Variation in Plant Substrates and its Consequences for Insect Vibrational Communication

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Abstract

Many insects and other arthropods communicate using plant-borne vibrational signals. Vibration transmission along plant stems imposes a frequency filter on signals, and may cause signal degradation from reflected waves. Furthermore, different plant species and plant parts can differ in their transmission properties. This variability in the communication channel may constrain the reliability of signals, with important consequences for the evolution of vibrational communication systems, as well as for researchers studying signal variation at an individual, population, or species level. In this study we estimate the magnitude of substrate-related variation in the mate advertisement signals of a treehopper (Hemiptera: Membracidae: *Umbonia crassicornis*). We used laser vibrometry to record the signals produced by 25 adult males on two different plant species, one host and one non-host. We recorded male signals on two plants per species; within each plant, signals were recorded simultaneously at two distances. We measured three spectral characteristics (dominant frequency, relative amplitude of the signals' high and low frequency components, frequency at the end of the signal) and two temporal characteristics (signal duration and click repetition rate). Spectral characteristics were influenced by the distance at which the signal was recorded, and this influence varied among plant species and individuals. Temporal characteristics were less influenced, although signal length was influenced by distance, an effect that varied among individual plants. Overall, the magnitude of the effects was small. Furthermore, there was significant within-individual repeatability of almost all signal traits across different plant substrates. Signal characteristics were thus reliably associated with individuals, even when they signaled on different plants.

Introduction

Many insects and other arthropods use substrate vibrations to communicate (Claridge 1985; Barth 1997; Cokl &Virant-Doberlet 2003) or to detect predators and prey (Barth et al. 1988; Pfannenstiel et al. 1995; Meyhofer et al. 1997). Among the substrates used to receive or transmit information, the stems and leaves of plants are the most widespread (Cocroft & Rodriguez 2005). Signal transmission through plant stems presents a number of challenges for vibrational communication, including severe frequency filtering of signals (e.g. Bell 1980; Michelsen et al. 1982; Magal et al. 2000), the frequency-dependence of propagation velocity (Michelsen et al. 1982) and in some cases temporal distortion from reflections (Michelsen et al. 1982; Miklas et al. 2001). These changes in transmitted signals can vary from plant to plant and from place to place on the same plant (Michelsen et al. 1982; Keuper & Kuhne

1983; Magal et al. 2000; Miklas et al. 2001; Cokl et al. 2005). There have been few attempts to systematically quantify the magnitude of substrate-induced changes in vibrational signals (Miklas et al. 2001; Sattman & Cocroft 2003; Henry & Wells 2004), but research with the pentatomid bug *Nezara viridula* illustrates the potential scope of the problem: on one plant, the length of signals in the 100-ms range differed by up to a factor of two, depending on the signal type and the distance from the source (Miklas et al. 2001).

Signal variation imposed by the transmission properties of the environment has important consequences for the ability of receivers to reliably assess variation among signalers. This kind of environment-dependent signal variation could contribute to the maintenance of genetic variation in signal properties within populations (Rodríguez & Greenfield 2003; Greenfield & Rodriguez 2004). Additionally, if some characteristics of vibrational signals are simply not repeatable across substrates, receivers would not be expected to evolve responses based on those characteristics. On the other hand, signal characteristics that are less substrate-dependent could provide more reliable cues for receivers. For example, substrate-independent characteristics of male mate advertisement signals would be subject to more consistent selection by female choice, and might diverge more rapidly. In addition, substrate-imposed signal variation poses a challenge for researchers attempting to compare signals among individuals, populations or species. Understanding how variation among substrates influences signals is thus important for understanding the evolution of vibrational communication systems.

Our goal in this study is to estimate the magnitude of changes that occur in plant-borne vibrational signals when insects are recorded on different plant species, different plant individuals, and at different distances within the same plant. We also estimate the effect of variation in substrates on the repeatability of different signal characteristics. Our approach was to use signaling insects (the treehopper *Umbonia crassicornis*) in a repeated-measures design in which the same individuals were recorded on different substrates. We chose to use actual insects rather than experimental simulation of a signaling insect using a playback device. Although mechanically generated stimuli provide greater control over the signal introduced into the substrate, use of real insects avoids the mass loading and concomitant changes in substrate properties imposed by use of mechanical devices. Furthermore, this design incorporates any variation introduced by differences in insect-sub-

strate impedance (see Michelsen et al. 1982) and by changes in the signaling behavior of the insects on different substrates (Miklas et al. 2001; Sattman & Cocroft 2003). Finally, in some cases mechanical stimulation of the stem can result in reflections not produced by signaling insects (Michelsen et al. 1982). In addition to recording insects on different individual host plants, we also explore the consequences for signal variation of the use of a non-host for signal transmission. For example, if changes in signals are greater on the non-host, this would provide a factor favoring host-specificity in mate-searching behavior. We measured signal changes over distances (≤15 cm) typical of many insect social interactions (Cocroft & Rodriguez 2005) and at which most measurements intended to characterize vibrational signals are made.
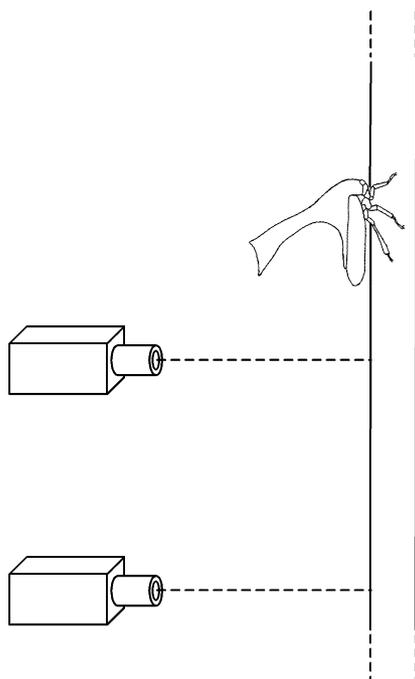
Methods

The treehopper *U. crassicornis* (Hemiptera: Membracidae) uses a range of host plants, primarily in the Mimosaceae (McKamey & Deitz 1996). Individuals produce plant-borne vibrational signals in a range of contexts: immature insects use short, broad-band signals to solicit maternal care (Cocroft 1996, 1999), and adult males produce complex frequency- and amplitude-modulated signals while searching for mates (Cocroft & McNett 2006). Mate-searching males will readily signal when placed on a new plant, and this behavior allowed us to characterize the signals of the same individuals recorded on multiple substrates. Individuals used in the study were drawn from a greenhouse colony maintained at the University of Missouri on a 16:8 L:D cycle. The colony was founded with repeated collections near Miami, FL, USA.

To explore the variation in signals produced on different plant substrates, we recorded vibrational signals from the same set of 25 adult male *U. crassicornis* on two distantly related plant species. One of the species is a woody host of *U. crassicornis* (Mimosaceae: *Albizia julibrissin*) and one is a woody non-host (Adoxaceae: *Viburnum lentago*). We used two potted plants (approx. 1 m tall) of each species. On each plant, we recorded each of the 25 males placed at the same location. Each male was induced to signal by playing a pre-recorded duet between a male and a receptive female through a loudspeaker (the airborne sound induced sufficient vibrations in the plant to elicit signaling). We recorded each signal simultaneously at two distances (approx. 5 cm and approx. 15 cm) from the signaling male. Thirteen of

the 25 males were recorded first on *Albizia* and the other 12 were recorded first on *Viburnum*. Because of the setup time required for positioning the laser, all males in a group were recorded on one plant before switching to the next plant, with recordings made for 1–2 plants/d. As a consequence, recordings of an individual male were made over a period of 3–4 d. The study was conducted in Jun. 2001.

Vibrational signals were transduced using two laser Doppler vibrometers (Polytec CLV 1000 with CLV M030 decoder modules; Polytec, Inc., Ann Arbor, MI, USA) at 5 mm/s/V sensitivity. The lasers were positioned in the same plane, perpendicularly to the plant stem and 25–50 cm from the plant surface. Insects were placed on the plant with their dorso-ventral axis in the same plane as the lasers (see Fig. 1 for an illustration of the recording setup). Environmental vibrations were minimized by placing plants and lasers on a Kinetic Systems Vibraplane isolation table. To increase reflectance of the plant stem, small pieces of reflective tape (approx. 1 mm^2) were attached at the recording locations. The laser output was high-pass filtered at 60 Hz using a Krohn-Hite model 3202 filter (Krohn-Hite Corp., Brockton, MA, USA), digitized at 44.1 kHz, and recorded and analyzed using Canary v. 1.2.4 (Cornell Lab of Ornithology, Ithaca, NY, USA). Recordings were made at an ambient temperature of 24 + 2.5°C.

Signal variables that were temperature-dependent as revealed by a linear regression were adjusted to a common temperature of 25°C.

The signals of *U. crassicornis* males contain two components produced simultaneously in different frequency ranges: a frequency- and amplitude-modulated tone or harmonic series in the 100–200 Hz range; and a train of broadband clicks in the 400–2000 Hz range (Fig. 2). We measured two temporal characteristics of the signals (signal duration and click repetition rate) and three spectral
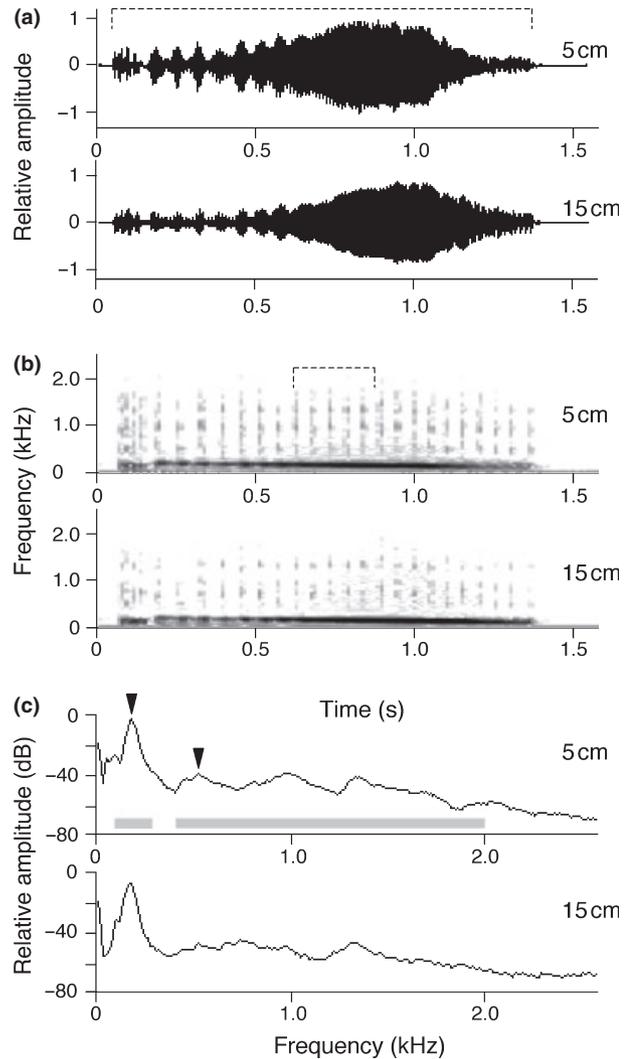
Fig. 2: Vibrational advertisement signal of a male *Umbonia crassicornis*, recorded simultaneously at 5 and 15 cm from the male. (a) Waveforms; (b) spectrograms; (c) amplitude spectra. Dotted lines in (a) and (b) show measurements of signal length and click rate, respectively. Filled triangles in (c) show the frequencies of peak amplitude in the lower and higher-frequency components (clicks) of the signal. The frequency ranges of these components are indicated by the gray bars under the upper of the two spectra

Fig. 1: Orientation of the laser vibrometers relative to the plant stem and the signaling insect (not to scale)

characteristics (dominant frequency, frequency at the end of the signal, and the difference in peak amplitude between the lower-pitched harmonic series and the higher-pitched clicks; see Fig. 2). Total signal length was measured from waveforms (Fig. 2a). Click rate was measured from five clicks in mid-signal from spectrograms (Fig. 2b); the value used was 5/the duration of five cycles, where one cycle started at the beginning of one click and ended just before the beginning of the following click. The dominant frequency was measured using a Fast Fourier Transform (FFT) as the frequency of highest amplitude in the amplitude spectrum (fft size = 16 384 points) of an entire signal, and falls in the range of the lower-pitched component. The final frequency was measured as the frequency of highest amplitude from the spectrum of the last 200 ms of the signal (fft size = 8192 points). The amplitude difference was measured as the difference in peak amplitude of the low-pitched and high-pitched components of the signal (see Fig. 2c). If, for example, higher frequencies were attenuated more quickly than lower frequencies, then this difference would be larger at 15 cm than at 5 cm.

Statistical analyses were conducted using SAS v 6.12. The experiment had a hierarchical within-subjects design: each male was recorded on two plant species, two individual plants within each species, and at two distances within each plant. At any one location, males produced a series of two to three signals, two of which were measured and averaged (we chose the first two signals produced, using the third signal only when the signal/noise ratio was poor for one of the first two). We used a MANOVA to examine the effect of plant species, plant individual and distance. The effect of plant species was tested over the interaction of individual males by plant species; the effect of distance and the interaction of plant species by distance were tested over males within species and distance; the effect of individual plant within species was tested over the interaction of individual males by individual plant within species; and the interaction of distance by plant individual was tested over the overall error term. A sequential Bonferroni correction (Rice 1989) was applied to the results to adjust the significance level for use of five variables.

In the above analyses, the recorded males were treated as a single sample, in order to ask how different plant substrates might change the way signals are produced or transmitted. We can also examine how substrates influence the variation among individuals: for example, does a male that produces a

relatively low-pitched or long signal on one plant also do so on other plants? We estimated the repeatability of each signal trait across the four substrates; repeatability describes the proportion of variation in a trait that is due to variation among rather than within individuals (Boake 1989). Because repeatability values incorporate measurements made at different times, it was not possible to isolate the effect of substrate variation alone on signal reliability (although this could be estimated using a different experimental design). Instead, our approach was to ask whether signal characteristics measured over a relatively short time period showed significant repeatability, even when individuals were recorded on different substrates. This test is thus to some degree one-tailed: to the extent that signal traits are not repeatable, this will be due to changes over time as well as to changes introduced by different substrates. To the extent that traits are repeatable, however, this would show that differences among substrates did not cause the signals to be unreliable indicators of individual characteristics. We obtained repeatability values as in Lessells & Boag (1987) from a MANOVA that included male identity, plant individual and plant species. Standard errors for repeatability estimates were obtained as in Becker (1984).

Results

There was no overall effect of plant species on signal properties (Table 1; see Appendix I for full MANOVA results). There was an influence of the distance at which signals were recorded and this effect varied depending on plant species and the individual plant. However, the magnitude of the effects was generally small (Table 1; Figs 2 and 3), and almost all signal variables were repeatable across the four different substrates.

Table 1: The effect of substrate-related variation on signal variables. For each variable or interaction with a significant effect, the maximum difference between means is shown (with the percent difference, based on the smaller mean); empty cells reflect variables for which there was no effect of substrate. For full results of the MANOVA, see Appendix I

|	Dominant frequency (Hz)	Final frequency (Hz)	Signal length (ms)	Click rate (Hz)	Amplitude difference (dB)
Species					
Plant	6.3 (4%)				
Distance		1.6 (1%)	9.5 (1%)		2.3 (8%)
Distance × Species	5.2 (3%)				4.2 (15%)
Distance × Plant	15.6 (10%)	8.4 (6%)	44.2 (3%)		8.9 (37%)

Temporal Features

The influence of substrate on temporal variables was relatively small. Signal length was influenced by distance from the signaler, and the effect of distance varied among individual plants; however, the difference was slight (Table 1; Fig. 3a). Measurements of click rate were not influenced by plant species, individual plant, or distance (Table 1; Fig. 3b).

Spectral Features

Substrate variation had a more pronounced effect on spectral features. The dominant frequency of the signal was not influenced by the plant species on which the insect was recorded, but it was influenced

by variation among individual plants within a species (Table 1; Fig. 3c). There was no overall effect of distance on dominant frequency, but there was an interaction between distance and plant species, as well as between distance and plant individual. The varying effect of substrate filtering on dominant frequency, which sometimes increased and sometimes decreased with distance, can be seen in Fig. 3c. The measurement of 'final frequency' (i.e. the dominant frequency of the last 200 ms of the signal) was less influenced by substrate, although there was a significant effect of distance, as well as an interaction between distance and individual plant (Table 1; Fig. 3d). The measurement of 'amplitude difference' (the difference in peak amplitude between the low-frequency component and the high-frequency
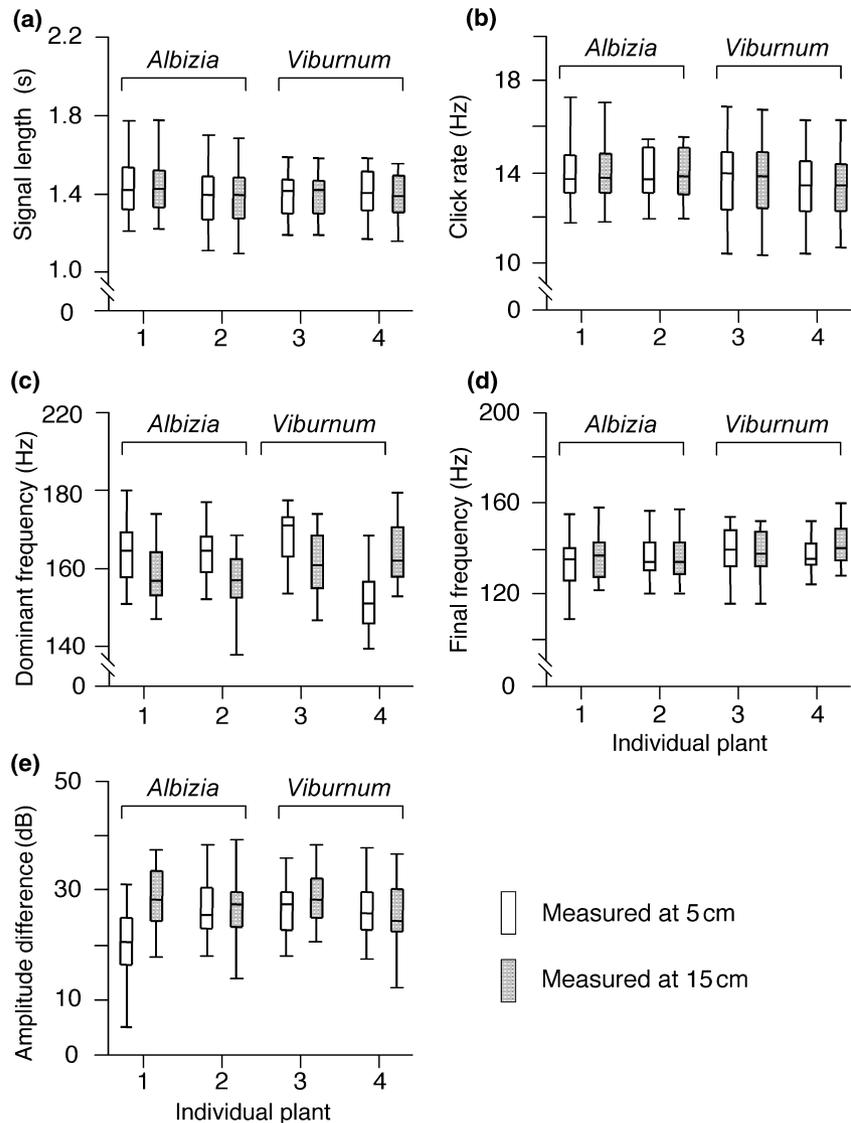

Fig. 3: (a–e) Boxplots showing variation (median, interquartile range) in signal measurements at two distances on different plant individuals and species

component) was more strongly influenced by substrate, with an effect of distance that differed between plant species and between individual plants within a species (Table1; Fig. 3e). The relative amplitude of lower and higher frequency components did not change in a consistent direction with distance among substrates (Fig. 3e).

Reliability of Signal Features Across Distance

The influence of distance from the source on signal measurements can also be evaluated by examining the correlation between measurements made simultaneously at two distances. Recordings of one male signal recorded at two distances are shown in Fig. 2 and correlations between measurements at the two distances are shown in Fig. 4. This figure again reveals the relatively small effect of distance on temporal measurements (Fig. 4a,b); measures of signal length and click rate at the two distances had a Pearson correlation coefficient of 0.99. Spectral measurements (Fig. 4c–e) showed more scatter and lower
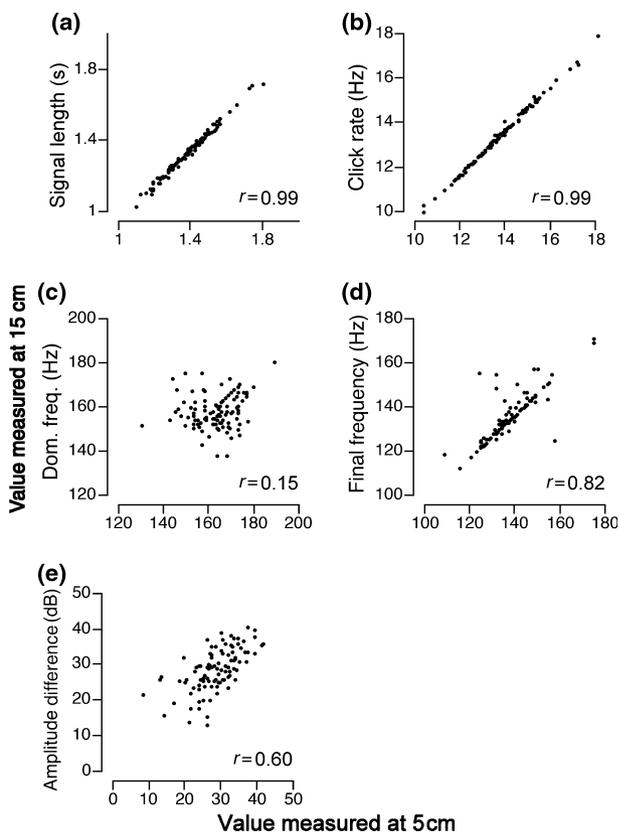
Table 2: Repeatability of male signal characteristics (±SE) across different individual plants and plant species, measured at 5 and 15 cm from the signaling male (n = 25 males, four observations per male). Based on the MANOVA, all signal characteristics exhibited significant repeatability except for final frequency measured at 15 cm (p = 0.0514)

	Repeatability	
Signal trait	5 cm	15 cm
Dominant frequency	0.158 ± 0.103	0.152 ± 0.103
Final frequency	0.209 ± 0.107	0.142 ± 0.102
Signal length	0.363 ± 0.111	0.353 ± 0.111
Click rate	0.246 ± 0.109	0.261 ± 0.110
Amplitude ratio	0.483 ± 0.105	0.526 ± 0.102

correlation coefficients; among the spectral features, the most consistent over distance was the final frequency (Fig. 4c).

Repeatability of Signal Features Across Substrates

Measurement of repeatability provides an indication of whether or not there are consistent differences among the signals of different males – in this case when the males were recorded on four different plant substrates over a period of 3–4 d. Almost all variables were significantly repeatable at both distances (Table 2), based on the MANOVA results. Surprisingly, the highest repeatability value at both distances was for the relative amplitude of high- and low-frequency signal components.

Discussion

Despite the widespread occurrence of vibrational signaling in insects – substrate vibrations are used in communication by an estimated 195 000 described species (Cocroft & Rodriguez 2005) – there have been few systematic studies of how variation among plant substrates influences the properties of vibrational signals. It is clear that plant stems and leaves act as frequency filters that can substantially alter the amplitude spectrum of a signal (Michelsen et al. 1982; Magal et al. 2000; Cokl et al. 2005). Furthermore, reflections can cause signal degradation under some conditions, altering the signal's amplitude envelope and increasing apparent signal length (Miklas et al. 2001). The influence of plant substrates on signal variation thus has important implications for insect communication, as well as for the measurement and characterization of insect vibrational signals. In this study, the influence of plant substrate on signal variation was estimated by

Fig. 4: (a–e) Scatterplots of measurements of *Umbonia crassicornis* male signals recorded simultaneously at two distances, with correlation coefficients indicated

recording the same set of insects on different plant individuals and species, with recordings made simultaneously at two distances within each substrate. The results showed that signal variation was influenced by plant-to-plant differences within a species; it also was influenced by distance from the source, with the effects of distance varying among plant species and among individual plants within a species. However, in spite of this variation, almost all signal variables showed significant repeatability across the four substrates on which males were recorded.

Measurements of the temporal features of signals were little influenced by variation among substrates. For one variable (the click repetition rate), there was no effect of plant species, plant individual or distance. Signal length measurements were unaffected by differences among plant species or individuals and there were only slight differences (on the order of 1%) among measurements made at different distances. Only by comparing measurements on one plant at one distance with those on another plant at another distance did signal length differences reach 5%. The implication is that temporal features detected at a consistent distance close to the source are relatively substrate-independent.

In contrast to the measurement of temporal variables, the measurement of spectral variables was more substantially influenced by variation in plant substrates. This was as expected, given the well-established result that plant stems act as frequency filters (Michelsen et al. 1982; Magal et al. 2000; Cokl & Virant-Doberlet 2003). Measurements of the dominant frequency of the tonal component of the signals, for example, varied by up to 6% on different plants of the same species even when measured at the same distance near the signaling insect. Larger differences would be expected for signals containing an even greater range of frequencies.

Differences among substrates can influence not only the properties of transmitted signals, but also the behavior of signaling insects. For example, when males of the host-specific treehopper *Enchenopa binotata* from *Ptelea trifoliata* host plants were recorded both on their native host and on a non-host, they produced significantly shorter signals, and fewer signals in a bout, on the non-host (Sattman & Cocroft 2003). When males of the pentatomid bug *N. viridula* were played female calling songs, their level of responsiveness differed when on a natural host than when on an artificial platform; furthermore, the difference in behavior between the two substrates varied between the two populations tested (Miklas

et al. 2001). By contrast, in the present study there was no clear evidence of such behavioral changes.

We measured the repeatability of signal variables as an index of how well signals reflected individual male identity across different substrates. Significant repeatability suggests the presence of genetic variation and thus the potential to respond to selection (Boake 1989). Substrate effects on signals are expected to introduce environmental variation into the values experienced by a receiver, reducing their repeatability. However, in this study both spectral and temporal variables showed significant repeatability across substrates. Importantly, because individuals were recorded over a period of 3–4 d, our estimates of repeatability were also influenced by variation within individuals over time; thus our results likely underestimate the repeatability of signals across substrates. This suggests that substrate-induced variation is less of a constraint on signal reliability than was anticipated.

One unexpected result was that the highest repeatability value was for a measure reflecting the relative amplitude of the high-and low-frequency components of the signal. What might explain the high repeatability of this seemingly very substrate-dependent feature? The high-frequency clicks in the signal are likely produced by tymbals (Ossiannilsson 1949), while the more tonal, low-frequency portion of the signal may be produced by the action of the thoracic muscles (Cocroft & McNett 2006). The amplitude of the low-frequency portion of the signal can vary substantially among males (De Luca, pers. comm.); if it is consistent within males and varies more than the amplitude of the clicks, then this could explain the high level of between-male variation relative to within-male variation for that feature.

For the treehoppers in this study, signal length did not change markedly with distance. In contrast, other studies (Keuper & Kuhne 1983; Miklas et al. 2001) found that the length of signals in the 100-ms range increased by a factor of 1.5–2 with distance. In one study, these changes occurred over a distance of 10 cm, less than that measured here (Miklas et al. 2001). What accounts for this difference in results? Three factors will influence the duration of a vibrational signal propagating along a stem. The first is strictly a measurement issue and may account for the slight (2–10 ms) decrease in signal length with distance measured on each plant in this study. The treehopper signals measured here have a gradual rise and fall (see Fig. 2a). If signal amplitude decreases with distance, the first and last few milliseconds of the signal can drop below the level of even small amounts of background noise, decreasing the

apparent length of the signal. In contrast, two other factors will cause an increase in signal duration. One is the dispersive nature of propagation of bending waves, for which transmission velocity increases with the square root of frequency (Michelsen et al. 1982). A signal with a range of frequencies will be longer at a distance than at the source, because lower frequencies will arrive after higher frequencies. Dispersive propagation accounts for at least some of the increase in the length of broadband katydid signals recorded on a grass stem (Keuper & Kuhne 1983). Dispersive propagation should have only a small influence on the duration of signals recorded near the signaler; e.g. the katydid signals increased in length by only 2 ms at 27 cm from the source (Keuper & Kuhne 1983) and no increase in signal length was detected at 15 cm in the present study. Dispersive propagation will cause changes in signals in proportion to the range of frequencies present in the signal, with larger changes expected in broadband signals, little change in narrowband signals, and no change in pure tone signals.

Another factor that may influence signal length and other temporal features of both broadband and narrowband signals is signal degradation from reflected waves. Reflections can in principle occur whenever a propagating signal encounters a large change in impedance, such as at the tips of the leaves or at the end of a cut stem (Michelsen et al. 1982; Cokl & Virant-Doberlet 2003). When this occurs, the waveform measured at a given location will include waves that have traveled by different paths and thus different distances from the source, altering and extending the amplitude envelope of the signal (Wiley & Richards 1978). Signal degradation from reflected waves accounts for the two-fold increase in duration of the relatively narrowband signals of pentatomid bugs recorded at a distance from the source in the study by Miklas et al. (2001). In the present study, there was no evidence of signal distortion from reflections, and in recordings of katydid signals on different substrates, reflections apparently occurred on some plants but not others (Keuper & Kuhne 1983). Substantial reflections are more likely to occur when the signal is of high amplitude at the source, when attenuation of propagating signals is low, and when there is a short distance between the signaler and the locations on the plant causing reflections. For small insects on relatively large host plants, reflections may not always be a problem. For example, we have seen minimal or undetectable signal degradation from reflections in recordings of membracid treehoppers in the lab and the field, or in vibrational playback experiments conducted at amplitudes characteristic of membracid signals.

In this study we did not measure the signals' absolute amplitude, which is highly substrate dependent. For example, the same insect will produce a lower-amplitude signal when recorded signaling on a thick stem than on a thin stem, other things being equal. Furthermore, substrates differ in the extent to which vibrational signals are attenuated during transmission: Keuper & Kuhne (1983) reported attenuation values of 50 dB/m for soft herbaceous stems vs. 15 dB/m for stiff woody stems. Signal amplitude is also greatly affected by differences among individual plant parts. For a bending wave that travels from a larger stem to a smaller stem, the vibration amplitude of the smaller stem must be higher if the total energy per unit length is to remain the same; accordingly, small side stems and leaves will generally vibrate at higher amplitudes than the main stem (see Keuper & Kuhne 1983).

The role of substrate-induced signal variation in insect communication in the field remains largely uninvestigated. As mentioned above, in our field recordings of treehopper signals, distortion from reflected waves was largely absent. On large woody hosts, where most signaling occurs on thin branches, substantial reflections from the roots are unlikely to be a problem because the signal amplitude will be greatly reduced once it reaches the main trunk; and reflections at the trunk-branch intersection may be small if the change in impedance is gradual. Some signal changes may be useful to the insects in locating the source; for example, measurements of the arrival time of high-frequency vs. low-frequency signal components would in principle provide insects with a means of estimating distance to the source (Michelsen et al. 1982). If plant effects on signals are consistent within a host species but differ between species, selection for efficient signal transmission (a component of the sensory drive hypothesis; Endler 1992; Henry & Wells 2004; Cocroft & Rodriguez 2005; Cokl et al. 2005) could result in signal divergence between species using different plant species or plant parts. Results of this study showed that although substrate-induced variation was significant for some signal traits, all traits measured were repeatable when males signaled on different substrates. In light of the potentially severe effects of transmission on the properties of vibrational signals (Michelsen et al. 1982), this result is unexpected. However, a reliable association of signal traits with individual signalers is a requirement if signals are to evolve in response to sexual or natural selec-

tion; and if one considers that vibrationally communicating insects have evolved a great diversity of signal forms (Cocroft & Rodriguez 2005), the finding that signal variation is repeatable even when individuals signal on different species of plants is less surprising.

Implications for the Measurement of Vibrational Signals

What can be done to minimize the influence of substrate on signal variation when comparing signals of different individuals, populations or species? One obvious solution is to use the same artificial recording substrate for all of the insects whose signals are to be compared. This approach works well for some insects (e.g. Henry 1985; Stewart et al. 1995). However, the examples of insects whose signaling behavior differs when on host plants vs. non-hosts (Sattman & Cocroft 2003), or on natural hosts vs. artificial platforms (Miklas et al. 2001), suggest that use of artificial recording substrates will not yield desirable results for all species. Therefore, before using this approach, it would be advisable to record a series of insects on both a natural host and the artificial recording substrate to be sure that signals and signaling behavior do not differ. It may be important to use a substrate that is relatively similar to a natural substrate, to avoid differences in insect-substrate impedance or in the relative position of the insect's legs and body during signal production. Furthermore, if different populations or species are to be compared, it should be borne in mind that they may differ in their responses to natural vs. artificial substrates (Miklas et al. 2001). When insects are host-specific and behave differently on and off their hosts, then the best method to ensure comparable measurements may be to use a different substrate for each species, recording each one on its own host.

Once a recording substrate has been chosen, recording signals close to the insect will minimize substrate-induced changes. Because artificial platforms are usually small, measurement distance is more of an issue when using natural hosts. Even when recordings are made close to the source, measurements of the relative amplitude in different frequency components will be substrate-dependent. Spectral measurements will only be substrate-independent if the signal is a pure tone, or if it is a frequency-modulated tone and measurements are made at a consistent 'landmark' in the signal [compare the results of this study for the frequency measured at the end of the signal vs. the overall dominant frequency of the entire signal; also see Sattman & Cocroft (2003)]. Whatever the recording substrate, all measurements should be made with the transducer and insect in the same locations. It is not always possible to ensure that the insect signals where the investigator wants it to, but variation in distance and plant part (e.g. leaf vs. stem) will translate into variation in signal properties.

Acknowledgements

Literature Cited

Barth, F. 1997: Vibratory communication in spiders: adaptation and compromise at many levels. In: Orientation and Communication in Arthropods (Lehrer, M., ed). Birkhauser Verlag, Basel, pp. 247—272.

Barth, F. G., Bleckmann, H., Bohnenberger, J. & Seyfarth, E. A. 1988: Spiders of the genus *Cupiennius* Simon 1891 (Araneae, Ctenidae) II. On the vibratory environment of a wandering spider. Oecologia **77**, 194—201.

Becker, W. A. 1984: Manual of Quantitative Genetics, 4th edn. Academic Enterprises Pullman, WA, USA.

Bell, P. D. 1980: Transmission of vibrations along plant stems: implications for insect communication. J. New York Entomol. Soc. **88**, 210—216.

Boake, C. R. B. 1989: Repeatability: its role in evolutionary studies of mating behavior. Evol. Ecol. **3**, 173—182.

Claridge, M. F. 1985: Acoustic signals in the Homoptera: behavior, taxonomy, and evolution. Annu. Rev. Entomol. **30**, 297—317.

Cocroft, R. B. 1996: Insect vibrational defence signals. Nature **382**, 679—680.

Cocroft, R. B. 1999: Parent-offspring communication in response to predators in a subsocial treehopper (Hemiptera; Membracidae: *Umbonia crassicornis*). Ethology **105**, 553—568.

Cocroft, R. B. & McNett, G. D. 2006: Vibrational communication in treehoppers (Hemiptera: Membracidae). In: Insect Sounds and Communication: Physiology,

Behaviour, Ecology and Evolution (Claridge, M. F. & Drosopoulos, S., eds). Taylor & Francis, Boca Raton, FL, pp. 305—317.

Cocroft, R. B. & Rodriguez, R. L. 2005: The behavioral ecology of insect vibrational communication. BioScience **55**, 323—334.

Cokl, A. & Virant-Doberlet, M. 2003: Communication with substrate-borne signals in small plant-dwelling insects. Annu. Rev. Entomol. **48**, 29—50.

Cokl, A., Zorovic, M., Zunic, A. & Virant-Doberlet, M. 2005: Tuning of plants with vibratory songs of *Nezara viridula* L (Heteroptera: Pentatomidae). J. Exp. Biol. **208**:1481—1488.

Endler, J. A. 1992: Signals, signal conditions, and the direction of evolution. Am. Nat. **139**, S125—S153.

Greenfield, M. D. & Rodriguez, R. L. 2004: Genotype × environment interaction and the reliability of mating signals. Anim. Behav. **68**, 1461—1468.

Henry, C. S. 1985: The proliferation of cryptic species in *Chrysoperla* green lacewings through song divergence. Fla. Entomol. **68**, 18—38.

Henry, C. S. & Wells, M. L. M. 2004: Adaptation or random change? The evolutionary response of songs to substrate properties in lacewings (Neuroptera: Chrysopidae: *Chrysoperla*). Anim. Behav. **68**, 879—895.

Keuper, A. & Kuhne, R. 1983: The acoustic behaviour of the bushcricket *Tettigonia cantans* II. Transmission of airborne-sound and vibration signals in the biotope. Behav. Proc. **8**, 125—145.

Lessells, C. M. & Boag, P. T. 1987: Unrepeatable repeatabilities: a common mistake. Auk **104**, 116—121.

Magal, C., Schöller, M., Tautz, J. & Casas, J. 2000: The role of leaf structure in vibration propagation. J. Acoust. Soc. Am. **108**, 2412—2418.

McKamey, S. H. & Deitz, L. L. 1996: Generic revision of the New World tribe Hoplophorionini (Hemiptera: Membracidae: Membracinae). Syst. Entomol. **21**, 295—342.

Meyhofer, R., Casas, J. & Dorn, S. 1997: Vibration mediated interactions in a host-parasitoid system. Proc. R. Soc. Lond. Ser. B Biol. Sci. **264**, 261—266.

Michelsen, A., Fink, F., Gogala, M. & Traue, D. 1982: Plants as transmission channels for insect vibrational songs. Behav. Ecol. Sociobiol. **11**, 269—281.

Miklas, N., Strtith, N., Cokl, A., Virant-Doberlet, M. & Renou, M. 2001: The influence of substrate on male responsiveness to the female calling song in *Nezara viridula*. J. Insect Behav. **14**, 313—332.

Ossiannilsson, F. 1949: Insect Drummers. A study on the morphology and function of the sound-producing organ of Swedish Homoptera Auchenorryncha. Opuscula Entomol. Suppl. **10**, 1—146.

Pfannenstiel, R. S., Hunt, R. E. & Yeargan, K. V. 1995: Orientation of a Hemipteran predator to vibrations produced by feeding caterpillars. J. Insect Behav. **8**, 1—9.

Rice, W. R. 1989: Analyzing tables of statistical tests. Evolution **43**, 223—225.

Rodríguez, R. L. & Greenfield, M. D. 2003: Genetic variance and phenotypic plasticity in a component of female mate choice in an ultrasonic moth. Evolution **57**, 1304—1313.

Sattman, D. A. & Cocroft, R. B. 2003: Phenotypic plasticity and repeatability in signals of *Enchenopa* treehoppers, with implications for gene flow among host-shifted populations. Ethology **109**, 981—994.

Stewart, K. W., Abbot, J. C., Kirchner, R. F. & Moulton, S. R. 1995: New descriptions of North American euholognathan stonefly drumming (Plecoptera) and first Nemouridae ancestral call discovered in *Soyedina carolinensis* (Plecoptera: Nemouridae). Ann. Entomol. Soc. America **88**, 234—239.

Wiley, R. H. & Richards, D. G. 1978: Physical constraints on acoustic communication in the atmosphere: implications for the evolution of animal vocalizations. Behav. Ecol. Sociobiol. **3**, 69—94.

Appendix 1: MANOVA results showing the effect of plant species, individual, distance and the relevant interactions on variation in vibrational signals.

Variance component	df	Dominant frequency			Final frequency			Signal length			Click rate			Amplitude difference		
		MS	F	p	MS	F	p	MS	F	p	MS	F	p	MS	F	p
Species	1	8.1	0.10	–	582.6	3.66	–	0.00002	0.00	–	10.3	4.31	–	50.1	2.53	–
Plant (species)	1	514.3	10.6	***	72.9	0.43	–	0.016	0.78	–	0.37	4.31	–	85.2	3.30	–
Distance	1	115.5	3.00	–	131.6	6.88	*	0.0045	51.25	**	0.011	2.84	–	271.1	53.69	**
Distance × Species	1	699.4	18.1	***	20.3	1.06	–	0.00006	0.65	–	0.013	3.23	–	171.2	33.91	**
Distance × Plant (sp.)	1	1128	33.8	***	115.8	6.30	**	0.0001	11.35	**	0.003	0.53	–	291.4	32.99	**
Male × Species	24	82.3			159.1			0.029			2.40			19.8		
Male × Plant (sp.)	48	48.4			168.5			0.021			3.38			25.8		
Male (species and distance)	48	38.5			19.1			0.000087			0.00402			5.05		
Error	48	33.4			18.4			0.000089			0.00497			8.83		

Significance level incorporates sequential Bonferroni correction (–, not significant; *p < 0.05; **p < 0.01; ***p < 0.001). No F-values are shown for variance components used only as error terms.