

Stridulation by *Jadera haematoloma* (Hemiptera: Rhopalidae): Production Mechanism and Associated Behaviors

ARIEL F. ZYCH,¹ R. W. MANKIN,^{2,3} JAMES F. GILLOOLY,¹ AND EVERETT FOREMAN²

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ABSTRACT The Hemiptera displays a notable diversity of vibratory communication signals across its various families. Here we describe the substrate and airborne vibrations (sounds), the mechanism of production, and associated behaviors of *Jadera haematoloma* Herrich-Schaeffer, a member of the family Rhopalidae. Adult males and females both produce short, stereotyped sound bursts by anterior–posterior movement of abdominal tergites I and II against a stridulitrum located on the ventral surface of the metathoracic wing. Sound bursts are produced by a single adult male or female when physically touched by another adult, and are strongly associated with being crawled on by the approaching individual, but are not produced in response to contact with other arthropods or when pinched with forceps. The propensity to produce sounds when crawled upon decreases during the mating season. These sound bursts by *J. haematoloma* likely are communication signals. Rhopalidae has been significantly absent from the vibratory communication literature until now. Although the sounds are produced using a mechanism common to vibratory communication systems in closely related Heteropteran Hemiptera, the sounds in these other species function primarily in courtship or in mother–daughter interactions, which suggests that the functions of stridulation and the behavioral contexts have diversified in the Heteroptera.

KEY WORDS insect communication, mating, stridulation, Heteroptera

Acoustic and vibratory communication is incredibly diverse and has arisen multiple times during the evolution of Hemiptera. The evidence in support of multiple evolutionary origins of vibratory communication in this group is quite extensive because of the characteristic morphology of diverse vibration-producing structures in several families (Ashlock and Lattin 1963, Schaefer 1980, Schaefer and Pupedis 1981, Polhemus 1994, Tishechkin 2006). Hemiptera occupy a wide array of niches and communicate in many different ecological and behavioral arenas (Cocroft and Rodriguez 2005). Comparisons of the structures used for vibratory communication among distantly related Hemipteran taxa may improve understanding of the behavioral and ecological pressures that drive acoustic convergence or diversification in insects. However, elaborating Hemiptera as a clade for studying acoustic evolution requires a good record of the diversity of signals and signal-producing structures, a record that is still incomplete. Here we investigate the vibrations produced by a well-studied member of the family

Rhopalidae (Hemiptera: Heteroptera), *Jadera haematoloma* Herrich-Schaefer, and describe for the first time the mechanism and behavior associated with vibrations in this family.

Jadera haematoloma is an abundant rhopalid throughout the continental United States that has been well-studied as an example of rapid evolution associated with host plant shift (Carroll and Boyd 1992). *Jadera* sp. play a role in reducing the seed productivity of a species of Sapindaceae (*Koelreuteria elegans* Laxmann) classified as a Class II Invasive by the Florida Exotic Pest Plant Council (Carroll et al. 2003, FLEPPC 2009). Rhopalids also are pests in countries where Sapindales such as Lychee and Longan are cultivated (Waite and Hwang 2002).

The opportunity to investigate rhopalid-produced vibrations came from a chance discovery that groups of *J. haematoloma* placed into a bucket for transfer from a field site to a laboratory produced audible sounds. A search of the literature revealed no information about sound production by this species, and only minimal information about substrate or airborne communication in Rhopalidae. In spite of their ecological and economic importance, a single recording of a male *Arhyssus hyoscyami* L. is the only documentation of sounds by species in this group (formerly *Corizus hyoscyami* L., Gogala 1990). Consequently, a study was initiated to: 1) record and analyze the sounds in a behavioral context, 2) describe the mechanism of sound production, and 3) explore whether sounds

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¹ Department of Biology, University of Florida, Gainesville, FL 32611-8525.

² USDA-ARS Center for Medical, Agricultural, and Veterinary Entomology, Gainesville, FL 32608.

³ Corresponding author, e-mail: richard.mankin@ars.usda.gov.

produced may be used for communication among members of the same species or as aposematic threats to potential predators.

The body parts used by other closely-related Hemiptera to produce vibrations provide clues to the mechanism that may be used by *J. haematoloma*. Rhopalidae lies within the infra-order Pentatomomorpha, a diverse group that includes Lygaeidae, Pentatomidae, Coreidae and other families with species known to communicate through vibrations (Henry 1997). In the Pentatomomorpha, a tergal plate formed by the fusion of abdominal tergites I and II commonly is associated with vibratory communication that may be used in Rhopalidae (reviewed by Virant-Doberlet and Čokl 2004, Gogala 2006). Two mechanisms of the tergal plate have been proposed as the vibrational mechanism. The first is as a plectrum used in conjunction with a wing stridulitrum (Leston et al. 1954, Ashlock and Lattin 1963, Schuh and Slater 1995). The second is as a "tymbal"; a bi-stable plate that pops in and out of two stable configurations, similar to that used by cicadas and planthoppers (Gogala et al. 1974, Gogala 2006). The tymbal mechanism was first hypothesized for Cydnidae, where Gogala 2006 and colleagues (showed that wax application between tergites I and II silenced low-frequency signals. Subsequent replications of this technique have turned up conflicting results [Lawson and Chu 1971, Numata et al. 1989]). Other vibrations in Pentatomomorpha have been attributed to a tymbal mechanism without direct observation or manipulation of the tergum (Schaefer 1980, Virant-Doberlet and Čokl 2004). Using *J. haematoloma*, it is possible to address not only the persistence of tergal plate involvement in sound production in Pentatomomorpha, but also determine whether it functions as a tymbal or a plectrum.

Before the sounds and sound-associated morphology described in this report could be contributed to the body of literature on the diversity of insect signals, it was important to distinguish between random or incidental sounds and those that may serve a role in communication. To disprove that sounds are produced randomly, we filmed interactions and then asked whether sounds were associated with specific behaviors and participants. We then identified intra- and inter-specific interactions that may play a role in communication, such as a defensive response to predator threats (Masters 1979) or as an attractive signal to conspecifics (Wenninger et al. 2009). This behavioral context will play an important role in our future understanding of how vibrations are used for communication in the Rhopalidae and other related families.

Materials and Methods

***J. Haematoloma* Collection.** Adult and immature *J. haematoloma* were field collected in July and August 2009 beneath large (>6-m crown height) specimens of the local host, Golden Raintree, *Koelreuteria paniculata* v. *bipinnata* Laxm, in northwest Gainesville (Alachua County) and on the University of Florida campus. Adults were captured from large canopy

aggregations by using a bag net beaten against branches containing seasonal aggregations (Zych 2010). Identification was verified with the help of members of the Florida State Collection of Arthropods in Gainesville, FL (Slater and Baranowski 1978, Schuh and Slater 1995). Groups of up to 100 adults and nymphs were housed in 2-liter plastic buckets with screen lids and given fresh host leaves and water from soaked wicks. Host seeds were omitted from enclosures to accurately reflect seed availability in the field at the time of collection (no intact seeds were present at the collection site). The buckets were held in a growth chamber maintained at 26°C with a photoperiod of 14:10 (L:D) h to simulate summer temperature and photoperiod. Individuals were housed for up to 2 wk before being returned to the field and replaced by new field collections. Acoustic vibratory, and video recordings of groups of individuals were conducted at 26°C and 60% RH in a vibration-shielded anechoic chamber (Mankin et al. 1996) at the Center for Medical, Agricultural, and Veterinary Entomology, Gainesville, FL.

Sound Recording and Analysis. To fully characterize the vibrations produced by *J. haematoloma*, it was important to record and describe both substrate and airborne (acoustic) vibrations, as well as identify variation among individuals. To capture both acoustic and vibratory recordings, nine tests were conducted with 10–12 individuals placed in a cylindrical cage (6 cm diameter by 10 cm height) made of 1-mm metal screen mesh for 30 min. An accelerometer (Brüel and Kjaer [B&K], Naerum Denmark) for recording substrate-borne vibrations (Wenninger et al. 2009) was clipped to the top edge of the screen lid. These accelerometer recordings were coupled with acoustic recordings by using a B&K microphone (Mankin et al. 2000) horizontally positioned atop a foam block 1 cm from the cage. Both acoustic and substrate-borne vibrations were recorded at the same time to determine whether they were produced synchronously and to determine how each differed from the other. All recordings were digitized and saved on a computer using a commercially available speech analysis system (Wenninger et al. 2009).

To determine whether sex or body length influenced variation in the sounds produced, a separate set of recordings were made of adults of known sex and body length. Body length was measured as the tip of the clypeus to the posterior edge of the last abdominal segment. Nineteen adults whose sex and body length were determined (Carroll and Loye 1987) were individually placed in the anechoic chamber setup with an individual who was silenced by waxing (see Experimental Manipulation of Stridulatory Apparatus below). We recorded three bouts of sound production for each individual to associate sound characteristics with body length measurements.

We analyzed the microphone and accelerometer signals by using Raven Pro version 1.3 (Cornell Lab of Ornithology 2008). The Raven software provided displays of oscillograms and spectrograms and measured the temporal patterns and amplitudes of individual sound impulses and groups (bursts) of impulses

(Mankin et al. 2008), the dominant frequencies of bursts, and the maximum frequency ranges of bursts.

Sound-associated Behaviors. To identify behaviors that may be associated with sound production, we filmed field-caught individuals while recording sounds, and asked whether or not sounds occurred more frequently when certain behaviors occurred. Preliminary observations showed that sounds were not produced outside of encounters between adults. Behavioral analysis was therefore restricted to encounters between individuals. Groups of four to ten adults were placed in flat rectangular plastic "interaction" containers (5 by 8 by 2 cm) with one of the two widest surfaces of the container replaced with a fine mesh screen that faced a B&K microphone as described above. Interaction containers were held vertically with one clear surface facing a SONY HD Handycam video camera, illuminated from 0.5 m above using a 120 W incandescent light bulb. Filming took place through the nonmesh face of the container at normal speed by using the HD macro setting. Fresh field-collected adult individuals were used for each recording session, and were allowed to acclimate for 30 min before the start of recording. Sound and video recording continued for 60 min.

To standardize among group interaction trials, we restricted this analysis to the first ten encounters after the 30-min acclimation period. Encounters were characterized as the period starting when two individuals at least one body-length apart initiate physical contact with any appendage, and ending the moment they move to greater than one body length apart. Encounters were then characterized according to the relative speed of individuals entering into the encounter, the sex of participants, the presence or absence of specific behaviors, and whether or not sounds were produced (by a signaler). The relative speed of each individual moving into and away from a physical contact was measured in terms of body length(s), and assigned to each participant according to "approaching" and "approached" as the individual moving with the greatest or least velocity at the start of an encounter (respectively). We categorized two encounter-specific behaviors in addition to recording the sex and speed of encounter participants. We defined "body rocking" as taking place when an individual rolled or rocked its entire body from side to side around the anterior-posterior axis. When the approaching individual crawled completely on top of the approached individual, we referred to this as "crawling onto" which was chosen over "mounting" to emphasize that no effort was made to associate this behavior with copulation. The relative speed of each individual moving into and away from a physical contact was measured in terms of body lengths per second, and assigned to each participant according to "approaching" and "approached" as the individual moving with the greatest or least velocity at the start of an encounter. The first ten encounters for each group were then pooled together totaling 100 scored encounters.

Ten groups of individuals were filmed in September 2009 before the mating season. Additional tests were

conducted in November 2010, after mating had begun, with the following modifications. To capture encounters that could result in mating, no acclimation period was possible, as individuals typically paired within several minutes after being placed in the containers together. Because of time constraints, only seven groups were recorded and analyzed. Because individuals moved considerably less upon being paired, two trials resulted in fewer than ten encounters.

Statistical Analyses of Behavioral Encounters. We pooled encounters across interaction trials to total 100 encounters in the nonmating season in 2009, and 63 encounters in the mating season in 2010. Trials were not pooled between seasons. This pooling was necessary to achieve adequate numbers of targeted behaviors to associate with sound production. Encounters were categorized by the presence or absence of target behavior, the approached sex, and the approaching sex. During the mating-season encounters were further categorized by whether or not the encounter resulted in mating between the two participants. A two-way contingency table with sound (+) or no sound (-) as the response variable was created for each of these encounter variables. A Fisher χ^2 (chi-squared) test then was used to determine if there was a significant difference in the number of encounters featuring a specific behavior or participant sex combination that resulted in signaling. Two-tailed χ^2 values are reported because they are a more conservative representation of the difference between two groups than a one-tailed test (Fisher 1922). Data analysis was completed using JMP statistical software (SAS Institute 2008).

Sound Production Mechanism. Using three approaches, we confirmed that *J. haematoloma* produced sound by use of a plectrum and stridulitrum, the most commonly found sound production mechanism in terrestrial Heteroptera. We first used a series of high-speed video recordings to identify structures that move specifically in association with sound production and that could function as a plectrum, the basic mechanism most common in terrestrial Heteroptera. After identifying a putative plectrum, we then captured high-resolution surface images of the articulating side of the hind wings to determine whether there was a stridulitrum that articulated with the putative plectrum. Lastly, we performed a series of loss-of-function manipulations to verify that we had correctly identified the structures involved in sound production. These consisted of removing suspected structures, or preventing their movement, and then recording any sounds produced by those individuals in conspecific interactions.

Plectrum Identification. To identify a putative plectrum, we searched for body part movements that corresponded to the rate and behaviors during brief periods of sound production. Individuals with wings removed were filmed using a high-speed video camera (250 frames/s) fitted with a macro lens under infra-red illumination (Autumn et al. 2006). Individuals were placed in five groups of four into small (10 by 8 by 4 cm) clear plastic enclosures, and recorded for 15-s

intervals encompassing acoustic activity. The specific behavioral context in which sounds were observed from behavioral trials made it possible to attribute recorded sounds to specific individuals being filmed. Because a single individual per interaction produces signals, film analysis was restricted to encounters in which the individual producing sounds was isolated within a frame to ensure that body part movements were correctly attributed to the stridulating individual. Synchronous high-speed video and sound recording was not available for this analysis, but video analysis was restricted to those 15-s periods of video in which sounds were being continuously produced. When repetitive movements were discovered, the cycle-rates were calculated using Photron Fastcam Viewer (Photron Limited 2006). This calculation was used in place of synchronous high-speed sound and video recording to determine whether the putative plectrum moved at a rate sufficient to generate the repetition rates observed during recording sessions.

Stridulitrum. Because initial inspection of the high-speed videos suggested that the moving part, or plectrum, associated with sound production was the plate formed by the fusion of abdominal tergites I and II, we considered whether the undersurface of the metathoracic wing possessed a stridulitrum that could be used in conjunction with the moving plectrum to produce sound. We used Scanning Electron Microscopy (SEM) to capture a surface image of the underside of the metathoracic wing. Images were obtained using a variable-pressure scanning electron microscope (Zeiss Evo MA10, Peabody, MA) pressurized at 70 Pa. The left metathoracic wing was removed from freeze-killed specimens by using forceps and then mounted on carbon tape dorsal side down. The remaining wings were removed using forceps, and the whole body placed venter-side down for images of the thorax and abdominal tergites I and II. One male and one female were included in the SEM-image set that were typical of the distribution of body lengths observed in the population. The images were similar, so only images of males are displayed in the figures. SEM images of the underside of the metathoracic wing and abdominal tergites were completed at the Thermal Ionization Mass Spectrometry and Scanning Electron Microscope Laboratory in the University of Florida Department of Geological Sciences.

Experimental Manipulation of the Stridulatory Apparatus. To verify that the structures identified using high-speed video analysis and SEM imaging are essential for sound production, we silenced individuals by removing the stridulitrum and preventing movement of the plectrum. Twenty males were collected that had previously been observed producing signals. Ten were chilled for 10 min in an airtight container at -10°C , and removed fore and metathoracic wings by pulling at the wing base slowly. After wing removal, individuals were allowed to rest and come to room temperature for 1 hr before being placed together in the interaction containers and recorded. Because wing removal did not result in complete silencing, melted paraffin wax was applied beneath the wings of

the remaining 10 males between abdominal tergites I and II after Gogala (1990). This prevented anterior-posterior movement of the abdominal tergites. Care was taken not to wax the wings to the thorax so that any role of wing movement in sound production independent of plectrum movement still could be observed. Individuals were checked after waxing to ensure that they still had complete use of all their legs, wings, and antennae and did not show any noticeable alterations of their behavior. After wax application, the 10 males were placed in the interaction container together and recorded to determine if muting was complete.

Acoustic Response to Threat Stimuli. To determine whether sounds may be used as defensive signals in response to threat, we recorded sounds produced during filmed interactions with ants, cockroaches, and simulated predation events. We used two types of threat stimuli: arthropod threats and simulated vertebrate predator threats. Two adult individuals were placed in a large petri-dish fitted with a screen lid and suspended in the same manner as the normal interaction trials and were allowed to acclimate for 10 min while being filmed and recorded. Threat stimuli consisted of either four large (>5 mm in length) carpenter ants (*Camponotus* sp.) or two American cockroaches (*Periplaneta americana* L.) of ≈ 10 -mm body length. After the acclimation period, the threat stimuli were introduced to the container, and interactions and sounds were recorded for 30 min. This procedure was repeated twice with new adults for each arthropod "threat."

Because these insects are highly chemically protected (Aldrich et al. 1990a), it is possible that other invertebrates do not pose a serious threat. Therefore, simulated vertebrate predator threats were conducted using isolated adult males in the sound chamber while recording with a microphone. Adult males were used because the interaction trials suggested they were the most likely to produce sounds in conspecific encounters. Six males were individually "attacked" by tugging on the legs and antennae with forceps on a stage in front of a recording microphone. A separate set of six adult males were individually picked up between the thumb and index finger and rolled between the fingers in front of the microphone four times for ≈ 30 s each.

Results

Sounds. Vibrations were detectable both in the substrate and as audible sound; each type of vibration was produced synchronously. These vibrations consisted of repetitions of a single short, stereotyped burst (corresponding to a phrase in Eliopoulos 2006) without any frequency modulation. Burst rate was highly variable among bouts (up to 40 per s), however, the structure of each discrete burst remained fixed for an individual. The frequency range of each acoustic burst was contained within 0.5 and 12 kHz (e.g., Fig. 1). The first dominant frequency band spanned 1–5.5 kHz. The second higher frequency band spanned 7.5–10 kHz. The frequency ranges of sounds and associated substrate-

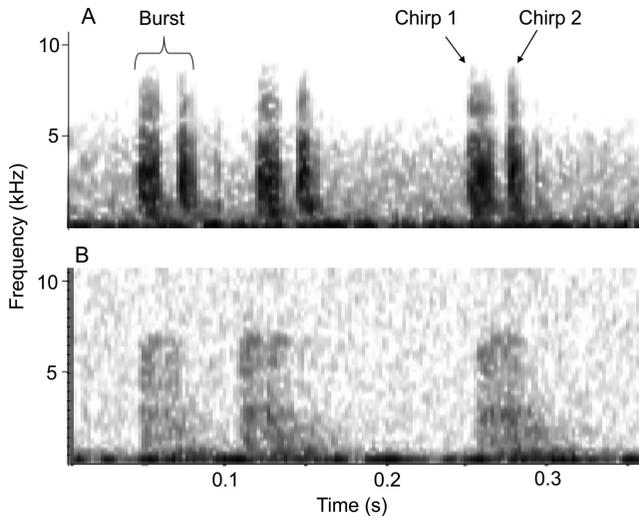


Fig. 1. Spectrogram comparisons of bursts produced by a male *J. haematoloma*, recorded simultaneously by a microphone (A, airborne sound) and an accelerometer (B, substrate vibration). The two-chirp couplet structure of each burst is less apparent in the substrate vibration. Darker shading indicates greater relative energy.

borne vibrations were within the detection range of insect vibration sensing organs found in other species of plant-dwelling Hemiptera (Čokl 1983, Shaw 1994).

Within a burst, there was a clear paired couplet structure in the airborne component of the signal that consisted of two short, distinct chirps (see terminology in Eliopoulos 2006). The first chirp consistently showed a higher amplitude (power) than the second (Fig. 1A). This two-chirp couplet, somewhat less apparent in the substrate-borne signal than in the airborne signal (Fig. 1B), was comprised of a series of 4–7 impulses per chirp, as in the example of Fig. 2.

The mean values of three sets of 20 bursts per measured individual ($N = 19$) were compared for the following analyses. Chirp interval was positively cor-

related with body length (linear, $N = 19$, $F = 17.54$, $r^2 = 0.5078$, $P = 0.0006$, Fig. 3). Though chirp interval was fixed for an individual ($SE \pm 0.2$ ms within a selected individual), burst rate varied widely from 1 to 40 bursts/s. In bouts of sound production showing high burst rate, the two-chirp couplet was always produced faster (less time between bursts), rather than decreasing the pulse interval within bursts.

Sound-associated Behaviors. All sounds recorded took place in a very specific behavioral context: they were exclusive to encounters between two adults of the same species. Sounds were only produced by a single interaction participant at the start of a physical encounter between two adults. Sounds were produced exclusively in this encounter context and were not made outside of periods of physical contact.

The individual that produced sounds, referred to here as the approached, was always the individual

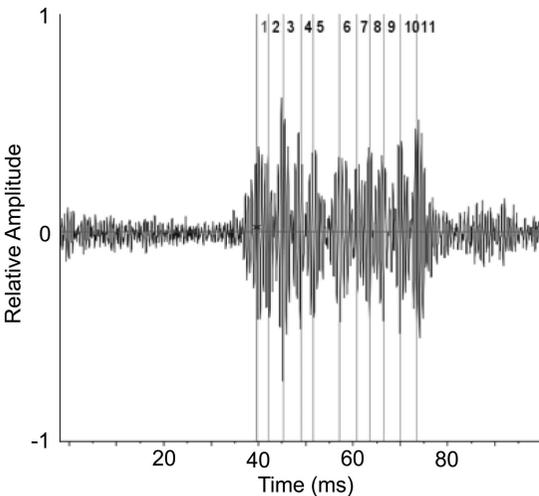


Fig. 2. Oscillogram of a single burst showing impulse structure in a microphone recording. Impulses 1–5, first chirp; impulses 6–11, second chirp in the burst.

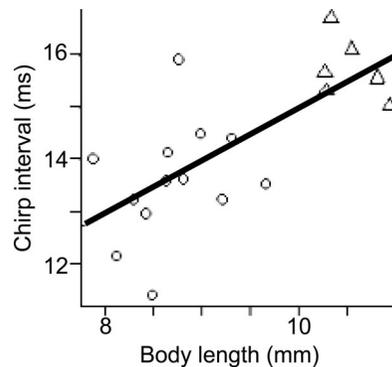


Fig. 3. Relationship between chirp interval, the time between the end of the first and the beginning of the second chirp of a burst, and body length of female (triangles) and male (circles) adult *J. haematoloma*. Solid line indicates the linear regression.

Table 1. Effect of specific combinations of sex and behavior of approaching male and female adults on the occurrence or absence of a signal by approached male and female adults during encounters before beginning of mating season and after the beginning of mating season

Encounter combinations	Pre-			Post-		
	N	χ^2	P	N	χ^2	P
Grouped by sex						
S \pm by \leftarrow sex when M or F \rightarrow	100	6.731	0.0159	63	1.239	0.2657
S \pm by \leftarrow sex when M \rightarrow	59	0.299	0.7381	42	1.952	0.1623
S \pm by \leftarrow sex when F \rightarrow	41	0.370	1.000	21	2.130	0.1444
\rightarrow sex when S \pm by M or F \leftarrow	100	16.246	<0.0001	63	16.247	<0.0001
\rightarrow sex when S \pm by M \leftarrow	52	6.405	0.0202	27	7.895	0.005
\rightarrow sex when S \pm by F \leftarrow	48	4.396	0.0489	36	11.197	0.0008
Grouped by behavior						
S \pm when \rightarrow crawls on \leftarrow	100	22.537	<0.0001	63	10.197	
S \pm when \leftarrow rocks body	100	2.912	0.243	63	11.599	0.007

P values from Fisher's two-tailed test performed using a χ^2 contingency table of the presence or absence of signals during N observations of each encounter type (df = 1), sorted by sex of participants or by presence or absence of listed behaviors.

\rightarrow , approaching; \leftarrow , approached; M, male; F, female; +, occurrence; -, absence; S, signal; Pre-, before beginning of mating season; Post-, after the beginning of mating season.

moving with the least relative velocity (body lengths/s) at the start of the encounter ($N = 100$). The individual moving with the most velocity, the approacher, was always silent; consequently, only approached individuals were signalers. These results suggest that signals are a direct response to approaches by other individuals and are not used in long or short-range mate attraction.

Nonmating Aggregations (2009 Trials). In trials conducted with specimens collected from nonfeeding, nonmating aggregations in 2009, certain target behaviors and sex combinations during encounters strongly increased the probability that an encounter produced sounds (Table 1). Sounds were produced in 100% of encounters where the approaching individual climbed on top of the signaler ($N = 19$). Approaching males crawled onto other individuals significantly more than approaching females, regardless of the sex of the signaler ($N = 100$, df = 1, $\chi^2 = 9.005$, $P = 0.0035$). Males also crawled onto other males more frequently than females. Because all encounters where an individual crawled on top of another resulted in sound production by the crawled-on individual, the male bias in crawling behavior is a reflection of male-bias in the sex of approachers during interactions which produced sounds. Signals also were produced much more frequently in encounters where the approached signaler shook his or her body laterally from side to side, a behavior that occurred more frequently when that individual was crawled upon ($N = 100$, df = 1, $\chi^2 = 13.020$, $P = 0.0062$). The strong association between being crawled upon and body rocking by the approached individual suggests that the function of sounds may be to discourage close physical contact.

Mating Aggregations (2010 Trials). In the seven trials observed after mating had begun in natural populations, pairings took place within the first 15 min of observation ($N = 12$). These pairings were initiated with physical contact, rapid mounting by the male, and subsequent coupling, similar to the mating sequence described by Carroll and Corneli (1995) for Oklahoma and Florida populations of *J. haematoloma*. Though

crawled-upon individuals signaled more frequently than in encounters where they were not crawled upon ($N = 63$, df = 1, $\chi^2 = 10.197$, $P = 0.0014$), sounds occurred in only 73.2% (31/40) of those encounters, not 100% as observed in the nonmating season. Furthermore, only 58.3% of mounting encounters that resulted in pairing ($N = 12$) produced sounds. Using a nominal logistic regression, we compared the response to being crawled upon during mating and nonmating seasons and found that the probability of producing sounds when crawled upon during the mating season was significantly lower than in the nonmating season ($N = 163$, df = 3, $\chi^2 = 41.023$, $P < 0.0001$).

In contrast to the encounters in trials using nonmating aggregations, the sex of the approached individual was not a significant predictor of sound production in trials during the mating season (Table 1). Whether or not an encounter between a male and a female produced sounds during the mating season was not related to mating outcome ($N = 40$, df = 1, $\chi^2 = 0.0049$, $P = 0.9443$).

Sound Production Mechanism. High speed video of individual interactions with wings removed showed rapid (15–25 cycles/s) anterior–posterior movement of the fused abdominal tergites I and II. During the period of rapid tergal movements, all other parts of the body remained isolated from movement (including head, mouthparts, legs, and thorax). This form of tergal movement only was observed in the approached individual for the five interactions filmed during the high speed video sessions. The rate of contractions (forward-backward) fell within the typical repetition rate observed when bursts are produced (25Hz). The anterior–posterior movement of the tergal plate was not accompanied by any deformation, depression, or folding of the tergites or corresponding sternites. Consequently, we can be reasonably certain that the tergal plate does not function as a tymbal to produce signals, as has been suggested by previous authors for this and other families in the Pentatomomorpha (Gogala et al. 1974, 1984; Virant-Doberlet and Čokl 2004).

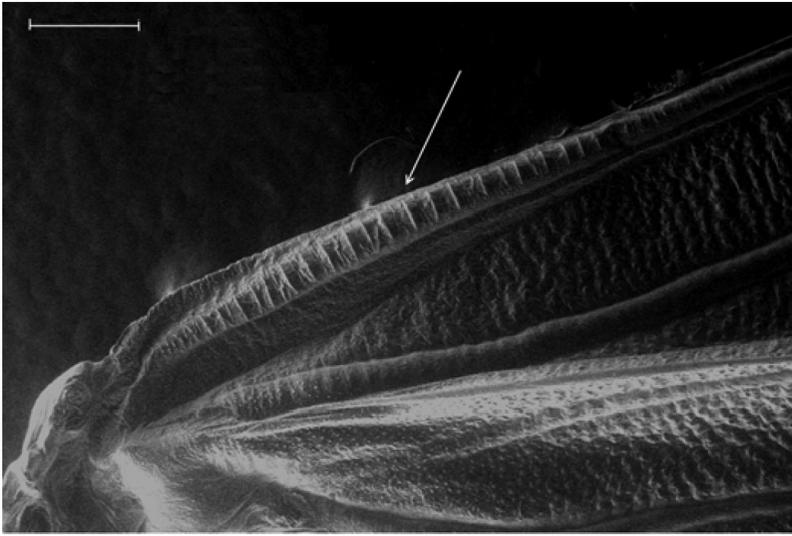


Fig. 4. Adult male *J. haematoloma*, SEM image of the ventral side of the left metathoracic wing. Arrow indicates stridulitrum on costal vein; scale bar, 200 μm .

SEM images obtained of the undersides of the right and left metathoracic-wings confirmed the presence of a stridulitrum located on the ventral side of the costal vein of the metathoracic wing (Fig. 4). The stridulitrum opposes a raised surface on the dorsal side of abdominal tergite I which, when moved along the observed anterior-posterior field of movement, follows the length of the stridulitrum against the grain of the stridulitrum teeth (Fig. 5). Stridultra were found in both male and female adults and were absent on the wing pads of nymphs.

The experimental loss-of-function manipulations supported the hypothesis that the wings act in concert

with abdominal tergites I and II to produce sounds. Experimental removal of the wings followed by the acoustic analysis revealed that wings are needed to produce high-frequency components of sounds, but not low-frequency components. Metathoracic wing removal eliminated all higher frequency components of signals, and reduced the two-chirp couplet structure to a single low-frequency chirp (0–1000 Hz, Fig. 6); however, it did not silence signals completely. Application of wax between the thorax and abdominal tergites I and II successfully silenced individuals by preventing anterior-posterior movement of abdominal tergites I and II. These observations suggest that

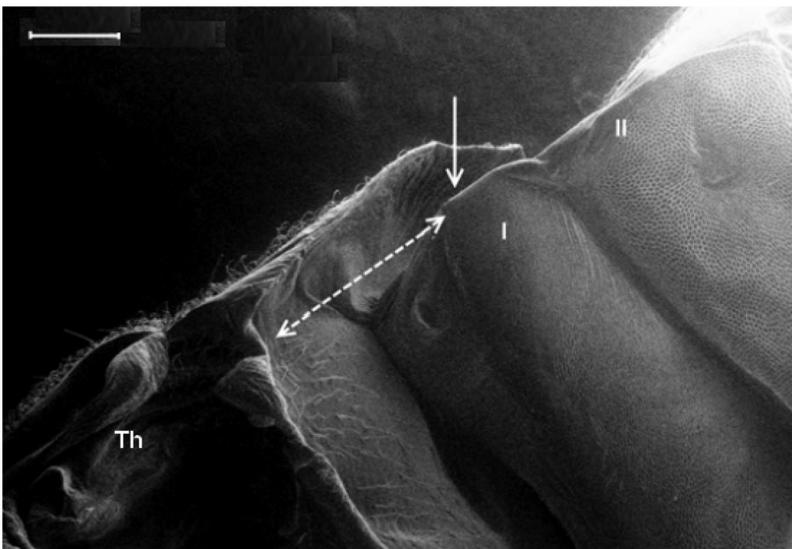


Fig. 5. Adult male *J. haematoloma* right abdominal tergites I and II. Dashed double-headed arrow indicates field of motion of abdominal segments along anterior-posterior axis, single headed arrow indicates plectrum surface. Th, thorax; scale bar, 200 μm .

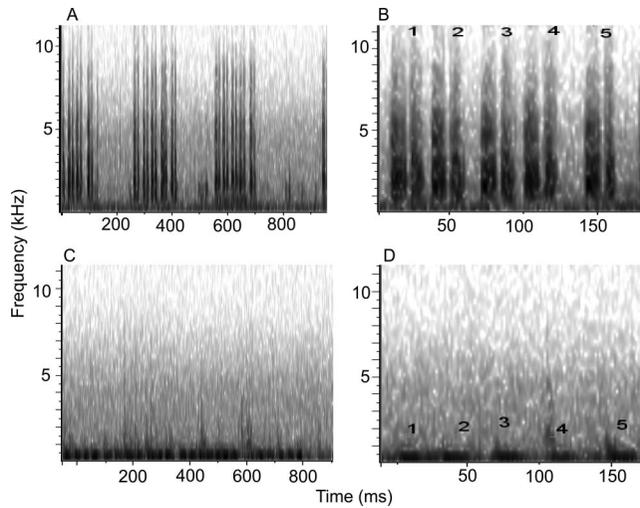


Fig. 6. Sample spectrograms of bursts produced by a male with wings (A, expanded view in B), and a male with wings removed (C, expanded view in D). Wing removal abruptly silenced all higher-frequency spectra, and eliminated the two-chirp couplet structure. Numbered labels mark the second chirp of each burst in B and the end of each burst in D.

the high-frequency two-chirp couplet is generated by the forward (first chirp) and backward (second chirp) movement of the tergal plate against a metathoracic wing stridulitrum. Without wings, the forward-reverse movements of the tergal plate no longer generate high-frequency sounds by rubbing against the stridulitrum. The remaining low-frequency percussive signals after wing removal corresponded to the impact of the tergal plate on the posterior edge of the metanotum.

Acoustic Response to Threat Stimuli. In the series of arthropod “threat” encounters, sounds were never produced in inter-species encounters, between *Jadera* and cockroaches ($N = 23$) or ants ($N = 17$). There was apparent release of chemical defense volatiles by each adult which caused the roaches and ants to clean themselves thoroughly. Pinching legs and antennae with forceps did not successfully elicit sounds from any males. Smothering between the thumb and index finger encouraged sound production by all individuals ($N = 6$) so long as constant pressure was applied. Defensive chemicals (toasty almond smell) could be smelled after the smothering treatment.

Discussion

We have shown that *J. haematoloma* produces substrate and airborne vibrations in a unique behavioral context by using similar morphological structures used by other closely related Hemiptera. The sounds presented here show superficial similarity to other Hemiptera that have short stereotyped signals without much signal modulation (Gogala 1984). There was a positive relationship between chirp interval and body length. *Jadera haematoloma* sounds lack the complex modulation or multiple song types that have been observed in the courtship rituals of some Pentatomidae (Čokl et al. 2001) and strongly differ from the continuous, heavily modulated sounds produced by

various Cicadidae (Drosopoulos et al. 2006), Membracidae (Rodríguez et al. 2004), and Psyllidae (Percy et al. 2004). A potential benefit of the broadband frequency range and minimal temporal modulation is that the signal can be easily identified and interpreted within a variety of different substrates. Typically, *J. haematoloma* are found aggregating on many different substrates, and there is considerable variation in how leaves, stems, branches or other structures transmit and attenuate signals compared with airborne transmission (Mankin et al. 2008).

The tergal plate and wing-stridulitrum mechanism found in *J. haematoloma* has been observed in several other members of the Pentatomomorpha; Piesmatidae (Leston et al. 1954, Jorigtoo et al. 1998) two lygaeid genera (*Piesma* and *Kleidocerys*, Ashlock and Lattin 1963); Cydnidae; and the Thyreocoridae (Gogala et al. 1974, Schaefer 1980) but is not similar to the tymbal mechanism observed in Sternorrhyncha. The stridulation shown here in absence of deformation of the tergal plate favors the plectrum hypothesis over the tymbal hypothesis for the role of the tergal plate in vibration production. The Cydnidae, which also possess a metathoracic wing stridulitrum and abdominal plectrum, differs from *Jadera* in sound complexity. *Jadera* produced only a single repeated burst, with fewer discrete harmonics detectable through the substrate, and did not demonstrate the diversity of context-specific songs observed by Gogala et al. (1974) in the Cydnidae. High-resolution recordings were unavailable for comparisons with sounds produced by the Lygaeidae, Tessaratomidae, Scutelleridae, Thaumastellidae, and Leptopodidae, which have similar wing and tergal plate morphology. The recordings by Gogala (1990) of adult male *Arhyssus hyoscyami* (Hemiptera: Rhopalidae) very closely resembled those recorded here; signals were succinct and highly stereotyped, however, the specified frequency range

was much lower than those recorded here (80 Hz), possibly because of differences in recording equipment. From these observations drawn from the literature, it is clear that a shared sound producing mechanism does not necessarily confer acoustic similarity.

The behavioral context of sound production suggests that sounds may be signals in response to an approach by another individual. Sound production began by an individual who was approached by a conspecific moving at greater speed. The specific behavioral context of sound production by *J. haematoloma* is unique because the sounding role in an encounter is determined by the relative speed of the individual, regardless of its sex. In most hemipteran communication systems, the signaler role is stereotyped by sex or age as is appropriate signaler identification during courtship and mother-offspring interactions (Cocroft 2001, Cocroft and Rodriguez 2005). Because two adult *J. haematoloma* are already touching when sounds are produced, and because it occurs in the absence of offspring, it is clear that sounds are not used as signals to attract conspecifics from a distance or to alert offspring to threats. On the contrary, increased repetition rates associated with being crawled upon and simultaneous body rocking behavior suggest that sounds are more common in proximate interactions. The association of sounds with close proximity between individuals and with body rocking suggests these vibrations are used to deter contact with conspecifics. Decreased acoustic response to being crawled upon during the mating season might suggest that signals are being used to indicate that a female or male is unreceptive to mating. However, the results show that during male-female encounters, sounds did not influence mating outcome during the mating season. Therefore further investigation into the influence of field density, food availability, and rates of cannibalism in the field are needed to address the seasonality of this behavior.

All inter-species interactions with other arthropods failed to produce sounds. Though handling the insects caused them to produce sound, all other threat stimuli failed to elicit signals. *Jadera haematoloma* is chemically protected, and may only experience real threat from larger vertebrate predators (Aldrich et al. 1990a, 1990b) which have been shown to respond negatively to aposematism in this species (Ribeiro 1989). These results support the hypothesis that sounds target nearby conspecifics and are not frequently used in inter-species interactions.

Our exploration of vibrations produced by *J. haematoloma* revealed that the vibration-producing structures are similar to other closely related Hemiptera. The tergal plate plectrum that *J. haematoloma* uses to produce sounds is shared by several other Heteroptera, supporting the hypothesis presented by Schaefer (1993) that the tergal plate plectrum is largely conserved in terrestrial Heteroptera with multiple independent origins of wing stridulating structures. However, there is still considerable acoustic variation among species using the same mechanism as *J. haematoloma*. Therefore, although sound producing struc-

tures may be conserved, the sounds themselves may not show phylogenetic signal. Instead, the function of signals and the behavioral context might be more important in shaping signal structure in this group of insects. Further exploration of signal diversity in target clades, and descriptions of each behavioral context would help to address this exciting question.

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