing is closely tied to the phenology of the host: the flow of water and sap through the host plant's vascular tissue triggers egg hatch each spring (Wood et al., 1990). The host plant species used by *E. binotata* are evolutionary diverse, coming from several families (Rutaceae, Leguminosae, Celastraceae, Fabaceae, Juglandaceae, Adoxaceae, Thymelaeaceae, Rhamnaceae, Magnoliaceae). Host plant species also occur in a wide range of habitats, including the forest canopy, understory, interior, and edge.

Communication in *E. binotata* is mediated through plantborne vibrational signals (Cocroft et al., 2008; Hunt, 1994). The vibrational communication system plays a central

role in pair formation through signal exchanges between males and females. Males search for females using a "call-fly" strategy, flying branch to branch and signaling to elicit a female response (Hunt 1994). Receptive females respond with a vibrational signal of their own that initiates local searching behavior in males. Structure of the male mating signal is strongly conserved across species but signals show considerable variation in quantitative traits (Cocroft et al., 2008). Frequency is a trait for which females exhibit strong, closed preferences, and it is the single most important signal trait for female mate recognition (Rodriguez et al. 2006). Variation in signal frequency also parallels host-plant use and shows greater variation among species than among individuals or populations (Cocroft et al., in prep). Species in the *E. binotata* complex are awaiting description, and will be referred to throughout this dissertation using their host-plant affiliation (e.g., *E. binotata* 'Cercis' for the species on *Cercis canadensis*').

Members of the *E. binotata* complex are ideally suited for addressing the issue of how natural selection for communication efficiency can influence signal evolution, and how this may lead to reproductive isolation. These species are a widely cited example of sympatric speciation through shifts to novel host plants (Coyne and Orr 2004; Wood and Guttman 1983, Wood 1993). Host shifts facilitate assortative mating and reproductive isolation through changes in life history timing, which are caused by differences in plant phenology (Wood 1980). Host shifts also result in divergent ecological selection: females experimentally transferred to non-natal hosts for oviposition experienced decreased survival and fecundity, and offspring suffered decreased survival (Wood and Guttman 1983). While these ecological barriers confer some reproductive isolation they do not entirely isolate the different host-related species (Cocroft et al., 2008). The differences

that have evolved in their vibrational mate attraction signals may be key traits in further isolating the species reproductively.

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Chapter 2

Wind-induced noise alters signaler and receiver behavior in vibrational communication

Abstract

Individuals communicating in any modality must do so in a complex environment, where many factors are capable of affecting the efficiency with which signals are transmitted or received. One particularly strong and ubiquitous factor is background noise, which can create signal detection problems that consequently affect signaling behavior. For the many insects that communicate by transmitting vibrations through plant stems and leaves, noise primarily comes from vibrations generated by wind. Previous studies of wind-induced vibrations in plants have characterized the spectral characteristics and transmission of noise. Here I investigate whether wind-related noise influences the communication behavior in males and females of *Enchenopa binotata* treehoppers (Hemiptera: Membracidae) that occur on the host plant *Ptelea trifoliata*. I use laboratory experiments to show that wind-induced noise reduces male signaling behavior and the probability that a female will respond. However, males adjust their signal timing such that communication can persist during low levels of noise. These results provide the first evidence that vibrationally-communicating insects modify their signaling behavior to mitigate interference from wind-induced vibrations.

Introduction

Individuals communicating in any modality are confronted with background noise that creates signal detection problems (Atema, 1995; Brenowitz, 1986; Endler, 1993; Forrest, 1994; Michelsen et al., 1982). Natural selection favors adaptations in senders and receivers that achieve spectral or temporal separation from noise, thereby allowing more efficient communication (Brumm and Slabbekoorn, 2005; Römer, 1993). Separation from noise can occur through changes in signal design (Foote et al., 2004; Slabbekoorn and Peet, 2003) or through changes in signal timing (Brown and Handford, 2003; Greenfield, 1988). Which solution is more efficient will depend, in part, on the relationship between the spectral and temporal properties of the noise and the properties of the signal.

Noise comes from both biotic and abiotic sources. The most common biotic source of noise in any communication modality is the presence of other signalers. Abiotic sources, on the other hand, may vary depending on the dominant mode of communication. For birds and primates that rely on acoustic communication wind can be a noise source (Ryan and Brenowitz, 1985; Waser and Waser, 1977). Fish that communicate with chemical signals can be affected by pollutants (Fisher et al., 2005), while those that communicate with electric signals may be affected by lightning (Hopkins, 1973). For the vast number of small plant-dwelling insects that communicate using vibrations, noise comes predominantly from wind (Barth, 1988; Cocroft and Rodríguez, 2005).

Wind moves branches and flutters leaves, inducing noisy vibrations throughout a plant. Even a slight breeze (< 1 m/s) can cause substantial noise (Figure 1a).

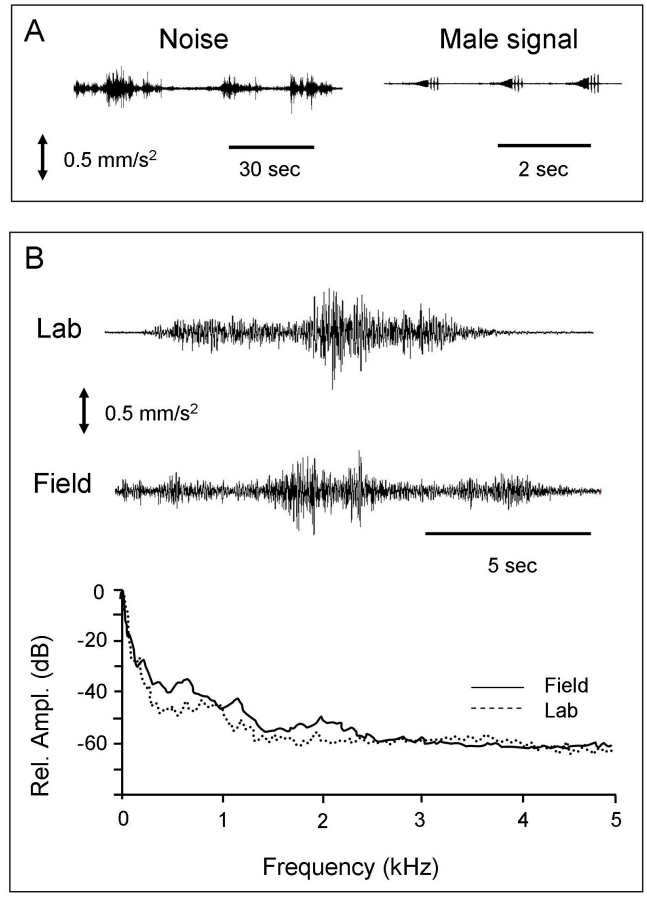

Figure 1) Wind-induced vibrational noise recorded from leaf petioles of *Ptelea trifoliata.* a) Field recording of vibrations produced by a light breeze (peak wind velocity = 1.1 m/s; average wind velocity = 0.5 m/s). b) Comparison of waveforms and amplitude spectra of field-recorded and lab-generated vibrational noise (peak wind velocity: 1.5 m/s for both).

These plant vibrations contain primarily low frequencies (<100 Hz), but may contain energy up to 20 kHz (Barth, 1988; Casas et al., 1998; Cocroft and Rodríguez, 2005). Wind-induced vibrations are a fluctuating noise source, with brief periods of calm (gaps) between periods of more intense noise (Figure 1a). At a given location, wind velocity may show not only unpredictable short-term variation, but also predictable daily highs and lows. Both temporal scales of variation are likely to be important for communication and may favor behavioral mechanisms that permit communication when noise levels are

low. If there is diel periodicity in wind-induced noise, an avoidance strategy might involve increased communication during quieter periods of the day. Diel patterns of signaling are seen in taxa that use other modalities, such as visual and acoustic communication (Andersson et al., 1998; Brown and Handford, 2003). If noise varies over shorter time scales, avoidance might involve signaling in silent gaps (Schwartz and Wells, 1983) and "listening in the valleys" (Buus, 1985). Given the presence of gaps in wind-induced vibrations on plants, signaling individuals could circumvent signal detection problems by communicating during quieter periods. Such gap detecting behaviors are found in a wide range of taxa, such as humans (e.g., Schneider and Pichora-Fuller, 1994), birds (e.g., Okanoya and Dooling, 1990), and insects (Greenfield, 1994).

In spite of the prevalence of vibrational signaling and a general recognition of wind as a major noise source (Cocroft and Rodríguez, 2005), no study has looked at signal timing in the vibrational modality with respect to wind-related noise. In this study I test the hypothesis that wind noise influences signaler and receiver behavior in a vibrationally-communicating insect. The *Enchenopa binotata* species complex (Hemiptera: Membracidae) is a clade of phloem-feeding insects that specialize on different host plants. Since these species are awaiting formal description, I refer to them using their host-plant names. Here I focus on *Enchenopa binotata* 'Ptelea,' the species that uses *Ptelea trifoliata* as its host plant. I measured the response to wind at two scales. First, I measured diel variation in wind velocity and male signaling behavior in natural populations. Then I tested males and females experimentally in the lab to determine their behavioral response to variation in wind-induced vibrations on a shorter time scale. If wind-induced vibrations in plants affect communication efficiency—the ability to

transmit information with minimal energy expenditure—males and females should alter their communication behavior to reduce noise-related costs.

Methods I: Male signaling

(a) Experimental animals

Mate searching *E. binotata* 'Ptelea' produce advertisement signals to which receptive females respond, forming a duet (Hunt, 1994; Rodríguez and Cocroft, 2006). The duetting response of both sexes provides an assay for playback experiments. Males and females used in experiments were from a greenhouse colony of lab-reared offspring, which originally derived from multiple (~12-15) adults collected the previous mating season from local populations. Individuals used in experiments were separated by sex prior to sexual maturation (5-7 weeks), maintained in sleeve cages on separate potted host plants and kept in a greenhouse at a daytime temperature of 28° C and a 13:11 day:night cycle.

(b) Diel periodicity in noise and natural signaling behavior

Diel variation in wind speed and male signaling behavior were measured during the mating season in July 2005. Female signals were not measured, since females signal only during a short period of receptivity and thus are rarely heard. These measurements were taken to determine: (1) when communication takes place during the day; and (2) what wind velocities would be appropriate for experimental tests. *Enchenopa binotata* 'Ptelea' form persistent aggregations of 2-12 individuals on leaf petioles at the distal branch tips of their host plant. Twelve host plants were monitored at three field sites (4

host plants per site). The three field sites were located within a 15 km^2 area of Boone County, Missouri, USA. *Ptelea trifoliata* occurs in locations that are likely to vary in local wind speeds, such as sheltered sites with low or broken canopies or open habitats along forest edges. To observe the insects' behavior at a range of wind speeds, host plants monitored at each site were found both in open and sheltered locations.

Signaling behavior of each aggregation and the adjacent wind speed were monitored for 12 hours (08:00-20:00 hrs). Signaling ceases after 20:00 (L.E. Sullivan, unpublished data). Behavior and wind speed were sampled for 2 minutes every 30 minutes. The largest male-biased aggregation on each host plant was chosen for monitoring, since these are most likely to engage in persistent signaling bouts (GDM, pers. obs.). Signaling was monitored with a Signal Flex SF30 Universal Tuner Pickup for each host plant. Although attaching a pickup to the plant never appeared to disturb an aggregation, to eliminate potential disturbance during the sample period pickups were placed within 10 cm of the focal aggregation at 07:30, and left throughout the day as long as ≥ 2 individuals remained. At all but two host plants, a single aggregation was monitored throughout the day. For two host plants, the individuals in the initial focal aggregation dispersed and a second aggregation on the same host plant was monitored. For these two host plants the pickups were moved between sample periods so as not to disrupt data collection. During the 2-minute sample period the pickup was plugged into a battery-powered Johnson JA-004 Mini-amp / speaker at a distance of 2-3 meters from the plant, and the total number of signals was counted by a field assistant, Lucia H. Luan (LHL) using a hand-held tally meter.

Wind speed was monitored in the immediate vicinity of the focal aggregation using a WindSonic ultrasonic anemometer (Gill Instruments, Hampshire, UK) mounted on a tripod. The anemometer was connected to a Dell 700m Inspiron laptop computer. Wind speed was acquired 4 times per second using WindCom software (Gill Instruments, Hampshire, UK). A peak and average velocity (m/s) reading was recorded for each of the 2-minute sample periods throughout the 12 – hour monitoring period, yielding 25 measurements of wind speed per day per host plant. These wind measurements were used as a guide in the experimental playbacks. A logistic regression was used to test whether the probability of male signaling depended on wind speed. The results of the likelihood ratio χ^2 – tests are reported for this and subsequent logistic regressions.

(c) Detecting signals in noise

Detecting signals in noise is a challenge for conspecifics and researchers alike. The potential for missing male signals was greatest while monitoring natural signaling behavior, since signals were monitored rather than recorded (see *d*, below). To address the potential for missed calls a hearing test was given to LHL under more stringent conditions than those encountered in the field. Wind was reproduced using a computer fan mounted onto a tripod (Figure 1b). Wind speed was monitored using the same anemometer and software described above (Methods Ib). Wind speed was maintained at a constant velocity of 1.5 m/s, the upper range of that commonly experienced by natural populations (see results).

The stimulus for each test consisted of a 2-minute series played from a Dell 700m Inspiron laptop running Raven software (v. 1.2; Cornell Bioacoustics Laboratory, USA),

amplified (Radioshack MPA-250 stereo amplifier), and transduced into the stem of a potted *Ptelea trifoliata* using a small magnet attached to the host plant stem and an electromagnet placed 1-2 mm from the magnet. Each test series contained 20 signals, sampled randomly (with replacement) from recordings of 17 males. The order of the signals was also randomized for each test. All 20 signals within a 2-minute series were set to the same RMS amplitude and presented to LHL in random order. To make signal timing unpredictable, signals were separated by randomly determined time intervals of 1-10 seconds. All three tests were conducted at the same wind speed, but each differed in male signal amplitude. The first test was conducted using a source RMS amplitude of 0.08 mm/s^2, equivalent to an average male signal measured at 2 cm or less on a leaf petiole. The second and third test attenuated the male signals by -6 dB and -12 dB, respectively. Signals were monitored using the same pickup and battery-powered mini-amp / speaker used for monitoring signals in the field (Methods Ib). The pickup was placed 50 cm from the magnet, a distance five times greater than the distance from focal aggregations in the field. The total number of signals was counted by LHL using a hand-held tally meter, as in the field. All information regarding each test and its corresponding stimulus (number of signals, signal intervals, test being administered) was unknown to LHL. Signals were either detected correctly amidst the background noise (a "hit"), detected when no signal was present ("false alarm"), or not detected (a "miss"). The combined conditions for this hearing test likely produced signal-to-noise ratios that were substantially lower than those experienced in the field: the wind velocity (1.5 m/s) was greater than most field conditions, male signal amplitudes at the source (magnet) were

either equal to or less than that of the average male, and the distance from signal source to the pickup was five times greater than the distance to focal aggregations in the field.

(d) Experimental test of gap detection

Playback experiments were conducted with lab-reared males in July 2005 to address their behavioral response to finer-scale fluctuation in wind-induced noise. Playback to a male of a male-female duet evokes a bout of signals, and provides an assay for testing whether males alter their signal timing. Males were tested in the lab and recorded, making researcher-related detection errors negligible since recordings allowed male signals to be readily identified in the spectrograms (Figure 2).

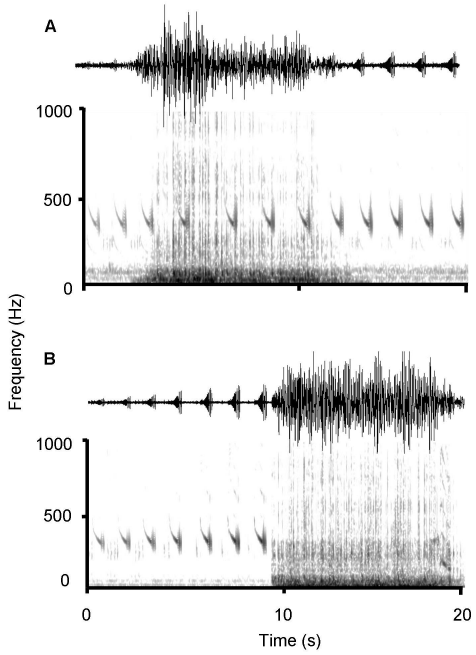

Figure 2) *Enchenopa binotata* 'Ptelea' males that continued signaling (A) or stopped signaling (B) during playbacks of wind-induced vibrational noise.

To test for gap detection, wind was reproduced using a computer fan wired to a switch. Two wind speeds were used, low (0.75 m/s) and high (1.50 m/s), and were

controlled by changing the distance between the test plant and the fan. Twenty five males were tested at each wind speed using a two-minute test series. The series was composed of four male-female duets used to evoke male signaling (Figure 3). The duets were

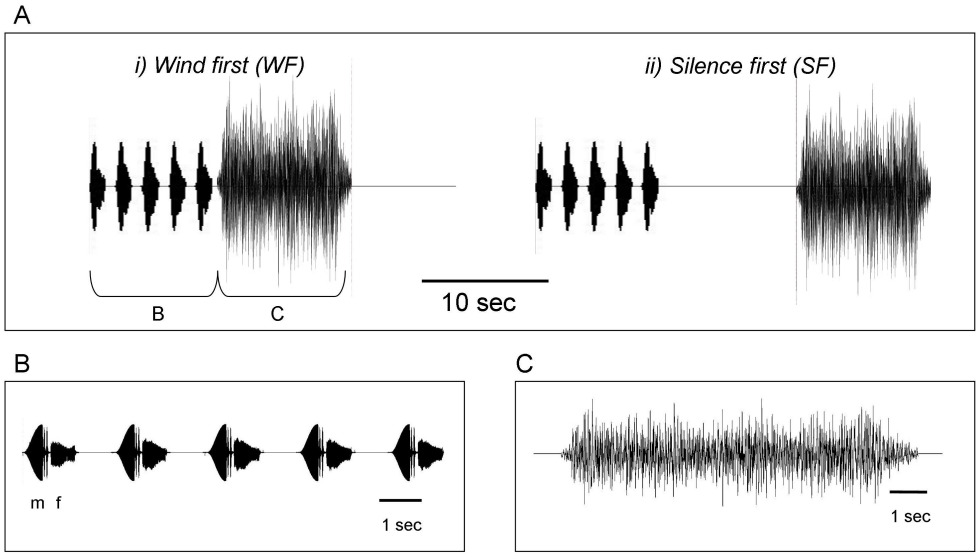

Figure 3) Example of stimuli used to test male responses to wind (A). The two stimuli differed in the order of wind and silence following a duet bout. (B) A duet with four alternating male and female signals was used to elicit male signaling. (C) Plant vibrations induced using a fan.

played from a Dell 700m Inspiron laptop running Raven software (v. 1.2; Cornell Bioacoustics Laboratory, USA), amplified (Radioshack MPA-250), and transduced into the stem of a potted *Ptelea trifoliata* using an electromagnet / magnet combination. The test stimuli both contained a 10 s wind burst and a 10 s silent gap, but in one the wind was first (WF) and in the other the silence was first (SF; Figure 3A). Males received each stimulus twice.

Individual males were transferred to the testing plant and allowed to settle for 2-3 minutes prior to the playback. Signals were recorded using a PCB 352A24 accelerometer connected to a PCB 480E09 ICP Sensor Signal Conditioner (PCB Piezotronics, New

York, USA), amplified using an M-Audio Mobile USB Pre amp (Avid Technology, Tewksbury, MA, USA), and acquired on a Dell 700m Inspiron laptop running Raven software (v. 1.2; Cornell Bioacoustics Laboratory, USA). I counted the number of signals during wind and during wind-free periods, and used an ordinal logistic regression to test whether the probability of male signaling is determined by the presence / absence of wind, wind speed, or a wind presence*wind speed interaction.

Methods II: Female response

(a) Stimulus design

Changes in communication behavior in lab-reared females were tested in late May / early June 2006. To exclude the possibility that individuals respond to air movement, rather than wind-induced vibrations, wind-noise for females was generated using playbacks of naturally-recorded wind-induced vibrations, rather than a fan.

Each female was tested using a control stimulus of a natural bout of four male signals and three test files that corresponded to three wind conditions (*no wind*, *low wind,* and *high wind*). Each test file was a two channel file with six male signal bouts in the left channel and six recorded bursts of wind-induced vibrations in the right channel. Each channel was played to a different electromagnet. Transducers were separated by 3 cm.

Male signals were computer-generated using signal parameters set to values of local population averages, and each bout contained four signals. The computer-generated signals used a constant frequency rather than the frequency sweep found in natural male signals, and they are nearly as effective in eliciting female response (Rodríguez et al., 2006). Each female thus received the same frequency, and any variation in response is

not due to variation in plant frequency-filtering. Peak amplitude was equalized for all male signals within a bout, but was varied between bouts using a custom-made program in Matlab (v. 6.5; Mathworks, Natick, MA). Signal amplitude for the six bouts was relative to that of a male at 1 cm and dropped in steps of 6 dB (0 dB, -6 dB, -12 dB, -18 dB, -24 dB, and -30 dB peak amplitude).

The six recorded wind bursts overlapped the six bouts of male signals. Wind stimuli were drawn from recordings of wind-induced vibrations (10-sec duration, n=37 wind bursts) in leaf petioles of 19 different *Ptelea trifoliata* host plants in the field. *Low wind* and *high wind* levels were obtained using ± 1 SD of the average RMS amplitude of the recorded wind bursts. These RMS amplitudes corresponded to an approximate peak wind speed of 1.0 m/s and 2.0 m/s for low and high, respectively. Six exemplars were chosen for each wind level. With an equal number of exemplars and signal bouts, females never experienced a wind exemplar more than once.

(b) Playback of wind-vibrations and recording of female response

The design described in the previous section was used to evaluate the effect of wind-induced noise on female response signals (Methods IIa). Each of the three test conditions used a separate two-channel stimulus. The *no wind* file contained silence in the right channel. The sequence of the three tests was randomized for each female. Preceding and following these three tests each female was played the control stimulus consisting of a natural bout of four male signals. With two control bouts and three tests consisting of 6 bouts each (Methods IIa, above), each female received 20 signal bouts.

Each female was placed on a leaf petiole of a small potted *Ptelea trifoliata* host plant (0.5 m tall) within 4 cm of an accelerometer. Using two electromagnets, male signal bouts were transmitted through one channel and bursts of wind-noise through the other channel. The magnets were placed 5 cm and 8 cm from the accelerometer on the opposite side from the female. The playback setup was the same as for males (Methods Id), except that stimuli were played back using Audacity (v.1.2.4) instead of Raven. The amplitude spectrum of each wind-burst was adjusted to compensate for plant filtering properties (Cocroft, 1996). The RMS amplitude of each wind burst was then calibrated to the low and high levels. The peak amplitude of male signal bouts was adjusted at the point on a leaf petiole where females would be released for testing. The highest peak amplitude level (defined as 0 dB) was 0.14 mm/s^2, equivalent to an average male at < 2 cm on a leaf petiole. The behavioral responses of twenty-five females were recorded using the same computer, accelerometer, pre-amp, and software described above for males (Methods Id). All females were within 2.5 cm of the accelerometer, except for one that settled 4 cm away. Female response was recorded as a binary variable with a positive response indicating a female responded to at least one of the four male signals in a bout. An ordinal logistic regression was used to test whether the probability of a female's response was predicted by male signal amplitude, wind level, or signal amplitude*wind level interaction. In addition to female responses that followed immediately after male signals, the number of non-duetting responses was monitored, which pertained to isolated responses not given in direct response to a male's signal.

Results

a) Detecting signals in noise

The conditions under which the hearing tests were given to LHL were more stringent than experienced in the field. Results of the first hearing test suggested that all males from the focal aggregation that signaled at average amplitude (0.08 mm/s^2 at 1 cm) would have been detected even with wind velocities at the high end of the range (error rate 0%, 20/20 hits). The second test assessed the error rate in detecting male signals that are at half the average amplitude (-6 dB) and resulted in about 5% error rate (19 hits, 1 miss). Only when male signals were attenuated by -12 dB did the hearing test reveal a significant jump in misses and false positives (6 hits, 14 misses, and 2 false positives). Only signals from either quiet or distant males would have contributed to error in my observations, and during wind-free periods these were rarely heard (see next section). These tests show that nearly all signals from the focal aggregation should have been detected with minimal error.

b) Male signaling behavior in the field

Most signaling occurred during the morning and evening when wind velocity was lowest (Figure 4). There was a marginally significant trend for an inverse relationship between wind speed and male signaling behavior (Table 1). Notable are the brief lulls in wind velocity and concurrent peaks in signaling at approximately 11:30 and 16:00 (Figure 4). The detected signals were almost exclusively produced by the focal aggregation: faint signals that may have come from more distant males were rarely heard during wind-free periods. Based on these field observations wind speeds of 0.75 m/s (low) and 1.5 m/s (high) were chosen to represent a point beyond which communication

may begin to decrease ("low;" see Figure 4, at 09:30), and where communication may nearly cease ("high;" see Figure 4, 14:00-16:00 hours).

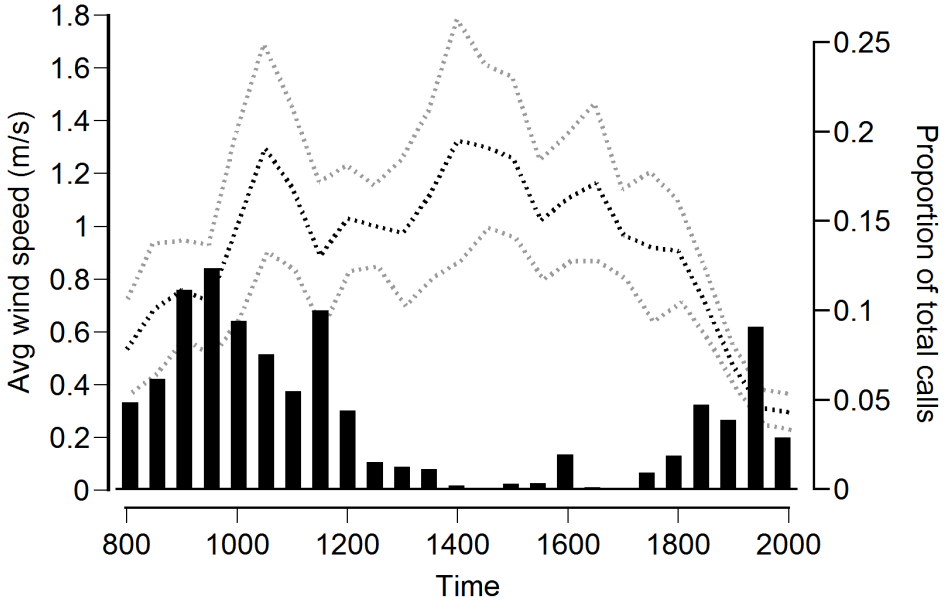

Figure 4) Diel variation in wind velocity and male signaling behavior. Average wind velocity (dotted line ± 95% CI), measured within 1 m of focal aggregation of signaling individuals. Male signaling behavior (bars) was inversely proportional to wind speed, peaking in the morning and evening when wind was low.

Table 1. The effect of wind speed, host plant individual, and time on male signaling (logistic regression).

Source	df	L-R Chi-square	p-value
Wind speed	1	3.5	< 0.061
Host plant	11	4.9	< 0.000
Time of day	24	0.3	0.0001

c) Experimental test of gap-detection

Males signaled significantly more during wind-free gaps than during wind bursts (Figure 5; Table 2), especially when the wind level was high. However, there was no interaction between the presence / absence of wind and wind level (Table 2).

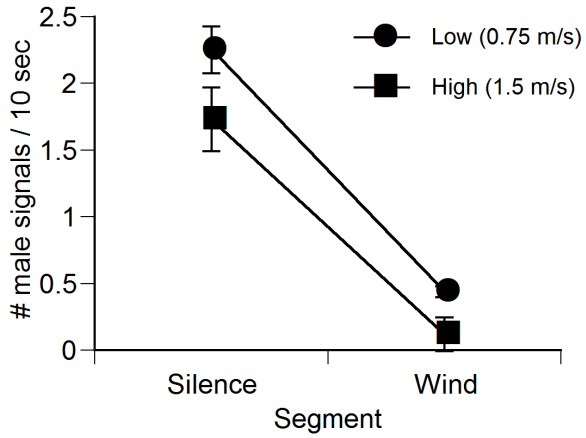

Figure 5) Inhibition of signaling by wind-induced noise: males signaled in the gaps after wind bursts, and their signaling rate was lower after high-velocity wind bursts.

Table 2. Results of an ordinal logistic regression testing whether the probability of male signaling behavior on a shorter time scale in the lab was predicted by the presence of wind, wind level, or an interaction between these two variables.

Source	df	Chi-square	p-value
Wind vs. gap	1	85.2	< 0.000
Wind level	1	4.9	0.03
Presence*Level	1	0.3	0.57

This result is interesting because if males had produced the same number of signals during the wind-free gaps present at both wind levels, an interaction would have resulted (i.e., had the "silent" periods been treated the same). The lack of an interaction indicates that the increased inhibition observed with the higher wind level continued after wind had ceased (Figure 5).

d) Female response

Females experienced 20 total signal bouts: six computer-generated bouts for each of three different wind levels, and a control bout from a natural male before and after the 18 test bouts. Females showed no evidence of habituation or decreased motivation during this time (Figure 6). There was also no significant difference in female response to the various wind exemplars during *low wind* (L-R chi-square = 5.5, df = 5, p = 0.36) and a nearly significant difference at *high wind* (L-R chi-square = 10.3, df = 5, p = 0.07).

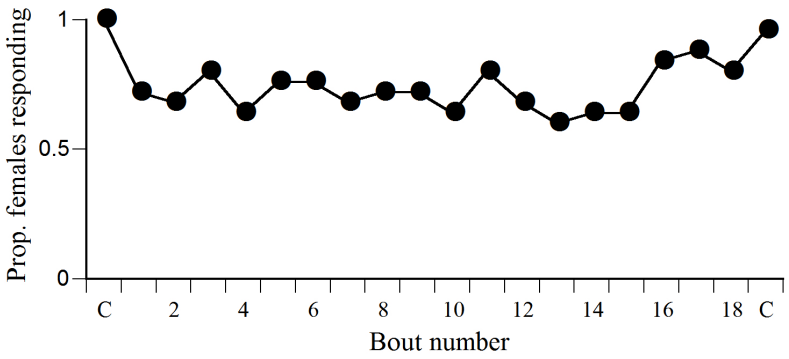

Figure 6) Females showed no evidence of habituation or decreasing motivation during presentation of 20 signal bouts.

Male signal amplitude, wind level, and a signal amplitude*wind level interaction were all significant predictors of female response probability (Table 3). During *no wind* the greatest proportion of females responded to the quietest signals (Figure 7). All

Table 3. Female duetting responses were influenced by the amplitude of the male signal, wind speed, and their interaction (logistic regression).

Source	df	L-R Chi-square	p-value
Amplitude level	5	13.3	0.02
Wind level	2	71.8	<0.0001
Ampl level*Wind level	10	90.7	<0.0001
Female	24	218.3	<0.0001

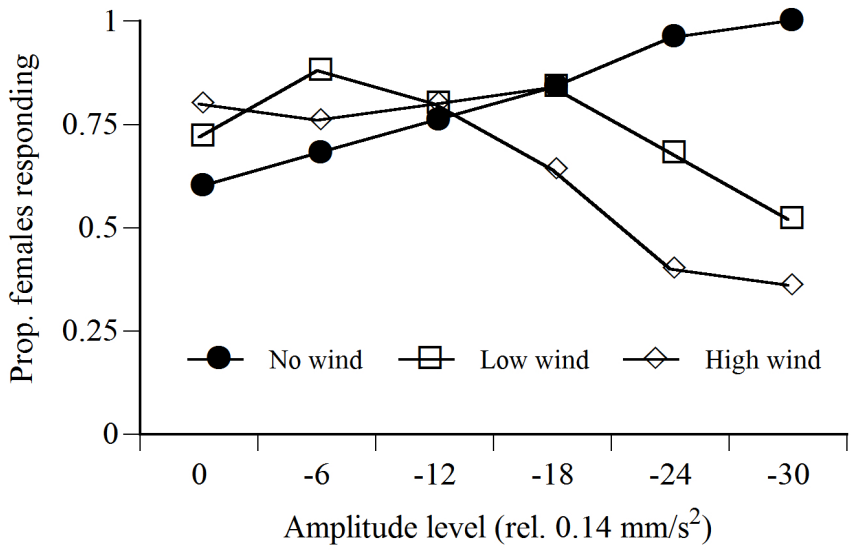

Figure 7) Females were less likely to duet with male signals in the presence of wind-induced vibrations, especially for male signals of lower amplitude. Male signal amplitude was relative to an average male from 2 mm on a leaf petiole. Note that only in the absence of noise did all females respond.

females responded to the male signals at – 30 dB (rel: avg male signal at < 2 cm), and all but one female responded to -24 dB. Female response never reached 100% when wind-induced noise was present, regardless of wind level. During *low wind* and *high wind* response to the quietest male signals dropped to 50% or less (Figure 7).

Discussion

Studies of the vibrational modality have suggested that wind-induced noise is an important feature of the communication environment (Barth, 1988; Cocroft and

Rodríguez, 2005; Cokl and Virant-Doberlet, 2003), but this is the first study to demonstrate an influence of wind on vibrational signaling behavior. Signaling by male *E. binotata* 'Ptelea' in the field was limited to the calmest periods of the day, and wind tended to reduce the probability of signaling behavior (Table 1). Although results of this correlational study cannot rule out other factors, such as temperature, a diel pattern of signaling during calmer periods is consistent with noise-avoidance behavior found in other taxa (Greenfield, 1988; Lengagne and Slater, 2002; Saxena and Kumar, 1980; Sun and Narins, 2005). Laboratory experiments showed a clear cause-and-effect relationship between wind and reduced signaling over a shorter time scale: males preferentially signaled during gaps in simulated wind and females responded less to male signals in the presence of wind.

In the absence of noise females responded less consistently to higher amplitude signals. This pattern likely reflects the long range function of male signals. The highest playback signal amplitude corresponded to that of a male in the immediate vicinity, a situation in which females need not respond. Female responses thus also appear to be used mostly in long-range interactions, functioning as localization beacons. In the presence of wind noise, females largely ceased responding to the lowest-amplitude signals, suggesting that wind decreases signal detection. Females also produced non-duetting responses (Figure 8), which are those not given immediately after a male's signal. These responses may provide a strategy for females to communicate receptivity in the presence of noise, to any male within range.

How important is wind as a source of background noise for vibrational communication on plants? For my study species, whose host plant occurs on edges and in

disturbed habitats, wind dominates the vibrational environment. A wind velocity of only 0.75 m/s was sufficient to evoke gap detection, suggesting this behavior may be common in natural populations. On average, the wind velocity measured in this study was at least

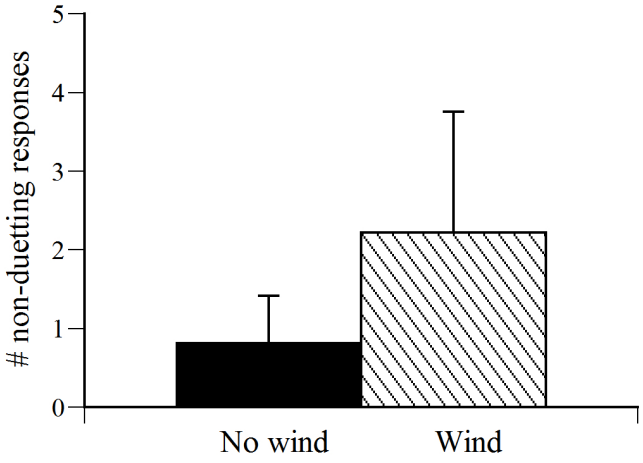

Figure 8) Females produced non-duetting responses (defined as those that did not immediately follow a male's signal) in the presence of wind-induced vibrations (paired $t(15)=1.6$, $p=0.13$).

that high for 75% of daylight hours. Higher wind velocities may inhibit communication altogether, especially during periods when wind speed never drops to zero (e.g., in Figure 4, note the near-absence of signaling between 1200 and 1800). In open habitats such as grasslands, wind may be even more significant as a source of selection on vibrational communication. In closed, forested habitats, wind speed will be lower in the herbaceous layer and the understory, and higher in the canopy (Wiley and Richards, 1982) where many vibrationally-communicating insect taxa (such as leafhoppers) are especially abundant.

A wealth of questions remains about the influence of wind on vibrational communication. At the most basic level, wind-induced vibrations result from the interaction between two components: wind and plant structures. How does variation in wind speed affect the vibration of a given plant structure? For example, higher wind speeds may raise the collision rate between plant parts, increasing the bandwidth of the (otherwise primarily low-frequency) induced noise and perhaps masking signals of a broader range of species. The spectral shape of wind-induced noise can vary between two structurally different plant species (Barth, 1988), and it can also vary between plant stems and leaf petioles (GDM, unpublished data). Within- and between-plant variation in noise may provide individuals another strategy to communicate during noise. Locations that have less relative noise would represent the spatial analog to temporal gaps in noise.

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Chapter 3*

(*published: McNett et al. 2006. The Journal of Comparative Physiology A, 2006, 192:1245-1251)

A method for two-dimensional characterization of animal vibrational signals transmitted along plant stems

Abstract

Conventional approaches to measuring animal vibrational signals on plant stems use a single transducer to measure the amplitude of vibrations. Such an approach, however, will often underestimate the amplitude of bending waves traveling along the stem. This occurs because vibration transducers are maximally sensitive along a single axis, which may not correspond to the major axis of stem motion. Furthermore, stem motion may be more complex than that of a bending wave propagating along a single axis, and such motion cannot be described using a single transducer. Here I describe a method for characterizing stem motion in two dimensions by processing the signals from two orthogonally-positioned transducers. Viewed relative to a cross-sectional plane, a point on the stem surface moves in an ellipse at any one frequency, with the ellipse's major axis corresponding to the maximum amplitude of vibration. The method outlined here measures the ellipse's major and minor axes, and its angle of rotation relative to one of the transducers. I illustrate this method with measurements of stem motion during insect vibrational communication. It is likely the two-dimensional nature of stem motion

is relevant to insect vibration perception, making this method a promising avenue for studies of plant-borne transmission.

Introduction

Many plant-dwelling insects use substrate-borne vibrations in communication and in detection of predators and prey (Cokl and Virant-Doberlet 2003; Virant-Doberlet and Cokl 2004; Cocroft and Rodríguez 2005; Casas and Magal 2006). Studies of substrate-borne vibrations have shown that in plant stems and leaves, these vibrations propagate in the form of bending waves (Barth 1998; Cocroft et al. 2000; Michelsen et al. 1982). As bending waves propagate along a stem, motion of the substrate is perpendicular to the direction of wave propagation (Cremer et al.1973). In theory other waves can occur in rod-like structures (Markl 1983; Michelsen et al. 1982), such as quasi-longitudinal waves, which produce particle motion in the same direction as wave propagation. However, only bending waves have been detected in plant stems (Michelsen et al. 1982, p. 277; Cocroft et al. 2000 and references therein), and longitudinal motion in the plant stem is ignored in this study.

Transducers commonly used to measure vibrational signals in plant stems, such as laser vibrometers or accelerometers, are only (or at least maximally) sensitive to motion along a single axis. Because of this, use of a single transducer leads to a consistent underestimation of signal amplitude, as usually it is not possible to know *a priori* how to position the transducer so that its axis of sensitivity coincides with the direction of maximum stem motion. Since bending waves propagate along the stem's length and

substrate motion is perpendicular to transmission direction, motion at any point occurs within a cross-sectional, two-dimensional (2-D) plane (gray ellipse, Figure 1). If I assume

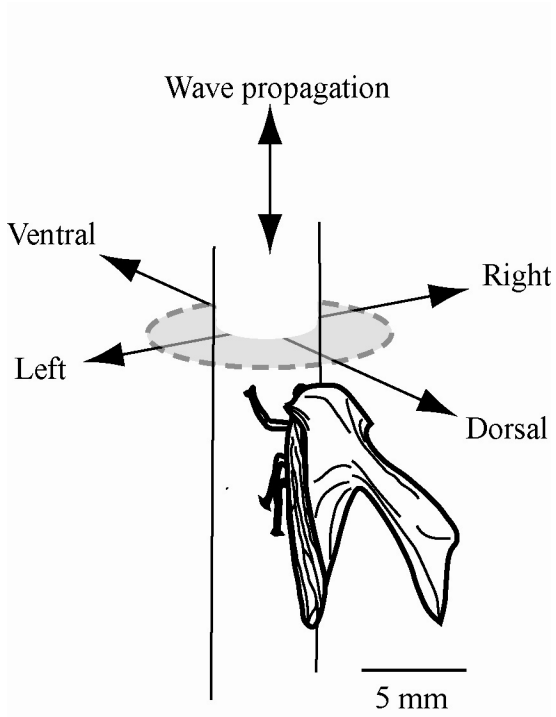

Figure 1. Vibrations in plant stems are transmitted as bending waves along the longitudinal axis of the stem, with stem motion perpendicular to the direction of propagation. Motion from bending waves at a given point on the stem occurs within a cross-sectional plane (dashed gray ellipse).

that it is equally probable for a plant stem to vibrate maximally along any axis within this plane, then it is unlikely that a transducer will be aligned by chance precisely along that axis. However, measurements made along any other axis will underestimate the real amplitude of vibration (Figure 2). The amplitude measured by the transducer is related to the real amplitude of stem vibration by the cosine of the angle between the axis of measurement and the axis of stem motion (Figure 2b). Underestimation of the real

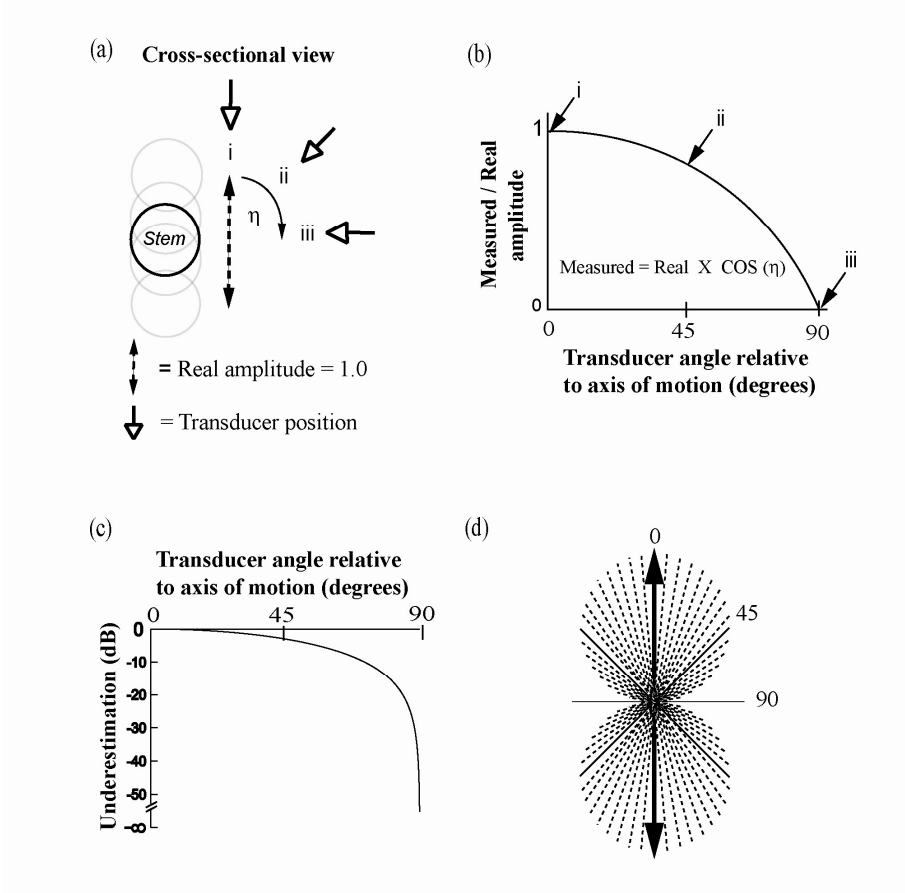

Figure 2. Illustration of the potential for measurement error when using a single transducer. (a) Stem motion (amplitude not to scale) is assumed to be uniaxial, and the transducer is assumed to be sensitive to motion along only one axis. (b) The measured amplitude is related to the real amplitude by the cosine of the angle (η) between the axis of stem motion and the transducer's axis of sensitivity (e.g. i, ii, iii). (c) The underestimation of amplitude (real/measured) that occurs with single-transducer measurements increases exponentially as transducer alignment approaches 90° relative to the axis of motion. (d) The length of the dashed lines represent the amplitude that would be measured if stem motion is uniaxial and single-transducer measurements are taken around the circumference of a stem in 5° increments. For reference, an additional solid line is placed at 45°.

amplitude of vibration increases exponentially as the angle between the axis of vibration and the transducer alignment increases (Figure 2c). Thus, in some situations the underestimation may be extreme, such as measuring little to no signal when in fact substantial vibration is present (e.g. Figure 2a, b, point iii).

In addition to underestimating the amplitude of vibration, a researcher using a single transducer may not accurately characterize stem motion. Stem vibration is likely to be perfectly uniaxial under very limited conditions, if at all. First, when a signaling animal vibrates a stem, it may impart motion along more than one axis. Furthermore, given factors such as the heterogeneity of stem architecture, it is possible that the properties of stem motion will change as a signal propagates (Virant-Doberlet et al. 2006). Previous investigators have provided evidence that measurement along a single axis may not completely describe stem motion (Michelsen et al. 1982; McVean and Field 1996; Virant-Doberlet et al. 2006). However, no formal method has been used to describe the path traced by a stem during transmission of vibrational signals.

The problem of characterizing stem motion within an x-y plane can be solved by applying a method used previously in the field of engineering (below; see also Bachschmid et al. 2004; Lee et al. 1997). The signals from the two transducers, aligned perpendicularly (Figure 3), provide the x and y coordinates of a point on the stem as its displacement (or velocity or acceleration, depending on the transducer) changes through time. The information obtained from these signals allows calculation of the true amplitude of vibration for any given frequency, as well as the orientation of the axis of vibration relative to one of the transducers. This method also provides the information necessary for describing stem vibration more complex than that of bending wave motion along a single axis.

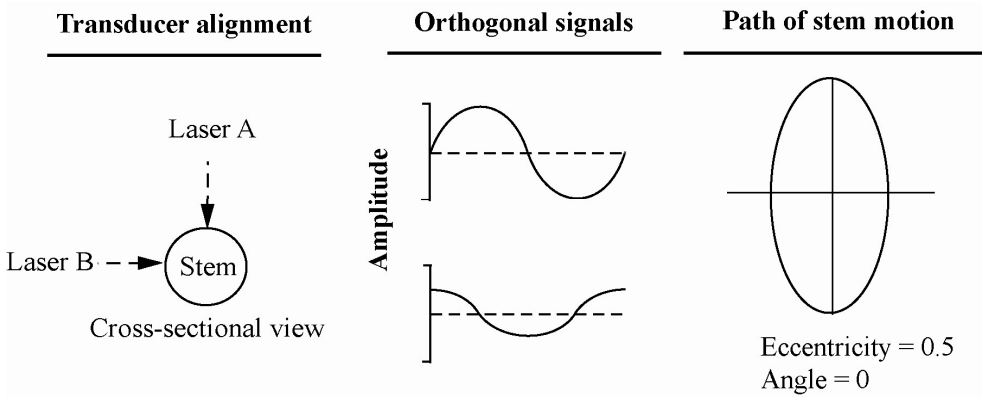

Figure 3. Orthogonal positioning of two transducers relative to a stem allows characterization of elliptical stem motion within a cross-sectional plane.

Here I show how the signals from two orthogonal transducers can be processed to characterize the path of stem motion in two dimensions. I then illustrate the use of this approach with an example of how a plant stem moves when vibrated by signaling insects. Finally, I discuss the situations in which orthogonal measurements may be especially useful, as well as some of the largely unexplored questions about vibrational communication raised by this more complete description of stem vibration.

Methods

Calculating the two-dimensional properties of stem motion

Consider the motion of a plant stem at a single frequency: two orthogonal sine waves, when plotted against each other, will define an ellipse whose properties depend on the relative amplitude and phase of the signals (Figure 3). The parameters of interest for each frequency in a signal can be obtained using amplitude and phase information from the signals, X(t) and Y(t), from the two orthogonally-aligned transducers. Because each

transducer is aligned along one orthogonal axis, together they define a coordinate plane whose axes will be rotated by some unknown angle relative to the to the major and minor axes of the ellipse (Figure 4). For my purposes, I am interested in four parameters: 1) the

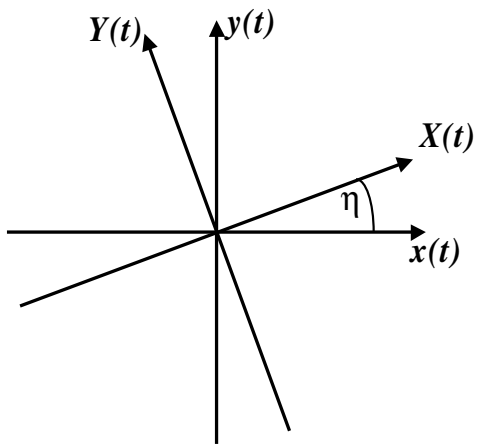

Figure 4. Rotation of the measurement axes *X(t)* and *Y(t)* through the angle η relative to the major and minor axes of the ellipse.

ellipse's major axis; 2) its minor axis; 3) its angle of rotation relative to one of the transducers; and 4) its eccentricity (obtained from the major and minor axes).

In the equations below, A and B represent the Fast Fourier Transforms (FFT) of X(t) and Y(t), each with real (Re[ ]) and imaginary parts (Im[ ]) corresponding to magnitude and phase. That is, A=2FFT(X(t))/N, and B=2FFT(Y(t))/N, where N is the number of points in the time record. The angle of rotation of the ellipse (η), arbitrarily defined relative to the first signal, X(t), can be found using the equations below (see McNett et al. 2006 for derivations) and the four-quadrant arctangent function (ATAN2) in Matlab (v. 6.5; Mathworks, Natick, Massachusetts):

$$\eta = \frac{1}{2}\left[ATAN2\left(2*\left(\mathrm{Re}[A]*\mathrm{Re}[B]+\mathrm{Im}[A]*\mathrm{Im}[B]\right), \left(|\mathrm{A}|^2 - |\mathrm{B}|^2 \right)\right)\right].$$

From this, the major (*a*) and minor axis (*b*) of the ellipse can be found using:

$$a = \sqrt{abs\left[\left(|A|^2 \cos^2 \eta\right) + \left(|B|^2 \sin^2 \eta\right) + 2\left(\mathrm{Re}\left[AB^*\right]\left[\sin\eta\cos\eta\right]\right)\right]},$$

$$b = \sqrt{abs\left[\left(|A|^2 \sin^2 \eta\right) + \left(|B|^2 \cos^2 \eta\right) - 2\left(\mathrm{Re}\left[AB^*\right]*\left[\sin\eta\cos\eta\right]\right)\right]},$$

where B^* is the complex conjugate of B.

Finally, for a given frequency, eccentricity is given as the ratio of the minor (b) and major (a) axes of the ellipse (eccentricity = b/a). Therefore, values for eccentricity are bounded by 0 (uniaxial) and 1 (circular).

Insect vibrational signals:

To illustrate the method outlined above, I characterize in two dimensions the plant stem vibrations produced by signaling insects. I recorded the substrate-borne sexual advertisement signals of each of 15 adult male treehoppers (Hemiptera: Membracidae: *Umbonia crassicornis*) on the stem of a 1-m-tall potted host plant (Mimosaceae: *Albizia julibrissin*). Insects were drawn from a greenhouse colony established with collections near Miami, Florida, USA.

To measure the signals I aligned two laser vibrometers (Polytec CLV 1000 with a CLV M030 decoder module; Polytec Inc., Auburn, MA) so that the beams were orthogonal to each other and to the longitudinal axis of the stem (Figure 3). All 15 insects were placed at the same position on the same individual plant, I recorded the signals of each treehopper at three locations: the first was within 1 cm of the insect (stem diameter 4.3 mm; this position referred to hereafter as *source*); the second was 10 cm from the

insect but on the same unbranched length of stem (3.5 mm diameter; referred to as *10 cm straight*); and the third was 5 cm from the insect, but on a petiole of a leaf arising from the stem (2.2 mm diameter; referred to as *5 cm branch*).

At the first two locations, *source* and *10 cm straight*, along the plant's vertical main stem, the beam of one laser (laser A) was aligned along the signaling male's dorso-ventral axis, while the beam of the other laser (laser B) was aligned along the male's left-right axis. At the third location, *5 cm branch*, the stem's cross-sectional plane is no longer comparable to the x-y coordinate system established at the source, so I arbitrarily chose to align laser B vertically with respect to gravity, leaving laser A in its original orientation; thus, in all three locations, laser A was aligned parallel with the tabletop. Small pieces of reflective tape (ca. 1 mm^2) were attached to the stem at each point of measurement to enhance laser beam reflectance. The temperature of the recording room was maintained at approximately 24°C (range 22.7-25.0 C).

Males of *U. crassicornis* were induced to signal by playing a pre-recorded male-female duet through a loudspeaker (Optimus) from a computer (Macintosh G4). The airborne signal from the loudspeaker was sufficient to induce vibrations in the stem and elicit signaling. The signals of all fifteen males were measured at a given location by placing each male in the same position and alignment on the stem. Then the lasers were re-aligned at the next location and measurements for each male were repeated, again at the same position and alignment on the stem. Between measurements, males were kept in a sleeve cage on a separate *A. julibrissin* plant. The output from the lasers was acquired on a Dell desktop computer using a National Instruments data acquisition board (44100 Hz sampling rate, 16 bit resolution) and a custom-made program written in Labview (v.

6.0; National Instruments, Austin, Texas). The laser signals were high-pass filtered at 70 Hz using a Krohn-Hite 3202 filter (-24 dB per octave, Krohn-Hite Corp., Brockton, MA). Signals produced by *U. crassicornis* males consist of a frequency-modulated sinusoid (100-200 Hz) lasting 1-2 seconds. Signals sometimes contain a series of higher-frequency pulses (< 2 kHz) (see Cocroft & McNett 2006), but these are not consistently produced and I did not measure them here.

Results

Underestimation of the real velocity

The amplitude of *U. crassicornis* signals differed between the two lasers (Figure 5) across all three locations, illustrating the influence of transducer alignment on the measurement of vibrational signals. The underestimation problem that results from variation in the measured signal can be illustrated by comparing single-laser measurements with the real velocity calculated using both laser signals. Because stem motion at *source* and *10 cm straight* was relatively uniaxial and in line with the insects' dorso-ventral axis, laser A illustrates a best-case scenario in which the transducer's axis of sensitivity is aligned with the major axis of vibration (the difference between real velocity and average measured velocity was close to 0 dB at both locations). In contrast, laser B is aligned perpendicularly to the major axis of stem motion, resulting in substantial underestimation (the difference between real velocity and average measured velocity was -13.8 dB at *source* and -12.5 dB at *10 cm straight*). At the third location, *5 cm branch*, the velocities measured by both lasers A and B underestimated the real velocity by 3-4 dB.

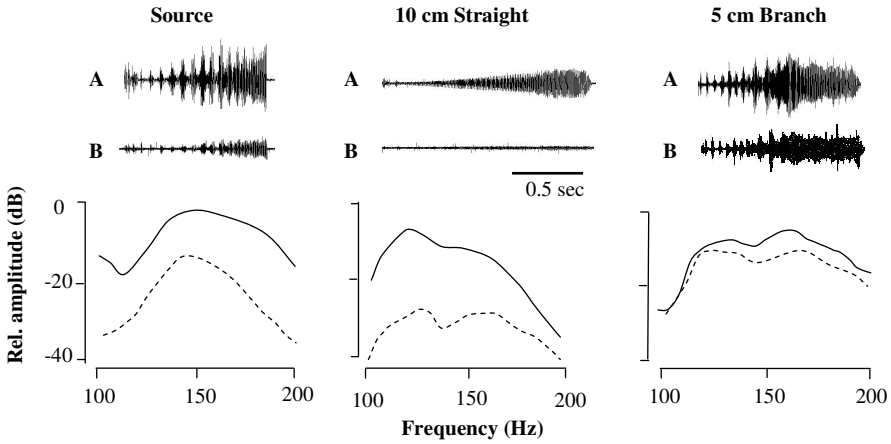

Figure 5. Signals of a male *U. crassicornis* recorded at three distances from the male using two orthogonally-positioned laser vibrometers (A, B). Waveforms from each laser, A and B, are shown with the corresponding amplitude spectra (A=solid line; B=dotted line) (see text). Differences between waveforms at a given location highlight the consequences of variation in transducer alignment.

Path of stem motion

For measurements made at the first two locations, *source* and *10 cm straight*, eccentricity was low (i.e., motion was nearly uniaxial) and relatively constant for frequencies within 10-20 dB of peak amplitude (Figure 6). Stem motion was more ovoid at the third location, *5 cm branch*.

At *source* and *10 cm branch*, the angle of rotation of the major axis of motion relative to laser A deviated little from 0° (Figure 6). That is, the axis of stem motion was approximately aligned with the male's dorso-ventral axis for all fifteen individuals. Angle of rotation did deviate from a 0° alignment at *5 cm branch*; however, as discussed above (see *Methods*) the x-y coordinate plane is not comparable once measurements were made

on a side branch. At each location, the angles of rotation were relatively consistent across frequencies within 10-20 dB of peak amplitude (Figure 6).

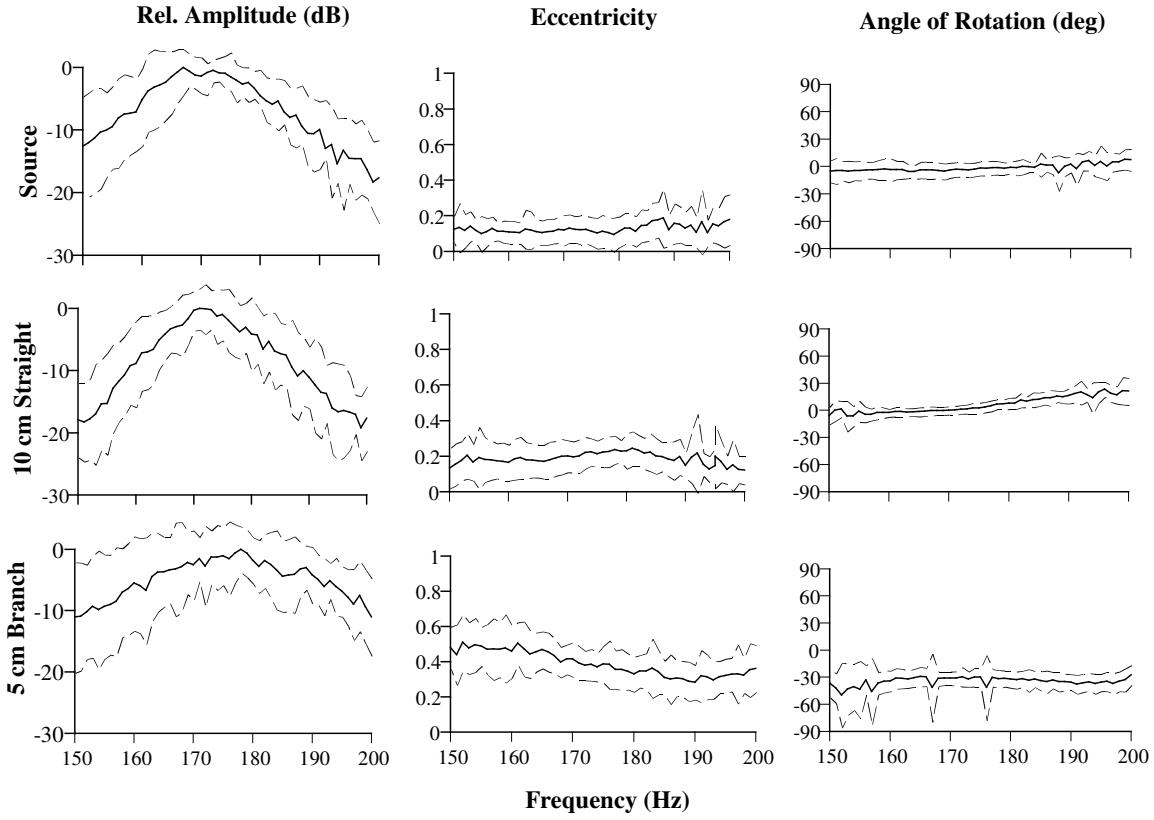

Figure 6. Amplitude spectrum, eccentricity, and angle of rotation of male *U. crassicornis* signals measured at three locations on a plant (x ± s.d., n=15 male signals). Note that at *source* and *10 cm straight* the angle of rotation relative to the insect's dorso-ventral axis is near zero and eccentricity is low. Eccentricity at *5 cm branch*, however, is greater.

Discussion

In this chapter I outline a method for accurately characterizing animal signals and other vibrations traveling along plant stems. This method, which uses spectral analysis of the signals from two orthogonal transducers, overcomes two limitations of measurements made with a single transducer. First, it allows calculation of the maximum vibrational

amplitude for bending waves in the stem, whether or not one of the transducers is aligned with the major axis of stem motion. Accurate measures of amplitude provide a more rigorous look at a variety of questions, such as the transmission properties of plant substrates. Second, this method provides a complete description of the elliptical path of motion at any one frequency, which provides an avenue for addressing a range of new questions related to animal vibrational signals in plant stems.

In some cases the extent to which a researcher using a single transducer will underestimate the major axis of vibration will be minimal: if stem motion at a given frequency is uniaxial, measurements made within 45° of the major axis of motion will underestimate its amplitude by ~ 3 dB at most (note that at 45° the measured amplitude is related to the true amplitude by cosine (45°) ≈ 0.7). However, if the measurement axis differs by more than 45° from the axis of motion, underestimation may be more dramatic; for example, at 75° underestimation will be ≈ 12 dB. If the path of stem motion at a given frequency is not uniaxial, then underestimation of the major axis of vibration is decreased, with no measurement error when the path is completely circular. Given these considerations, use of orthogonally-aligned transducers will be most important for measuring vibrational amplitude when motion is uniaxial or nearly so, but the axis of this motion cannot be predicted. While the present study used two laser vibrometers, contact vibration sensors such as accelerometers could also be used, at least in situations where the additional mass loading is not problematic.

It would also be possible to use the methods described here using orthogonal measurements of repeatable, mechanically-generated signals made at different times with a single transducer. This would require a reference signal that would allow the two

measurements to be compared using a common time base. Use of a single transducer in this way is probably limited to non-contact methods of vibration detection, since use of contact methods such as accelerometers will likely impose different mass-loading effects when the transducer is moved 90° around the stem, effectively reducing the repeatability of measurements.

Measurements made around the circumference of plant stem have shown the presence of signal energy along each axis measured (McVean and Field 1996), as well as changes in the distribution of that energy as the signal propagates (Virant-Doberlet et al. 2006). Virant-Doberlet et al. (2006) suggested that such changes might provide a receiver with information about its distance from the vibration source. Interpreting such measurements is difficult, however, because signal energy can be measured at most angles even when motion is uniaxial (see Figure 4d), and because the phase information is lost between successive measurements. The method described here, by providing information about the path of stem motion, will facilitate research into questions such as whether the eccentricity of the elliptical motion increases with distance from the source. It would be of interest to investigate how stem motion changes when signals are reflected, as a consequence of impedance changes in the transmission channel (Michelsen et al. 1982).

Receptor sensitivity and 2-D stem motion

If vibration perception is influenced by motion along more than one axis, then describing stem motion within a 2-D coordinate plane may be important for understanding how vibrations are transmitted and perceived.

Sensitivity to orthogonal axes of motion in a vibrational stimulus is important in vibration localization in some scorpions (e.g., Brownell and Farley 1979) and spiders (Barth and Geethabali 1982). Orthogonal sensitivity also appears to be important in insects (Sandeman et al. 1996), although the question has not been widely examined. Insects have multiple receptors for detecting vibrations (Markl 1983; Kalmring 1985; Yack 2004), among which the subgenual organ has been particularly well studied. In honeybees, the subgenual organ responds to both dorso-ventral and left-right motion, with greater sensitivity to the former (Rohrseitz and Kilpinen 1997). This differential sensitivity implies that the axis of motion of a vibrational stimulus is an important feature of vibration perception. Insects possess subgenual organs in each leg, and in a free-standing insect on a plant stem the legs are arranged in a variable spatial array (Virant-Doberlet et al. 2006). Integration of sensory information from multiple receptors may allow discrimination between uniaxial and more ovoid or circular 2-D motion. Addressing these questions provides fertile ground for further research, and takes us a step closer to understanding how plant-borne vibrations contribute to social and ecological interactions in natural environments.

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Chapter 4*

(*in review: Behavioral Ecology)

Host shifts favor vibrational signal divergence in *Enchenopa binotata* treehoppers

Abstract

For specialized herbivorous insects, shifts to novel host plants can have dramatic evolutionary consequences. If mating traits diverge, assortative mating can develop between ancestral and novel host populations and facilitate speciation. Mating signals may diverge under a variety of scenarios. Signal differences may be a consequence of divergence in correlated traits, such as body size. If local communication environments differ, mating signals may also diverge through selection for enhanced transmission. I tested these hypotheses using two closely related species in the *Enchenopa binotata* complex of treehoppers. Each member of this complex specializes on a different host plant species. Their communication modality may make signal divergence likely after a host shift: like many plant-dwelling insects, *Enchenopa* communicate using substrate-borne vibrations for which the plant itself is the transmission channel. Each species' mating signal is a relatively pure tone, and differences between species in signal frequency are critical for mate recognition. While no support was found for a correlated selection hypothesis, I found support for a signal transmission hypothesis: both species use a signal frequency that transmits well in their contrasting communication

environments, suggesting host shifts may favor signal divergence and ultimately, behavioral isolation.

Introduction

Plant-feeding insects constitute 40% of all animal species (Bush and Butlin, 2004; Strong et al., 1984), and are thought to have diversified through shifts to novel host plants (Feder et al., 1988; Funk et al., 2002). Host shifts lead to divergent selection, assortative mating, and potentially reproductive isolation between populations on the ancestral and novel hosts. This can happen when plant phenology differs between hosts, leading to an allochronic shift in life-history timing (Wood and Keese, 1990). Host fidelity may also contribute to reproductive isolation (Bernays, 1998; Feder et al., 1994; Jaenike, 1990; Via, 1999; Wood, 1980). However, these reproductive barriers may not completely eliminate interactions between host-associated populations (Drès and Mallet, 2002). Additional isolation may come from traits associated with mating, such as mate attraction signals. Divergence in such traits favors assortative mating and may lead to reproductive isolation (Boughman, 2002; Coyne and Orr, 2004; Schluter and Price, 1993; West-Eberhard, 1983). Only a few studies have addressed plant-related changes in insect mating signals (e.g., Etges and Ahrens, 2001; Landolt and Phillips, 1997), but if host shifts alter the nature of selection on mating signals, this could increase the likelihood that host shifts result in speciation.

Here I address hypotheses to explain mating signal evolution related to host shifts. I use two closely related species in the *Enchenopa binotata* species complex (Hemiptera: Membracidae), which is a clade of host-specialist insects that occur sympatrically

throughout much of the eastern U.S. (Cocroft et al., 2008; Wood, 1993). Species in the *Enchenopa binotata* complex communicate using substrate-borne vibrational signals transmitted through the leaves and stems of their host plant, as do many other plant-feeding insects (Cocroft and Rodríguez, 2005; Virant-Doberlet and Cokl, 2004). Male mating signals in the *E. binotata* complex all consist of a pure-tone 'whine' followed by a series of pulses, but vary in several traits, particularly frequency (Cocroft et al., 2008). Frequency also is the single most important signal trait for female mate recognition, because signals of different species differ more in frequency than in other signal traits (Cocroft et al., 2008), and females strongly prefer the signal frequencies of conspecific males (Rodríguez et al., 2006). I investigate the ultimate causes of frequency differences in two species within the complex: the species that uses Eastern redbud (Fabaceae: *Cercis canadensis*) and the species that uses wafer ash (Rutaceae: *Ptelea trifoliata*). The species on *Cercis canadensis* communicates using a lower signal frequency than the species on *Ptelea trifoliata* (Figure 1). Species in the *E. binotata* complex are awaiting description, and will be referred to here using their host-plant affiliation (i.e., *E. binotata* 'Cercis' and *E. binotata* 'Ptelea').

For host-specialist insects like *E. binotata*, the host plant plays a central role in nearly every aspect of the life cycle, including communication and mate-searching behavior. This intimate relationship suggests at least four hypotheses that could account for mating signal variation following a host shift. First, signal variation could be an immediate consequence of signaling on a new substrate. Previous research has shown this not to be the case in *E. binotata*: when males are moved between different host plant species, signal frequency remains unchanged – that is, frequency in these tonal signals is

a property of the signaller, not the substrate (Cocroft et al., 2006; Sattman and Cocroft, 2003). Second, signal variation could be an immediate consequence of developing on a novel host plant. Previous research has also shown this not to be the case for *E. binotata*: reciprocal transplant experiments that reared *E. binotata* 'Ptelea' on two different hosts showed little or no influence on signal frequency (Rodríguez et al., 2007).

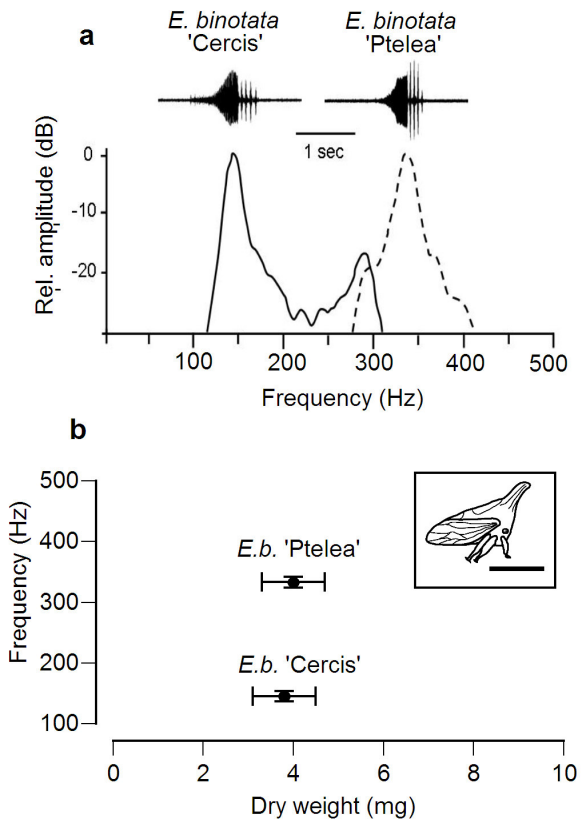

Figure 1. Variation in male mating signal frequency and dry weight for *E. binotata* 'Cercis' and *E. binotata* 'Ptelea.' (a) Waveforms of each species' signal with the corresponding amplitude spectra, showing the frequency difference between species. (b) Variation in dry weight (mg ± s.d.) and frequency (Hz ± s.d.) within each species and a drawing of a representative male (inset; males of both species similar; scale bar = 3 mm). Dry weight was not significantly different between species (t_{113} = 1.83, p=0.07; mean dry weight ± s.d.: *E. binotata* 'Cercis' = 3.8 mg ± 0.70, n=56; *E. binotata* 'Ptelea' = 4.0 mg ± 0.70, n=59).

Here I test two additional hypotheses for how host shifts could lead to signal frequency differences between *E. binotata* 'Ptelea' and *E. binotata* 'Cercis.' First, I test the hypothesis that frequency differences are a consequence of changes in body size. Among vibrationally-communicating insects, larger species have lower-frequency signals, although there is considerable scatter around the best-fit line (Cocroft and De Luca, 2006). Body size can change as a result of host shifts (Messina, 2004), and if the larger of the two *Enchenopa* species has a lower frequency, this would be consistent with the hypothesis that the difference in frequency is a by-product of a change in body size. In contrast, if the two species do not differ in size, or if the species with the lower frequency is smaller, this hypothesis is rejected. Second, I test the hypothesis that signal frequencies have diverged as a result of adaptation to host plants with different signal transmission properties. For the many plant-feeding insects that communicate with vibrations, the stems and leaves of host plants represent the transmission environment. Plant tissues act as frequency filters that attenuate signals and limit the long range transfer of information (Bell, 1980; Bradbury and Vehrencamp, 1998; Michelsen et al., 1982). If there is selection on males to use a signal frequency that propagates with little loss through the substrate, and/or on females to favor frequencies that allow males to be detected and assessed from a greater distance, then populations on hosts with different physical properties might be under selection for use of different optimal signal frequencies.

The hypothesis that frequency differences are a consequence of adaptation to host plants with different transmission properties makes two predictions. The first is that the hosts of species with different signal frequencies must differ in their signal transmission

properties. Transmission properties can be represented by plotting attenuation as a function of frequency (see below). These transmission curves are analogous to adaptive landscapes (Simpson, 1944), where a peak in the attenuation curve represents a frequency range of optimal signal transmission. Transmission curves are likely to differ between hosts because of variation in physical structure and plant mechanical properties (Michelsen et al., 1982; Read and Sanson, 2003). However, if transmission curves do not differ between the host plants of two species with different signal frequencies, then the hypothesis is rejected.

The second prediction is that, given differences in transmission properties of the host plants, male signal frequency should match the peak of optimal transmission, thereby maximizing long range transmission and signal detection (Endler, 1992; Schluter and Price, 1993). Studies of the green stink bug have shown that the frequency of its vibrational signals matches the transmission curves of some of its common host plants (Cokl et al., 2005; Miklas et al., 2001), while a study of two lacewing species, one of which signals on conifers and the other on herbaceous plants and grasses, found no match (Henry and Wells, 2004). However, no study has yet investigated the role of sensory drive in signal divergence where it would be most expected: closely related species, each restricted to a single host plant. If there is a signal-environment match, then signals should transmit better through the substrate where they are typically used than through other substrates, such as plant modules where the insects do not occur, or the substrate used by closely related species. In contrast, if signals do not match the optimum frequency more closely in the environment in which they are used, then adaptation to different host plants cannot explain the divergence in frequency.

Methods

Study system

Members of the *Enchenopa binotata* species complex are a widely cited example of sympatric speciation through shifts to novel host plants (Coyne and Orr, 2004; Wood, 1993; Wood and Guttman, 1983). These species occur sympatrically throughout much of the eastern U.S. on host plants that are evolutionarily diverse, being represented in several different plant families (Rutaceae, Celastraceae, Fabaceae, Juglandaceae, Adoxaceae, Thymelaeaceae, Rhamnaceae, Magnoliaceae). Pair formation in *E. binotata* is mediated by their plant-borne vibrational signals (Cocroft et al., 2008; Hunt, 1994). Males use a "call-fly" strategy while searching for mates, producing advertisement signals to which receptive females respond. Female response then stimulates males into a localized search. An ongoing phylogenetic and phylogeographic study (Snyder, Lin and Cocroft, in prep.) indicates that the two species used in this study are very closely related within the complex, forming a clade along with the *E. binotata* species on *Liriodendron*.

Body size measurements

To test the hypothesis that the difference in frequency between *E. binotata* ‘Cercis’ and *E. binotata* ‘Ptelea’ is due to a difference in body size, I compared dry body weight between 56 males of *E. binotata* 'Cercis' (1-3 males from 39 different host plants) and 59 males of *E. binotata* 'Ptelea' (1-3 males from 31 different host plants). Males were collected in and around Columbia, Boone Co, Missouri, then dry frozen, thawed, and air dried before being weighed on a Mettler AB54S electronic balance to the nearest 0.1 mg.

Signaling sites

Before testing the hypothesis that the frequency difference between *E. binotata* 'Cercis' and *E. binotata* 'Ptelea' reflects adaptation to different host plant transmission properties, it was crucial to determine where on the host plant communication takes place. Behavioral observations were made in 2003 and 2004 throughout the breeding seasons on host plants found within a 20 km^2 area in Boone County, Missouri, USA, in local community parks and natural recreation areas. To identify specific plant stems and branches used for transmitting signals I clipped onto host plant stems a Signal Flex SF30 Universal Tuner Pickup, and monitored signaling behavior using a battery-powered Johnson JA-004 Mini-amp / speaker. For some stems and branches, communication was inferred if multiple individuals were observed on the stem three or more times throughout the breeding season. The rationale for this inference is that adult treehoppers spend most of their time feeding, and there is no spatial separation between feeding and signalling sites (Shugart, Backus and Cocroft, unpub. data). Consequently, if males and females are present on a stem during the mating season, signalling is almost certainly occurring on that stem.

The average stem diameter used by *E. binotata* 'Ptelea' = 2.8 mm ± 0.9 SD (range 1.5 – 5.4 mm, n=86), and the average petiole diameter = 1.2 mm ± 0.4 SD. The average stem diameter for *E. binotata* 'Cercis' = 2.7 mm ± 0.9 SD, and the average petiole = 1.2mm ± 0.4 SD (range 1.4 – 5.4 mm, n=169).

Frequency attenuation curves of the host plants

I tested the predictions of the signal transmission hypothesis by comparing plant transmission properties between host plant species. Each *E. binotata* species was found almost exclusively on the stems and leaf petioles of its host plant, although the species differed with respect to the plant part most frequently used (see Results). Stems and leaf petioles thus represent the environments to which signal adaptation would be expected. I measured the filtering properties of both plant parts for both host plant species. One branch of approximately 1 m in length (measurement includes both stems and leaves) was removed from twenty different plants for each host plant species. The branches removed were those known or inferred to have been used by signaling *Enchenopa*. Branches were cut, capped with a water vial, brought into the lab, and clamped at the base in the same spatial orientation as in the field. To ensure that lab-based measurements reflected those expected under natural conditions I conducted preliminary tests in spring / summer of 2003 on potted host plants within the size range of those used by the insects. I tested for changes in transmission properties due to cutting a stem and applying a clamp to its base for mounting it in the lab. Attenuation curves for distal stem portions were robust to this procedure (GDM, unpublished data). Additionally, since conducting transmission measurements required 3—3 ½ hours per stem, I tested for drift in transmission properties over time; this was negligible for a 4 hour period. For each branch, I measured the transmission properties for one leaf petiole (*C. canadensis*, mean length=3.3 cm, n=20; *P. trifoliata*, mean length=5.7 cm, n=20) and one woody stem (both species, mean length=20 cm, n=20). For both species, the average stem distance is approximately 20 cm (*C. canadensis*: 19.2 ± 3.0 SD; *P. trifoliata*: 20.2 ± 4.0 SD) between the point on a stem with the largest diameter used by the insects and the point

with the smallest diameter used by the insects. I thus report transmission functions on the stem as the relative attenuation occurring over 20 cm.

To measure attenuation I used a 5-second band-limited noise stimulus (100-1000 Hz) imparted into the stem with an ET-132-203 Electrodynamic Shaker (Labworks, CA) that was placed in contact with the base of the stem. The noise stimulus was played from a Macintosh G3 computer using SoundEdit software (v. 16), high-pass filtered at 60 Hz (Krohn-Hite 3202 filter, -24 dB per octave, Krohn-Hite Corp., Brockton, MA), and amplified (Pioneer A-305 stereo amplifier). Amplitude compensation was made at the base of the stem to ensure that each frequency had equal energy once imparted (Cocroft, 1996). The transmitted noise stimulus was recorded on a separate Macintosh G3 (44100 Hz sampling rate, 16 bit resolution) using a National Instruments data acquisition board and a custom-written program using Labview (v. 6.0; National Instruments, Austin, TX). Recordings were analyzed using a custom-written program in Matlab (v. 6.5; Mathworks, Natick, MA).

Measurements of the transmitted stimulus were made using a method detailed elsewhere (Chapter 3); only the essential points are given here. This method involves processing the signals of two orthogonally-aligned transducers. Plant stems vibrate in two dimensions as signals propagate but transducers are maximally sensitive along one axis. Common methods that use a single transducer therefore, will often underestimate the actual vibrational amplitude since it is unlikely the transducer will be aligned with the major axis of vibration. Two orthogonally-aligned transducers establish a two-dimensional plane and allow accurate measurements of vibration amplitude for a given frequency. I arranged the laser beams of two laser vibrometers (Polytec CLV 1000 with a

CLV M030 decoder module; Polytec Inc., Auburn, MA) orthogonally to each other and to the long axis of the stem or leaf petiole being measured. To obtain an attenuation curve, I took a ratio of amplitude values (i.e., transmission function) across a range of frequencies (100-1000 Hz) spanning the frequencies used by the *E. binotata* complex (~140-500 Hz). The amplitude values used in the transmission function were derived from the two orthogonally-aligned transducers. The transmission functions I obtained represent the filtering properties between two points separated by 20 cm length of woody stem, or two points along the entire length of a leaf petiole (petiole length varies between host plant species, see above). One transmission function was thus obtained for one stem and one petiole per tree, for twenty trees of each host plant species. The peak of the transmission function obtained for each woody stem and petiole per branch was set to 0 dB relative to the frequency that transmitted best through that plant part.

Attenuation by environment

I assessed adaptation to the local environment in male signals of both *E. binotata* species by comparing signal attenuation across transmission environments. I used the measured transmission curves to estimate the attenuation that a sample of individual male signals (n=20 for each species) would experience relative to the best frequency in a given plant part. I used the peak frequency of each male signal to obtain an attenuation estimate. All signals used were obtained from males collected from populations near Columbia, Boone County, Missouri.

Results

Body size measurements

Males of *E. binotata* 'Cercis' and *E. binotata* 'Ptelea' did not differ significantly in dry body weight (Figure 1b). Instead, there was a slight trend in the opposite direction: the lower-frequency *E. binotata* 'Cercis' was slightly smaller than higher-frequency *E. binotata* 'Ptelea' (Figure 1b). There is thus no support for the hypothesis that size differences are responsible for the difference in frequency in these two species.

Signaling sites

Both species preferred the distal portions of branches, as do other membracids (Price and Carr, 2000), instead of basal positions nearer the trunk. Within these distal branch portions, however, individuals preferentially occupied different plant parts (Figure 2). *Enchenopa binotata* 'Cercis' was found more often on the stems of its host

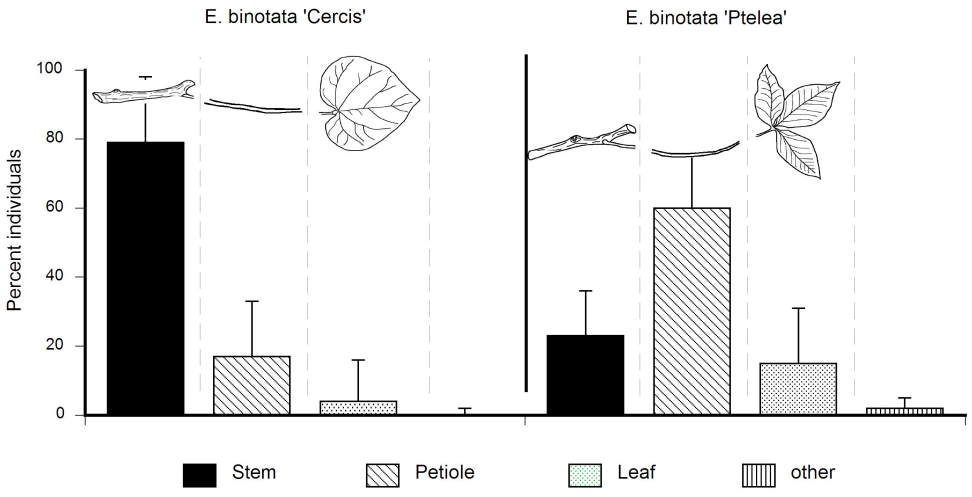

Figure 2. Signaling environments of *Enchenopa binotata* adults within each host plant. *Enchenopa binotata* 'Cercis' (n=33 host plants) primarily communicates through the stems of its host plant, while *E. binotata* 'Ptelea' (n=20 host plants) primarily communicates through leaf petioles.

plant while *E. binotata* 'Ptelea' was found more often on leaf petioles. Furthermore, use of a particular plant part was more consistent in *E. binotata* 'Cercis': the percentage of individuals found in the preferred environment was greater in *E. binotata* 'Cercis' than in *E. binotata* 'Ptelea.'

Frequency attenuation curves of the host plants

The shape of the transmission curves differed between plant parts and plant species. For *Cercis canadensis*, stems transmitted low frequencies with the least attenuation (Figure 3), whereas leaf petioles transmitted higher frequencies with the least attenuation. For *Ptelea trifoliata*, stems also transmitted lower frequencies with the least attenuation (Figure 3), while leaf petioles transmitted mid-range frequencies with the least attenuation. Because the two species use not only different host plants but also different plant modules, they encounter very different communication environments. The first prediction of the hypothesis that signal divergence is due to adaptation to different host plant properties – that the transmission environments of the two treehopper species differ -- is thus supported.

Attenuation by environment

Attenuation estimates confirmed the qualitative match between signal frequency and the transmission curve of a given plant part (Figure 3). The signals of each species experienced the least attenuation in the plant part predominantly used on that species'

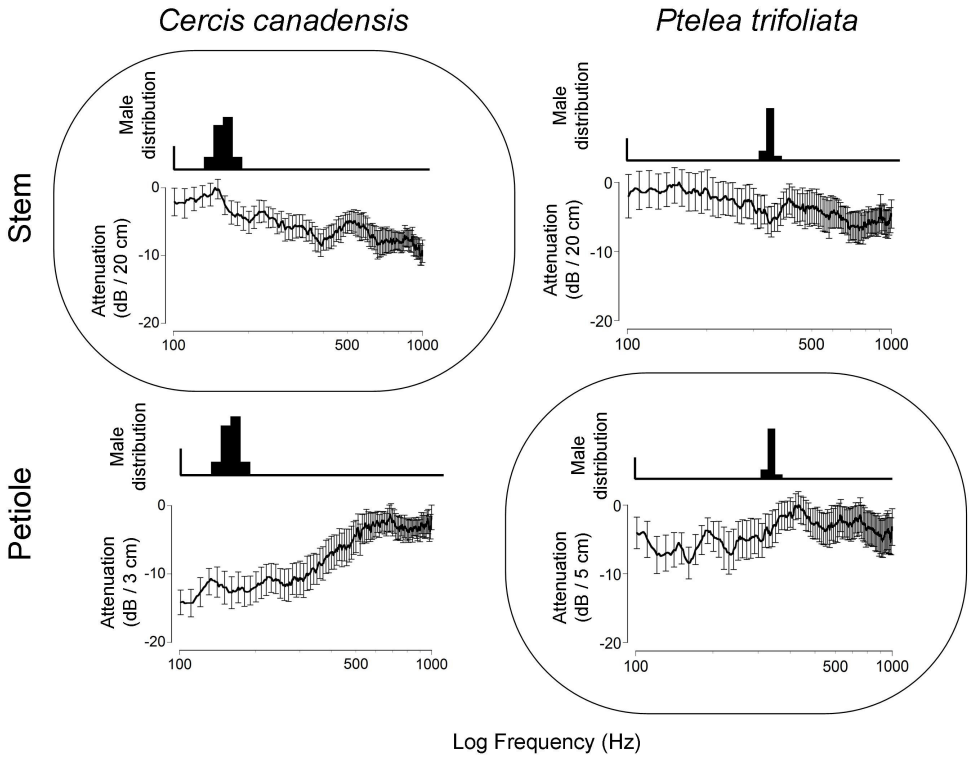

Figure 3. Transmission function curves (attenuation per distance ± standard error) showing differential filtering in stems and petioles of *Cercis canadensis* and *Ptelea trifoliata* (n=20 stems, 1 from each of 20 plants). Transmission curves are relative to the frequency transmitting with the least attenuation (0 dB) in that plant part. Histograms above each curve represent the distribution of male signal frequency. The boxed curve for each species represents the transmission properties of the plant module predominantly used for communication.

own host plant (Figure 4). The signals of *E. binotata* 'Cercis' performed dramatically better when transmitted through the woody stems this species commonly uses, than in the leaf petioles it rarely uses. Signals of *E. binotata* 'Ptelea' also performed best on the petioles where communication more often takes place, although differences in attenuation are less pronounced (Figure 4) because the average transmission curves are flatter (Figure 3). The signals of each species also performed significantly better in their own transmission environment than that normally used by the other species (Figure 4),

supporting the second prediction of the hypothesis that signal divergence is a result of adaptation to different host plant properties.

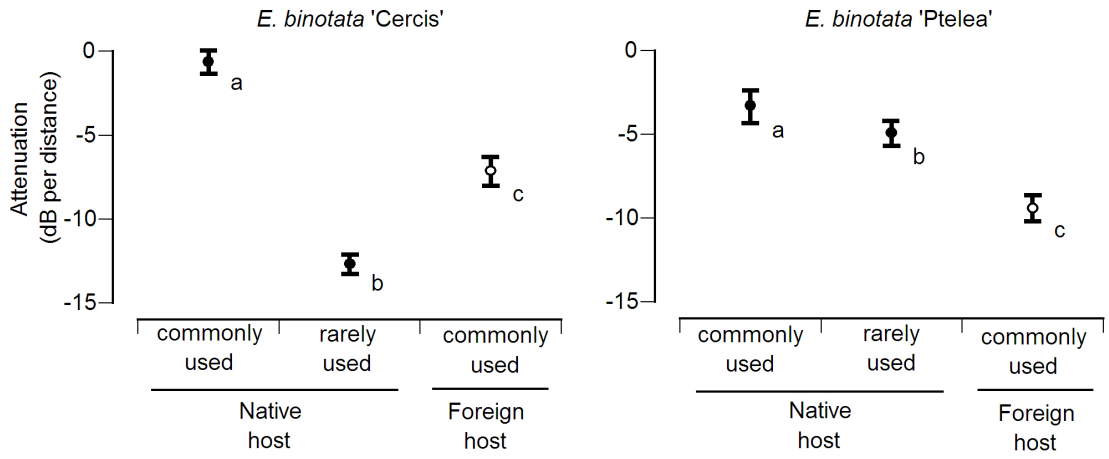

Figure 4. Signals of each species transmit best in the host plant part typically used for communication. Relative attenuation (dB ± standard deviation) of the mating signals of *E. binotata* 'Cercis' (n=20 males) and *E. binotata* 'Ptelea' (n=20 males). For each species, the average attenuation for three plant parts is given. Filled circles represent signal attenuation on the native host plant, in the plant part commonly used (e.g. woody stems for *E. binotata* 'Cercis') and the plant part rarely used (e.g. leaf petioles for *E. binotata* 'Cercis'). Open circles represent signal attenuation on the other host plant, in the plant part commonly used by the other *Enchenopa* species. Attenuation differed significantly across plant parts for both species (*E. binotata* 'Cercis': $F_{2,57} = 2021.4$, $p < 0.0001$; *E. binotata* 'Ptelea': $F_{2,57} = 357.8$, $p < 0.0001$; symbols with unique subscripts are significantly different).

Discussion

I found support for both predictions of the hypothesis that signal differences between these two closely related species are due to adaptation to the signal transmission properties of their respective hosts. First, transmission properties differ between plant environments, in this case, between plant parts as well as host plant species. Second, the frequency of each species transmits with the least attenuation in its own plant

environment. I found no support for the hypothesis that the differences in signal frequency are a by-product of size differences, since body size did not differ between the two species. Two other hypotheses, that frequency differences are a result of signaling or developing on a different host plant, have been rejected in previous studies (Table 1).

Table 1. Four hypotheses to explain mating signal variation as a result of shifting to novel host plants. References indicate the status of each hypothesis in accounting for frequency differences in the *Enchenopa binotata* species complex.

Hypothesis	Prediction(s)	Supported?	Reference
Substrate effect	(i) Signals differ when produced on non-host	no	Sattman & Cocroft 2003; Rodriguez et al. 2007
Developmental plasticity	(i) Signals differ when reared on non-host	no	Rodriguez et al. 2007
Correlated evolution	(i) Differences in body size that influence signal traits	no	(tested in present study)
Signal transmission	(i) Transmission differs between hosts (ii) Signal frequency matches local transmission properties	yes	(tested in present study)

Of the four hypotheses emphasized here to explain frequency differences between *Enchenopa* species, only the signal transmission hypothesis is supported. In addition to selection from female mate choice (Rodríguez et al., 2006), host plant transmission properties are a possible agent of signal evolution, suggesting that shifts to novel host plants can favor divergence in vibrational mating signals. The use by males of frequencies that transmit well through host plant tissues could benefit both males and females. Because female *E. binotata* 'Ptelea' invest substantial amounts of time in mate assessment (LS Sullivan, pers.comm.), they might benefit from the ability to detect males

from greater distances, thereby gaining more information about the availability of mates. Males would gain from obtaining a larger signal active space.

An alternative hypothesis for a signal-environment match is it results from behavioural feedback – i.e., that individuals choose an environment that transmits their signal with little attenuation. In the absence of other causes of frequency change, I would expect signal frequency to remain the same after a host shift, because the insects would choose an environment within the new host that has the same signal-transmitting properties as the old host. Any changes in signal frequency after a host shift would have to arise from other causes such as developmental plasticity; however, experiments with *E. binotata* 'Ptelea' revealed that developing on a different host does not alter signal frequency (Rodriguez et al. 2007). Although individuals are likely to choose favourable sites for signal transmission, where available (e.g.'s, Bennet-Clark, 1987; Elias et al., 2004; Heindl and Winkler, 2003), males will be constrained in their choice of signalling sites by the distribution of females. Females, in turn, are likely to be under fecundity selection, maximizing access to nutritional resources (Roff, 1992) rather than to male signals. Accordingly, given that (1) I have found no evidence for immediate changes in signal frequency after a host shift (Sattman and Cocroft, 2003); (2) within the *E. binotata* complex, frequency differences are closely associated with changes in host use (Cocroft et al., 2008); and (3) male choice of signalling sites is likely to be constrained by the distribution of females, I feel that the most likely explanation for the signal-environment match in the two species examined here is that it is due to selection on signal frequency arising from host plant transmission properties.

Whether plant environments can favor vibrational signal divergence has been an open question (Cocroft and Rodríguez, 2005). Widely held beliefs about vibrational signals predict convergence in signal design, rather than divergence. For example, it has been assumed that plant-dwelling insect species should use broadband signals for efficient transmission (Bradbury and Vehrencamp, 1998; Michelsen et al., 1982). This idea is based on the expectation of unpredictable plant filtering properties: a wide bandwidth will allow at least some frequencies to reach the intended receiver. Also, studies that have tested the predictions leading to signal divergence have produced mixed results. Henry and Wells (2004) found no support for signal-environment matching or differential transmission in two green lacewing species (*Chrysoperla* spp.) that use very different assemblages of plant substrates. In contrast, studies of the cosmopolitan green stinkbug (*Nezara viridula*) have shown that it uses a frequency range that transmits well on some of its common host plants (Cokl et al., 2005; Miklas et al., 2001).

The present study is the first to address the signal transmission hypothesis using large sample sizes, host-specialists, methods for accurately measuring signal amplitude, and clearly defined communication environments. The lack of consensus from previous studies may result from the absence of one or more of these features. First, most studies have reported estimates of plant transmission properties based on very small sample sizes, which may not accurately characterize the signal environments encountered by a population of insects. Second, all previous studies have involved taxa that use multiple plant species, which complicates predictions about signal adaptation. Third, although plant stems vibrate in two dimensions during vibration propagation, previous studies have used approaches that measure stem vibration in only one dimension. Such an approach is

likely to underestimate signal amplitude and reduce the accuracy of measured transmission curves (Chapter 3). Finally, behavioral observations in the field have not usually been available to precisely identify the signaling environment to which adaptation should be expected.

Substrate-borne vibrational communication is widespread in plant-feeding insects (Claridge, 1985; Cocroft and Rodríguez, 2005; Henry, 1994; Virant-Doberlet and Cokl, 2004). This is the first study to support the hypothesis that changes in host plant use can impose natural selection on insect vibrational signals, and it may be significant that support was found in strict host specialists (this study) but not in a pair of species that use a range of different plants (Henry and Wells, 2004). Generalist species may face a very different selective environment, especially in the variability of signalling substrates, and a hypothesized adaptation to such unpredictability is to use signals containing a broad band of frequencies, which may fare better in the 'frequency lottery' than signals that use a narrow band of frequencies (Michelsen et al., 1982). However, at least one host generalist (the green stinkbug, *Nezara viridula*) uses narrow-band signals. Its signal frequency is well-matched to the transmission properties of some of the common hosts in its introduced range, and the use of a narrowband signal is hypothesized to be an adaptation to a frequency 'window' around 100 Hz that occurs in a number of the plant substrates used by this species (Cokl et al., 2005; Miklas et al., 2001). To understand the nature of selection imposed by host plants on signal traits in generalist species, then, it may be important to measure multiple hosts. Much more work will be needed, however, before generalizations can be made about how vibrational communication systems evolve in response to the environments created by the tissues of living plants.

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Chapter 5

Discussion

Summary of results

My research has focused on two sources of selection on the communication systems of insects that use plant-borne vibrations: wind-induced noise and plant transmission properties. In Chapter 2 I showed how vibrational signaling behavior of *E. binotata* treehoppers is influenced by wind-induced vibrations. For insects communicating through plants, wind is probably the major source of abiotic noise. Males and females of *E. binotata* 'Ptelea' responded behaviorally when presented with wind-induced vibrational noise. Males preferentially inserted their signals into brief gaps. Females were less likely to respond to male signals masked by wind, so males exhibiting gap-detecting behavior will be more likely to elicit a response from receptive females. Females exhibited another behavior that may be adaptive in a windy environment: in addition to responding immediately to male signals overlapped by wind, females produced non-duetting signals during silent gaps. I hypothesize that these additional signals may increase a female's likelihood of recruiting multiple males from which to choose a mate.

The adjustments by males and females are temporal in nature and can be distinguished from spatial adjustments. Individuals may use elevated song perches in acoustic communication (Mathevon et al., 1996; Wilczynski et al., 1989), or conspicuous postures and favorable lighting conditions in visual communication (Endler and Thery, 1996; Marchetti, 1993). Elias et al. (2004) has shown that vibrationally-communicating

spiders prefer substrates that afford minimal attenuation of signals. It is unknown whether insects using plant-borne vibrations choose signaling sites with favorable conditions for communication, but such adjustments would be expected. For example, larger plants may have wind-exposed and sheltered portions; the latter may provide individuals a low-noise communication environment.

My research is at the intersection between sensory drive and ecological speciation in its focus on the role of natural selection on communication signals and in the evolution of reproductive isolation. Reproductive character displacement (RCD) results in the evolution of reproductive isolation through natural selection to avoid mismating (Howard, 1993; Servedio and Noor, 2003). Reproductive isolation is adaptive since it increases an individual's fitness by preventing costly mating mistakes. The insight that has be gained from an RCD perspective is witnessed both through the empirical work on RCD (Höbel and Gerhardt, 2003; Jang and Gerhardt, 2006; Nosil et al., 2007), and also recent theoretical work on the topic (Goldberg and Lande, 2006; Pfennig and Ryan, 2006; Servedio and Noor, 2003). This work has consistently shown that natural selection on communication-related traits can confer rapid reproductive isolation and may play an important role in speciation.

Reproductive character displacement can also be viewed as a form of sensory drive. This is a novel view of RCD that has not been recognized previously. In the original formulation of sensory drive, Endler (1992) recognizes the role of other individuals in the communication environment, but considers predators rather than other signalers. Moreover, since sensory drive is not explicitly a speciation hypothesis, it is focused more generally on the joint evolution between signaler and receiver in a given set

of environmental conditions, rather than divergence and the evolution of reproductive isolation. Other signalers are a form of noise—and hence, an agent of selection—in the communication environment. Viewing RCD as a form of sensory drive has the consequence that reproductive isolation may not necessarily be adaptive, but rather, may be a by-product of evolving greater efficacy in a noisy environment.

In addition to the influence of noise and transmission properties on vibrational communication, it is important to recognize that other factors are certainly important. These include sensory exploitation by predators or parasites (Casas and Magal, 2006; Zuk and Kolluru, 1998), impedance mismatches between signaler or receiver and the transmitting substrate (Michelsen et al., 1982), or other noise sources such as rain (Barth, 1988; Casas et al., 1998). Future research will determine the importance of these factors in the evolution of communication in the *Enchenopa binotata* complex.

Plants as a biotic signal environment

Vibrational communication in phytophagous insects is unique because the transmission environment is a living organism. Throughout my dissertation I have treated the insect-plant relationship as a one-way interaction, with plants imposing selection on their herbivores' vibrational communication system. However, plants also respond to the presence of herbivores. The interesting question that results: How might a plant's response to herbivore activity affect vibrational communication?

Plants exhibit bewildering complexity and range in how they respond to stresses in their environment (Awmack and Leather, 2002; Jones and Coleman, 1991)—responses that also change considerably in space and time (Karban and Myers, 1989). In general,

however, the transmission of vibrations in rod-like structures can be predicted by comparing the elasticity (i.e., stiffness, or "Young's modulus") and the density (mass) of the transmitting substrate. Vibrations transmit better in plant stems that are elastic relative to their density (Michelsen et al., 1982); that is, substrates that are stiff but not too massive. To have the effect of reducing the efficacy of vibrational communication, plants would benefit from a response that increases mass relative to stiffness. Plants do vary widely in stiffness, even when controlling for variation in density (Gibson and Ashby, 1997), suggesting such a response may be possible. The cost for a vibrationally-communicating herbivore is due to the fact that more force is required to impart a vibration (Cremer et al., 1973; Michelsen et al., 1982).

Plants respond to insect herbivores in a variety of ways, but most often the target is herbivore feeding success (Jones and Coleman, 1991). The response to herbivores has the potential to affect communication, especially when that response involves changes in plant morphology. The type of response to an herbivore and whether this response is morphological will depend strongly on the relative availability of carbon and nitrogen, or the C:N balance (Jones and Coleman, 1991). This tradeoff is further complicated by the fact that insects are often nitrogen-limited (Huberty and Denno, 2005). Although the C:N balance in plants is itself affected by a wide range of factors, it provides a useful way of categorizing potential mechanical responses. Carbon-based responses are often structural, and may include increases in cuticle or cell wall thickness (e.g., Raupp, 1985), or increases in fiber or lignin content (e.g., Wainhouse et al., 1990). Because of their structural role, carbon-based responses would be strong candidates for plant responses that affect signal transmission. An exception might include the use of soluble carbon to

dilute the relative availability of nitrogen (Awmack and Leather, 2002). Nitrogen-based responses to herbivores, on the other hand, commonly involve secondary compounds that deter herbivore feeding. Since these responses typically are not structural, they would be less likely to affect signal transmission.

Plants respond not only to the presence of herbivores, but also to mechanical stimulation, such as wind (Grace, 1977; Mitchell, 1996; Niklas, 1992; Telewski, 2006). Often a response to wind will be morphological, and therefore, similar to the carbon-based responses mentioned above that serve to decrease herbivore feeding success. Common responses include an increase in stem and leaf petiole diameter (e.g., cuticle or sclerenchyma tissue) (Grace, 1977). Plant responses to wind may also be biochemical, which some studies have shown can also decrease herbivore feeding success (Cipollini Jr., 1997). These results suggest that in terms of feeding performance, herbivores should select plants that occur in calm environments. Furthermore, from the vibrationally-communicating herbivore's perspective, this is precisely the type of low-noise environment that would also favor communication.

Future research on the communication environment

By focusing on the communication environment it becomes clear that there are many ways that natural selection can affect communication. Countless studies have demonstrated a correlation between factors of the environment and communication systems. The acoustic modality has been particularly well studied (Blumstein and Daniel, 1997; Forrest, 1994; Patten et al., 2004; Römer and Lewald, 1992; Wiley, 1991). Habitat-based selection on communication signals has also been heavily studies in the

visual modality (Allender et al., 2003; Endler and Thery, 1996; Leal and Fleishman, 2002). The bias towards the acoustic and visual communication may result, in part, from our own reliance upon these modalities. My work has extended our understanding of the communication environment in the vibrational modality, though many questions remain. Below I offer a few suggestions for future research regarding communication environments.

i) Signal polymorphisms

Although reproductive isolation can evolve rapidly, speciation is typically a slow process relative to our ability to study it. As a result, considerable insight has been gained from studies that catch divergence in its earliest stages (Coyne and Orr, 2004). Polymorphic traits have long been recognized as providing a window into the early stages of divergence (Levene, 1953; Ravigné et al., 2004; Wilson, 1989). Signal polymorphisms (e.g., Arnegard et al., 2005), therefore, represent an overlooked, but potentially fruitful direction for studying the early stages of signal divergence. Furthermore, should the design of signal morphs be closely related to their communication environments, this would represent an excellent opportunity for studying the role of the communication environment in signal divergence and reproductive isolation.

ii) Micro-habitat variation

In addition to emphasizing the early stages of divergence, the presence of polymorphic traits also stresses the importance of fine-scale variation in the environment.

The very maintenance of polymorphisms is thought to depend on environmental heterogeneity (Hedrick, 1986; Ravigné et al., 2004; Smith and Skúlason, 1996). Biologically relevant habitat variation might get underestimated if the communication environment is not parsed fine enough. Without considering plants as modular units (Chapter 4), transmission differences in the plant vibrational environment would have been less pronounced (G.McNett, unpublished). An unexplored example might exist with wind-induced noise within a particular host plant: portions of a plant that face a prevailing wind likely experience greater levels of noise than "leeward" portions. As a result, herbivore populations may be sub-divided into groups that are specialized for communicating through different plant environments.

iii) Effects of noise on signal evolution

Noise, particularly of anthropogenic origin, has been a recent focus in studies of acoustic communication. Most of the studies related to noise have shown how noise affects signaling behavior (reviewed in Brumm and Slabbekoorn, 2005). Given the pervasiveness and abundance of abiotic and biotic noise sources, comparatively less research has shown how noise can affect signal evolution. Classic examples of behavioral phenomena include the Lombard effect in birds, which involves the production of higher amplitude signals when noise is present than when it is absent. The Lombard effect represents a short-term behavioral adjustment. How might noise affect the long-term evolution of signal structure? Of course, it must be recognized that the *capacity* to make behavioral adjustments is a product of evolution. Particularly interesting is the influence noise has on multi-modal or multi-component signaling (Candolin, 2003). Noise may

favor the evolution of signal complexity or signal divergence since detection depends strongly on environmental conditions. For example, if a more specific signal fails to reach a receiver, a more general signal may be more effective ('efficacy back-up hypothesis', Hebets and Papaj, 2005). Might a more tonal signal (e.g., a single frequency or saturated color) be "backed up" by a broadband or white / black signal?

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VITA

Gabriel Dion McNett was born May 22, 1974 in St. Marys, Ohio to Wayne and Nancy McNett. Gabe graduated in 1992 from Memorial High School in St. Marys, Ohio, and spent his first year of college at the University of Toledo where he was an education major. He transferred to The Ohio State University in September 2003 and finished a bachelor's of science (BS) degree in December 1997. After his BS, Gabe spent three years gaining research experience in a variety of locations. He lived out of tents, cabins, and research stations from sub-arctic Canada, to Australia and southern Ohio. Eventually Gabe began his Master's research at the University of California in San Diego in summer 2000, and finished two years later in fall 2002. At this time, Gabe moved to the University of Missouri to begin working on his Ph.D. Eleven semesters later, December 2007, he earned his doctorate degree. Currently, Gabe is working as a Visiting Assistant Professor of Biology at Westminster College in Fulton, Missouri. He hopes to find a job teaching at a small liberal arts college in the Midwest.