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ARTICLE

Acoustics of the mountain pine beetle (*Dendroctonus ponderosae*) (Curculionidae, Scolytinae): sonic, ultrasonic, and vibration characteristics

A.J. Fleming, A.A. Lindeman, A.L. Carroll, and J.E. Yack

Abstract: Acoustic signaling is widespread in bark beetles (Scolytinae), although little is known about the physical characteristics of signals, how they are transmitted, and how they differ among behavioural contexts. Signals were studied in the male mountain pine beetle (*Dendroctonus ponderosae* Hopkins, 1902) during stress, male–female, and male–male interactions. Sounds are broadband with significant energy in the ultrasound (peaks between 15 and 26 kHz) and low amplitude (55 and 47 dB SPL at 2 and 4 cm, respectively), indicating that signaling functions at close range. Signal trains vary among contexts primarily in the proportions of chirp types. Chirps were categorized as being simple or interrupted, with the former having significantly lower tooth strike rates and shorter chirp durations. Stress chirps are predominantly simple with characteristics resembling other insect disturbance signals. Male–female interactions begin with the male producing predominantly interrupted chirps prior to gallery entrance, followed by simple chirps. Male–male (rivalry) chirps are predominantly simple, with evidence of antiphonal calling. Substrate-borne vibrations were detectable with a laser-doppler vibrometer at short distances (1–3 cm), suggesting that sensory organs could be tuned to either air or substrate-borne vibrations. These results have important implications for future research on the function and reception of acoustic signals in bark beetles.

Key words: acoustic, mountain pine beetle, communication, *Dendroctonus ponderosae*, vibration.

Résumé : Si l'utilisation de signaux acoustiques est répandue chez les scolytes, les caractéristiques physiques des signaux, leurs modes de transmission et leurs variations selon le contexte comportemental demeurent méconnus. Ces signaux ont été étudiés chez le dendroctone du pin ponderosa (*Dendroctonus ponderosae* Hopkins, 1902) dans des contextes de stress et d'interactions mâle–femelle et mâle–mâle. Il s'agit de sons à large bande, notamment dans l'ultrason (pointes entre 15 et 26 kHz), et de faible amplitude (55 et 47 dB SPL à 2 et 4 cm, respectivement), indiquant que les signaux fonctionnent sur de courtes distances. Les trains de signaux varient selon le contexte, principalement pour ce qui est des proportions des différents types de piaulements. Les piaulements ont été catégorisés selon qu'ils étaient simples ou interrompus, les premiers étant caractérisés par des fréquences de battements significativement plus faibles et des durées moins longues. Les piaulements de stress sont généralement simples, présentant des caractéristiques semblables à celles d'autres signaux de perturbation chez les insectes. Les interactions mâle–femelle commencent par la production par le mâle de piaulements généralement interrompus avant l'entrée dans la galerie, suivie de piaulements simples. Les piaulements associés aux interactions mâle–mâle (rivalité) sont principalement simples, présentant des signes de chant antiphonique. Des vibrations transmises par les substrats étaient décelables avec un vibromètre Doppler à laser sur des courtes distances (de 1 à 3 cm), ce qui laisse croire que les organes sensoriels pourraient percevoir des vibrations transmises tant par l'air que par les substrats. Il s'agit de résultats importants pour ce qui est d'orienter les travaux de recherche futurs sur la fonction et la réception des signaux acoustiques chez les scolytes. [Traduit par la Rédaction]

Mots-clés : acoustique, dendroctone du pin ponderosa, communication, *Dendroctonus ponderosae*, vibration.

Introduction

Acoustic signaling is widespread in bark beetles (Scolytinae) (Barr 1969; Lyal and King 1996). There exists a great deal of interspecies variability with respect to how signals are generated, which gender produces the signals, and which behaviours are associated with signaling (Barr 1969; Lyal and King 1996). Sound production has been implicated in a broad range of functions including attraction, acceptance, cooperation, species recognition, courtship, territoriality, and defence (Barr 1969; Ryker and Rudinsky 1976; Lyal and King 1996). Despite the ubiquity and purported importance of acoustic signals in bark beetles, little is known about their physical properties and how these properties vary among behaviours. Furthermore, nothing is known about possible sound or vibration receptors. To advance research on the neuroethology and behavioural ecology of bark beetle acoustics, it is important to understand how signals are propagated in the natural environment and what information is available to recipients. This study uses the mountain pine beetle (*Dendroctonus ponderosae* Hopkins, 1902) as a model to probe these issues.

The life history of *D. ponderosae* (Fig. 1*a*) is relatively well known (reviewed by Safranyik and Carroll 2006). Its populations are prone to dramatic fluctuations, and outbreaks occasionally erupt over large forested landscapes. Most species of the genus *Pinus* L. within the range of *D. ponderosae* are susceptible to attack, but lodgepole pine (*Pinus contorta* var. *latifolia* Engelm. ex S. Watson) and ponderosa pine (*Pinus ponderosa* Douglas ex P. Lawson & C.

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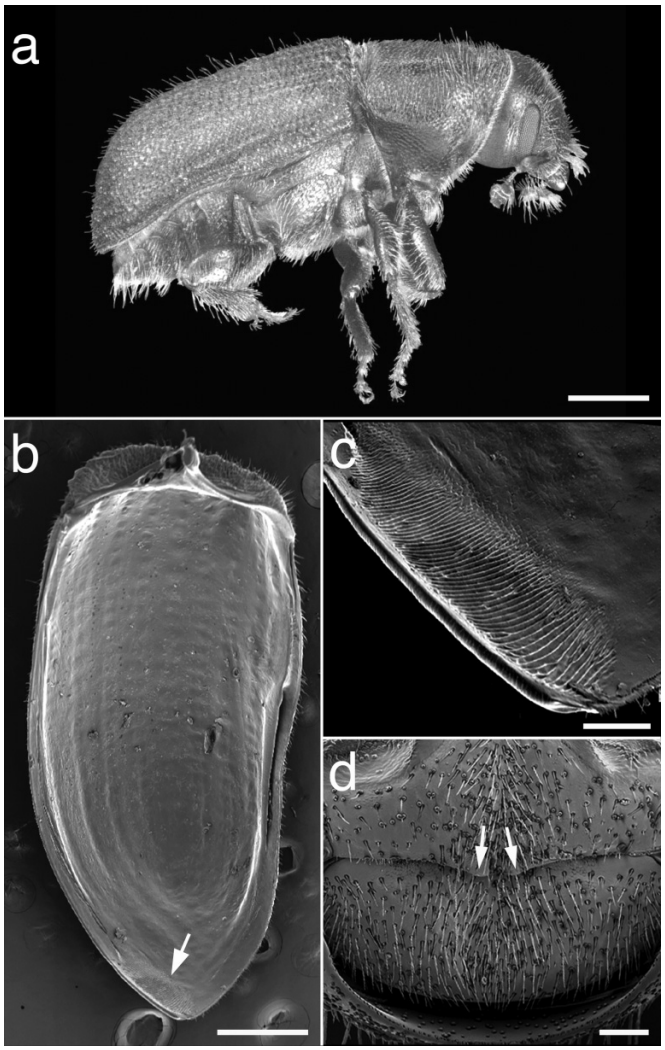
Fig. 1. (*a*) A male mountain pine beetle (*Dendroctonus ponderosae*). Scale bar = 1 mm. (*b*–*d*) Scanning electron micrographs of sound-producing structures. (*b*) Inner surface of the left elytron, with an arrow indicating the location of the pars stridens. Scale bar = 600 μm. (*c*) Enlargement of the pars stridens showing the individual teeth. Scale bar = 100 μm. (*d*) Dorsal view of the seventh abdominal tergite, with arrows pointing to the processes of the plectrum. Scale bar = 100 μm.



Lawson) are considered its primary hosts. Female *D. ponderosae* select host trees and initiate colonization. As they bore through the bark into the phloem of a potential host tree, females produce aggregation pheromones that attract mainly male beetles. Upon arrival at the tree, male beetles also emit aggregation pheromones that attract additional females. At close distances, acoustic signaling appears to become the predominant form of communication during social interactions (Ryker 1988).

Acoustic signals have been studied in *D. ponderosae* from Oregon (USA) with respect to the sound-producing mechanisms, and some temporal features associated with different behaviours. Males produce sounds using an elytro-abdominal mechanism, whereby a plectrum (a sclerotized portion of the posterior margin of the seventh abdominal segment) is scraped against the pars stridens (a series of teeth located on the underside of the left elytron) (Figs. 1*b*–1*d*) (Michael and Rudinsky 1972). Sounds generated by males have been variously categorized as courtship, stress, attractant, or rivalry signals, and have been implicated to function in a variety of contexts, including cooperation, courtship, greeting, aggression, and premating recognition (e.g., Michael and Rudinsky 1972; Rudinsky and Michael 1974; Ryker and Rudinsky 1976; Yandell 1984). Females have similar sound-producing organs to males, but with fewer teeth on the pars stridens (Rudinsky and Michael 1973) and have been reported to produce simple chirps when defending a gallery or during initial interactions with males, and short clicks while forming egg galleries (Rudinsky and Michael 1973; Ryker and Rudinsky 1976). Previous studies of *D. ponderosae* signals have broadly categorized chirps as being "simple" or "interrupted" and have primarily described chirp duration, number of tooth strikes per chirp, and tooth strike rate (Michael and Rudinsky 1972; Ryker and Rudinsky 1976; Yandell 1984). Although these studies have provided valuable insight into the diversity of temporal patterns that occur in the behavioural repertoire of *D. ponderosae*, and how these patterns might vary between populations, there is often a lack of clarification on how signals were sampled, quantified, and categorized. For example, Yandell (1984) concluded that male stress and rivalry signals, but not male attractant signals, differed between populations of *D. ponderosae* colonizing three different species of host pine; however, no information was given on chirp type (simple or interrupted) or sample size. Ryker and Rudinsky (1976) studied temporal characteristics of signals associated with different behaviours, but no information was provided on how simple chirps were distinguished from interrupted chirps and how chirps were sampled. This lack of information, which is characteristic of many bark beetle acoustic studies, makes it difficult to compare signals between different behavioural conditions, or between populations. In addition, details on spectral and amplitude properties of airborne sounds, and whether signals are propagated as substrate-borne vibrations, are generally lacking for any bark beetle species. Understanding the characteristics of signals and how they are transmitted would provide insight into the function of signals in bark beetles and provide a necessary step towards new research on the neuroethology and reception of acoustic signals.

The specific objectives of this study are to (*i*) characterize the temporal, spectral, and amplitude features of airborne sounds produced by *D. ponderosae*; (*ii*) compare signals produced in different behavioural contexts; and (*iii*) determine if signals are transmitted as substrate-borne vibrations.

Materials and methods

Beetles and morphology

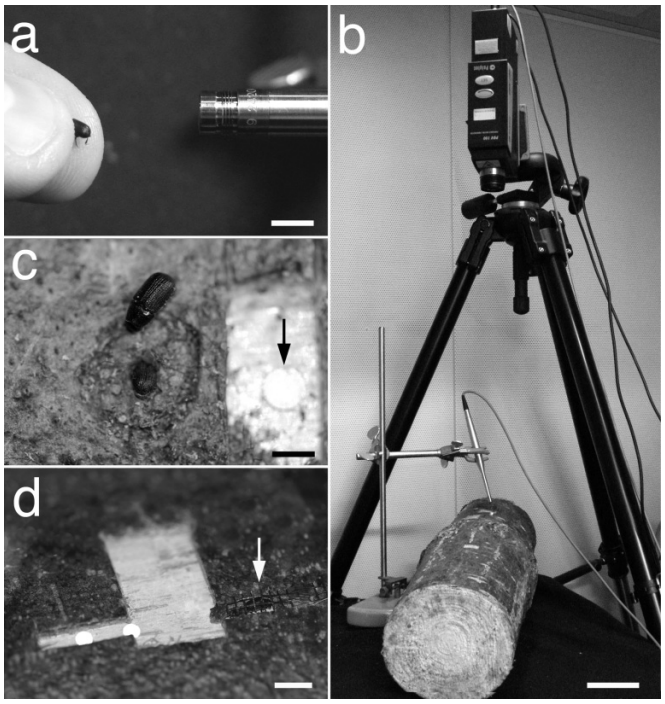
Beetles were obtained from naturally infested bolts of lodgepole pine collected just south of Merritt, British Columbia, Canada (49°52′31.51″N, 120°53′34.9″W), during 2007 and 2008. Bolts were stored in double-sealed 15 US gallon (1 US gallon = 3.78541 L) containers at 3–5 °C, and when adults were needed, the containers were transferred to a secure insect-holding facility maintained at 21–25 °C at Carleton University. Sex was determined initially by lightly squeezing individuals and noting the presence or absence of audible chirps (McCambridge 1962). Following experiments, beetles were preserved in fixative (Chauthani and Callahan 1966) and sex was confirmed by examining the dimorphism of the seventh abdominal tergite (Lyon 1958). Following experiments, all bolts were autoclaved and incinerated. Voucher specimens are held at Carleton University.

Scanning electron micrographs of male stridulatory structures were obtained by dissecting elytra and abdomens, placing them on aluminum stubs, sputter coating with gold–palladium, and examining with a JOEL JSM-6400 scanning electron microscope.

Recording procedures

Acoustic signals were recorded under three different conditions: "stress", "male–female", and "male–male" interactions. No individuals were used twice. All recordings were made in a

Fig. 2. Methods for recording sounds and vibrations. (*a*) Stress sound recordings were obtained by lightly pinching the prothorax of a male mountain pine beetle (*Dendroctonus ponderosae*) held at various distances from a microphone. Scale bar = 5 mm. (*b*) Set up used to record signals from interacting beetles. Substrate-borne vibrations, sounds, and behavioural interactions were recorded simultaneously using a laser vibrometer, a microphone placed 1 cm from the interacting individuals, and a camcorder with a second microphone (not shown). Scale bar = 10 cm. (*c*) Detail of setup for male–female interactions. A male approaches a female, whose posterior end protrudes from the entrance hole (see also supplementary video).[1] An arrow points to a reflective laser target disc on the phloem layer. Scale bar = 3 mm. (*d*) Detail of the setup used to record male rivalry interactions. A small arena was cut in the bark to expose the phloem. Males were restrained in a section of this arena (arrow) and laser target discs were placed at varying distances from the beetles to record vibrations. Scale bar = 6 mm.



walk-in type acoustic chamber maintained at 22.0 ± 2.0 °C (mean ± SE).

Stress

Eleven males were induced to signal using methods described in Ryker and Rudinsky (1976), by grasping the animal between the thumb and the index finger and lightly pinching the pronotum and head while avoiding the elytra (Fig. 2*a*). Sounds were recorded using a ¼″ (1 inch = 2.54 cm) condenser microphone (model 4939, Brüel & Kjær (B&K), Nærum, Denmark) at distances of 1, 2, 4, 8, and 10 cm, amplified with a B&K Nexus conditioning amplifier (model 2690), and recorded to a data recorder (FR-2; Fostex, Los Angeles, California, USA) at a sampling rate of 192 kHz.

Male–female interactions

Recordings of interactions between seven male–female pairs were conducted on freshly cut bolts of red pine (*Pinus resinosa* Aiton). A female was placed near a predrilled hole (2 mm), where she was secured for 24 h by placing an empty gel capsule over the hole. Once she had begun constructing a gallery (noted by the accumulation of frass surrounding the entrance hole), the gel capsule was removed and a randomly selected unmated male was placed 1–2 cm from the gallery entrance. The interaction was recorded simultaneously using a microphone and laser vibrometer (Fig. 2*b*) for several minutes or until signaling was no longer detectable (see also supplementary video).[1] Sounds were recorded using the B&K microphone (described above) positioned 1 cm from the entrance hole. Substrate-borne vibrations were recorded from the phloem layer that was exposed by removing a small portion of the bark layer 1 cm from the entrance hole (Fig. 2*c*). A disc of reflective tape (0.25 cm) was securely attached to the phloem surface by its adhesive backing and vibrations recorded using a laser-doppler vibrometer (PDV 100; Polytec, Waldbronn, Germany). Laser signals were recorded to a data recorder (PMD 671; Marantz, Los Angeles, California, USA) at a sampling rate of 44 kHz. Behaviours were monitored in five trials using a camcorder (HDRHC7; Sony, Tokyo, Japan) with a second microphone (ECM-MS908C; Sony) positioned adjacent to the preparation and connected to the camera microphone jack.

Male–male interactions

Fourteen male–male pair interactions were staged using a modification of the Ryker and Rudinsky (1976) method. A narrow holding arena was created in the phloem layer by cutting away a section of the bark and covering the arena with a mesh cage to prevent the beetles from escaping (Fig. 2*d*). Directly adjacent to this arena a larger section of the bark was cut away to place the laser discs. One male was placed in the arena first, and when he stopped producing handling-related stress signals, the other male was added. Sounds were recorded using the B&K microphone (described above) positioned 1 cm from the arena. Vibrations were recorded from laser target discs placed in the uncovered extension of the arena at 1 and 3 cm from the beetles.

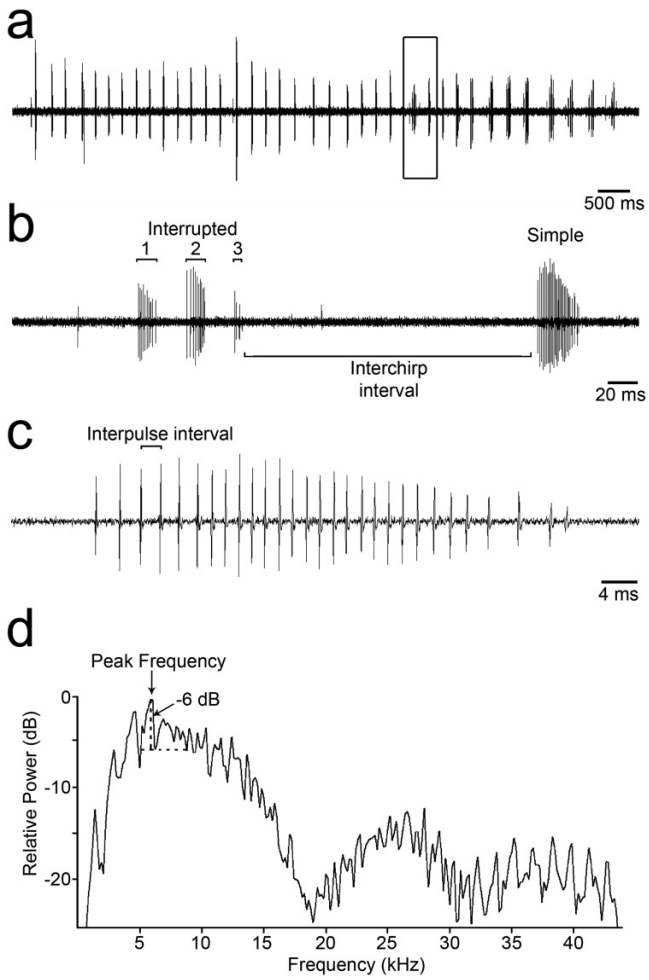
Signal measurements and analyses

Temporal measurements

Temporal characteristics including chirp rate, chirp type (simple or interrupted), chirp duration, interchirp interval, number of tooth strikes per chirp, tooth strike rate, and intertooth strike interval were measured from sound files using Avisoft SAS Lab Pro (Avisoft Bioacoustics, Berlin, Germany). A chirp, following terminology used in previous bark beetle studies (Barr 1969), is defined as a train of stridulatory pulses. Each pulse (that we call a tooth strike) is assumed to result from a "tooth" on the pars stridens being plucked by the plectrum, although to date, the precise relationship between the sound pulses (tooth strikes) and the sound-production mechanism has not been experimentally confirmed. Chirp rates were calculated during periods of consecutive chirp production (chirp trains) by dividing the number of chirps by the duration of the chirp train. Chirps are generally categorized as being simple or interrupted; a simple chirp comprises one series of regularly spaced tooth strikes, whereby an interrupted chirp has two or more components interrupted by brief periods of silence (Ryker and Rudinsky 1976). In the vast majority of chirps analyzed, the distinction between simple and interrupted was obvious. However, there existed cases where there were "stray" tooth strikes at either end of a simple chirp, and so for clarity, we defined interrupted chirps as having chirp components with intertooth strike intervals of at least 3 ms and consisting of two or more tooth strikes. This decision was based on observing thousands of chirps, as well as plotting frequency histograms of interspike intervals on a subset of simple and interrupted chirps (data not shown). The tooth strike rate was measured by dividing the

[1]Supplementary video is available with the article through the journal Web site at http://nrcresearchpress.com/doi/suppl/10.1139/cjz-2012-0239.

Fig. 3. Acoustic parameters measured in this study. (*a*) A representative chirp train produced by a male mountain pine beetle (*Dendroctonus ponderosae*) following a disturbance. Box encloses two chirps expanded in *b*. (*b*) An interrupted chirp with three components a simple chirp showing the temporal parameters measured. (*c*) Simple chirp from *b* expanded to show individual tooth strikes. (*d*) A representative power spectrum showing the spectral parameters measured. The bandwidth was measured at 6 dB below the dominant frequency.



number of tooth strikes by the duration of the entire chirp. Intertooth strike intervals were individually measured using Avisoft. Temporal characteristics analyzed are illustrated in Figs. 3*a*–3*c*.

Spectral measurements

Spectral characteristics including dominant frequency and bandwidth at –6 dB were measured from sounds recorded at 1 cm from the source for all three conditions. In male–female interactions, where the male enters the gallery after a period of time, only chirps recorded prior to gallery entrance were analyzed (see also supplementary video).[1] Sounds were high pass filtered at 500 Hz to remove background noise. In the case of vibration recordings, the signals were not always easy to detect above background noise, and filtering was achieved by applying a spectral subtraction of the noise profile using Wavepad acoustic program version 3.20 (NCH Swift Sound, Bruce, ACT, Australia). Spectra were produced using a 512-point Fast Fourier Transform Hamming window in Raven Bioacoustics Research Program (Cornell Laboratory of Ornithology, Ithaca, New York, USA). Spectral characteristics analyzed are illustrated in Fig. 3*d*.

Amplitude measurements

Sound-level recordings were made using the B&K microphone (described above). Voltages (peak-to-peak in mV) of chirps were measured on an oscilloscope (THS720A; Tektronix, Richardson, Texas, USA) at 2 and 4 cm. A continuous pure tone centered at the mean dominant frequency (~10 kHz) was generated with a Tabor Electronics 50MS/s Waveform Generator WW5061 (Tel Hanan, Israel) and broadcast through a Horn Tweeter (GT-1016, response 3.5–40 kHz). The volume was adjusted until the output voltage was equal to that of the chirps emitted by the beetles. The dB·peSPL values at the specified distances were measured with a Brüel & Kjær sound level meter type 2239 placed at the same location as the microphone. Chirps were also classified as having ascending, descending, or bell-shaped amplitude envelopes according to their amplitude modulation. Amplitudes of solid-borne vibrations were measured as voltages directly from the analogue output of the laser and converted to vibrational velocities (mm/s) according to the instrument calibration chart.

Analyses

Temporal and spectral features of the first five simple and first five interrupted chirps were compared within each behavioural context using a paired *t* test and between contexts using a one-way ANOVA. Proportions of simple and interrupted chirps sampled from the entire train were also compared between conditions and are presented in Table 1. Five videotaped male–female trials were analyzed to monitor interchirp intervals and chirp type during the course of the first 90 s in an encounter.

Results

Males produced chirps under conditions of stress, while interacting with females, and while interacting with other males, and females produced chirps occasionally during interactions with males. Although previous researchers have reported clicks in females (e.g., Ryker and Rudinsky 1976), we could not reliably distinguish between clicks and incidental noise caused by the beetles interacting with the substrate. Our analyses therefore focus on male chirps, with brief mention of how they were distinguished from those of females (see below). In agreement with previous studies, we found that chirps could usually be divided into simple and interrupted. But in contrast to previous reports, we found that both chirp types were associated with each behavioural condition. Therefore, to make meaningful comparisons with values in previous literature, we analyzed separately the first five chirps of each chirp type per condition (Tables 1 and 2).

Airborne sounds

Stress

Upon "attack", males generated a train of chirps that typically continued until animals were released. During a train, chirps were produced at a mean rate of 2.1 ± 0.3 chirps/s. The majority of chirps were simple (Table 1). Ten of 11 individuals produced predominantly simple chirps (Figs. 4*a*–4*d*), with 6 of the 10 individuals producing simple chirps exclusively. Simple chirps were significantly shorter than interrupted chirps (Table 1), with the latter having from two to five components. Both chirp types had a similar number of tooth strikes, although interrupted chirps tended to include more (Table 1). Interrupted chirps had significantly lower mean tooth strike rates (Table 1) and higher mean intertooth strike intervals (Table 1). When analyzed for their spectral content, stress chirps had three prominent peaks ranging between 9 and 60 kHz, with interrupted chirps tending to have higher dominant frequencies (Figs. 4*a*–4*d*, Table 1). Sound levels measured at 2 and 4 cm were 55.7 and 47.1 dB SPL, respectively. At 10 cm, the sounds were no longer detectable by our microphone. Chirps with descending amplitude envelopes were the predominant type in most males. Moreover, two individuals produced an

Table 1. Comparison of simple and interrupted chirp types of the mountain pine beetle (*Dendroctonus ponderosae*) within and between stress, male–female, and male–male chirp contexts.

				Temporal				Spectral	
Chirp context	Chirp type	*n*	Proportion (%)	Tooth strikes (no./chirp)	Tooth strike rate (no./s)	Intertooth strike interval (ms)	Chirp duration (ms)	Dominant frequency (kHz)	Bandwidth at –6 dB (kHz)
Stress	Simple	11	93.3	17.4±1.8	828.5±59.8	1.4±0.1	21.8±1.7	15.6±2.8	14.6±1.9
	Interrupted	7	6.7	27.9±4.1	593.2±89.5	2.2±2.8	56.3±8.0	18.3±5.8	18.2±3.4
Male–female	Simple	7	31.2	21.3±3.5	786.0±63.7	1.4±0.1	30.0±6.8	26.0±4.6	12.8±1.8
	Interrupted	7	68.8	35.0±3.5	433.1±18.8	2.6±0.1	90.0±6.5	21.9±5.9	13.5±1.8
Male–male	Simple	14	75.9	16.9±2.2	709.5±40.2	2.6±1.0	28.8±6.3	17.4±1.6	14.6±1.5
	Interrupted	11	24.1	22.2±2.2	464.0±44.7	2.6±0.2	56.1±8.5	26.4±3.4	24.2±3.6
Comparison between chirp types				Significance level of comparison (paired *t* test)					
Stress				NS	<0.01	<0.01	<0.01	NS	NS
Male–female				<0.01	<0.01	<0.01	<0.01	NS	NS
Male–male				NS	<0.01	NS	0.02	<0.01	<0.01
	Comparison between contexts			Significance level of comparison (ANOVA)					
	Simple			NS	NS	NS	NS	0.05	NS
	Interrupted			0.02	NS	NS	0.02	NS	NS

Note: Sample size represents the number of individuals sampled; the first five chirps were analyzed per chirp type per individual, except in cases where individuals produced fewer than five chirps of a given chirp type. Also, proportions of chirp types were sampled from all signals produced for each individual. Data presented are mean ± SE. NS, not significant.

Table 2. Comparison of current data to previous literature of chirp types of the mountain pine beetle (*Dendroctonus ponderosae*) within and between stress, male–female, and male–male chirp contexts.

	Stress		Male–female		Male–male	
Publication	Mean	Variability*	Mean	Variability*	Mean	Variability*
Tooth strike (no.)						
Michael and Rudinsky (1972)†	30.2	25–38	24.5	12–52	—	—
Ryker and Rudinsky (1976)‡	27.2	±1.0	41.0	±1.2	36.6	±1.1
Yandell (1984)§	24.7	±1.4	34.8	±1.7	28.6	±1.2
Current data (simple)‖	17.4	±1.9	21.3	±3.5	16.9	±2.3
Current data (interrupted)‖	27.9	±4.1	35.0	±3.6	22.2	±2.3
Tooth strike rate (no./s)						
Michael and Rudinsky (1972)†	840	550–990	238	113–480	—	—
Ryker and Rudinsky (1976)‡	1007	±35	407	±8	411	±8
Yandell (1984)§	960	±23.4	425	±11	626	±20.4
Current data (simple)‖	828.5	±59.9	786.0	±63.7	709.5	±40.2
Current data (interrupted)‖	593.2	±89.5	433.1	±18.8	464.0	±44.7
Chirp duration (ms)						
Michael and Rudinsky (1972)†	38.4	25.1–60.0	143	95–185	—	—
Ryker and Rudinsky (1976)‡	28.0	±1	154	±6.0	147	±6.0
Yandell (1984)§	27.0	±1.3	138	±6.7	59	±6.4
Current data (simple)‖	21.8	±1.8	30.0	±6.8	28.8	±6.4
Current data (interrupted)‖	56.3	±8.0	90.0	±6.5	56.1	±8.4

*For most literature standard error (SE) was used as the indicator of variability, except in the case of Michael and Rudinsky (1972) where range was given.

†Data taken from Michael and Rudinsky (1972) was for trials done at room temperature for consistency with recording procedures done in other literature.

‡Ryker and Rudinsky's (1976) "attraction" trial data was used in the male–female category for consistency with other literature.

§Only the data recorded from beetles taken from ponderosa pine (*Pinus ponderosa*) were used from Yandell's (1984) study for consistency with other literature.

‖Data analyzed from the first five simple or first five interrupted chirps, as in Table 1.

equal number of chirps with descending and bell-shaped envelopes, while two more produced predominately chirps with bell-shaped envelopes. Thus, there appears to be individual differences with respect to the shape of the chirps.

Male–female interactions

Video replays of trials were used to monitor which signals were associated with different stages of the encounter, and care was taken to include only signals made once the male came into contact with the female's frass and none produced as a result of handling stress. Males signaled consistently throughout the trials and females signaled intermittently (Figs. 5*a*–5*d*, 6*a*–6*h*). Putative female chirps were simple and distinguishable from simple chirps of males by their shorter duration, decreased number of tooth strikes, and longer interval between tooth strikes (Figs. 6*a*–6*h*), as previously reported by Ryker and Rudinsky (1976). Males signaled in all trials and all males produced both simple and interrupted chirps; however, the majority of chirps were interrupted (Table 1). Throughout the trials, males tended to decrease their production

Fig. 4. Simple stress chirps from a mountain pine beetle (*Dendroctonus ponderosae*) recorded with a microphone at 1 cm. (*a*) Oscillogram illustrating a series of 14 chirps. Black dots mark chirps expanded in *b*. (*b*) Four chirps from *a* shown at an expanded time scale and with a corresponding spectrogram. The black dot indicates the chirp expanded in *c*. (*c*) A single chirp from *b* showing individual tooth strikes. (*d*) Power spectrum of the chirp presented in *c*.

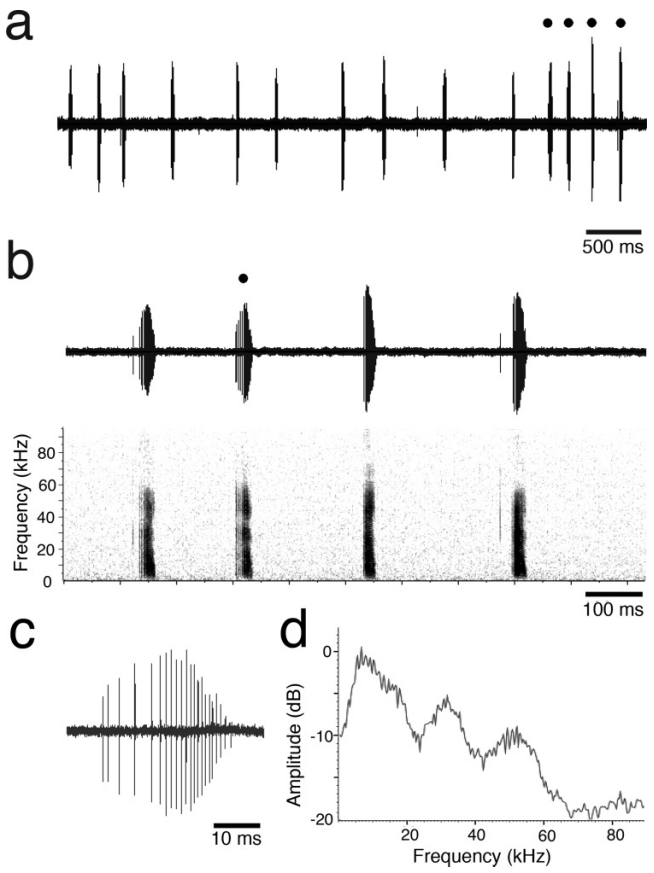
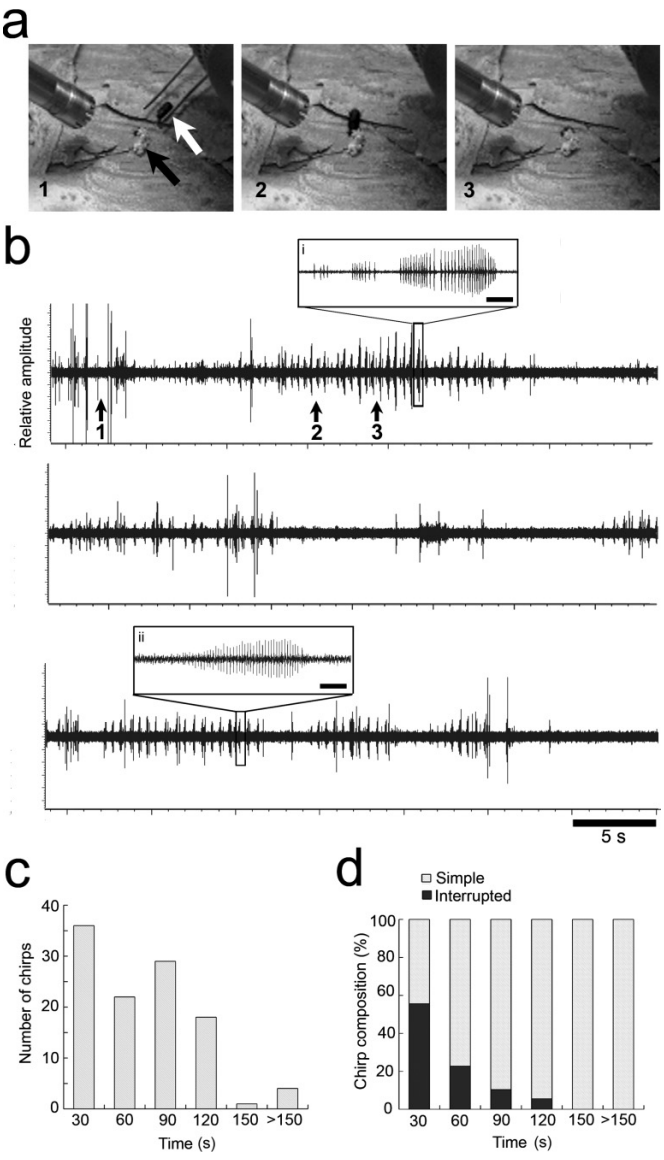


Fig. 5. A typical mountain pine beetle (*Dendroctonus ponderosae*) male–female trial. (*a*) A sequence of signaling showing bouts of chirping interrupted with bouts of silence. Top panel shows the position of the male (white arrow) with respect to the female's entrance hole indicated by the presence of frass and wood shavings (black arrow). Numbers correspond to those on the oscillogram in *b*. As the male enters the gallery, he produces predominantly interrupted chirps (shown in the first inset in *b*, scale bar 20 ms). Later in the trial, the chirps are predominantly simple (second inset in *b*, scale bar 20 ms). In this trial, signaling does not continue after ~2 min of chirping. (*c*) Number of chirps occurring during the course of the trial. (*d*) Type of chirps produced over the course of the trial.



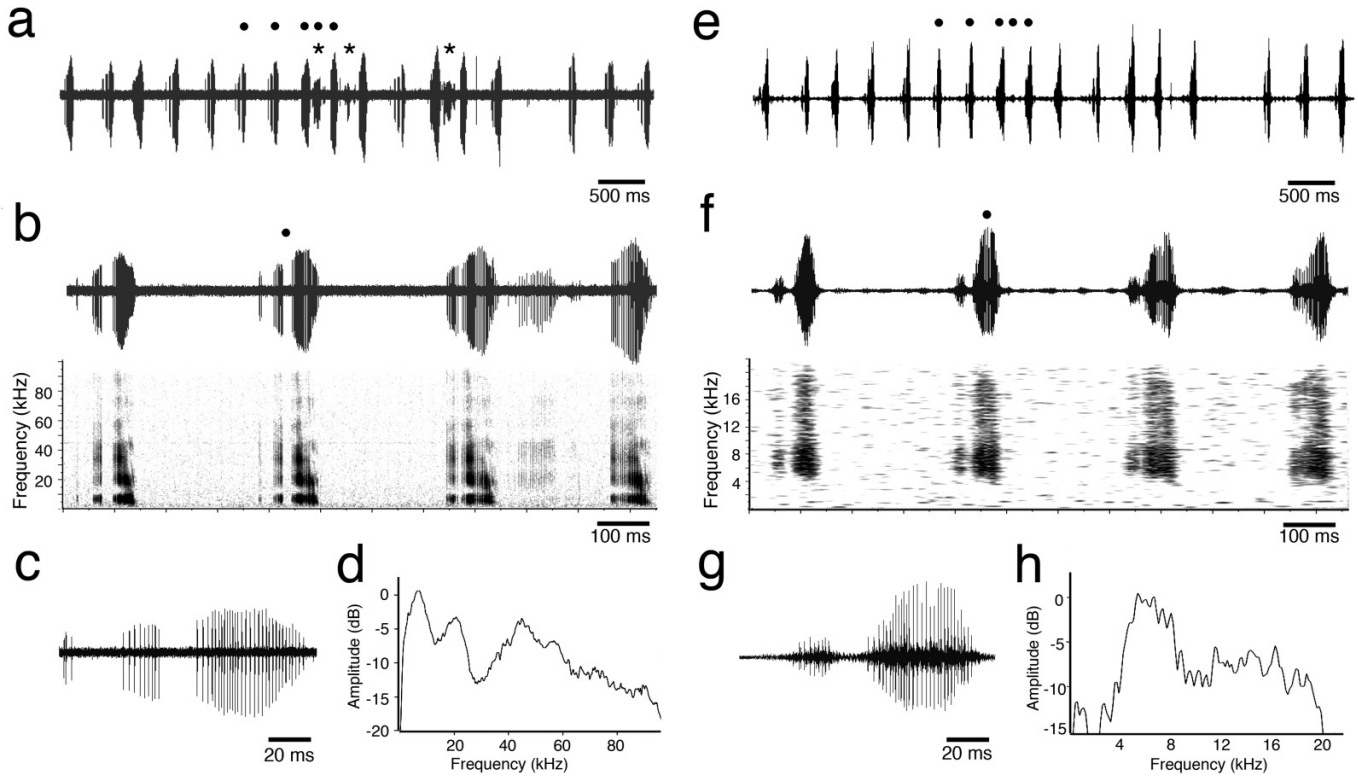
of interrupted chirps. Initially, as they approached and entered a female's entrance hole, the majority of chirps produced were interrupted (mean (±SE) number of interrupted chirps = 85.0% ± 7.1%); by 90 s into the trial, they had changed to producing almost an equal number of simple and interrupted chirps (mean (±SE) number of interrupted chirps = 59.2% ± 20.4%), although this difference between early and late proportions was not significant (t = –1.16, p = 0.33). Signaling effort decreased later as well, with individuals tending towards larger interchirp intervals at 90 s (mean (±SE) interchirp interval at 0 s: 0.477 ± 0.04 s; at 90 s: 0.625 ± 0.15 s; t = –0.99, p = 0.38) (Figs. 5*a*–5*d*).

Temporal and spectral characteristics were measured from male signals prior to his entering the entrance hole. Simple chirps were significantly shorter and had fewer tooth strikes than interrupted chirps (Table 1). Interrupted chirps had significantly lower tooth strike rates and longer intertooth strike intervals than did simple chirps (Table 1). As seen in the stress signals, chirps were broadband, with up to five peaks ranging from 6.5 to 75.0 kHz; however, dominant frequencies were marginally higher than seen in the stress condition and, unlike for stress chirps, here simple chirps had slightly higher dominant frequencies than interrupted chirps (Table 1). Again amplitude modulations were individualistic; approximately half of individuals produced signals exclusively with amplitude envelopes that were descending (57%), whereas 29% of individuals had amplitude envelopes that were bell-shaped. Only one individual produced a mix of envelope types in the chirps analyzed, the others were consistent with envelope patterning regardless of chirp type.

Male–male interactions

When two males interacted in a small arena, both began to stridulate (Figs. 7*a*–7*h*). It was not possible to distinguish between chirps of the two individuals, although qualitative observation frequently revealed signals of two different amplitudes and temporal patterns indicating one male responding to another (Figs. 7*a*–7*h*). In all 14 trials, simple chirps were recorded and these

Fig. 6. Sound and vibration signals recorded during a mountain beetle (*Dendroctonus ponderosae*) male–female interaction. (*a*–*d*) Sounds produced during a male–female interaction at 1 cm from the microphone. (*a*) Oscillogram illustrating a train of 17 interrupted chirps by the male. Three simple chirps of lower amplitude, putatively generated by the female, are marked with asterisks. Black dots mark five chirps that are expanded in *b*. (*b*) Five chirps from the train in *a* shown at an expanded time scale and with a corresponding spectrogram. Black dot marks an interrupted chirp expanded in *c*. (*c*) An interrupted chirp from *b* showing individual tooth strikes and three chirp components. (*d*) Power spectrum of the interrupted chirp shown in *c*. (*e*–*h*) Corresponding vibrations recorded on the phloem layer at 1 cm from the interacting individuals. Note that neither the first chirp component of the male interrupted chirp, nor the female chirp, is visible in the vibration recordings.



comprised 76% of all chirps (Table 1). No interrupted chirps were seen in 3 out of 14 trials. Simple chirps were significantly shorter than interrupted chirps and had a significantly higher tooth strike rate; they also tended to have fewer tooth strikes (Table 1). There was no difference in the intertooth strike intervals between interrupted and simple chirps (Table 1). Dominant frequencies ranged from 9 to 70 kHz with interrupted chirps having significantly higher dominant peaks than simple chirps (Table 1). As seen in the stress context, chirps with descending amplitude envelopes were the dominant type, with >85% of trials containing predominantly this envelope pattern.

Substrate-borne vibrations

Vibrations were recorded on the phloem layer during male–female and male–male interactions. When compared with sounds that were recorded simultaneously, it was observed that lower amplitude signals, such as the first component of interrupted chirps in males or female signals, were often not detected by our laser even at distances of 1 cm (see Figs. 6*a*–6*h*, 7*a*–7*h*). During male–female interactions, vibrations of simple chirps recorded at a distance of 1 cm had a dominant frequency of 4.9 kHz with up to three additional peaks of 8.7, 12.3, and 15.7 kHz. Interrupted chirps were similar in their spectral characteristics, with dominant frequencies at 4.6, 8.1, 12.0, and 14.2 kHz. During male–male interactions, vibrations were recorded on the phloem layer at distances of 1 and 3 cm. At 1 cm, simple chirps had a dominant frequency of 6.7 kHz with two additional peaks at 11.9 and 17.8 kHz. Interrupted chirps were similar, with peaks at 7.1, 12.9, and 17.9 kHz. Vibrations were low amplitude, with a velocity of 2.7 ± 0.9 mm/s (mean ± SE) measured at 1 cm.

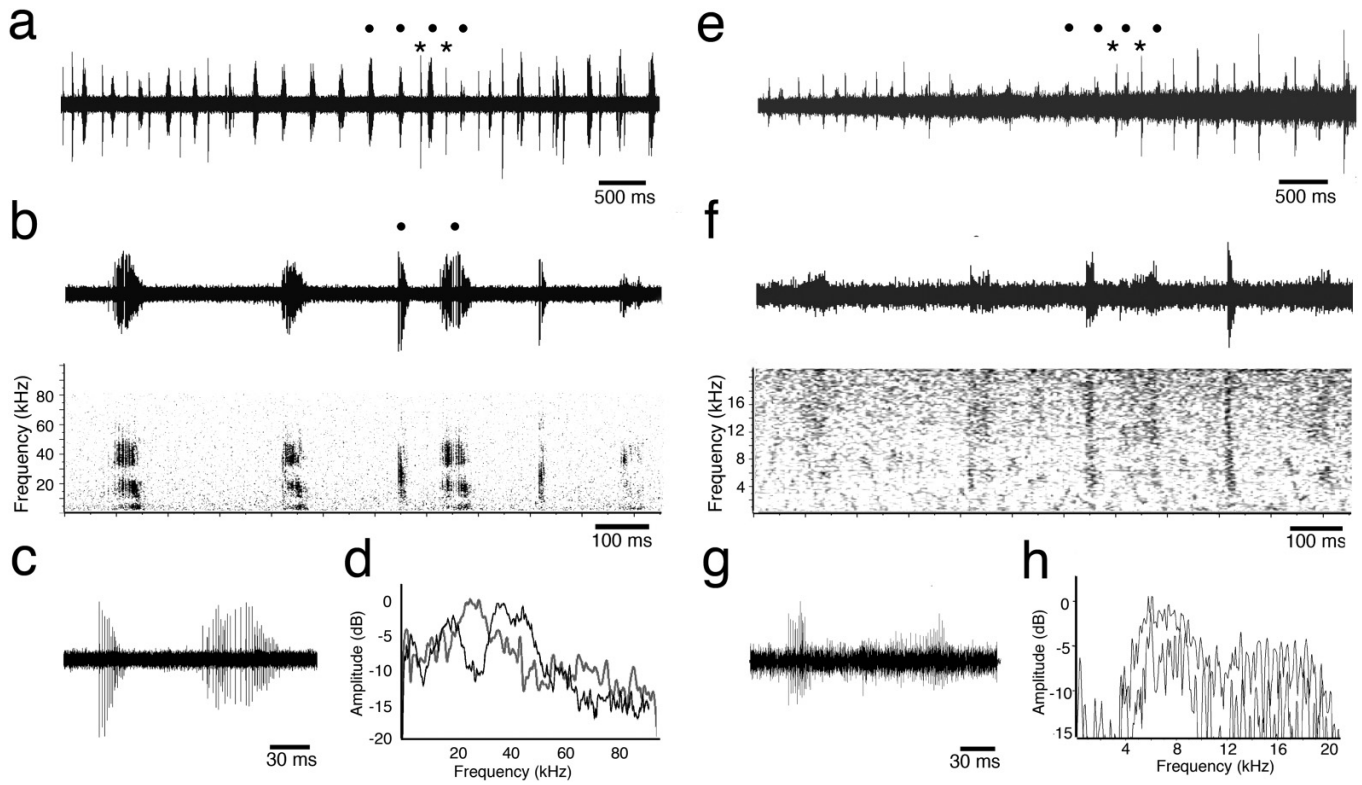
Discussion

Although the chemical ecology of bark beetles has been studied extensively (e.g., Byers 1989), research on acoustic communication has lagged behind and basic questions concerning the function of signals and receptor mechanisms remain largely unanswered. We discuss how characterizing the physical properties of signals is an important first step towards answering proximate and ultimate questions about bark beetle acoustics, compare our results with previous studies, and propose new directions for research.

Stress signals

Chirps were consistently evoked when male *D. ponderosae* were held by the pronotum. This response to a general disturbance is referred to as the "stress" chirp and has been previously reported for male *D. ponderosae* (e.g., Michael and Rudinsky 1972; Ryker and Rudinsky 1976; Yandell 1984) (Table 2). Our results demonstrate that stress chirps are predominantly (but not entirely) simple. Previous studies describe stress chirps as being simple; however, it is difficult to determine whether interrupted chirps were completely absent from stress chirp trains in these studies, or were just not included in the analyses, because details on how chirps were sampled are lacking. Our values for tooth strike rate and chirp duration of simple stress chirps are in general agreement with those previously reported for stress chirps in other studies, but we report a lower mean number of tooth strikes (Table 2). We

Fig. 7. Sound and vibration signals recorded during a mountain pine beetle (*Dendroctonus ponderosae*) male–male interaction. (*a*–*d*) Sounds recorded 1 cm from the microphone. (*a*) Oscillogram illustrating a series of simple chirps being produced by (purportedly) two males. Black dots indicate signals produced by one male and asterisks represent the second male. (*b*) Six chirps from the train in *a* shown at an expanded time scale, with a corresponding spectrogram. (*c*) Two simple chirps from *b* showing individual tooth strikes. (*d*) Spectra of the two chirps representing the two males shown in *c*. (*e*–*h*) The corresponding vibrations recorded on the phloem layer at 1 cm from the interacting individuals.



have also characterized for the first time the spectral and amplitude properties of these signals in *D. ponderosae*, showing that they are broadband signals with energy extending across sonic and ultrasonic ranges, and that they attenuate rapidly with distance and are therefore likely utilized for close-range communication.

Although stress signals are often assumed to function as a deterrent against predators (Barr 1969; Ryker 1988), this hypothesis remains untested for the most part in any bark beetle species. One prediction is that signals are evoked by disturbance, which is supported in our study. A second prediction is that the signals will have attributes characteristic of antipredator acoustic signals in other insects. Insect disturbance sounds targeted at predators typically have simple temporal patterns, comprising short bursts of tooth strikes (~80 ms long) produced at a rate of about 5–10 sound bursts (i.e., chirps) per second, are broadband and are not loud (between 10 and 60 dB SPL at 10 cm) (Masters 1980). Masters (1980) also notes that temporal patterns are irregular, and this variability is attributed to the insect struggling while it is making the sounds. The stress signals of *D. ponderosae* reported in this study share many of these attributes, suggesting that these signals could target a broad range of predators at close range. A third prediction is that stridulating beetles will escape a predator more readily than would a non-stridulating beetle. Lewis and Cane (1990) found that clerid beetle predators dropped six-spined engraver (*Ips calligraphus* (Germar, 1824)) females significantly more frequently than they dropped naturally mute males, and 91% of drops occurred while the female stridulated vigorously. However, this finding was not supported in a similar study in pine engravers (*Ips pini* (Say, 1826)) (Sivalinghem 2011). An alternative hypothesis is that stress chirps may be directed at conspecifics to recruit help from mates, or as an alarm. Future studies should involve experiments with a variety of live predators or conspecifics under natural conditions to test hypotheses regarding the function of stress signals.

Male–female interactions

What is the function of signaling during male–female encounters in *D. ponderosae*? In bark beetles in general, the founding sex attracts the opposite sex with pheromones, and once the nonfounding sex arrives at the gallery entrance, it begins to stridulate (see also supplementary video).[1] In *Dendroctonus* spp., it has been shown that these early signals comprise a high proportion of interrupted chirps and have been broadly termed "attraction" signals (see Ryker and Rudinsky 1976). Studies of these signals show that they induce the female to cease production of aggregation pheromones (e.g., Rudinsky and Michael 1972). Furthermore, there is evidence that in the absence of stridulation, the male is often refused entry to the gallery (Ryker and Rudinsky 1976). However, this could indicate many different potential functions of the act of stridulation in males, and researchers have variously said these "pre-entry" signals function to "announce the arrival of the stridulating sex" (Barr 1969), in species recognition (e.g., Yandell 1984), in aggression towards the female (e.g., Ryker and Rudinsky 1976), and in "premating recognition" (e.g., Ryker and Rudinsky 1976). Yet another possibility is that males signal at the gallery entrance to indicate to the female that he is not a predator, as predatory beetles from the family Cleridae have been shown to orient towards bark beetle aggregation pheromones (Raffa and Dahlsten 1995). Regardless, these early signals do not appear to function in attraction per se and should therefore not be called attraction signals. Similarly, signals that occur following entry have been called courtship signals in *D. ponderosae*, and have been

reported as a change from a train of interrupted chirps during the early signaling period pre-entry to simple chirps. However, there is little evidence to suggest that they function in courtship because they are reported to continue after copulation (Ryker and Rudinsky 1976). Therefore, although we also observed a switch to a lower proportion of interrupted chirps following entry to the gallery, we refrain from using the terminology "attractant" and "courtship" unless we are referring to previous literature. To clarify the function of these signals, future studies should attempt to simultaneously record behavioural interactions and signals over the long term. Such recordings could be achieved using phloem sandwiches (Kinn and Miller 1981), where beetles construct galleries in phloem tissue between glass plates. In addition, the variability seen in envelope patterns, spectral properties, and interspike intervals should be evaluated to explore the adaptive significance of signal variation, especially because each of these properties has implications for honest signaling and female choice.

Comparing our results with previously studies, we found a similar number of tooth strikes per chirp, with our interrupted chirps being most like previously reported values. This is interesting because most interrupted chirps are produced early on during male–female interactions, and the number of tooth strikes has been suggested to reflect species recognition during mating (Yandell 1984). Second, our animals produced chirps of much shorter duration than seen previously (the interrupted chirps from the current study, which were the longest, were 90 ms, on average, compared with previously reported durations of 138–154 ms). Third, our animals show a large difference in tooth strike rate between simple and interrupted chirps, and only the values for interrupted chirps (433/s) are similar to the values reported previously for attractant chirps (238–425/s), indicating that previous studies excluded simple chirps from their analyses. These differences could be attributable to a number of things, including differences in sample sizes, how the chirps were sampled and selected from the train, temperatures at which recordings were made, or indeed, they may reflect differences in populations. Regardless, an important point that we can take home from this is that to make meaningful within- and between-population comparisons, future studies must be careful to define how signals were sampled and categorized.

Male–male interactions

In our study, when two males were confined they produced both simple and interrupted chirps, with a higher proportion of simple chirps. This finding was similar to results reported by Yandell (1984), who noted both interrupted and simple chirps when recording from rival males as they interacted in the presence of female frass, but contrary to Ryker and Rudinsky (1976), who only reported interrupted chirps when two rival males were confined in troughs either with or without the presence of female frass. There is also a large amount of variability in the three temporal properties reported (number of tooth strikes, tooth strike rate, and chirp duration) among the three previous studies (Table 2). This wide variation may be due to the different experimental setup for male–male trials in each study—differences existed in terms of the presence or absence of female frass, whether the interactions took place in an enclosed trough or near a female entrance hole, and whether or not one of the two males was silenced. It may also be that wide variations in signals naturally exist. This would be the case if male–male signals have a function that is fulfilled simply by producing acoustic emissions in the correct behavioural context, and whose temporal and spectral characteristics are therefore not under stabilizing selection, as suggested by Yandell (1984). Again, future studies should explain specifically how chirps were sampled, and under what experimental conditions, to compare within and between populations.

The functional significance of rivalry in males must be examined more carefully. Previous studies and ours show that males do respond to one another regardless of whether they are in the presence of a female. However, it is unclear how this "rivalry" relates to males interacting under natural situations. McGhehey (1968) argues that in *D. ponderosae*, first males that have been introduced to a gallery the previous day will respond to a second male by blocking the gallery entrance and stridulating, and that this functions to warn the second male that the gallery is already occupied. Signals were not recorded during that study, and it would be interesting to repeat a similar experiment while recording signals to test hypotheses regarding the function of these male–male interactions. In our study, we noted that males appear to call back and forth to one another, and this is reminiscent of antiphonal calling between two rival males in other animals (e.g., Greenfield and Minckley 1993). The significance of male–male acoustic interactions could be further analyzed by studying the signals produced under natural conditions and by employing playback and ablation studies to test hypotheses on the function of these signals.

Sound or vibratory communication?

Coleoptera possess a diversity of stridulatory structures, and it is assumed that many species use these signals for conspecific communication (Wessel 2006). Yet at present, very little is known about sound or vibration reception in beetles. Tympanal ears have been identified in the tiger beetle (genus *Cicindela* L., 1758) (Cicindelidae) (Spangler 1988; Yager and Spangler 1995) on the tergum of the first abdominal segment, and in a few scarabs (Scarabidae, Dynastinae) on the cervical membrane behind the head (Forrest et al. 1997). In both, hearing organs are thought to function in bat detection, and in tiger beetles, possibly also in conspecific communication (Yager and Spangler 1995). There is no evidence to the best of our knowledge for hearing or vibration receptors in all other beetle families.

In bark beetles, morphological or physiological evidence for acoustic sensory organs is currently lacking. However, there is some behavioural evidence that beetles respond to acoustic signals. For example, Rudinsky et al. (1973) showed that females of the Douglas-fir beetle (*Dendroctonus pseudotsugae* Hopkins, 1905) responded to male "attractant" chirps by releasing pheromone. Sounds were played back through a piezoelectric ceramic disk pressed to a silicon rubber gasket over an opening in a glass chamber facing the screened ends of vials. Although these airborne sounds could have stimulated vibration receptors by vibrating the walls of the vial, it is also possible that the females were picking up the airborne sounds. In other experiments, it is evident that females are responding to the acoustic signals of males, as silenced males are not allowed entry (e.g., Ryker and Rudinsky 1976 for *D. ponderosae*). Again, it is difficult to know if these signals are transmitted through the air, because the male is often in direct contact with the female as he is entering the gallery. There is also some indirect behavioural evidence that bark beetles are communicating through solid substrates, such as a provocation of simple chirps in female *Dendroctonus* spp. by other females boring nearby in the phloem layer (Rudinsky and Michael 1973), or through direct contact, such as the observation that male *D. ponderosae* will push and nudge the female before, and stroke her after, entry into the gallery (Ryker and Rudinsky 1976). Our study of signal characteristics in *D. ponderosae* shows that both airborne and substrate-borne vibrations are available to conspecifics at the distances they would normally be interacting, and these close signals could be detected using tympanal ears, near-field sound detectors (e.g., Johnston's organs), or vibration receptors (e.g., subgenual organs) (reviewed in Yack 2004). It should be noted that the poor signal quality of substrate-borne vibrations compared with airborne sounds, could be due to attenuation through the wood, but could also be attributable to our recording method (i.e., using a laser). We recommend that future studies using different types of vibration sensors (see Cocroft and Rodríguez 2005) should be con-

ducted to further explore the characteristics of substrate-borne vibrations.

Conclusions

We have demonstrated that *D. ponderosae* generates simple and interrupted chirps that vary in their physical properties between behavioural conditions, and that signals are transmitted at close range as sonic and ultrasonic signals through the air and as vibrations through the phloem layer of pine trees. The results of this study on the characterization of the acoustic signals lay the groundwork for further research on the potential receptor mechanisms. The capacity to detect ultrasound in a phloeophagous beetle such as *D. ponderosae* could be instrumental not only in social interactions, but also in finding host plants, as drought-stressed trees have been shown to produce ultrasonic emissions as a result of cavitations of the xylem (Mattson and Haack 1987; Haack et al. 1988). Future studies should focus on standardizing nomenclature and sampling parameters for collecting acoustic signals to better understand how signals vary between individuals, behaviours, and populations. Moreover, experimental manipulation of signals should be conducted to determine whether signals provide information about signaler fitness. Also, neuroanatomical and neurophysiological, as well as playback studies, should be incorporated to gain insight into the unexplored terrain of the bark beetle acoustic sensory system.

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