

# Acoustic communication in the pine engraver bark beetle: do signals vary between behavioural contexts?

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**Abstract.** Acoustic communication is taxonomically widespread in bark beetles and is proposed to play an important role in a variety of social and defensive behavioural contexts. Yet our understanding of how signals vary between contexts is currently limited. The present study tests the hypothesis that acoustic signals vary between behavioural contexts in the female pine engraver beetle *Ips pini* (Say) (Coleoptera: Curculionidae: Scolytinae). Female *Ips pini* produce acoustic chirps using a vertex-pronotal stridulatory organ. Randomly sampled chirps generated under three contexts (i.e. distress, predation and premating) are compared for their duration, number of pulses, interpulse intervals, pulse rate and amplitude envelope shapes. The results obtained show that, during premating events, chirps are significantly longer in duration and tend to have a higher proportion of descending amplitude envelopes than chirps occurring during distress and predation events. Chirps produced during distress and predation conditions are indistinguishable from one another. By contrast to the results from previous bark beetle studies, no support is found for categorizing chirps as ‘interrupted’ or ‘uninterrupted’ types based on temporal patterns. The functional significance of context-dependent variation in chirp characteristics is discussed. Previous studies on acoustic communication in bark beetles are limited as a result of a general lack of objective sampling and measurement criteria for characterizing signals. Recommendations are outlined for future studies on the functions and evolution of acoustic communication in bark beetles.

**Key words.** Acoustic, amplitude envelope, behaviour, chirp, Coleoptera, distress, envelope shape analysis, *Ips pini*, signalling.

## Introduction

Acoustic communication by airborne sounds and solid-borne vibrations is widespread amongst insects (Dumortier, 1963; Greenfield, 2002; Cocroft & Rodríguez, 2005). Signals are associated with most aspects of insect life history, including but not limited to attracting and choosing mates, locating food, and defence against conspecifics or predators (Alexander, 1967; Ewing, 1989; Greenfield, 2002; Yack, 2016). There is extensive research on how signal characteristics vary between and within species, as well as the factors leading to this variation

(Alexander, 1961; Gerhardt & Huber, 2002; Greenfield, 2002; Sueur, 2005). Between species, signal variation is studied mostly on calling songs in relation to species recognition and female choice (Gerhardt & Huber, 2002; Greenfield, 2002, 2016; Boulard, 2005; Heller, 2005; Henry, 2005; Hoikkala, 2005; Sueur, 2005; Stewart & Sandberg, 2005). Within species, studies on signal variation focus on the calling and courtship songs that communicate information about the condition of signallers (Greenfield, 2002; Tregenza *et al.*, 2006). Within species, acoustic signals can also vary between behavioural contexts.

Many insects are reported to signal in a variety of behavioural contexts, including various stages of mating, aggressive or other social interactions (Alexander, 1961; Gerhardt & Huber, 2002; Stölting *et al.*, 2002; Guerra & Mason, 2005; Conrad *et al.*, 2010; Balakrishnan, 2016). Empirical studies characterizing variation

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between signals produced in different contexts are lacking for most insects, although examples are available for cicadas (Sueur & Aubin, 2004), crickets, (Zuk *et al.*, 2008) and *Drosophila* (Ritchie *et al.*, 1998; Hoikkala, 2005). Understanding the signalling repertoires of a species provides important information about the communicative functions of different signal traits and allows us to develop hypotheses on the function and evolution of communication signals. In the present study, an objective and quantitative approach is taken to sample and characterize context-dependent signals of bark beetles (Scolytinae).

Bark beetles (Coleoptera: Curculionidae: Scolytinae) are ecologically and economically important insects that play a key role in forest ecosystems, promoting carbon and nutrient recycling (Vega & Hofstetter, 2014). Some aggressive species, however, can be devastating parasites, causing billions of dollars in damage to the global forest industry (Vega & Hofstetter, 2014). As a result of their importance, extensive research focuses on their sensory ecology and life-history traits (Vega & Hofstetter, 2014). Most of this research is on chemical communication, leading to improvements in our understanding of their biology, as well as pest control methods (Coulson, 1979; Raffa, 2001; Raffa *et al.*, 2008). Another key form of communication for these insects is acoustic communication, although this sensory modality has received comparatively little attention.

Acoustic signalling in bark beetles is taxonomically widespread and is proposed to play important roles in many aspects of life history (Barr, 1969; Lyal & King, 1996). Many species are reported to signal in more than one behavioural context, although whether chirps vary between contexts is poorly understood as a result of a lack of quantitative analysis of signal characteristics for most species (Fleming *et al.*, 2013; Lindeman & Yack, 2015; Yturralde & Hofstetter, 2015). Several studies refer to different signal types, such as 'attraction', 'pre mating', 'rivalry' or 'distress' chirps, although whether chirp characteristics differ between contexts requires verification (Barr, 1969; Ryker & Rudinsky, 1976; Oester *et al.*, 1978; Ryker, 1988). Other studies refer to two types of chirps: simple and interrupted in different contexts but, yet again, the distinction between these two lacks quantification (Michael & Rudinsky, 1972; Ryker & Rudinsky, 1976). Understanding how signals vary between contexts can be further complicated when signals are recorded under artificial conditions that may not represent natural conditions (Wilkinson *et al.*, 1967; Swaby & Rudinsky, 1976; Yturralde & Hofstetter, 2015). The present study takes an empirical approach to sampling, analyzing and comparing characteristics of chirps in different behavioural contexts using the pine engraver beetle, *Ips pini* (Say) as a model.

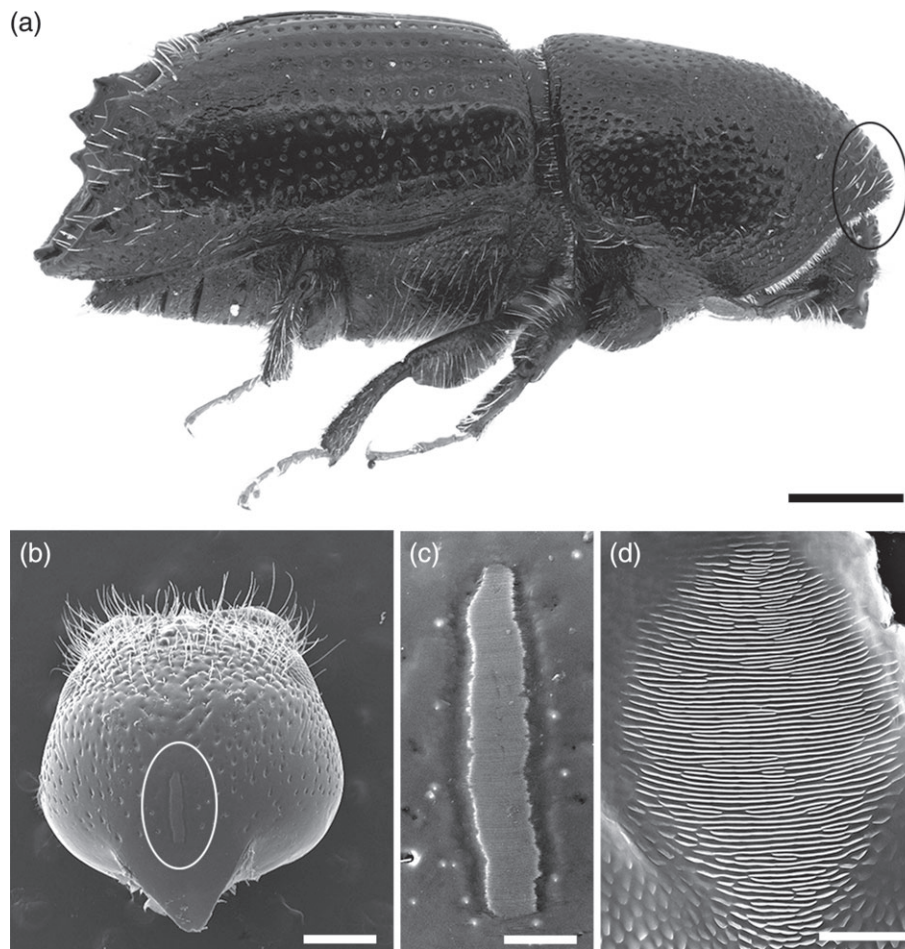
*Ips pini* occurs across North America, breeding beneath the bark of various pine and spruce trees (Thomas, 1961; Barr, 1969). Colonization is initiated by males by attacking damaged or weakened trees, excavating the initial nuptial chamber and releasing pheromones to attract both females and other males to the tree (Pureswaran *et al.*, 2000). As is described for most *Ips* species, *I. pini* have a polygynous mating system (Thomas, 1961; Schmitz, 1972; Kirkendall, 1983; Reid & Roitberg, 1994) in which harems are formed by males by attracting two or three females to the entrance of the nuptial chamber (Swaby & Rudinsky, 1976). As the females arrive, entry to the chamber

is usually blocked by the male. Copulation occurs subsequent to successful entry, after which egg galleries are excavated by the females. The galleries remain occupied by both the male and the females until the end of the egg-laying process (Reid & Roitberg, 1994; Robertson, 1998). Studies of the chemical sensory ecology of *Ips* spp. are reported in detail (Teale *et al.*, 1991; Seybold *et al.*, 1992; Miller *et al.*, 1996; Robins & Reid, 1997; Robertson & Roitberg, 1998; Pureswaran *et al.*, 2000; Salle & Raffa, 2007). Although acoustic communication is reported for several species, comparatively less is known about this sensory modality.

Sound production is reported for both male and female *I. pini* (Oester & Rudinsky, 1975; Swaby & Rudinsky, 1976); however, only females are described to possess stridulatory organs (Barr, 1969). *Ips pini* possesses a vertex-pronotal type of stridulatory organ comprising the pars stridens and the plectrum, both consisting of a series of ridges or 'teeth'. The pars stridens is located on the dorsal surface of the head, with the plectrum near the anterior end of the undersurface of the pronotum (Barr, 1969) (Fig. 1). The pars stridens is considered to represent the more complex of the two structures and to be the primary resonating structure involved in sound production (Barr, 1969; Swaby & Rudinsky, 1976). The plectrum is simpler with fewer and less organized ridges (Swaby & Rudinsky, 1976). By rubbing the pars stridens against the plectrum through a nodding motion of the head, chirps are produced (Barr, 1969; Swaby & Rudinsky, 1976).

*Ips pini* females are reported to signal acoustically in different behavioural contexts: during distress (handling), attraction (female introduced to male) and 'rivalry' (multiple females in an egg gallery) (Swaby & Rudinsky, 1976). Swaby & Rudinsky (1976) report that certain temporal signal characteristics are context-dependent. However, a lack of objective criteria for quantifying signal characteristics, including how chirps are defined, what criteria are used to define 'interruptions' and how chirps are sampled for analysis, renders the results inconclusive and precludes validation or comparison with other studies. It is also important to note that *I. pini* 'stress' chirps, similar to those for many other bark beetle studies, are elicited under artificial conditions, and therefore characteristics may not reflect those of chirps produced under natural stressful situations, such as when being attacked by a predator. The assessment of context-dependent signal variation requires objective quantification of signal traits to replace the more arbitrary analysis methods used in previous studies.

The purpose of the present study is to test the hypothesis that acoustic signal characteristics in *I. pini* are context-dependent. Objective methods are used to sample chirps between three contexts (distress, predation and pre mating) and to compare their temporal characteristics: duration, number of pulses and average interpulse interval (IPI). The traditional handling method is used to simulate artificial stress in distress trials, as well as predation by natural predator, aiming to assess differences between sound characteristics in artificial and natural conditions. In addition to these temporal characteristics that are commonly used to characterize acoustic signals in insects, to the best of our knowledge, the present study is also the first to quantitatively analyze the amplitude envelope shape of chirps using curve-fitting of



**Fig. 1.** Vertex-pronotal stridulatory organ of female *Ips pini*. (a) Lateral view of the beetle showing the general region of the stridulatory organ (circled region). (b) Dorsal view of the head with the rest of the body removed and the pars stridens highlighted. (c) Higher magnification of the pars stridens on the vertex. (d) Plectrum shown on the ventral side of the anterior pronotum. Scale bars: (a) 500  $\mu\text{m}$ , (b) 250  $\mu\text{m}$ , (c, d) 50  $\mu\text{m}$ .

nonlinear regressions. The study also includes a comparison of the findings with those of previous studies, as well as recommendations for future research on bark beetle acoustic analysis.

## Materials and methods

### Animals

*Ips pini* were collected at Herbert's Corner (Carleton University Forest, Ottawa, ON, Canada) and the Central Experimental Farm Arboretum (Ministry of Agriculture and Agri-Food Canada (AAFC), Ottawa, ON, Canada) between April and September during 2011 and 2014. Adult beetles were collected using Lindgren traps (Contech Enterprises Inc., Canada) baited with ipsdienol and lanierone. Traps were hung on red pine (*Pinus resinosa*), white pine (*Pinus strobes*) and jack pine (*Pinus banksiana*) trees. Males were distinguished from females by the presence of an enlarged third declivity spine (Wood, 1982). Separated males and females were stored in plastic containers with moist paper towels and phloem shavings and kept at 5–8 °C

for a maximum of 2 weeks until used in one of the procedures described below. Voucher specimens were preserved in 90% ethanol and stored at Carleton University.

Checked beetles *Thanasimus dubius* (Coleoptera: Cleridae: *Thanasimus*), common predators of *I. pini* (Aukema & Raffa, 2004), were also collected from the above mentioned traps. Individuals were placed in separate plastic containers covered with moist paper towels and kept at 5–8 °C as above until later use during the predation trials.

### Morphology

To image the sound-producing structures in females, beetles were prepared for scanning electron microscopy by separating the pronotum and head from the rest of the body using an insect pin. Specimens were mounted on aluminum stubs and double-coated with gold–palladium (Hummer VII SEM Sputtering System, Anatech Ltd, Alexandria, Virginia) prior to imaging using a variable pressure scanning electron microscope (Tescan Vega II XMU; Czech Republic).

### Signalling contexts

Sounds were recorded under three different contexts (i.e. distress, predation and premating) using a customized condenser microphone (model CMPA-P48/CM16; Avisoft, Germany) and stored as .wav files to a data recorder (model FR-2; Fostex, Boonton, New Jersey; sampling rate: 192 kHz). All trials were conducted in a sound-attenuated chamber (model C-14A MR; Eckel Industries of Canada, Canada) at temperatures of 20–22 °C.

**Distress.** To assess any differences between signals produced during natural predation (see below) and artificial stress conditions, females were stimulated to signal in accordance with a procedure used to elicit distress signals in other *Ips* spp. (Swaby & Rudinsky, 1976) and bark beetle studies (McCambridge, 1962). This condition is referred to in the present study as distress. Each female was held by the abdomen and sounds were recorded 1 cm from an Avisoft condenser microphone (Fig. 2a). Distress signals were recorded for 26 females.

**Predation.** Predation trials were used to represent natural stress conditions. These trials were conducted by placing an individual *I. pini* female in a glass Petri dish with a checkered beetle that had been food deprived for 48 h (Fig. 2b). Acoustic signals produced during attacks were recorded with an Avisoft condenser microphone placed approximately 2 cm above the beetle, and signals were stored as .wav files to a Fostex data recorder. All predation trials were conducted in a sound attenuated chamber and videotaped (Handycam HDRHC5/HC7; Sony Corp., Beverly Hills, California). Predation trials were conducted on 19 female *I. pini*. Predators were food deprived for 48 h prior to the trial. Multiple predators were used but, in a few cases, the same predator was used for different trials.

**Premating.** Premating trials were conducted on red pine log bolts (40–50 cm in length) sealed on each end with paraffin wax to prevent dehydration. Prior to conducting premating trials, each log was inoculated with four to 15 males. Individual males were placed in drilled holes (diameter ~0.5 cm), spaced 8–10 cm apart. A 1.5-mL microcentrifuge tube (with the conical base cut off) was placed over the hole and sealed with reusable adhesive putty (Staples Canada Inc., Canada) to prevent escape. All males were given 48 h to build nuptial chambers prior to introducing females. Inoculated logs were kept at room temperature (22–24 °C) in an insect rearing facility.

On the day of recording, an inoculated log was transported to a sound attenuated chamber, and a female was introduced near the entrance hole. An Avisoft microphone was placed 2 cm from the hole (Fig. 2c) and all recorded signals were stored using a Fostex data recorder. These trials were recorded for up to 6.5 min of the encounter, after which time signalling subsided. Premating trials were conducted on 19 females, all paired with different males.

### Sound analysis

Up to 10 chirps per female were analyzed for each behavioural context. Because, currently, there is currently no generally accepted quantitative definition of a chirp for bark beetles, it was defined as ‘the shortest sound which appears unitary to the human observer’s unaided ear’ (*sensu* Broughton, 1963). The beginning and end points of chirps were defined using the RAVEN PRO, version 1.4 beta (Cornell Laboratory of Ornithology, Ithaca, New York) as the first and last pulse distinguishable from the background. Chirps were sampled by generating random times in EXCEL (Microsoft Corp., Redmond, Washington) and selecting the chirps within the sound file closest to those times.

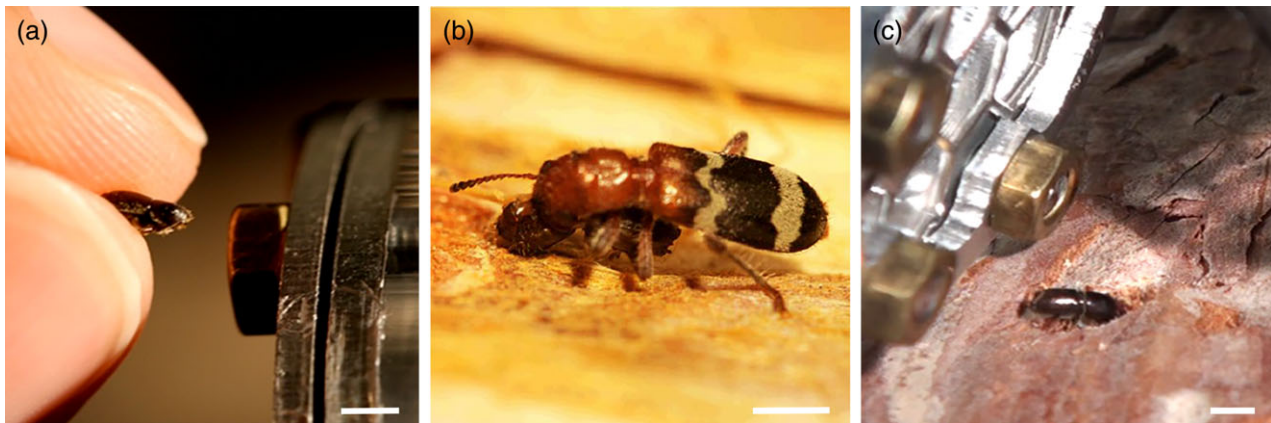
Each chirp was analyzed for specific temporal and amplitude characteristics. Spectral characteristics were not analyzed in the present study as a result of the different sound environments in which beetles were recorded. Temporal characteristics measured included: chirp duration, number of pulses and IPI (Fig. 3). Pulse rate was calculated using the chirp duration and the number of pulses. These characteristics were measured with Avisoft Bioacoustics Sound Analysis and Synthesis Laboratory program (AVISOFT-SAS LAB PRO, version 4.53; Avisoft).

A quantitative method was developed to analyze the proportions of different amplitude envelope shapes of chirps. This required quantification of both the envelope shape and the different shape types. The amplitude envelope was obtained for each chirp as amplitude versus time curves using the amplitude and time values of each pulse within the chirps. Three basic shape types were identified: ‘bell-shaped’, for chirps that have the highest amplitude in the middle portion; ‘ascending’, for chirps with pulses that gradually increase in amplitude towards the end; and, finally, ‘descending’, which is the opposite, where pulses generally decreased in amplitude (see Results). A sample of 80 chirps (separate from the sample used for the three behavioural conditions) was gathered by two observers and for identification of the best representatives of the three shape types (24–30 for each shape). The amplitude envelopes of these chirps were then used in TABLE CURVE 2D (Systat Software Inc., San Jose, California) software to generate model curves for the three types. Finally, these functions were fitted on the original sample of chirps for the different behavioural contexts using the same software (TABLE CURVE 2D) to sort the amplitude envelope shape of the chirps into categories.

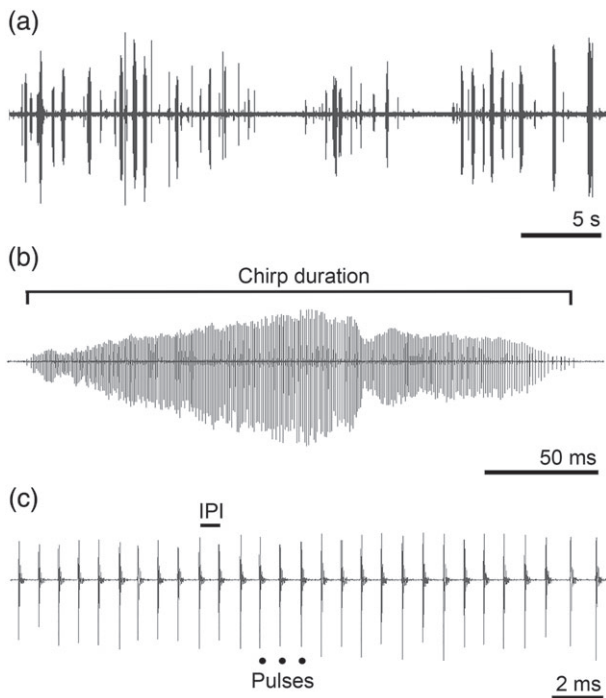
### Statistical analysis

Previous studies were able to distinguish two types of chirps in *Dendroctonus* species based on regular interruptions of the same duration: simple (without any interruptions) and interrupted (Lindeman & Yack, 2015). Interruptions within chirps were also claimed to hold importance for *I. pini* (Swaby & Rudinsky, 1976). In the present study, it was predicted that, if there were distinct chirp types based on temporal pulse patterns (i.e. simple or interrupted), the frequency distribution of interpulse interval values collected from all chirps would be binomial: the first peak being the average IPI and the second peak would be a result of the regular interruptions of similar length in a high number of





**Fig. 2.** Experimental set-up for the three behavioural contexts. (a) Distress: *Ips pini* females were held between the fingers with the head facing the microphone. (b) Predation: beetles were paired with natural predator (*Thanasimus dubius*) in a Petri dish. (c) Premating: females were placed near the entrance of a nuptial chamber with a male inside. Scale bars: (a–c) 2 mm. [Colour figure can be viewed at [wileyonlinelibrary.com](http://wileyonlinelibrary.com)].



**Fig. 3.** Temporal measurements of female *Ips pini* chirps. (a) Chirp train recorded during distress (handling). (b) Single chirp with measurement of chirp duration shown. (c) Individual pulses within a chirp with measurement of interpulse interval (IPI) shown.

chirps. The frequency distribution of IPI values derived from chirps selected from all contexts was recorded and subjected to curve-fitting to recognize the model of frequency distribution (TABLECURVE 2D); the frequency distribution models were selected based on parsimony, high  $F$ -values (and mean squares) and steep increases in  $r^2$  with model complexity.

Assessment of differences in temporal characteristics was performed by first averaging all parameters (duration, number

of pulses and average IPI per chirp) for each individual. These averages were used for statistical analysis. A canonical variate analysis (CVA) was performed to determine whether significant differences are present between contexts (PROC CANDISC; SAS Institute, Cary, North Carolina). Individual analysis of variance (ANOVA) and Fisher's least significant difference test ( $P < 0.05$ ) per parameter were used to determine which parameter differs significantly (PROC GLM; SAS). Normality and homoscedasticity assumptions were checked before CVA and ANOVA and no data transformation was necessary (PROC UNIVARIATE; SAS).

The shapes of amplitude envelopes per time were subjected to regression analyses again using the curve-fitting procedure of TABLECURVE 2D and by selecting the most suitable models based on the same criteria used to recognize the models for frequency distribution of IPI. A chi-squared contingency table was run to determine whether the proportion of each envelope shape was independent from the context (PROC FREQ; SAS). A Kruskal–Wallis test was subsequently used to analyze differences between contexts regarding envelope shape proportions (PROC NPARIWAY; SAS).

## Results

### General chirp characteristics and types

Females produced chirp trains (Fig. 3a) in all three behavioural contexts tested. Chirps consisted of a pulse-train (Fig. 3b). All chirps sampled under all three contexts were first analyzed together to assess the range of chirp characteristics, as well as to determine whether there is quantitative evidence for dividing chirps into simple and interrupted categories. In total, 588 chirps from 64 females were analyzed. There was a wide range in the temporal characteristics across all chirps (Table 1). To assess whether chirps could be categorized based on regular interruptions, a frequency distribution analysis of IPIs was performed to determine whether interruptions of similar duration were regularly occurring across all chirps sampled (Fig. 4a)

or in at least one of the behavioural contexts (Fig. 4b–d). The analysis showed that only unimodal distributions can be fitted to the IPI data, suggesting that there are no regular interruptions of similar length within the chirps produced in the observed conditions (Fig. 4). One hundred percent of the chirps fit one of the three basic shapes (ascending, descending, bell-shaped) (Fig. 5) defined by curve-fitting. This indicates that these three shape categories are sufficient for quantitative analysis of amplitude envelope shapes between contexts (see below).

#### Context-dependent chirp characteristics

Females produced chirps in all three behavioural contexts tested. Light pinching of the female abdomen elicited distress chirps. When grasped by the mandibles of *T. dubius* predators, females exhibited struggling behaviour characterized by erratic leg movements, at the same time as nodding their heads up and down, with opened mandibles, and with the head nodding eliciting chirps. During premating trials, all females admitted into male chambers produced acoustic signals.

#### Temporal characteristics

Significant differences were observed in chirp characteristics between behavioural contexts. CVA analysis showed that distress and predation were undistinguishable at the same time as being significantly different from premating (approximated  $F_{6,110} = 3.56$ ,  $P < 0.05$ ) (Fig. 6a). Individual ANOVA tests confirmed the previous analysis and showed that only duration was significantly different between premating and the other two contexts ( $F_{2,59} = 8.03$ ,  $P < 0.05$ ) (Fig. 6b). Average temporal measurements from the three contexts are summarized in Table 1.

#### Amplitude envelope

The chi-squared contingency table showed that frequency of each envelope shape differed between the three contexts ( $\chi^2 = 33.70$ ; d.f. = 4;  $P < 0.05$ ). The Kruskal–Wallis test showed that the ‘descending’ shape was significantly more frequent in the premating context (Fig. 7).

### Discussion

The results of the present study show that acoustic chirps in female pine engraver beetles are consistently produced in the three contexts tested: distress, predation and premating. The results show significant differences between the temporal and amplitude envelope characteristics of chirps in different contexts, suggesting that context-dependent signalling is present in this species. The functional significance of variation in chirp characteristics between contexts is discussed and recommendations for future comparisons of signal characteristics in bark beetles are provided.

#### Do *I. pini* produce different chirp types?

Bark beetle species have been proposed to exhibit two chirp types based on regular interruptions: simple (or uninterrupted) and interrupted [e.g. *Dendroctonus* spp.: Michael & Rudinsky, 1972; *Ips* spp.: Oester & Rudinsky, 1978]. However, currently, there is little quantitative evidence that such types exist. In *I. pini*, the distribution of IPIs is unimodal for all chirps whether analyzed together, or for the three different contexts. Therefore, in disagreement with previous *Ips* spp. studies (Oester & Rudinsky, 1978), the results of the present study do not support the hypothesis that *I. pini* produces categorically different chirp types, as has been confirmed for at least one *Dendroctonus* species (Lindeman & Yack, 2015). *Ips pini* has a completely different sound-producing mechanism from *Dendroctonus* species (Barr, 1969). Males of stridulating *Dendroctonus* species possess an elytra-tergal type mechanism, where the pars stridens is located on underside of the elytra, and the plectrum is a single pair of ridges on the seventh abdominal tergite (Lyon, 1958). This conceivably allows for finer motor control of the plectrum, resulting in a more precise mechanical manipulation of interruptions (Lindeman, 2016). Such fine motor control necessary for inserting regularly spaced interruptions may not be feasible for *I. pini*. Different chirp types cannot be ruled out entirely for *I. pini* because these may exist in other behavioural contexts not tested in the present study. Accordingly, at present, this research does not support previous categorization of chirps into distinct types.

#### Distress signalling under natural and artificial conditions

Most bark beetle species, including *I. pini*, are reported to generate ‘distress’, ‘stress’ or ‘disturbance’ signals (Barr, 1969; Ryker & Rudinsky, 1976). Signals are elicited by holding the insect between fingers and squeezing slightly (Swaby & Rudinsky, 1976). This method, however, is not representative of any natural stressful conditions, such as predation, and it is possible that the sound characteristics do not represent natural conditions. Unfortunately, there is only one reported study that tests acoustic behaviour during predator attack and it does not include analysis of emitted signals (Lewis & Cane, 1990). In the present study, natural stress conditions are created by pairing the beetles with one of their natural predators, *T. dubius*, and the recordings from these trials are compared with those emitted during handling. No significant differences are found between chirps recorded under natural and artificial stress conditions; therefore, it is concluded that, in *I. pini*, handling is an accurate representation of stress and can be considered a valid means of evoking distress signals for future studies.

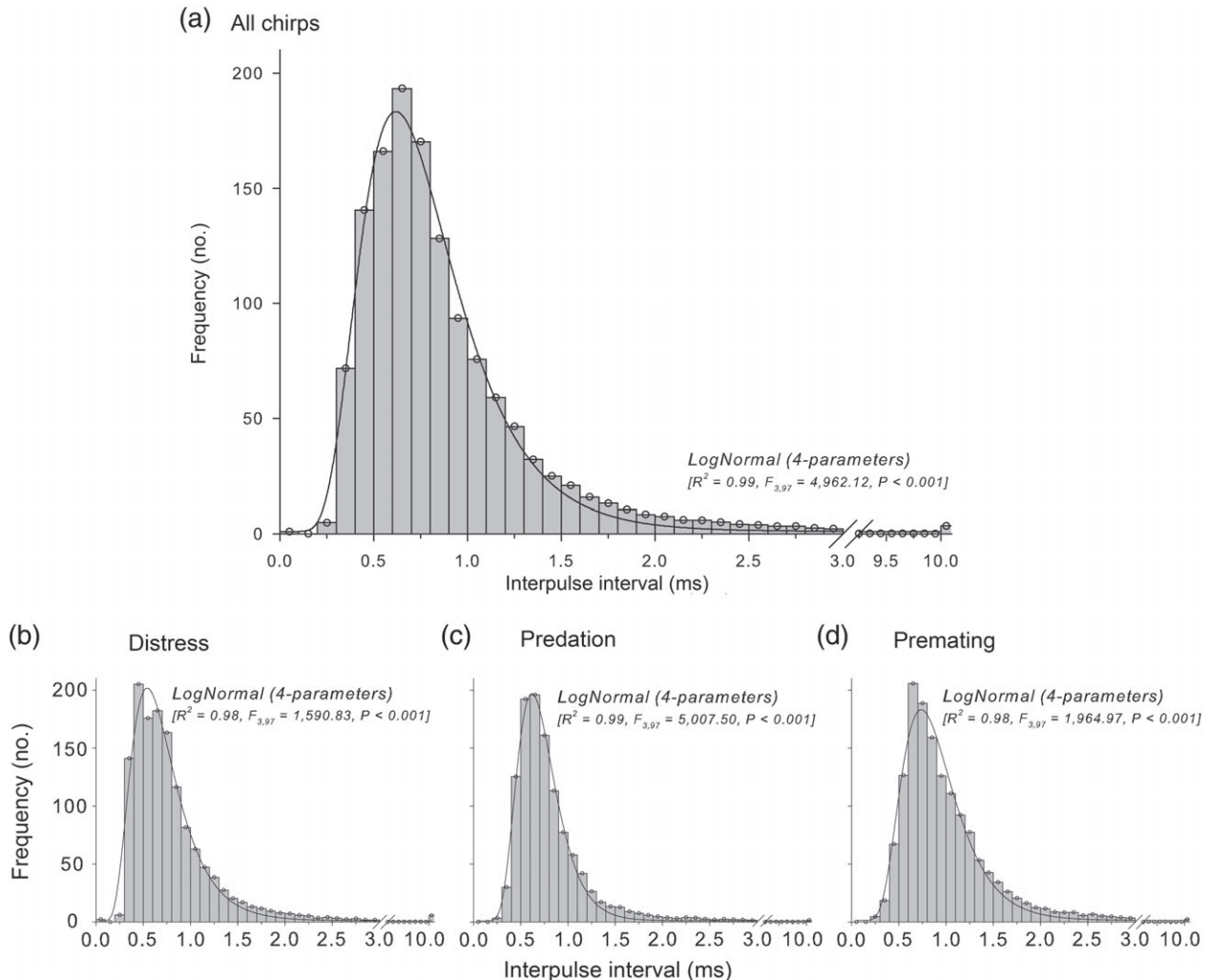
#### Premating chirps

Chirps produced during premating are significantly longer than chirps during distress or predation. However, the number of pulses and the average IPIs are not significantly different between contexts. This might be the result of the small increase

**Table 1.** Temporal characteristics of *Ips pini* chirps recorded in different behavioural conditions.

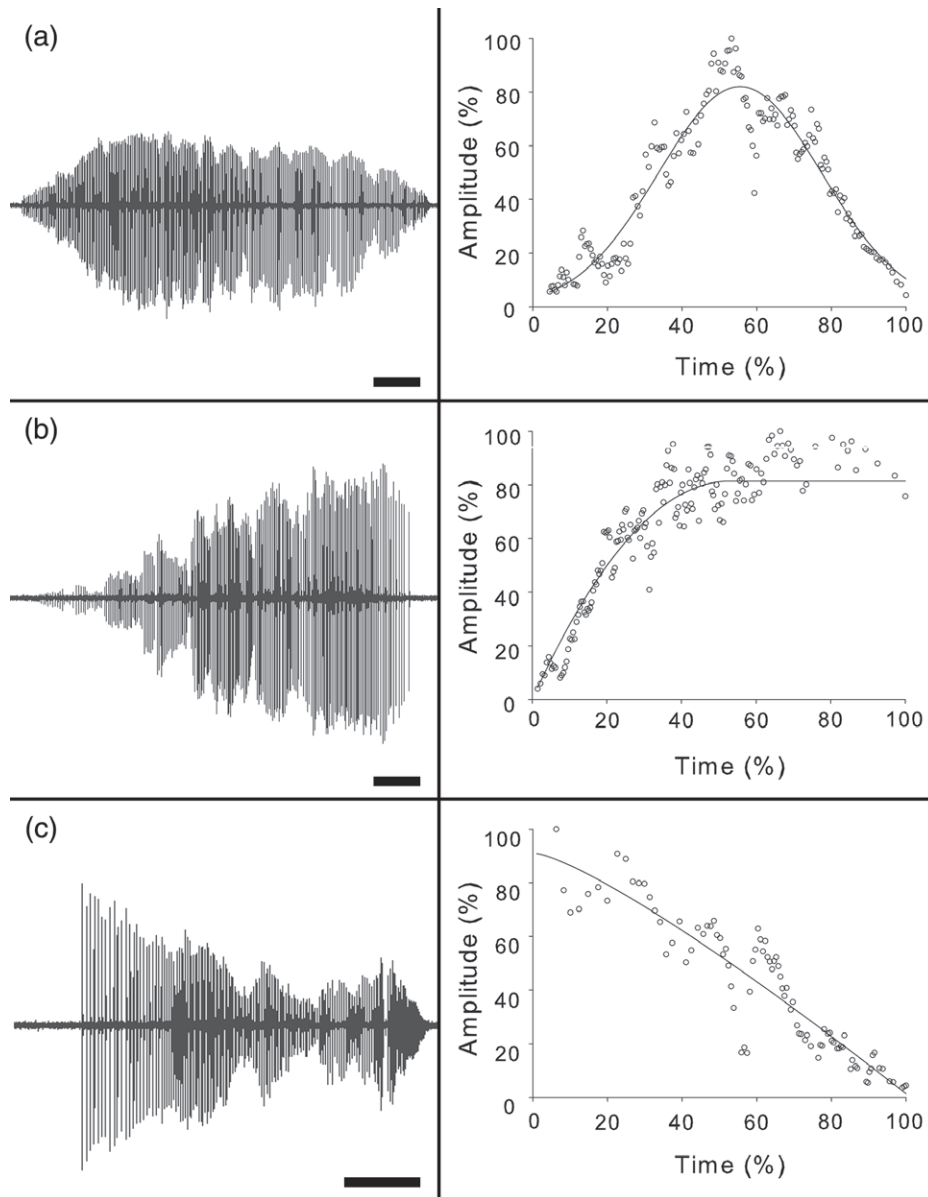
Context	Chirp duration (ms)		Number of pulses		IPI (ms)		Pulse rate (number pulses s <sup>-1</sup> )	
	Range	Mean ± SEM	Range	Mean ± SEM	Range	Mean ± SEM	Range	Mean ± SEM
All	29.2–501.3	137.3 ± 2.6	14–385	147.31 ± 2.78	0.02–135.49	0.94 ± 0.01	79.75–2703.72	1160.76 ± 20.24
Distress	29.2–429.4	122.3 ± 5.4	14–369	145.76 ± 7.34	0.02–135.49	0.95 ± 0.06	79.75–2703.72	1223.99 ± 37.06
Predation	38.9–328.3	133.1 ± 6.8	19–305	137.66 ± 6.45	0.17–35.70	0.92 ± 0.05	288.94–2233.51	1241.59 ± 30.54
Premating	33.5–501.3	159.7 ± 8.9	17–385	152.91 ± 6.91	0.19–48.95	1.06 ± 0.06	146.34–2094.54	1007.19 ± 28.42

IPI, interpulse interval.

**Fig. 4.** Frequency distribution histogram of *Ips pini* chirp interpulse intervals showing unimodal distribution in all behavioural contexts. (a) All chirps. (b) Distress. (c) Predation. (d) Premating.

in the number of pulses combined with a slightly lower rate, or it could suggest that, during premating interactions, females increase chirp duration by introducing random interruptions within a chirp. During premating interactions, when acoustically signalling, females also engage in jostling behaviour by repeatedly pushing against the male elytral declivity with their frons, and occasionally biting and scraping male elytral spines (Schmitz, 1972). It is possible that these physical

interactions can introduce random interruptions, causing longer chirp durations. However, the exact mechanism of generating longer duration premating chirps is not clear and requires further investigations with respect to signalling mechanisms. Regardless of how they are produced, longer signals can play an important role during mate choice in insects and this may also be the case for bark beetles.



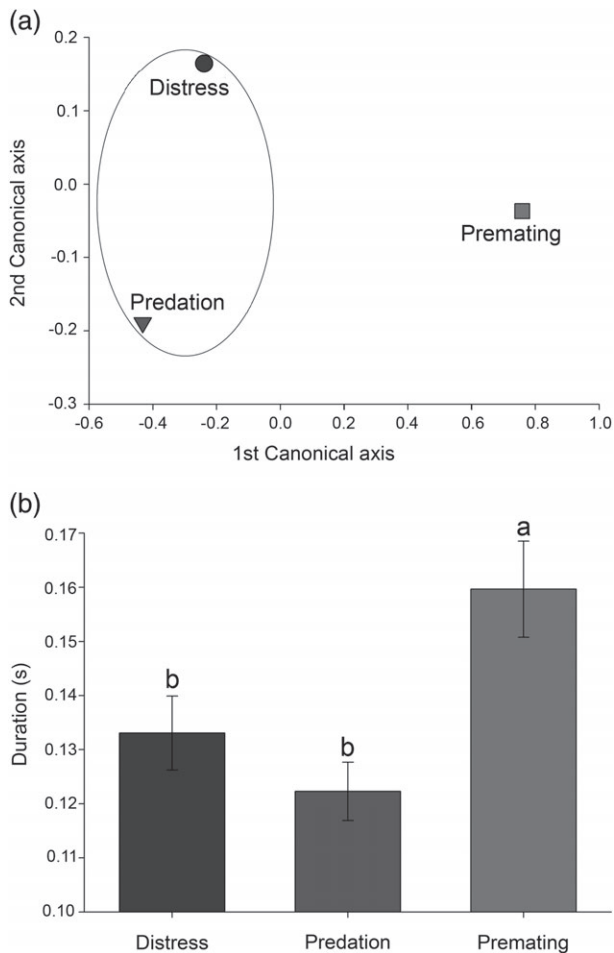
**Fig. 5.** Basic amplitude envelope shapes of female *Ips pini* chirps calculated by curve-fitting on selected representative chirps, shown with examples. (a) Bell-shaped. (b) Ascending. (c) Descending. Scale bars: (a–c) 20 ms.

Longer signals are easier to detect (Pohl *et al.*, 2013) and can provide information about signaller quality. Signal characteristics that provide honest information about the phenotypic and/or genetic quality of the signaller are usually under directional selection and show much variation between individuals (Johnstone, 1995; Pomiankowski & Møller, 1995). Longer signals are often more costly than shorter ones (Prestwich, 1994) and are shown to be more attractive to the opposite sex in other acoustic animals, such as field crickets (Hedrick, 1986), lesser wax moths (Jang & Greenfield, 1996), tree frogs (Gerhardt *et al.*, 2000) and spiders (Parri *et al.*, 2002). It is hypothesized that, in bark beetles, premating signals provide information about the physical attributes of a signaller (Byers *et al.*, 2010; Lindeman & Yack,

2015), as well as a higher effort in signalling, may relate to better physical condition, as shown for other acoustic insects (Bertram *et al.*, 2006).

The present study shows that female *I. pini* signals have a broad range of chirp durations, and may be highly variable between individuals. However, future studies should examine variations in signal characteristics between females, as well as whether these traits can provide information about quality and/or species. By contrast, distress signals, which presumably function for momentarily deterring predators, are generally shorter in duration and more intense (Masters, 1980). The effectiveness of these signals is usually short-lived; predators can become habituated to longer signalling. Therefore, producing long distress



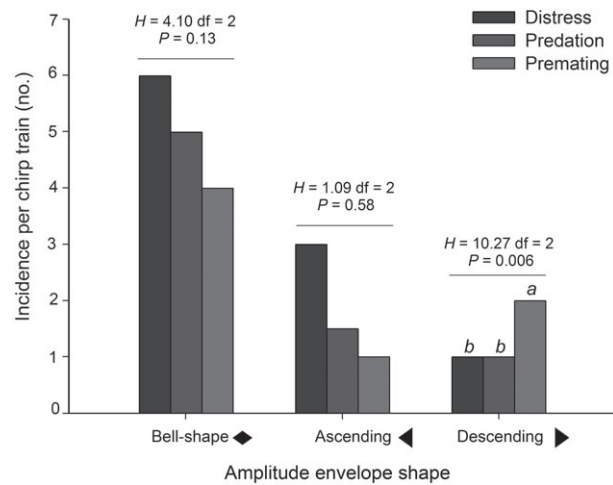


**Fig. 6.** Statistical results showing significant differences between the temporal chirp characteristics from the three behavioural contexts. (a) Canonical variate analysis diagram graph showing pre-mating chirps being significantly different from other chirps, whereas distress and predation are undistinguishable; the symbols are centroid of treatments representing class mean canonical variates and the large ellipsis grouping then indicate lack of significant difference (approximated  $F$ -test at  $P < 0.05$ ), based on the Mahalanobis distance ( $D^2$ ) between class means (i.e. behavioural contexts). (b) Bar graph of the mean  $\pm$  SEM chirp duration in the three behavioural contexts; different lowercase letters at the top of each bar indicate significant differences by Fisher's least significant difference test ( $P < 0.05$ ). Duration was significantly longer during pre-mating conditions.

signals can be counterproductive. The effectiveness of distress signals in bark beetles remains unknown, and untested empirically (but see Lewis & Cane, 1990).

#### Amplitude envelope shape

The amplitude envelope shape of insect sounds can be as important as the temporal attributes (Ronacher, 2016). In grasshoppers, species specific amplitude modulation is directly related to the sound-producing mechanism of the given species



**Fig. 7.** Bar graph showing the occurrence of the three basic amplitude envelope shape types in the three behavioural contexts. Bell-shaped chirps were the most common in all contexts. Relative occurrence of descending chirps was significantly higher during pre-mating than in the other two contexts.

and is important for the listener (von Helversen & von Helversen, 1998). In the grasshopper *Chorthippus biguttulus*, changing the amplitude shape decreases the attractiveness of that signal: signals with a descending shape are very attractive, whereas signals with an inverted shape (ascending) are rejected (Schmidt *et al.*, 2008). Despite the importance of amplitude envelope shape, quantitative methods for the analysis of the amplitude-time envelope shape with respect to assessing insect acoustic signals are not reported.

To analyze the relative amplitude characteristics of *I. pini* chirps, a curve-fitting based method is developed. By quantifying the amplitude envelope using the software to match with pre-defined curves, we minimize subjectivity. The results show that 'bell-shaped' is dominant for all contexts, whereas 'descending' is significantly more frequent during pre-mating conditions. The change in the envelope shape might be the result of a different signalling motion during pre-mating. For example, during pre-mating signalling, females might only stridulate using part of the pars stridens. This suggests that females may have some control over the envelope shape, which possibly requires effort. In turn, these descending chirps could be preferred by the male. It would be important to determine whether these insects are capable of detecting changes in the envelope shape. However, to date, there is no information available on the sound detecting organ of bark beetles. Furthermore, studies on the morphology and kinetics of the sound-producing mechanism might show why 'bell-shaped' appears to be the dominant envelope shape for these species. These findings suggest that the amplitude envelope can possibly be part of the context-dependent signal characteristics of *I. pini* chirps, and it is recommended that this chirp characteristic be considered in future acoustic analyses.

It is important to note that, at present, there is only indirect behavioural evidence to indicate that bark beetles are capable of detecting airborne sound (Rudinsky *et al.*, 1973) and it

is possible that acoustic signals are transmitted as solid-borne vibrations. Although this would mean that some characteristics of the sounds may be transformed or lost during the transfer between substrates, differences in chirp duration and the amplitude envelope of sounds are reported to be translated into vibrations (Fleming *et al.*, 2013; Lindeman, 2016), meaning that the observed significant differences would most probably be translated as well.

Bark beetles produce acoustic signals in several different behavioural contexts, although little is known about how these signals vary, partly as a result of a lack of objective and quantitative methods used to characterize acoustic signals in this group. The present study takes a first step in applying quantitative analytic methods to sample and analyze signal characteristics in the bark beetle *I. pini*. The results show that premating chirps are of longer duration than defensive chirps, supporting the hypothesis that signal characteristics vary between conditions. It is recommended that future comparative studies on bark beetle acoustics follow similar methods to facilitate meaningful comparisons of acoustic communication signals within and between populations of this economically and ecologically important insect group. Finally, it is recommended that more research focuses on context-dependent signal variation in other insect groups because this knowledge will provide insights into the selection pressures on insect communication signals in general.

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