

Characterization and Comparison of Substrate-Borne Vibrational Signals of *Chlorochroa uhleri*, *Chlorochroa ligata*, and *Chlorochroa sayi* (Heteroptera: Pentatomidae)

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ABSTRACT The substrate-borne vibrational songs produced by phytophagous stink bugs of the genus *Chlorochroa* (Heteroptera: Pentatomidae) were recorded and compared. Sex- and species-specific vibrational songs were recorded from both sexes of three stink bug species of agricultural importance, the congeners *Chlorochroa uhleri* Stål, *Chlorochroa sayi* (Stål), and *Chlorochroa ligata* (Say). The temporal and spectral characteristics of the songs were determined, along with the context in which they were produced relative to contact between signalers and receivers. Vibrational communication started with production of the calling song by *C. ligata* females (FS-1), and by males of *C. uhleri* (male song [MS]-1) and *C. sayi* (MS-1). Males of all three species produced courtship songs (MS-2), whereas only *C. ligata* and *C. sayi* produced rivalry songs. Copulatory songs were produced by all three species, but they could not be attributed to either sex. Several of the songs share similarities in their fine-scale time units that seem to be rearranged either temporally or by behavioral context in generating songs associated with different behavioral functions. The relationship of the *Chlorochroa* spp. songs to the songs of other stink bug species is discussed.

KEY WORDS substrate-borne vibrational signal, acoustic communication, Pentatomidae, sex-specific signal

A rapidly growing body of research has revealed that intraspecific vibrational communication is widespread in insects (Drosopoulos and Claridge 2006). Vibrational signals mediate a variety of behaviors, including mate location and mate choice, intrasexual competition, alarm, defensive behaviors, predator-prey interactions, recruitment to food, and complex social interactions (Čokl et al. 1999, Hill 2001, Virant-Doberlet and Čokl 2004). Among phytophagous stink bug species of the subfamily Pentatominae (Heteroptera: Pentatomidae), sexual communication mediating pair formation is a bimodal process whereby long-distance attraction is mediated by sex pheromones (Borges et al. 1987; Aldrich et al. 1987, 1991; Ho and Millar 2001a,b; McBrien and Millar 2003; Millar 2005), and shorter-range mate location and recognition are mediated by species-specific substrate-borne vibrational songs (Čokl et al. 1999, 2000a,b, 2004; Čokl and Virant-Doberlet 2003; Miklas et al. 2003). It is also likely that the behavioral context and species specificity of acoustic signals is a requirement for reproductive isolation (Stumpner and von Helversen 2001).

Phytophagous stink bugs are distributed worldwide, are highly mobile, and can be sporadic to chronic pests

of a variety of agricultural products, including cotton (*Gossypium hirsutum* L.), grains, legumes, alfalfa (*Medicago sativa* L.), and perennial crops such as tree fruits and nuts (McPherson and McPherson 2000). Conservative estimates of annual economic losses caused by stink bugs in the United States easily exceed \$100 million (McPherson and McPherson 2000). Furthermore, because modern integrated pest management (IPM) strategies have resulted in decreased use of broad-spectrum insecticides in favor of more target-specific control methods, a resurgence of secondary insect pests (Fairchild 2004), including stink bugs, has taken place (Barbour et al. 1990). The lack of accurate, reliable, and uniform monitoring procedures, combined with the insects' high mobility and reemerging pest status, provides considerable motivation for developing a better understanding of their reproductive biology, particularly if aspects of this biology can be exploited or manipulated in IPM programs. Identifying and understanding stink bug acoustic signals could aid in the development of rapid detection methods for determining when a particular crop is at risk.

The primary objective of this work was to characterize the vibrational songs of the congeners *Chlorochroa uhleri* Stål, *Chlorochroa sayi* (Stål), and *Chlorochroa ligata* (Say). These three species were chosen for several reasons. First, all three are endemic to and widely distributed throughout western North America, with geographic ranges and host plants that over-

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lap substantially. Second, two of the species (*C. sayi* and *C. uhleri*) are very similar in color and morphology, such that they present virtually the same visual image (Buxton et al. 1983, Panizzi 1997, McPherson and McPherson 2000). Third, *C. uhleri* and *C. ligata* males seem to produce pheromone blends that are chemically indistinguishable (Ho and Millar 2001b). Collectively, these factors would hypothetically present opportunities for cross-attraction between heterospecifics. That we have not observed cross-breeding or successful hybridization of these congeners suggests the presence of one or more additional reproductive isolation mechanisms. Thus, our second objective was to compare the characteristics of the vibrational songs produced by the three species, particularly those used during the longer range phase of acoustic communication (calling phase), for potential species specificity. We discuss similarities of spectral and temporal characteristics of *Chlorochroa* songs with those of other pentatomine species.

Materials and Methods

Insects. Laboratory colonies of *C. sayi* and *C. uhleri* were started and regularly augmented with all life stages of nymphs and adults, field-collected from Russian thistle (*Salsola iberica* Sennen & Pau), black mustard (*Brassica nigra* L.), California buckwheat (*Eriogonum fasciculatum* Benth.), and summer cypress [*Kochia scoparia* (L.) Roth] throughout Riverside County from April 2002 to March 2004. A laboratory colony of *C. ligata* was started in spring 2003 from specimens collected by Christian Krupke of Washington State University Tree Fruit Research and Extension Center, Wenatchee, WA. All insects were reared in a controlled environment room at $26 \pm 1^\circ\text{C}$, 55% RH, and a photoperiod of 16:8 (L:D) h. Voucher specimens have been submitted to the University of California, Riverside Entomology Museum.

The rearing methodology follows that described for *Chlorochroa* spp. by Ho and Millar (2001a,b) with minor changes. Adult bugs were kept in glass-topped wooden cages with 15-cm-diameter mesh-covered air vents cut into the sides. Nymphs and adults were fed raw peanuts (*Arachis hypogaea* L.), sunflower (*Helianthus annuus* L.) seeds, organically grown green beans (*Phaseolus vulgaris* L.), and a bouquet of seasonally available host plants in water, including alfalfa, various field mustards (*B. nigra*, *Brassica campestris* L. and London rocket, *Sisymbrium irio* L.), California buckwheat, shepherd's purse (*Capsella bursa-pastoris* L.), and summer cypress, depending upon availability. Eggs were collected from the breeding colony every other day, and they were reared in covered petri dishes with food until nymphs reached the third instar. Instars 3 through 5 were reared in 15-cm-diameter plastic tubs. Newly eclosed adults were individually isolated in plastic cups with cardboard lids, and they were allowed to attain sexual maturity (≥ 10 d after the final molt) before being used in experiments. All life stages received the same diet, but seasonal weeds used

in containers apart from the main colony were not kept in water. Food for all insects was changed every other day.

Recording Vibrational Signals and Correlation with Specific Mating Behaviors. All recordings were made with virgin, sexually mature bugs between 0800 and 2000 hours (2–14 h after the start of photophase) in a laboratory room held at $25 \pm 3^\circ\text{C}$ and $50 \pm 5\%$ RH. The room was illuminated with fluorescent lights with color temperatures of 3500, 4100, and 6500 K (Sylvania Octron, 32-W lights) to simulate the daylight spectrum. Songs were recorded from bugs placed on the membrane of a 10-cm-diameter low-midrange loudspeaker (80–15,000-Hz frequency response, impedance 8 Ω , Radio Shack 40-1197, Fort Worth, TX), laid flat on a vibration-damping table. A plastic cylinder made from a two-cup Sterilite plastic container with the bottom cut off was fixed to the metal housing of the speaker with Quake-Hold museum putty (Conservation Resources International LLC, Springfield, VA) to act as a containment field. The bottom 2 cm of the cylinder was coated with Fluon (AGC Chemicals Americas Inc., Bayonne, NJ) applied on the inside edge to prevent bugs from escaping.

Signals were digitized and stored on the hard drive of a Pentium3 PC computer via external sound card (24 bit, 96 KHz, 100 dB SNR, Sound Blaster Extigy, Creative Labs Inc., Stillwater, OK) by using Cool Edit Pro 2.0 software (Adobe Systems, San Jose, CA). Each sound file was subjected to a noise reduction algorithm within the software with a fast Fourier transformation size of 4096, precision factor 7, smoothing 1, and transition width 0. Temporal and spectral characteristics of songs were measured with Sound Forge version 6.0 software (Sonic Foundry Inc., Madison, WI). All song recordings were initiated with mixed sex, conspecific pairs placed on the loudspeaker. Recording proceeded until at least 10 samples of a given song were taken, or 4 h had passed. Bugs that did not sing within a 4-h period were not counted.

The specific functions of songs were inferred from the behavioral context of production during preliminary observations on London rocket plants, and with reference to stereotypical mating behaviors as outlined previously for other stink bugs (Fish and Alcock 1973, Borges et al. 1987) and confirmed for *C. sayi* (Ho and Millar 2001a) and *C. uhleri* and *C. ligata* (Ho and Millar 2001b). Calling songs were identified as such on the basis that they were the only signals initiated at long distances (i.e., male and female on separate stems of London rocket, before physical contact). Calling song classification was further corroborated in that these were the only songs to be initiated and/or continued for longer periods (i.e., several minutes) in the absence of other insects during loudspeaker experiments. For each song, the sex singing was determined by removing one member of a mixed sex pair during song production.

Terminology and Statistical/Comparison Procedures. Terminology used to describe signals follows the description provided for *Murgantia histrionica* Hahn by Čokl et al. (2004). Pulses were defined as

Table 1. Temporal and spectral characteristics of the calling, reply, and rivalry songs of *C. uhleri*, *C. sayi*, and *C. ligata*

Species	Song	Type	Duration (ms)	Repetition time (ms)	Dominant frequency (Hz)	Bandwidth (Hz)
Calling songs						
<i>C. uhleri</i>	MS-1 ♂	P	1,716 ± 295 (353/19)	22,117 ± 10,612 (336/19)	93 ± 9 (353/19) ^b Upper (f): 110 ± 12 ^b Lower (f): 79 ± 13 ^b	48 ± 16 (336/19)
<i>C. sayi</i>	MS-1 ♂	PT	1,090 ± 204 (300/15)	11,754 ± 611 (300/15)	108 ± 16 (300/15)	77 ± 22 (300/15)
<i>C. ligata</i>	FS-1 ♀	PT/SF	4,917 ± 3,657 (380/19) 59 ± 12 (380/19) ^a	19,609 ± 13,793 (380/19)	98 ± 14 (380/19) 87 ± 13 (380/19) ^a	81 ± 19 (380/19)
Reply songs						
<i>C. uhleri</i>	FS-1 ♀	P	392 ± 108 (100/5)	766 ± 107 (100/5)	82 ± 7 (100/5)	36 ± 8 (100/5)
	FS-2 ♀	PT	1,433 ± 1,017 (55/3)	3,588 ± 3,047 (55/3)	91 ± 8 (57/3)	45 ± 12 (57/3)
<i>C. sayi</i>	FS-1 ♀	PT	5,217 ± 2,395 (37/7) 84 ± 20 (37/7) ^a	n/a (transient reply to male)	93 ± 9 (37/7) 87 ± 6 (37/7) ^a	62 ± 19 (37/7)
<i>C. ligata</i>	MS-1 ♂	PT	1,710 ± 261 (20/1)	27,670 ± 11,394 (20/1)	89 ± 5 (20/1)	74 ± 13 (20/1)
Rivalry songs						
<i>C. sayi</i>	R-1 ♂	P (long)	516 ± 172 (240/12)	n/a (acoustic duel)	127 ± 9 (240/12)	23 ± 8 (240/12)
		P (short)	173 ± 48 (240/12)	n/a (acoustic duel)	97 ± 13 (240/12)	69 ± 16 (240/12)
<i>C. ligata</i>	R-1 ♂	P	1,284 ± 332 (400/20)	n/a (acoustic duel)	126 ± 18 (400/20) ^b Upper (f): 146 ± 17 ^b Lower (f): 117 ± 15 ^b	78 ± 28 (400/20)

Songs were recorded from bugs on a loudspeaker cone. Data are presented as means ± SDs, of the numbers of signals analyzed (N), and the number of insects producing songs (n); n/a, not applicable.

^a Visible subpulses.

^b Frequency modulation: peak frequency of an entire pulse, and upper and lower frequency extremes.

unitary homogenous parcels of sound waves of finite duration (Broughton 1963). Pulse trains were defined as pulses arranged into repeated subunits that were either temporally distinct or amplitude modulated so as to seem to be fused, or semifused subunits of such parcels of sound waves. We measured pulse durations (the time between the onset and end of a pulse or pulse train above random noise), and pulse repetition times (the time between onsets of two consecutive pulses or pulse trains of the same kind). We also measured dominant frequency, bandwidth at 20 dB below the dominant peak, as well as the upper and lower frequency limits of frequency-modulated signals.

Data are presented as means ± SDs of the numbers of signals analyzed (N), and the number of bugs (n) from which the signals were obtained. Mean values for temporal parameters were highly variable such that normality and homoscedasticity could not be obtained, even through data transformation. Additionally, when subjected to nonparametric tests (Kruskal-Wallis one-way analysis of variance [ANOVA] on ranks), calling songs were separated into too many subgroups within populations for meaningful statistical comparisons. Therefore, calling song comparisons are based only upon general qualitative and quantitative data.

Results

Vibrational Songs of *C. uhleri*. Analysis of songs emitted by 35 males and eight females resulted in the identification of two female songs (FS-1 and FS-2) and two male songs (MS-1 and MS-2). A copulatory song (CPS; n = 6) also was found, but it could not be reliably attributed to either sex. In every case but one

(n = 30), the male initiated singing with either MS-1, or MS-2 song (upon proximity or contact with the female). Both female songs were initiated in the presence of a male, and typically continued for <2 min after the male was removed.

Male song 1 (MS-1; Table 1; Fig. 1a–c) had a calling function in this species. It was composed of individual, frequency-modulated pulses. The dominant frequency decreased throughout the duration of each pulse, with upper and lower frequency limits of 110 ± 12 and 79 ± 13 Hz, respectively (N = 353, n = 19). A second harmonic was regularly present, with third and fourth harmonics occurring infrequently. The repetition time was highly variable. Therefore, an arbitrarily chosen duration of 1 min between pulses defined the maximum repetition time.

Both female songs were produced as responses to the male MS-1. FS-1 (Table 1; Fig. 2a–c) was composed of individual pulses of relatively short duration and fast repetition rate, with a dominant and a second harmonic that converged with the dominant peak by the end of each pulse. A third harmonic was infrequently present. FS-1 continued for up to 2 min after the male was removed.

Female song 2 (FS-2; Table 1; Fig. 2d–f) was recorded three times. It was composed of a series of fused, amplitude-modulated pulses lasting several seconds in duration. Pulses were treated as separate units when intervals between them were longer than 100 ms. A second harmonic was regularly present, whereas a third harmonic was infrequent. Females emitted FS-2 over relatively short periods (<2 min), except during one instance of alternation (duetting) with a male lasting ≈12 min.

The *C. uhleri* male courtship song (MS-2; Table 2; Fig. 4a–d) occurred during close-range courtship be-

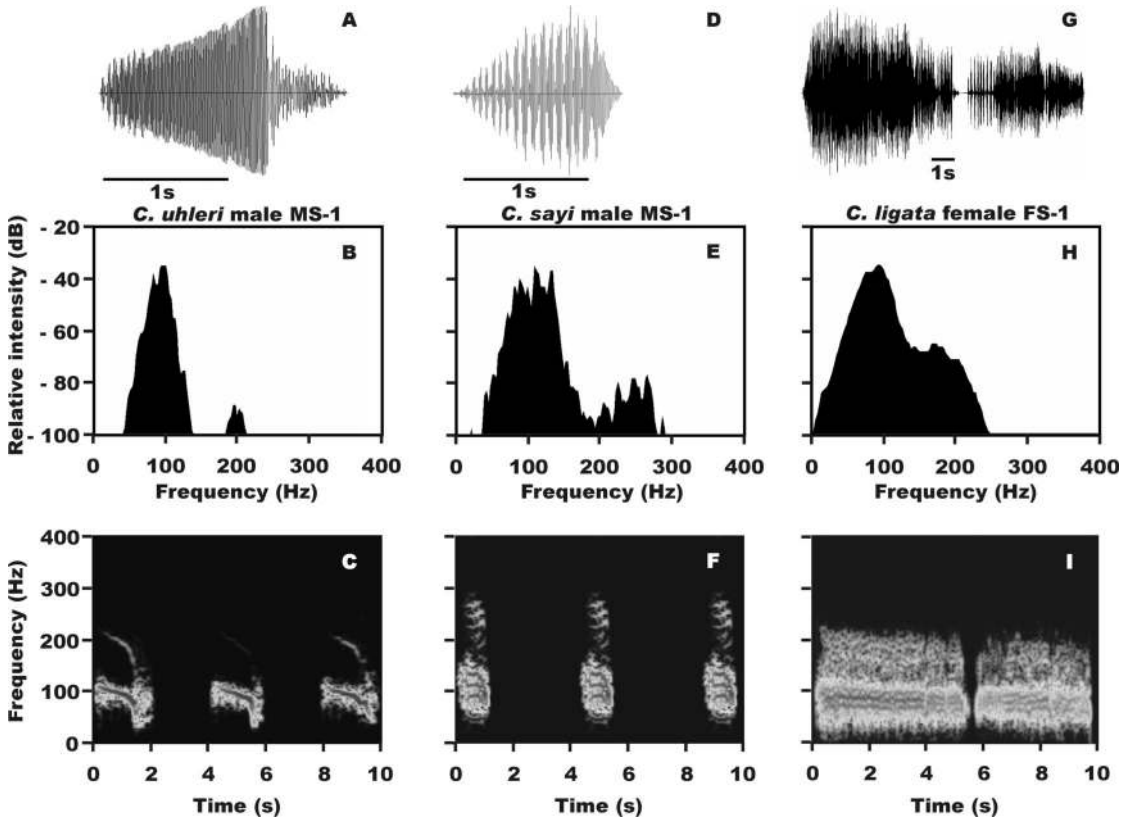


Fig. 1. Calling songs of the three *Chlorochroa* spp. *C. uhleri*: MS-1 as (A) an oscillogram of an individual pulse, (B) the frequency spectrum of one pulse, and (C) a sonogram of several pulses. *C. sayi*: MS-1 as (D) an oscillogram of an individual pulse train, (E) the frequency spectrum of one pulse train, and (F) a sonogram of several pulse trains. *C. ligata*: FS-1 as (G) an oscillogram of an individual pulse train, (H) the frequency spectrum of one pulse train, and (I) a sonogram of one pulse train.

havior. In 17 of the 19 successful recordings from males, MS-2 was emitted almost immediately after a male came in proximity to a female. A male would typically walk directly toward the female and begin producing MS-2 while initiating typical courtship behaviors such as antennating and head-butting the female's abdomen (Fish and Alcock 1973, Borges et al. 1987). The song has a complex pattern of two irregularly repeated pulses of similar frequency but with different duration and amplitude characteristics. The longer pulses were either separated by 1–10 of the shorter pulses, or fused. The shorter pulses had lower amplitude and highly regular repetition rate (except for three instances in which they were all fused for the duration of the experiment). They regularly occurred in groups of 1–10, but they also could be repeated consecutively in groups of 50 or more.

A copulatory song (CPS; Table 2; Fig. 6a–c) was characterized by distinctly separated pulses of relatively low amplitude. This song was produced only during copulation. We could not determine the singing sex, because vibrations ceased immediately upon interfering with the insects in any way.

Vibrational Songs of *C. sayi*. Analysis of singing behavior performed by 42 males and seven females

resulted in the identification of one female song (FS-1) and three male songs (MS-1, MS-2, and R-1). Copulation occurred between three pairs and one song (CPS) that could not be attributed to either sex was produced during this phase. In every instance, the male initiated singing, with either MS-1 (soon after proximity to a female, or after the female was removed) or the MS-2 song (upon proximity or contact with the female).

Male song one (MS-1; Table 1, Fig. 1d–f) had a calling function and was triggered by the presence of a female. After her removal, the repetition rate of the pulse trains decreased and became highly variable. Therefore, an arbitrarily defined duration of 1 min between pulse trains determined the maximum repetition time.

The *C. sayi* first female song was a pulse train (FS-1; Table 1; Fig. 2g–i), and it appeared as a transitional response to MS-1. It was composed of a series of short pulses with variable repetition rate leading to their fusion into pulse trains of up to 11 s in duration. Signals separated by intervals longer than 1 s were defined as separate and complete song units, similar to the *C. ligata* female calling song. In cases where individual subunits of the pulse train were distinguishable from

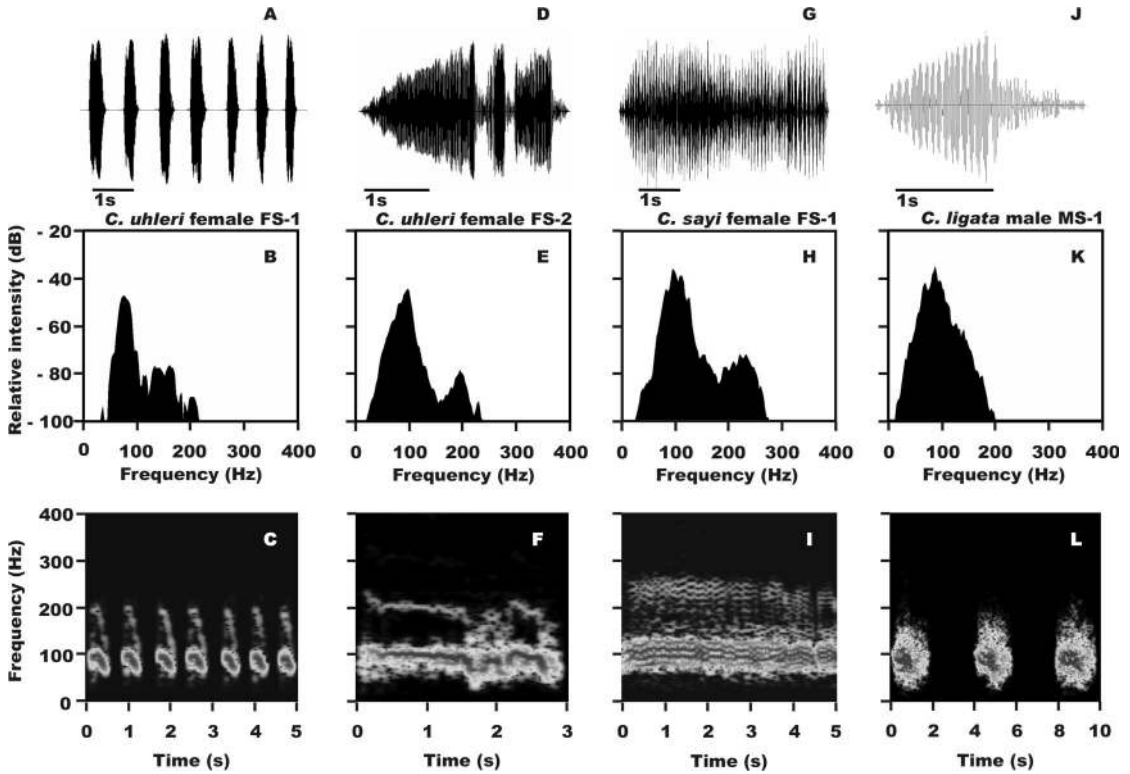


Fig. 2. Reply songs of the three *Chlorochroa* spp. *C. uhleri*: FS-1 as (A) an oscillogram of several pulses, (B) the frequency spectrum of one pulse, and (C) a sonogram of several pulses. *C. uhleri*: FS-2 as (D) an oscillogram of one pulse train, (E) the frequency spectrum of one pulse train, and (F) a sonogram of one pulse train. *C. sayi*: FS-1 as (G) an oscillogram of an individual pulse train, (H) the frequency spectrum of a pulse train, and (I) a sonogram of an individual pulse train. *C. ligata*: MS-1 as (J) an oscillogram of an individual pulse train, (K) the frequency spectrum of a pulse train, and (L) a sonogram of several pulse trains.

each other, they were measured for pulse duration and dominant frequency.

The *C. sayi* rivalry song (R-1; Table 1; Fig. 3a–d) was produced when a second male was added to a courtship-engaged mixed sex pair, and it continued after the female was removed for as long as the two males remained together. The song consisted of two types of pulses that differed in their duration and frequency characteristics (Table 1).

The male courtship song (MS-2; Table 2; Fig. 5a–c) was produced by males when close to females. Males moved directly to contact with females and began courtship behavior together with the emission of the MS-2 song. This song was unlike the courtship songs of the other two *Chlorochroa* species tested in that it was composed of short-duration pulses throughout, repeated at a very fast rate, with some pulses being entirely fused. These pulse trains were regularly pro-

Table 2. Temporal and spectral characteristics of the courtship and copulatory songs of *C. uhleri*, *C. sayi*, and *C. ligata*

Species	Song	Type	Duration (ms)	Repetition time (ms)	Dominant frequency (Hz)	Bandwidth (Hz)
Courtship song						
<i>C. uhleri</i>	MS-2 ♂	P (long)	567 ± 241 (240/12)	1,010 ± 593 (239/12)	70 ± 13 (240/12)	103 ± 20 (240/12)
		P (short)	129 ± 40 (317/16)	285 ± 77 (317/16)	78 ± 11 (317/16)	91 ± 19 (317/16)
<i>C. sayi</i>	MS-2 ♂	PT/SF	116 ± 35 (275/13) ^a	n/a (continuous)	82 ± 13 (275/13) ^a	103 ± 25 (275/13)
<i>C. ligata</i>	MS-2 ♂	P (long)	625 ± 223 (394/20)	1,875 ± 884 (377/20)	62 ± 10 (394/20)	95 ± 19 (394/20)
		P (short)	114 ± 29 (400/20)	302 ± 86 (400/20)	82 ± 16 (400/20)	103 ± 26 (400/20)
Copulatory song						
<i>C. uhleri</i>	CPS	PT	127 ± 24 (120/6)	246 ± 47 (120/6)	94 ± 23 (120/6)	113 ± 34 (120/6)
<i>C. sayi</i>	CPS	PT	78 ± 36 (20/1)	134 ± 34 (20/1)	106 ± 16 (20/1)	107 ± 37 (20/1)
<i>C. ligata</i>	CPS	PT	106 ± 24 (60/3)	211 ± 38 (60/3)	80 ± 6 (60/3)	77 ± 17 (60/3)

Songs were recorded from bugs on a loudspeaker cone. Data are presented as means ± SDs, of the numbers of signals analyzed (N), and the number of insects producing songs (n); n/a, not applicable.

^a Visible subpulses.

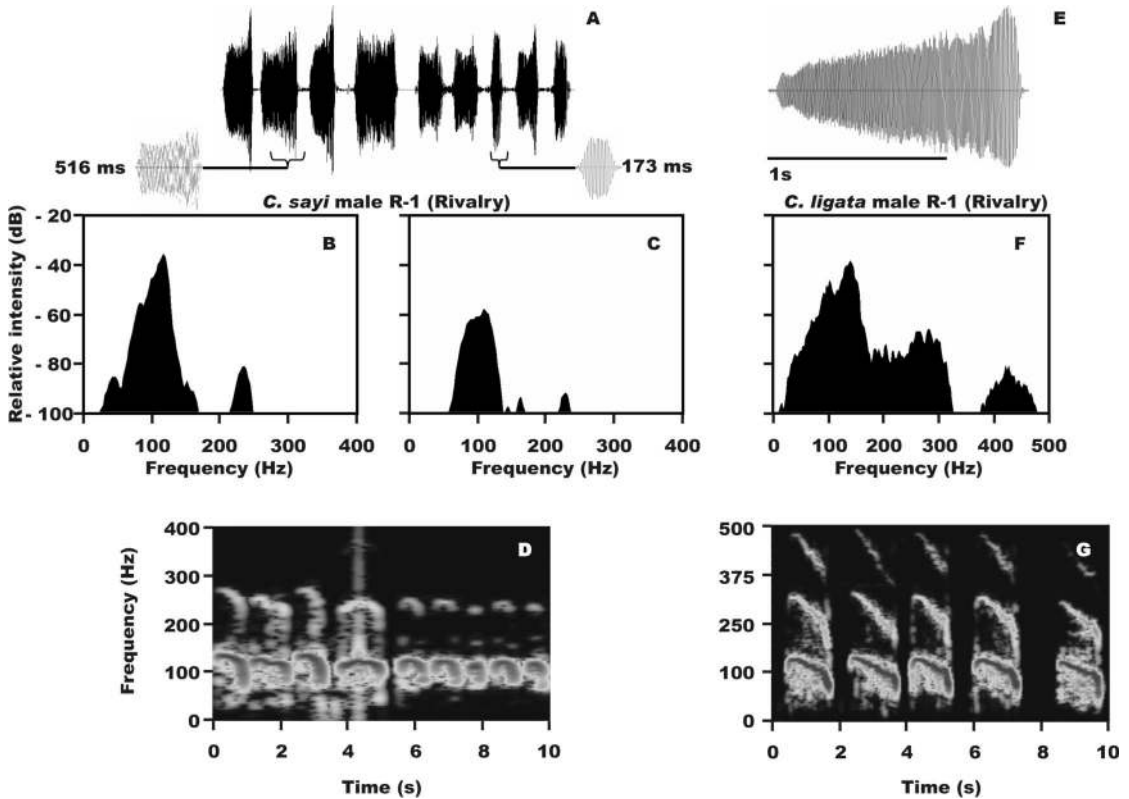


Fig. 3. Rivalry songs of two *Chlorochroa* spp. *C. sayi*: R-1 as (A) an oscillogram of several pulses performed by two competing males, (B) the frequency spectrum of one long pulse, (C) the frequency spectrum of one short pulse, and (D) a sonogram of several pulses. *C. ligata*: R-1 as (E) an oscillogram of one pulse, (F) the frequency spectrum of one pulse, and (G) a sonogram of several pulses.

duced in bouts longer than 2 min. Where individual pulses were distinguishable from each other, they were measured for duration, dominant frequency, and bandwidth. In two instances, the pulse train subunits were fused throughout the duration of song production.

The *C. sayi* copulatory song (CPS; Table 2; Fig. 6d–f) occurred only during copulation. The signaling sex could not be determined.

Vibrational Songs of *C. ligata*. Analysis of singing behavior of 41 males and 19 females resulted in the identification of one female song (FS-1) and three male songs (MS-1, MS-2, and R-1). A copulatory song (CPS) occurred on only one occasion. The rivalry song (R-1) was regularly produced upon introduction of a second male to a courtship-engaged pair.

Female song 1 (FS-1; Table 1; Fig. 1g–i) had a calling function. It was initiated by females in the presence or absence of males and was repeated for >20 min after a male was removed from the loudspeaker. FS-1 was emitted also in response to MS-2, before the removal of the male. FS-1 was composed of a series of short pulses with rapidly increasing repetition rate, which resulted in their fusion into pulse trains of up to 17 s in duration. We used intervals between signals longer than 1 s to define complete song units.

Male song 1 (MS-1; Table 1; Fig. 2j–l) was composed of single, amplitude-modulated pulse trains with an extended tail with decreasing amplitude. The song was recorded only on one occasion, in response to a singing female, by the last remaining male in a declining colony. The pulse train repetition rate was highly variable.

Males emitted the rivalry song (R-1; Table 1; Fig. 3e–g) upon introduction of a second male to a courtship-engaged, mixed sex pair. It was composed of individual, frequency-modulated pulses, with the dominant frequency decreasing throughout the duration of each pulse. A second harmonic peak was always present, with third and fourth harmonics occurring infrequently. Although individual pulses were apparent, determination of the signaler for any given pulse was impossible. This song continued for as long as two males remained together, even if the female was removed. Signaling between rival males was recorded for up to 12 h with only short breaks (≈ 30 s to 2 min) between repeated bouts of singing.

The male courtship song (MS-2; Table 2; Fig. 4e–h) was temporally and spectrally similar to the *C. uhleri* male courtship song. It had a complex temporal pattern consisting of two irregularly mixed pulses with similar frequency characteristics but different dura-

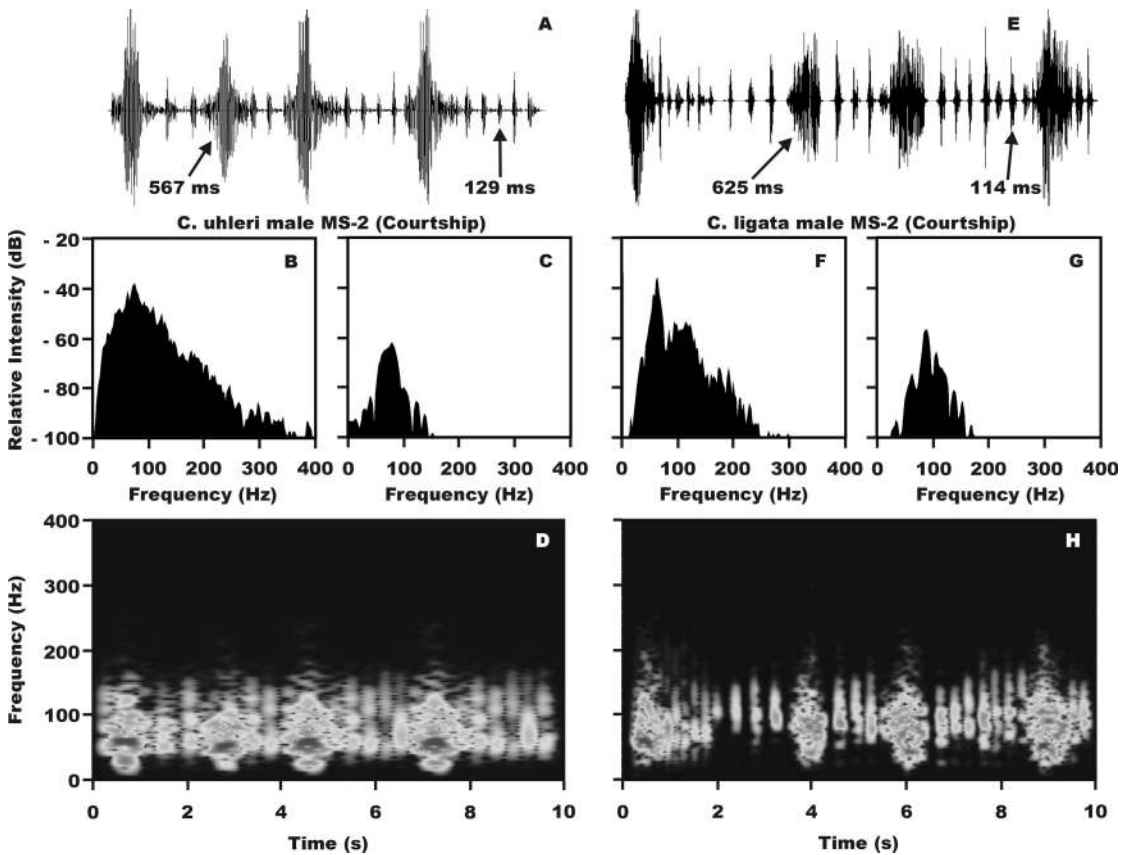


Fig. 4. Courtship songs of two *Chlorochroa* spp. *C. uhleri*: MS-2 as (A) an oscillogram of both pulse types, (B) the frequency spectrum of one long pulse, (C) the frequency spectrum of one short pulse, and (D) a sonogram of both pulse types. *C. ligata*: MS-2 as (E) an oscillogram of both pulse types, (F) the frequency spectrum of one long pulse, (G) the frequency spectrum of one short pulse, and (H) a sonogram of both pulse types.

tion and amplitude. The longer pulses occurred individually, were not fused, and were separated by 1–10 of the shorter pulses. The shorter pulses of lower amplitude and regular repetition rate most often occurred in groups of 1–10, although sequences of >50 could be recorded as well.

A copulatory song (CPS; Table 2; Fig. 6g–i) was recorded on only one occasion. It was characterized by low-amplitude, short pulses as in *C. uhleri*. This song was produced only during copulation, and the sex of the singing insect could not be determined.

Discussion

Comparison of different song types within and across stink bug taxa suggests that a limited number of basic temporal and spectral building blocks for different songs exist, delimited by factors such as bug size, the mechanical properties of the vibration production and reception organs, and the transmission properties of the host plant. The basic song units are arranged in different ways by each sex and species to form highly stereotyped signals, with the species, sex, and behavioral context all contributing to song specificity (Čokl

et al. 2001, Virant-Doberlet and Čokl 2004). Although spectral characteristics are restricted, the presence or absence of harmonics, frequency modulation, and differing signal bandwidths are variables that also may contribute to production of unique signals. It is unlikely that the dominant frequency contributes significantly to signal specificity for *Chlorochroa* spp. given that the peak frequencies and bandwidths of all the songs we observed overlap considerably between individuals, and among song unit replicates produced by the same individual. Species specificity of insect vibrational signals generally arises from differences in the temporal patterns of signals such as duration, repetition times, and amplitude modulations (Čokl et al. 2000a, Stumpner and von Helversen 2001, Miklas et al. 2003). Furthermore, varying degrees of reproductive isolation imparted through differing temporal patterns of congruous songs have been explored among geographically isolated populations of the southern green stinkbug *Nezara viridula* (L.) (Ryan et al. 1996, Jeraj and Walter 1998, Čokl et al. 2000b, Miklas et al. 2003, Hrabar et al. 2004, Virant-Doberlet and Čokl 2004). Such patterns also have been shown to be under direct genetic control (Virant-Doberlet et al. 2000). How-

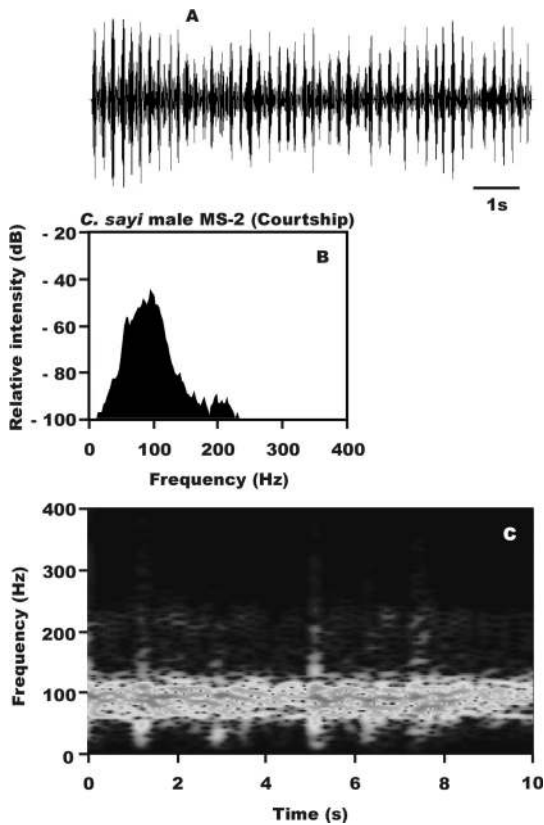


Fig. 5. Courtship song of *C. sayi* MS-2 as (A) an oscillogram of a continuous pulse train, (B) the frequency spectrum of one subpulse, and (C) a sonogram of a continuous pulse train.

ever, species recognition based upon temporal differences between vibratory songs does not prevent intra- and interspecific hybridization (Kon et al. 1988, Čokl et al. 2000b, Moraes et al. 2005).

Our results reveal the same trends in variation among spectral parameters, syntax, and behavior-linked song repertoires as those found among other stink bugs, but with highly variable temporal parameters within populations. There are several possible explanations for this. First, signaling behavior can be affected by both physical environment and/or diet. During experiments, our insects were restricted, in pairs or as individuals, to an obviously unnatural environment with no natural external stimuli present. Under natural conditions, the bugs are gregarious and subject to a multitude of incidental vibrations. For *N. viridula*, the physical presence of a bug of the opposite sex or its acoustic replies has an effect on duration or repetition rate of songs (Čokl et al. 2001, McBrien et al. 2002, Čokl and Virant-Doberlet 2003). We observed our insects producing signals in response to incidental acoustic sources (i.e., voices and mechanical noises) that might indicate a tendency for bugs to send out interrogatory signals in response to nonspecific vibrations of the proper frequency range. Furthermore,

during observations on plants, calling songs occurred with repetition times almost exclusively in the 5–10-s range (unpublished data) as opposed to the wider distribution we found during loudspeaker experiments (Fig. 8).

Some insect songs also are reported to follow seasonal patterns of production (Jeraj and Walter 1998). The *Chlorochroa* bugs we used in these experiments produced signals most reliably during the period between August and September, when field weeds and organically grown beans in our colonies' diets were fresh and plentiful. Another possibility is that all currently recognized components of songs are collectively important in ways that are still unrecognized or not well understood (McBrien and Millar 2003).

We classified one song as the primary calling song for each species based on both our preliminary observations on plants and the fact that these were the only songs ever to be initiated in the absence of prior contact with other insects. Two of these were male songs (*C. uhleri* MS-1 and *C. sayi* MS-1), and one was a female song (*C. ligata* FS-1). Temporally, pulse durations of the male-produced calling songs of *C. uhleri* and *C. sayi* were more similar to each other than either of them was to the female calling song of *C. ligata* (Figs. 1 and 7). Furthermore, all three calling songs were radically different qualitatively. The *C. uhleri* MS-1 song was an individual, continuous pulse whereas the *C. sayi* MS-1 song was a pulse train, and the *C. ligata* FS-1 song was a semifused pulse train. Interestingly, in one instance a *C. uhleri* male produced an MS-1 song as a sequence of pulse trains instead of uniform pulses, such that it seemed structurally similar to that of the *C. sayi* MS-1 song. This suggests that vibrational units of similar duration are emitted at higher or lower rates resulting in different degrees of fusion of subunits into the observed waveforms (e.g., songs composed of pulses versus pulse trains). During transmission through plants, vibratory pulses of high repetition rate can also become fused because of their prolongation due to echoes and induced resonant vibration (Miklas et al. 2000).

The repetition times of pulses or pulse trains varied considerably, but the majority of these were repeated in the range of every 5–15 s (Fig. 8). Repetition times in this range are consistent with songs produced by other stink bugs (Čokl et al. 2000b, 2001, 2004; Pavlovčič and Čokl 2001; McBrien et al. 2002).

All calling songs triggered vibrational responses from the opposite sex conspecifics. Although we note distinct qualitative differences between calling songs, when viewed as a whole against all songs within each congeneric repertoire, it seems that pulse subunits (the building blocks of syntax), and the call-reply roles between species and sexes can be rearranged to create novel communication interactions. For example, the calling and response contexts of *C. ligata* (FS-1 → MS-1) and *C. sayi* (MS-1 → FS-1) were reversed for qualitatively very similar songs. It is also noteworthy that for two of the three species (*C. uhleri* and *C. sayi*), males were both the pheromone-producing sex and the sex that initiated singing.

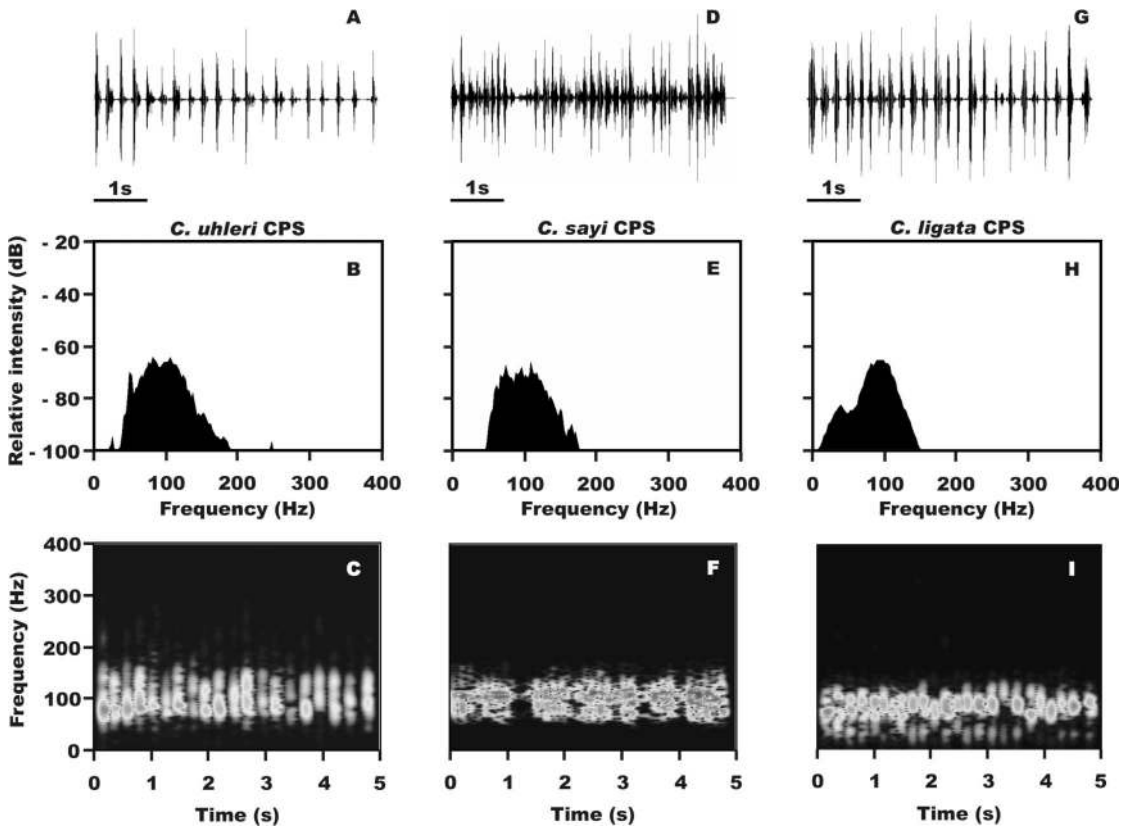


Fig. 6. Copulatory songs of the three *Chlorochroa* spp. *C. uhleri*: CPS as (A) an oscillogram of several pulses, (B) the frequency spectrum of one pulse, and (C) a sonogram of several pulses. *C. sayi*: CPS as (D) an oscillogram of several pulses, (E) the frequency spectrum of one pulse, and (F) a sonogram of several pulses. *C. ligata*: CPS as (G) an oscillogram of several pulses, (H) the frequency spectrum of one pulse, and (I) a sonogram of several pulses.

This phenomenon seems to extend to other *Chlorochroa* songs as well. For example, the *C. sayi* male-produced courtship song (MS-2) is similar to the *C. sayi* female's reply song (FS-1), but very different from the male produced courtship songs of *C. uhleri* and *C. ligata*, which are similar both temporally and spectrally. The latter two songs also seem similar to the male courtship song of *Holcostethus strictus* (F.) (Pavlovčič and Čokl 2001). In contrast, the *C. sayi* male courtship song is primarily composed of short pulses, but when listening closely to certain recordings, one can hear increases in amplitude (occurring in conjunction with the male head-butting the female) and fusion of these short pulses into what sound like precursors of the longer, higher intensity pulse types found in *C. uhleri* and *C. ligata* male courtship songs. For other pentatomine stink bugs studied to date, courtship songs show the highest degree of species specificity and complexity (Čokl and Virant-Doberlet 2003), but this does not seem to be the case for the *Chlorochroa* spp. studied here.

Rivalry songs were observed only from *C. ligata* and *C. sayi* males. For both species signals were exchanged between males, often in rapid succession, and continued for as long as those males remained together. Both

species' rivalry songs were temporally and spectrally different (Table 1; Fig. 3). Ritualized signal interactions may be a means of avoiding the elevated costs and risks of fighting while still achieving the same outcome as a physical contest, or they may provide a means for the female to assess males by the energetic costs of the acoustic duel (Greenfield 2002).

To date, the literature on pentatomine vibrational signaling contains little as to the function of the copulatory song. Eberhard (1991) presented the thesis that for some insects these types of signals function as cryptic female choice mechanisms. That is, specifically for insects, several female-controlled processes (e.g., control of duration and termination of copulation; sperm storage, movement, and nourishment; egg maturation) must occur if copulation is to result in fertilization. Because continued courtship after intromission can serve only to induce postintromission processes, it is likely that copulatory signals affect one or more of these female-controlled processes, with benefits accruing to the signaling male (e.g., greater volume of sperm transferred, resulting in increased probability of paternity). The copulatory songs of our *Chlorochroa* spp. seem superficially similar, but they

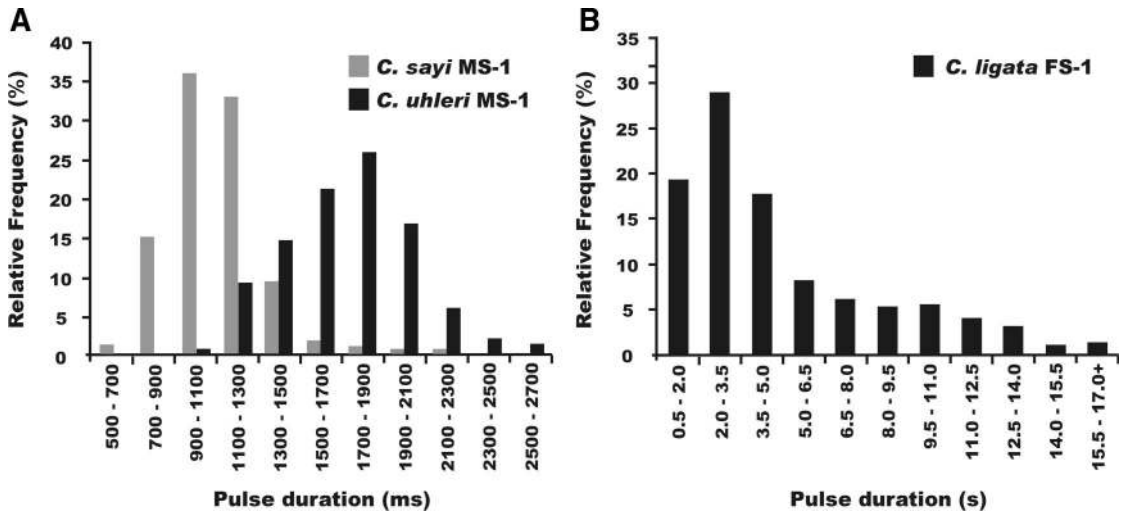


Fig. 7. Relative frequency histograms of the subdivided range of pulse duration values of calling songs produced by (A) *C. sayi* and *C. uhleri* males and (B) *C. ligata* females. Two separate graphs are presented due to large differences in time scales.

have distinct temporal and spectral differences (Table 2; Fig. 6).

Ultimately, because the three *Chlorochroa* spp. are distinct species that cannot hybridize, we must assume that some form of interspecific mating barrier exists. Otherwise, cross-attraction to and courtship of heterospecifics would be costly, both in terms of energy expended in mate location, and the considerable energy expended in producing vibrational signals. Superficially, it would seem as though the potential for cross-attraction was substantial, because these congeners are sympatric over much of their ranges

(McPherson and McPherson 2000), and they share many host plants (G.J.B., personal observation). Furthermore, they all share one or more male-produced pheromone components, and they have similar diurnal rhythms (Ho and Millar 2001a,b). In contrast with *N. viridula* and *Nezara antennata* Scott, two sympatric species in which interspecific mating was observed to occur under natural conditions (Kon et al. 1988), we have never observed this phenomenon or even courtship display between *Chlorochroa* congeners in the field or in the laboratory. This is true also for heterospecific virgin pairs confined in rearing containers

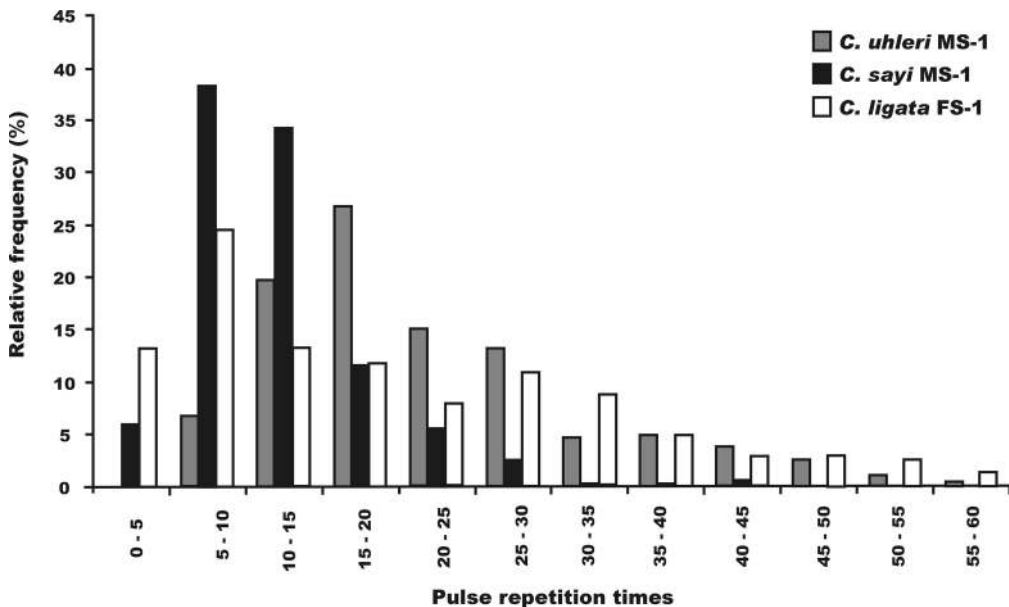


Fig. 8. Relative frequency histogram of the subdivided range of repetition time values of the calling songs of *Chlorochroa* species.

(G.J.B., personal observation), conditions under which conspecific pairs mate readily and rapidly. Thus, it seems likely that species-specific and sex-specific vibrational songs play a key role in prezygotic reproductive isolation.

In summary, mate location and recognition by phytophagous stink bug species seems to be mediated by a series of overlapping signals of different types. Volatile, male-specific pheromones seem to act over longer ranges, serving to bring the sexes together on the same substrate (Borges et al. 1987). Once on the same plant, substrate-borne vibrational signals produced by both sexes take over as the primary signaling modality that aids in bringing the sexes together within the complex architecture of the plant. As they come to each junction point in the plant canopy, bugs straddle the junction to discern which branch the vibrational signal is coming from, and then move up that branch (Ota and Èokl 1991, Cokl et al. 1999). This iterative decision-making process at each junction point allows male bugs to follow the most direct path to a signaling female within the maze-like structure of the plant. Typically, multiple junctions will have to be traversed to bring the two bugs together, and so successful mate location is critically dependent on the continuous production of guiding signals. Thus, during mate location, males and females exchange signals continuously, with the male's calling song being answered by the female. Furthermore, there is evidence to suggest that the olfactory and vibrational signals are not independent because vibrational signals from females increase pheromone production by males (Miklas et al. 2003). Conversely, the male pheromone has been reported to stimulate production of calling songs by females (Miklas 2002). Then, once males and females are in proximity or in contact, mate recognition and the initiation of copulation are mediated by a different set of signals, including the male and female courtship songs. Contact chemical signals also may be used in mate recognition, because there are differences in the cuticular lipid profiles of male and female bugs (Borges et al. 2003, Colazza et al. 2007). It is also possible that visual signals play a role in mate recognition, particularly because some stink bug species are brightly colored with vivid patterns, and because the reproductive behaviors seem to peak during the photophase (e.g., *Chlorochroa* spp., Ho and Millar 2001a,b; *M. histrionica*, Zahn et al. 2008), when visual signals would be most useful. In sum, the multitude of overlapping signals of different modalities provides minimal opportunities for mistakes, thus ensuring a robust and reliable mate location and recognition system for each stink bug species.

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