Acoustic signalling in the destructive bark beetle genus *Dendroctonus* (Curculionidae: Scolytinae) with emphasis on *Dendroctonus valens*

by

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ABSTRACT

Acoustic signalling is common in bark beetles (Curculionidae: Scolytinae) but has been underappreciated in the literature, resulting in many unanswered questions regarding their acoustic ecology. The goal of this work was to answer some outstanding questions in the economically important *Dendroctonus* genus using mainly *Dendroctonus valens.* *Dendroctonus* spp. produce complex and variable acoustic signals in many contexts. Addressing the question of why they signal, I found that during courtship the ultimate function of signals was linked to female mate choice. Not only did chirp characteristics correlate with signaller fitness, but females differentiated between and made decisions about potential mates based on chirp variability in courtship song performances. The question of how chirp variability is produced was then addressed. I found the proximate mechanism of chirp production is a form of 'spring stridulation', where elastic potential energy is stored as fuel for stridulation. Altering the number of times the energy store was reloaded during a chirp led to the variable pulse pattern of the distinct chirp varieties. Finally, I addressed the question of sexual dimorphism in sound production. I noted that females produced sound in the contexts of disturbance and territoriality and further that they do so by way of an alternative mechanism to that of sound production in males. However, female sounds were erratic and rare, and their presence did not evoke a conclusive behavioural response in conspecifics, leading to an overall lack of support for a communicative function of female sound. My thesis research forms the most comprehensive story of the ultimate and proximate nature of sound production in both sexes of a destructive group of tree-killing bark beetles.

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CO-AUTHORSHIP STATEMENTS

Several pieces of this thesis document have been published or are being assembled for publication. Chapter 2 is published in *Behavioural Processes* (Lindeman and Yack, 2015). Chapter 3 is in preparation for submission to the *Journal of Experimental Biology* (authors: Lindeman and Yack). Chapter 4 is being prepared for submission to the *Canadian Journal of Zoology*, expected to be submitted within the upcoming year (authors: Lindeman and Yack). Both Appendices are also being prepared for publication. Appendix A is expected to be submitted for publication this fall (expected journal/submission date: *The Canadian Entomologist,* Fall 2015; authors: Lindeman and Yack). Appendix B is expected to be submitted for publication this winter (expected journal/submission date: the *Journal of Economic Entomology,* Winter 2016; authors: Lindeman and English). For data from all chapters and appendices, some of the experiments were designed collaboratively between Dr. Jayne Yack and myself, while others I designed independently. I conducted all experiments and data collection, analysed all data, generated all figures with feedback from Dr. Yack, and drafted all initial and final manuscripts. Dr. Yack facilitated all data collection by providing advice, funding and laboratory equipment and by using contacts to obtain beetles either directly, as was the case for beetles imported from Alberta, or through requesting permission for beetle collection locally. Furthermore, Dr. Yack provided great insight into the field of acoustic communication, gave input on the organization of data and figures for the manuscripts and assisted with writing. Finally, I provided the project goal and bark beetle acoustics expertise that directed the computer program for this project

and determined the variables of interest, while the program itself was written by Kent English.

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CHAPTER 1: A general introduction to bark beetle life history and acoustic communication

1.1 Introduction

Bark beetles (Coleoptera: Curculionidae, Scolytinae) are a group of sub-cortical feeding insects comprising a broad subfamily of hundreds of genera and more than 6,000 species (KnÍŽek and Beaver 2004). Most of their lives are spent within plants as most species are either phloeophagous (phloem feeding) or xylomycetophagous (feed on ambrosia fungus cultivated in their galleries) (Raffa et al. 2015). Each species is selective of the hosts they colonize and the particular location of colonization on the host, with distinct adaptations suited to their colonization preferences (Rudinsky 1962; Wood 1982). The vast majority of bark beetle species colonize weakened or dead trees (i.e. are saprophytic; Raffa et al. 1993; Raffa et al. 2015). These insects are productive members of their communities as the primary decomposers of dead woody tissue, and influence nutrient, carbon and nitrogen cycling and plant biodiversity (Kirkendall 1983; Kurz et al. 2008; Raffa et al. 2008). However, there exist a few bark beetle species that are facultative parasites; these are normally saprophytic animals but are capable of parasitizing stressed live plants (Raffa et al. 1993; Raffa at al. 2015) or even healthy plants under outbreak conditions. These predatory species aggregate en masse on healthy trees, and girdle the tree through the construction of galleries along the tree's phloem (Wood 1982). Insects in general kill more trees each year than all other natural factors combined (Anderson 1960), but it is the bark beetles along with ambrosia beetles (Platypodinae) that stand alone in their destructive capacity (Wood 1982). Within those predatory groups there are only an influential few that can become primarily destructive to forests resulting in the devastation that gives bark beetles their

notoriety, for they are capable of causing damage to coniferous forests on such a scale the result is worth billions of dollars in economic loss (Kurz et al. 2008; Raffa et al. 2008). Species of the genus *Dendroctonus* are unquestionably among the most destructive (Hopkins 1909; Wood 1963; Six and Bracewell 2015).

1.2 Ecology of the genus *Dendroctonus*

> "The aggressiveness [of *Dendroctonus*] has marked them as the greatest tree killers known"
>
> - Wood (1963)

There are 19 species that make up the genus *Dendroctonus*, 17 of which are native to North and Central America (Six and Bracewell 2015). All species infest coniferous hosts, principally representatives of the genera *Pinus, Picea, Pseudotsuga* and *Larix* (Wood 1963; Wood 1982). *Dendroctonus* spp. are mainly host-specialists. Most use only one host species, but a few use more, such as *Dendroctonus valens* which can colonize more than 20 species of host tree (Wood 1982; Salinas-Moreno et al. 2004). The exception is during outbreaks, when almost any conifer may exhibit signs of attack (Wood 1963; Reed et al. 1986; Huber et al. 2009). The geographic distribution of each *Dendroctonus* species is thus generally limited to the range of its host(s), and thus for the polyphagous *D. valens* it includes most of North and Central America (Wood 1982; Owen et al. 2010). *Dendroctonus valens* has also recently extended its range to include parts of China, where it has become a major pest following its accidental introduction to the Shanxi Province in 1998 (Yan et al. 2005).

Within their given range, adult *Dendroctonus* females use chemical cues to actively seek out suitable hosts and are responsible for individual attacks (Wood 1963; Raffa et al. 1993). Once a host is located the female beetle tunnels into the bark of the tree while releasing an aggregating pheromone that effectively escalates the attack (Raffa et al. 1993). The host trees have both constitutive and induced defences (reviewed in Krokene 2015), which individual females are remarkably capable of dealing with during attack (Wood 1963). Mass attacks over short periods of time coupled with fungal inoculation (brought in by the beetles) can cripple a tree's defences (Krokene 2015). The pitch and frass resulting from the excavation are pushed out of the entrance hole creating pitch tubes, the size, colour and character of which can be used in the identification of the species colonizing the tree (Wood 1963). As males emerge they orient towards the female pheromone; *Dendroctonous* species employ the prevalent mating strategy among bark beetles where each gallery is established and guarded by an individual female and a male partner will arrive later and join her (Kirkendall 1983). Once the male-female interaction has begun a second pheromone is released, the anti-aggregation or masking pheromone, which signals other beetles to stay away (Raffa et al. 1993). This masking pheromone has been found to be released by the female in response to acoustic signalling by the male (in at least two species, Rudinsky and Michael 1972; Rudinsky et al. 1973; Rudinsky et al. 1976).

Once the pair has formed, they will work together to construct an egg gallery under the bark of the host tree with bouts of digging being interrupted by bouts of courtship and mating (Rudinsky 1962; Wood 1963). The larvae, once hatched, feed on

the cambial layer digging out tunnels known as “larval mines” perpendicular to the female gallery in solitary species, or create “larval chambers” as is the case for the gregarious *D. valens* larvae (Wood 1963). The number of generations observed per year can be anywhere from one to five or more, and flight activity begins with rising temperatures in early spring and is more or less continuous throughout spring and summer, depending on the species and the region (Wood 1963). Tree pathology occurs due to the adults and larvae feeding as well as the associated fungus, both of which completely block the phloem of the infested tree, causing the death of any tissues above the damage (Rudinsky 1962). The destructive capability varies between species.

1.3 Bark beetle sensory ecology: a brief literature overview

Although the field of bark beetle ecology has received intense interest in the literature in general (cf. Rudinsky 1962), the most studied aspect of bark beetle sensory ecology is chemical signalling, which is the bark beetle’s primary modality of signalling (Raffa 2014). Likely this field has gained so much attention owing to the importance of the bark beetle's chemical ecology in achieving pest status, as this sensory channel mediates complex intra- and interspecific interactions with sympatric bark beetles, host plants, symbiotic mites and fungi and predators and parasitoids (Byers 1983; Borden 1989; Byers 1989; Klepzig et al. 2001; Sauvard 2004; Raffa et al. 2008; Bentz et al. 2010). Chemosensation is used in many contexts, including recognizing terpene cues used for host selection and pheromone communication used in warning, aggregation, anti-aggregation, sexual attraction and rivalry (Wood and Vité 1961; Rudinsky and Michael

1972; Moeck et al. 1981; Wood 1982; see also review in Raffa et al. 2015). These chemical signals mostly function in long-distance communication.

Bark beetle acoustic sensory ecology, by comparison, has been underappreciated in the literature despite the pervasiveness of acoustic communication among many bark beetle species during close-range activities. The acoustic signals are produced by bark beetles through stridulation, and have had implicated functions in attraction, courtship, spacing, and defence (Barr 1969; Wood 1982; Lyal and King 1996). Stridulation involves a file (pars stridens) stimulated by a scraper (plectrum), sending a sound emitter into vibration. These vibrations are potentially capable of being transmitted through both air and substrate. Three different stridulatory mechanisms have been found among bark beetles - elytro-tergal, vertex-pronotal and gula-prosternal - named after the anatomical regions where they are found (Barr 1969). Acoustic communication is purported to play a major role in the life history of many bark beetle species owing to its presence in a variety of contexts, including when disturbed, during intersexual premating interactions and intrasexual rivalry interactions (Ryker 1988). Yet, compared to the extensive research on chemical communication in these beetles, comparatively little is understood about their acoustic communication, including the function of signals in any bark beetle species in any context and the mechanics of sound production.

1.4 What is known of the 'what', 'why' and 'how' of *Dendroctonus* acoustic signals

Dendroctonus species are an interesting group to address ultimate and proximate hypotheses regarding bark beetle sound production, because they produce complex acoustic signals which vary both between and within species. Most research in *Dendroctonus* spp. acoustic signals has focused on characterizing the signals and describing how they vary between contexts (e.g. Ryker 1988; Fleming et al. 2013). Sounds have typically been characterized by their temporal domain (e.g. Ryker 1988). Chirps have been identified as belonging to one of two categories based on a qualitative analysis of their pulse pattern: 'simple' chirps, defined as comprising a series of relatively regularly spaced tooth strikes, and 'interrupted' chirps, defined as having two or more components interrupted by brief periods of silence (e.g. Ryker and Rudinsky 1976b). In addition to categorizing the chirp types, signals have primarily been described in terms of duration, number of tooth strikes per chirp, and tooth strike rate (e.g. Michael and Rudinsky 1972; Rudinsky and Michael 1974; Ryker and Rudinsky 1976a,b). While these studies have provided valuable insight into the diversity of temporal patterns that occur in the behavioural repertoire within and between *Dendroctonus* species, there is often a lack of clarification on how signals were sampled, quantified and categorized. A recent study of *Dendroctonus ponderosae* (Fleming et al. 2013) has gone further to characterize the signals than any other study of a *Dendroctonus* beetle by including descriptions of spectral and amplitude domains, and also by addressing the question of pathways of transmission by considering both the airborne and substrate-borne components of the sound.

While the characteristics of signals have been broadly described, the function of male *Dendroctonus* signals in various contexts has remained the subject of debate. Historically, researchers have drawn conclusions regarding the function of each signal type based on the context in which it occurred, with distinct functions assigned to the distinct chirp varieties. For instance, interrupted chirps were found to be associated with aggression (both intra- and intersexual) and thus hypothesized to signal aggressive intent, while simple chirps were hypothesized to function in courtship (Ryker and Rudinsky 1976a,b). However, the earlier hypotheses were based on studies that did not consider whole acoustic performances, but rather examined small samples of signalling within a given context. While this earlier work led to hypotheses on signal function that pigeon-holed function by chirp type, Fleming and colleagues (2013) were the first to take long term sampling of performances and show that both simple and interrupted chirp varieties are produced during each of three contexts: disturbance, male-female interactions, and male-male interactions. Their evidence of heterogeneity of signal type within each context suggested that signal type on its own does not have a specific contextual meaning. Further investigation is required before any conclusions on function can be drawn.

Similar to current unresolved questions surrounding of the function of signals, there is much to be determined concerning the mechanics of acoustic signal production. All *Dendroctonus* bark beetles are elytro-tergal stridulators, with this one stridulatory organ used to produce the two distinct kinds of chirps, simple and interrupted. The sound producing organ has been identified as a file located near the sutural margin on

the underside of the elytra, predominately but not exclusively on the left elytron, and a protruding structure located medially on the seventh abdominal tergite which is presumed to be the plectrum (Barr 1969). However, the mechanism behind the interaction between these two structures to produce sound has never been examined. Sound has been proposed to be produced by moving the last few segments of the abdomen in a posteriad stroke (using either haemolymph pressure, Gibson 1967, or muscular contraction, Carlysle et al. 1975) to cause the plectrum to strike the file in chirp production (Hopkins 1909; Michael and Rudinsky 1972). However, very little is known or has been tested regarding the chain of sound production, from muscle power to mechanical vibration of the sound-producing structure to acoustic loading of this source to sound radiation (Bennet-Clark 1995). Likewise, nothing is known of the mechanics of incorporating variability into signals. These outstanding questions each need to be addressed for a full understanding of sound production in *Dendroctonus*.

1.5 Thesis overview

This doctoral research program was designed to follow the logical steps needed to tell a comprehensive story of why and how *Dendroctonus* spp. produce acoustic signals. Questions both ultimate and proximate in nature were addressed.

Ultimate questions are those that address the higher purpose or reason for the behaviour, and they include such questions as: why did the behaviour of producing sounds evolve? What information do the sounds encode? What functions do the sounds perform? These are questions addressed in Chapter 2, which directly tested hypotheses

to explain the function of male-produced signals during male-female interactions at the gallery entrance using *D. valens*. In this section, the problem of variable signals was approached in a quantitative way to provide an objective definition of simple and interrupted chirps for the first time in the bark beetle literature. Once this was accomplished, the ultimate function of the two signal types was addressed through experiments testing two hypotheses: that acoustic signals are honest indicators of male condition and that females choose males based on signal characteristics. Overall, the results of Chapter 2 led to the conclusion that simple and interrupted chirps have quantitatively distinct properties which female *D. valens* are capable of discriminating between to select better mates.

Proximate questions address how the behaviour is produced, including: what is the mechanism for producing chirps? And how is the important variability in chirp type generated? Thus, the goal of Chapter 3 was to test hypotheses regarding the mechanics of variable sound production in male *D. valens*. First, the body structures involved in sound production were experimentally verified. Then, hypotheses regarding the sound production mechanism were developed from what is known for well-studied ensiferan stridulators - crickets and katydids - including hypotheses regarding the mechanisms underlying stimulation and vibration of the sound emitter. The tests of these hypotheses led to the conclusion that *D. valens* males employ a form of spring stridulation to produce chirps and introduce chirp variability.

Chapters 2 and 3 focused exclusively on male-produced acoustic signals, but within *Dendroctonus* there are several species for whom the presence female acoustic

signalling has been proposed. Consequently, the goal of Chapter 4 was to address the question of female sound production in two such species, *D. valens* and *D. ponderosae*. It was found that some *D. valens* females did produce sound during contexts of disturbance and female-female interactions, suggesting that females are capable of sound production. It also appears to be likely that females produce these sounds by way of a completely different mechanism than the male-produced chirps. However, female sound production was rare and irregular in the contexts examined, and tests of whether female sounds could influence the behaviour of conspecifics were inconclusive. Consequently, there was no decisive evidence to suggest that the females' sounds functioned as acoustic signals. This completed the picture of sound production in both sexes of the genus.

Over the course of this dissertation work, two further points were appreciated: (1) the importance of a laboratory-reared colony of a study specimen to research, and (2) that destructive bark beetles have greatly impacted the lives and welfare of many people, and understanding their sensory ecology could provide important information to guide future management practices. For this reason, two appendices were incorporated that addressed research on these two topics. The goal of Appendix 1 was to develop a laboratory colony of *D. valens* that could be followed from the time of successful mating through to emergence of young adults. The goal of Appendix 2 was to develop an application that uses the acoustic signals produced by bark beetles as a means of species identification so that they might be monitored by their acoustic signals while under the bark of logs.

The body of research conducted over the past four years has led to an overarching view of the key role that the acoustic sensory system plays in the life history of *D. valens*. This knowledge can in turn be used to direct future studies on bark beetle acoustic ecology and on the application of acoustic technology to the management of destructive bark beetles.

CHAPTER 2: What's the password? Female bark beetles (Scolytinae) grant males access to their galleries based on courtship song

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2.1 Abstract

Acoustic signals are commonly used by insects in the context of mating, and signals can vary depending on the stage of interaction between a male and female. While calling songs have been studied extensively, particularly in Orthoptera, much less is known about courtship songs. One outstanding question is how potential mates are differentiated by their courtship signal characteristics. We examined acoustic courtship signals in a new system, bark beetles (Scolytinae). In the red turpentine beetle (*Dendroctonus valens*) males produce chirp trains upon approaching the entrance of a female's gallery. We tested the hypotheses that acoustic signals are honest indicators of male condition and that females choose males based on signal characteristics. Males generated two distinct chirp types (simple and interrupted), and variability in their prevalence correlated with an indicator of male quality, body size, with larger males producing significantly more interrupted chirps. Females showed a significant preference for males who produced interrupted chirps, suggesting that females distinguish between males on the basis of their chirp performances. We suggest that interrupted chirps during courtship advertise a male's size and/or motor skills, and function as the proverbial 'passwords' that allow him entry to a female's gallery.

2.2 Introduction

Close-range courtship signals may be the most influential type of acoustic signal in mating decisions in insects (Fitzpatrick and Gray 2001; Rebar et al. 2009). Whereas calling songs precede courtship songs and operate in far-range attraction of mates,

courtship songs occur once males and females are in close proximity, and they exhibit variability that infers they are condition-dependent and can convey information on mate quality (Alexander 1961; Fitzpatrick and Gray 2001; Zuk et al. 2008). Courtship songs have been found to be important in prompting females to mate across a broad range of taxa, including in Orthoptera (such as in field crickets, e.g. Burk 1983; Balakrishnan and Pollack 1996; Nelson and Nolen 1997), Diptera (such as in *Drosophila* species, e.g. Liimatainen et al. 1992), and Coleoptera (such as for bark beetles, e.g. Wilkinson et al. 1967; Barr 1969; Ryker and Rudinsky 1976b). In many *Drosophila* species, where courtship signals have been extensively studied, male courtship song has been found to be an important target for sexual selection (Ritchie et al. 1998). However, it has yet to be firmly established whether signals can be used by females as a basis for distinguishing between males in other insect groups. This is at least partly owing to the many difficulties in observing courtship songs in either natural or artificial conditions (Sueur and Aubin 2004; Tregenza et al. 2006). Bark beetles (Scolytinae) provide a unique avenue to study the role of courtship songs in mating decisions because it is easy to elicit the production of courtship songs in captivity, and rejected mates are forcibly denied access to mating galleries.

The function of acoustic courtship signals in bark beetles has been the subject of debate (see Fleming et al. 2013). Muting experiments in several species have provided strong evidence that these signals are integral to mating, as muted individuals have a significantly reduced chance of successful mating (e.g. Wilkinson et al. 1967; Barr 1969; Ryker and Rudinsky 1976b). However, the specific functions of courtship signals are not

understood. It has been variously hypothesized that they function to announce the arrival of the stridulating sex (Barr 1969), in aggression towards the female (Ryker and Rudinsky 1976b), in "premating recognition" (Ryker and Rudinsky 1976b), in species recognition to avoid inbreeding (e.g. Yandell 1984), or in species recognition as an anti-predation mechanism (Raffa and Dahlsten 1995). There is presently no experimental evidence to accept or discard any one particular hypothesis. Using the red turpentine beetle (*Dendroctonus valens*), this study is the first formal test of the function of these courtship signals in bark beetles.

Dendroctonus valens are members of the most destructive genus of bark beetles (Hopkins 1909; Wood 1963; Six and Bracewell 2015) and in recent years have become significant economic pests in China (Yan et al. 2005). This species employs a mating strategy prevalent among bark beetles - serial monogamy - and galleries are established and guarded by individual females and a male partner will arrive later and join her (Kirkendall 1983). To ensure the arrival of potential mates, bark beetles do not use acoustic calling songs but rather rely on chemical communication. During gallery construction, females release attractant pheromones as a form of long-range communication with males (Zhang and Sun 2006). Upon arrival, males produce signals called chirps which function in close-range communication (Ryker and Rudinsky 1976a). Bark beetles produce sound through stridulation, where the teeth on a file, the pars stridens, are excited by a plectrum (Barr 1969). In *D. valens* there exists an elytra-abdominal stridulatory structure (Fig. 2.1). *Dendroctonus valens* chirps have been categorized into two types: simple and interrupted. Simple chirps have been defined

subjectively as comprising one series of regularly spaced tooth strikes while interrupted chirps have two or more components interrupted by brief periods of silence (Ryker and Rudinsky 1976b). In previous literature, *D. valens* chirp types were assigned meaning based on the behaviour they were associated with: simple chirps were observed during disturbance and courtship and so speculated to function in those contexts, while interrupted chirps were observed during intrasexual interactions and were speculated to have a rivalry function (Ryker and Rudinsky 1976a). However, interrupted chirps were also observed during intersexual interactions but were not considered to play a role in that context (Ryker and Rudinsky 1976a). The function of signals in various contexts was never empirically tested.

The purpose of this study was to test hypotheses on the function of male courtship signals. The first hypothesis is that signals are honest indicators of signaller quality. We predicted that individual variability would exist in chirp characteristics, and that this variability would be related to male quality. We chose body size as our indicator of quality because, in bark beetles, size is correlated with various measures of fitness (e.g. McGhehey 1971; Botterweg 1982; Anderbrant 1989; Reid and Roitberg 1995; Evenden et al. 2014). The existence of honest indicators of mate quality is an important consideration for determining whether or not mate choice is significant in a given system (Andersson 1994; Maynard Smith and Harper 2003). Therefore, we also hypothesized that acoustic signals are involved in female choice. Earlier studies involving silenced individuals have shown that acoustic signals in general play an important role in successful mating (e.g. Wilkinson et al. 1967; Barr 1969; Ryker and Rudinsky 1976b). In

our study we moved beyond this to examine whether variability between males' acoustic performances would provide a basis for a female to choose one male over another. Bark beetle life history typically enables a high mate encounter rate (Vité et al. 1972) and a cost to mating (e.g. serial monogamy can reduce future mating opportunities, Anderbrant 1989), thus promoting a sexual selection strategy rather than random mating (Kokko and Monaghan 2001). Additionally, it was previously shown in another *Dendroctonus* species that females prefer to mate with larger males, demonstrating the presence of female choice in this system (Reid and Baruch 2010). Thus, we predicted female *D. valens* would be choosy over mates, and that their choice would be based on some aspect of the acoustic signal related to body size.

2.3 Methods

Animals

Adult *Dendroctonus valens* (Curculionidae: Scolytinae) were collected from May-September of 2011-13 at several locations near Ottawa, Ontario, Canada (Limerick Forest, Spencerville, 44.876248,-75.636419; the arboretum at the Ottawa Central Experimental Farm, 45.391021,-75.70489; Carleton Lands, Manotick, 45.183882,-75.604673; and outside Petawawa, 45.853530, -77.536156). Collection was done using Lindgren funnel traps baited with *D. valens* lure (Contech, British Columbia, Canada). Animals were kept at Carleton University, and stored in vials at 5-10^{o}C until use. Bolts of red pine (*Pinus resinosa*) were obtained by cutting fresh trees taken from Carleton lands

into bolts (~60cm long, ~15cm diameter) and sealing the ends with wax to prevent desiccation and mould infestation. These bolts were then used for female gallery construction and male-female interactions. Only one trial was performed per bolt. Voucher specimens are held at Carleton University.

Scanning electron microscopy

Scanning electron micrographs were taken of the stridulatory organs (elytra-abdominal) of nine males, 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. Images were used to calculate the number of teeth on the pars stridens. The teeth are clearly defined ridges and easy to identify; however, they are difficult to count because a given tooth is not always continuous across the width of the file and may bifurcate (Fig. 2.1). Therefore, an estimate of the number of teeth was taken by counting the teeth that occur along a straight line down the middle of the file.

Recording procedures

Recordings of interactions between 30 male-female pairs were conducted on bolts of red pine (individuals were never reused; 25 trials were done with "intact" males and five trials with "muted" males - see below). A female was placed on a predrilled hole (˜2 mm deep, exposing the phloem) where she was secured under an empty gel-

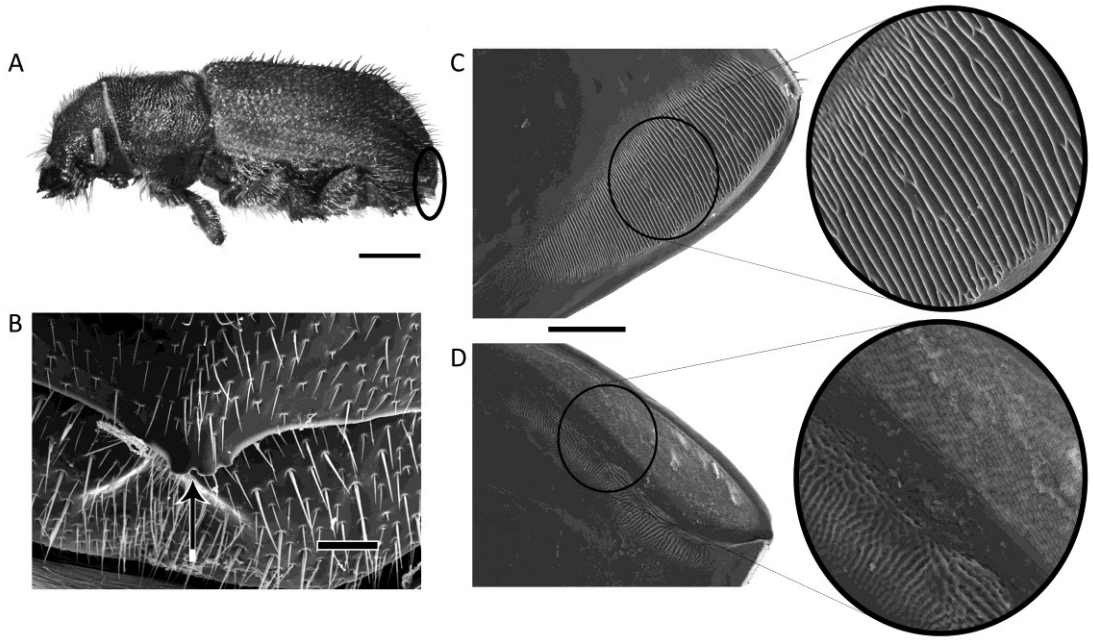

Figure 2.1. Light and scanning electron microscope (SEM) images of male *Dendroctonus valens.* (A) Light microscope image of a male, with circled area indicating the location of the stridulatory organ; scale bar = 1000 µm. (B) SEM of the 7th and 8th abdominal tergites, showing the plectrum (arrow); scale bar = 100 µm. (C) SEM of the file (pars stridens) located at the posterior tip of the underside of the left elytron. (D) SEM of the posterior tip of the underside of the right elytron. C and D scale bar = 100 µm.

capsule. Gallery construction could be observed by the accumulation of frass surrounding the entrance hole, and after at least 24 hours of construction the gel capsule was removed and a randomly selected male was placed next to the gallery entrance (unconfined). Sounds were recorded using an Avisoft condenser microphone (model CMPA-P48/CM16, Berlin, Germany) positioned directly above the gallery entrance at 4 cm, and recorded onto a data recorder (FR-2, Fostex, Los Angeles, USA) at a sampling rate of 96 kHz. Sound recording lasted at least 30 minutes, with some individual performances lasting longer, in which case recordings lasted up until the point at which the performance was deemed to be over (see below). Simultaneous video recordings were taken using a camcorder (HDRHC7, Sony, Tokyo, Japan) connected to an external, second microphone (ECM-MS908C, Sony). All recordings were performed in a walk-in type acoustic chamber maintained at 22 ± 2^{o}C.

Acoustic signal measurements and analysis

Temporal characteristics were analyzed over the entire time spent signalling (performance) using Avisoft SAS Lab Pro (Avisoft Bioacoustics, Berlin, Germany). The characteristics measured were performance duration and signal composition (as in simple or interrupted chirps), chirp duration, inter-chirp interval, number of tooth strikes per chirp, tooth strike rate, and inter-tooth strike interval. A chirp is defined as the smallest unit of sound distinguishable by the human ear (Broughton 1963). Performance duration is characterized by the period of time following introduction for which the male signals. Males would have periodic bouts of stopping and starting

signalling during a performance, and the performance was deemed to be complete if a male remained silent at the gallery for longer than 10 minutes, or if a male withdrew from the gallery entrance (as happened in cases where the female disallowed entry). Chirp duration varied greatly (especially between simple and interrupted chirps, see below); therefore, calculating the number of chirps per time period would not give an accurate measurement of effort for chirp rate, because longer interrupted chirps would need to be produced much more rapidly (with smaller intervals between chirps) to achieve the same number of chirps per time period as the shorter simple chirps. Therefore, we measured the intervals between chirps as the method of calculating chirp rate. Chirp rate was thus measured as the inverse of the intervals between chirps (inverse taken to reflect positive effort; i.e. a larger interval between chirps means less effort, and vice versa). A tooth strike is defined as a single discrete sound pulse produced when a tooth on the pars stridens is struck by the plectrum (Michael and Rudinsky 1972). Inter-tooth strike intervals were individually measured using Avisoft.

Differentiating simple and interrupted chirps

The distinction between simple and interrupted chirps has been qualitatively described in previous literature, where one discrete chirp without gaps was classified as being simple, and any chirp that was subjectively assessed to contain gaps was classified as interrupted (e.g. Michael and Rudinsky 1972). To reduce error, we devised a quantitative way to define chirp type based upon the minimum inter-tooth strike interval that could be considered a "gap" to classify chirps as interrupted. To do so we

assembled frequency histograms for inter-tooth strike intervals to identify the largest inter-tooth strike interval common to both chirp types (see Results). All chirps that contained intervals larger than that point were deemed interrupted. Using this method we were able to quantifiably categorize chirp type for the entire performance of all 25 signalling males. We then analyzed the differences between the two chirp types by comparing the number of tooth strikes per chirp, chirp duration, and the proportion of teeth recruited in chirp production (where the proportion of teeth recruited is the number of tooth strikes per chirp compared to the average number of teeth determined by SEM). This was a paired comparison done only for individuals who produced at least 10 simple and 10 interrupted chirps during their performance (n=13).

Signal variability within animals over time

To examine consistency in signalling effort, we investigated how dynamic male chirping behaviour was over the course of a performance. Because individuals varied in performance duration, we examined these variables with respect to where they occurred over the course of the performance. Therefore, the beginning of the performance was ascribed the value zero, and the last chirp in the performance was ascribed the value one. The performance was then divided up into intervals of 0.02 (i.e. each interval accounting for 2% of the performance), with all chirps investigated based on which interval they fell into and thus the time they occurred relative to the performance as a whole. Within each interval, the average chirp rate, interrupted to simple chirp ratio, and total number of chirps were calculated. The average for each

section across all males was then taken. For this analysis, all chirps from all 25 trials with intact males were included.

Signal variability between animals and its relationship to body size

Body size, measured as the pronotum width, was measured using a light microscope (Olympus SZX12, Tokyo, Japan) equipped with a camera (Zeiss AxioCam MRc5, 1.4 megapixels, 1388 x 1040, Oberkochen, Germany) connected to a computer equipped with Zeiss AxioVision digital Image processing software (Oberkochen, Germany) for 18 males (13 intact and five muted males). Body size was then correlated with features of male performance - duration, total number of chirps, presence and proportion of interrupted chirps, and chirp rate.

Female preference

We calculated the percentage of intact males that were rejected from female entrance holes. Male rejection was occasionally obvious, with the female visibly pushing the male out of the gallery entrance and the male retreating. Sometimes females remained invisible beneath the bark, in which case male rejection was inferred by the male retreating after a period of trying to enter the gallery while signalling. We also calculated the time it took for males to enter the gallery, using disappearance under the frass as an estimate of acceptance time (calculated for 19 males whose entrance was clearly visible during video analysis; n=13 individuals which produced interrupted signals and n=6 individuals which did not).

We conducted an additional experiment with males that were surgically silenced (n=5) and calculated the rejection rate for muted males. This was done in order to increase the sample of individuals who would not signal interrupted chirps, as well as to examine whether muting would negatively affect the probability of acceptance in *D. valens,* as it does in other bark beetle species (e.g. Wilkinson et al. 1967; Barr 1969; Ryker and Rudinsky 1976b). Muting was done by cutting the posterior margin of the elytra, the region that contains the file on the left and right elytron, using extra fine point dissection scissors. Muted males were observed for abdominal movements during disturbance indicating an attempt to produce sound (because males will stridulate during disturbance; Ryker and Rudinsky 1976a). Males will reliably produce disturbance chirps when disturbed by pinching the head and pronotum, thus the absence of chirp sound was confirmed by sound recording during disturbance using the same recording methods described above, and viewing the resulting waveform of the recording using Raven Bioacoustics Research Program (Cornell Laboratory of Ornithology, Ithaca, NY, USA). Additionally, video and audio recordings during trials confirmed that silenced males did not produce chirps during interactions with females.

Statistical analysis

Paired sample t tests for unequal variances were used to analyze differences between characteristics of interrupted and simple chirps. A linear regression analysis investigated the relationship between signal characteristics and body size. In female choice experiments, the rejection rate between normal and muted male groups was

assessed using a Fisher's Exact Test. All probability tests were two tailed and had α set at 0.05 (SPSS Inc., Version 19, Chicago IL, USA).

2.4 Results

Differentiating simple and interrupted chirps

Simple and interrupted chirps were distinct from one another in a number of respects (Fig. 2.2). Simple chirps contained almost exclusively inter-tooth strike intervals less than 5 ms (99.6%) (Fig. 2.2A,B). Interrupted chirps also comprised inter-tooth strike intervals that were primarily less than 5 ms; however, more than one quarter (27.9%) were greater than 5 ms (Fig. 2.2C,D). These >5 ms intervals account for the gaps seen in the interrupted chirps. Interrupted chirps contained significantly more tooth strikes (t_{12}=-7.57, $p<0.001$; Fig. 2.3A) and were significantly longer than simple chirps (t_{12}=-8.27, $p<0.001$; Fig. 2.3B). The duration of interrupted chirps was strongly correlated with the number of components it contained (R^2=0.93, $p<0.001$; Fig. 2.3C). Males had on average 71 ± 2 teeth (n=9), but the mean number of teeth used per chirp was only 15 and 26 for simple and interrupted respectively. If one "tooth strike" is the stimulation of one tooth on the file, comparative analysis suggests that even during longer interrupted chirps fewer than half of the available teeth on the file are used. These results are interesting because they suggest that although males are conceivably capable of producing long

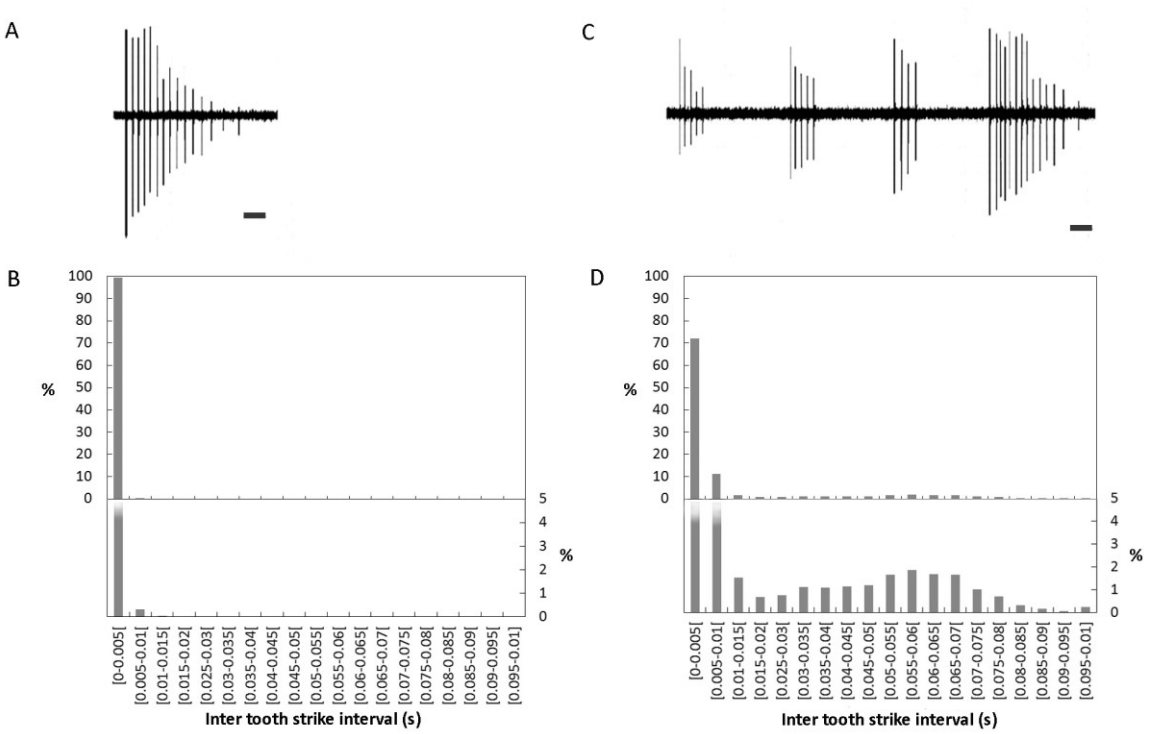

Figure 2.2. Comparison of simple and interrupted chirps. (A) Example of a typical simple chirp; scale bar = 10 ms. (B) Panel chart demonstrating the frequency of inter-tooth strike intervals in simple chirps sampled from 8 individuals during male-female interactions. The top panel summarizes the overall frequencies, while the bottom panel enhances minor differences in the lower 5% of frequencies. (C) Example of a typical interrupted chirp. Red turpentine beetle interrupted chirps were observed to contain 1 - 10 gaps, separating the chirp into 2 - 11 components. This particular interrupted chirp contains 3 gaps and 4 corresponding components; scale bar = 10ms. (D) Panel chart demonstrating the frequency of inter-tooth strike intervals in interrupted chirps sampled from the same 8 individuals during the same male-female interactions as in the simple chirp analysis.

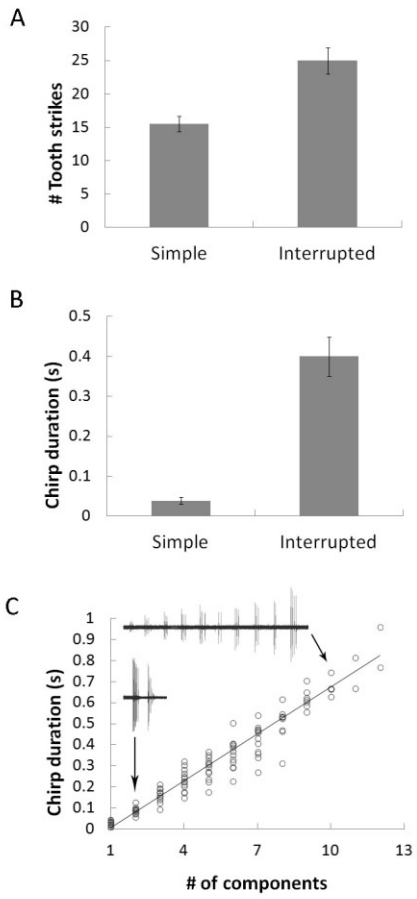

Figure 2.3. Comparison of simple and interrupted chirp properties including (A) the average number of tooth strikes per chirp and (B) the average chirp duration. (C) There was a strong positive correlation between the number of components contained in the chirp and its duration (1 component = simple chirp; multiple components = interrupted). Insets illustrate examples of chirps with 2 and 10 components.

simple chirps, they do not. Rather, they switch to a categorically different type of chirp, the interrupted chirp, which may advertise his skill in addition to his size.

Variability within individuals: Chronological variation in male courtship chirp performance

Performances were dynamic in the sense that the majority of individuals produced both simple and interrupted chirps, while varying the ratio of interrupted to simple chirps produced at different stages of an encounter. This is a novel concept; previous studies assumed only one signal type was important to the context and did not consider the mix of signals over the course of a performance. Signalling effort is greatest in the first half of the encounter, where we see the highest chirp rate (average rate ± SE during the first half of an encounter: 1.03 ± 0.1 s^{-1}; during the second half of an encounter: 0.60 ± 0.1 s^{-1}; Fig. 2.4A), and more interrupted chirps (average 62.2 ± 9.4 % of chirps during the first half of an encounter, as opposed to 43.7 ± 14.4 % during the second half of an encounter; Fig. 2.4B).

Variability between individuals: Presence/abundance of interrupted chirps and relationships to body size

Not all males produced interrupted chirps during courtship. Of the 25 trials with intact males, five males (20%) produced exclusively simple chirps, and there was wide variation in the proportion of interrupted to simple chirps produced by males (Fig. 2.5A). Larger males tended to have shorter performances before acceptance (i.e. they stopped

signalling sooner, r^2=0.46, p=0.11; Fig. 2.5B), but they produced more chirps overall (r^2=0.53, p=0.07; Fig. 2.5C), although this trend was not significant. Larger males produced chirps at a significantly higher rate (r^2=0.63, p=0.02; Fig. 2.5D), with a significantly larger portion of those chirps being interrupted (r^2=0.60, p=0.04; Fig. 2.5E). Additionally, larger males tended to produce interrupted chirps with more components (r^2=0.54, p=0.06; Fig. 2.5F).

Female preference

Not every male was accepted by the female. In the 25 trials of intact males, 23 males were accepted (92%); additionally, in the five trials with muted males, three were accepted (60%) (Fig. 2.6A). Moreover, females differed in their acceptance rate of non-manipulated males that had different signalling behaviours: 100% of males that produced interrupted chirps were accepted by females whereas those males that were rejected produced only simple chirps (i.e. 40% of males that produced exclusively simple chirps were rejected) (Fig. 2.6B). These acceptance rate differences based on a male's chirping performance (interrupted, simple or mute) are significant (Fisher's Exact Test, p<0.01).

Males that produced interrupted chirps had an easier time at entry. In each of the trials where males performed exclusively simple chirps, including those where the male was eventually accepted, we saw instances of active resistance of the female towards the male. Figure 2.7 illustrates an example from one such trial where the female's body can be seen at the entrance hole blocking the male's access. Although this

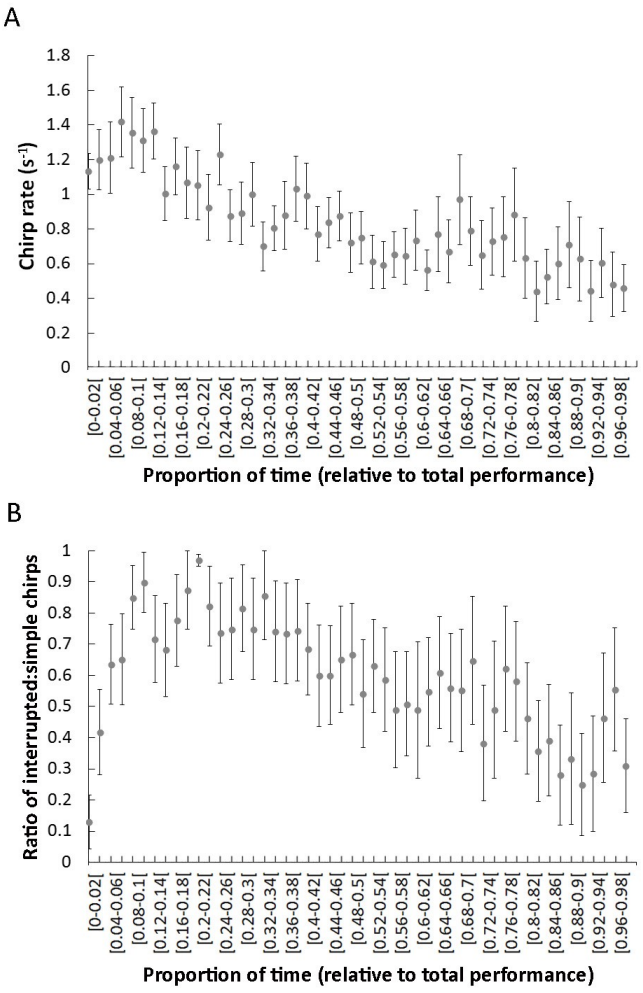

Figure 2.4. Dynamic signalling: changes in performance over the course of an encounter. Because every individual's performance was of a different duration, we averaged data ± SE for every 2 % interval relative to the whole performance for all 25 intact males during male-female trials. (A) Over the course of the performance, individuals decreased their chirp rates. (B) During the initial stages of an encounter (the first 5% of a performance), males produced predominantly simple chirps, but switched to mainly producing interrupted chirps throughout the first half of the performance. By roughly halfway through their performance, males tended to switch back to producing a majority of simple chirps.

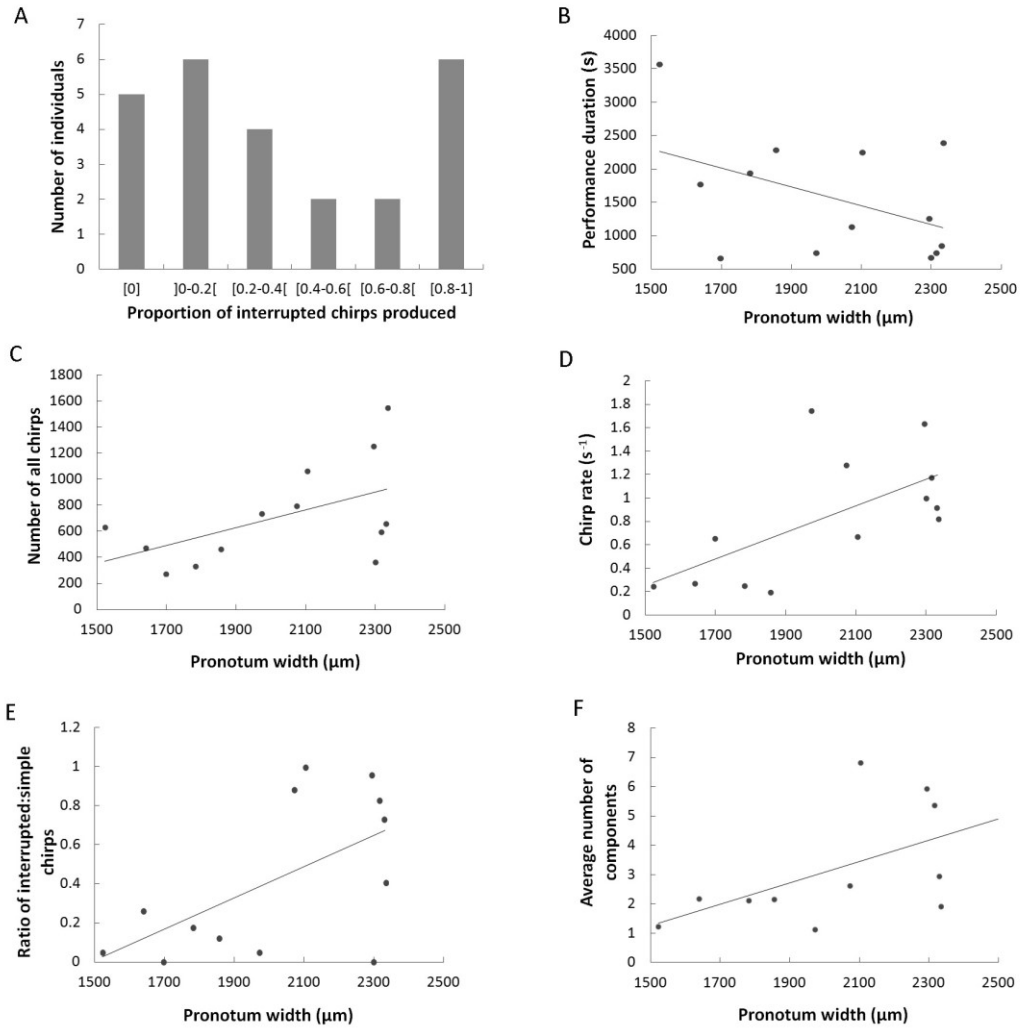

Figure 2.5. Measurements of between-male variability. (A) Each male's chirp train varied in terms of the proportion of interrupted chirps performed. Although most of the males (total n=25) produced at least some interrupted chirps, 5 males produced exclusively simple chirps. Correlation between body size (pronotum width) and (B) performance duration, (C) the total number of chirps in a performance, (D) average chirp rate for entire performance, (E) ratio of interrupted to simple chirps for entire performance and (F) average number of components per interrupted chirp. Sample size equals 13 for B-E, with 11 males producing at least some interrupted chirps while two produced exclusively simple chirps; in (F) only interrupted chirps are included (n=11).

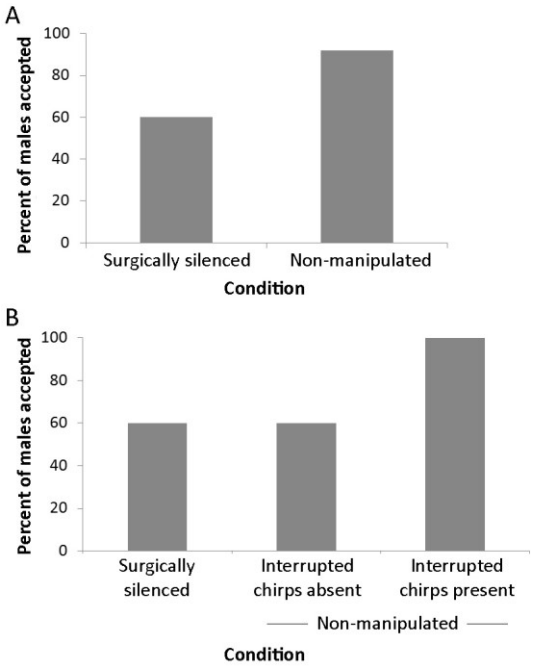

Figure 2.6. Relationship between male success and signalling performance. (A) Males that were surgically silenced (n=5) had less success at being admitted to galleries than their non-manipulated, signalling counterparts (n=25). (B) Data for surgically silenced males (n=5) is compared to data for intact males that produced exclusively simple chirps (n=5) and intact males that produced at least some interrupted chirps (n=20). Individuals that did not produce interrupted chirps had equal occurrences of rejection to those that were muted; meanwhile, all individuals that produced interrupted chirps successfully entered the gallery.

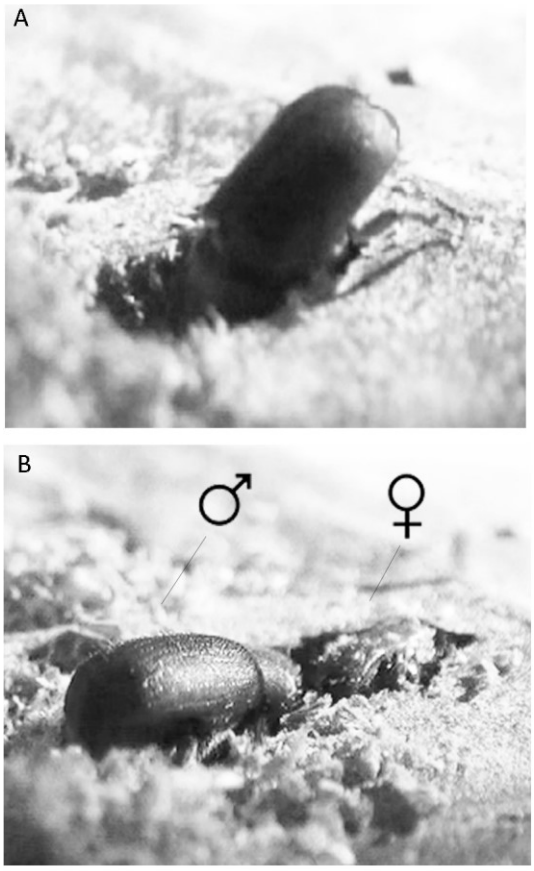

Figure 2.7. Video frames of female blocking a silenced male. (A) The silenced male begins his initial attempt at entering the gallery. (B) At 2 minutes into the interaction, the female emerges and pushes the male backwards, completely blocking the entrance to the gallery.

blocking behaviour was seen in trials where the male produced interrupted chirps as well, females yielded more quickly to males that performed interrupted chirps (average time for males to enter ± SE: interrupted chirps present: 19.8 ± 7.2 minutes; silenced males + those producing exclusively simple chirps: 82.7 ± 31.6 minutes).

2.5 Discussion

Acoustic courtship songs in insects are believed to play an important part in successful mating, but the extent to which females use these signals to choose males remains unclear (Sueur and Aubin 2004; Tregenza et al. 2006). As predicted, male courtship songs in the red turpentine bark beetle contained individual variability, supporting the idea that they convey information on male condition. Furthermore, we found support for a link between acoustic performance and male body size, an indicator of quality in bark beetles. Our results support the hypothesis that courtship signals function in female choice.

Male courtship signals vary between individuals

We demonstrated that male performance during courtship is not uniform. The production of interrupted chirps was the rule, rather than the exception, with 80% of individuals having produced at least some interrupted chirps. This is in contrast to original reports that during courtship *D. valens* males produce a homogeneous performance of simple chirps (Ryker and Rydinsky 1976a). Moreover, there was a great deal of variability between individuals in terms of the duration of their signalling

performance, the total number of chirps produced, the rate at which they produced them, the ratio of interrupted to simple chirps, as well as the attributes of the interrupted chirps, such as number of components (Fig. 2.5). This variability was related to signaller body size. Body size in bark beetles is positively correlated to various features of fitness (see below); therefore, the information present in the chirps relating to body size could be a valuable resource for the female.

Female choice

We found that differential mate acceptance exists in *D. valens*, with not all males being granted access to a gallery. This variation in a male's ability to acquire a mate was linked to the presence or absence of interrupted chirps in their courtship song repertoire. One hundred percent of those males that produced interrupted chirps were granted access to the galleries. Meanwhile, males without any interrupted chirps in their performance (either owing to having simple-only performances or to having been muted) were admitted only 60% of the time (Fig. 2.6). Although earlier muting studies in other bark beetle species have suggested that signalling plays a role in successful mating by showing that silenced males are often refused entry by the female (e.g. Wilkinson et al. 1967; Barr 1969; Ryker and Rudinsky 1976b), our results provide evidence addressing the outstanding question of whether females can discern between signal characteristics, and which characteristics are meaningful to the female. The majority of males produced interrupted chirps, and those males were invariably admitted into the gallery; conversely, the smaller group of males without interrupted chirps had large variability in

admittance. Thus, both the prevalence of interrupted chirps and the females' responses to performances lacking interrupted chirps strongly suggest that interruptions are an important consideration to females when making mate choice decisions.

One question that remains from this study is why was there such a high acceptance rate, with even some males producing exclusively simple chirps permitted to enter the female's gallery? It could be that female *D. valens* are operating a fixed threshold tactic (Reid and Stamps 1997), where a threshold level of stimulation is required before she will admit a male (e.g. stimulation thresholds to elicit a behavioural response as seen in crickets; Marsat and Pollack 2010). We found that males with more attractive performances (i.e. those that included interrupted chirps) were granted access into galleries sooner; likewise, larger males performed for shorter periods of time (Fig. 2.5B). It may be that males with less attractive performances (i.e. fewer to no interrupted chirps) needed to compensate by signalling for a longer period of time, or by producing additional tactile or pheromone stimulation (Candolin 2003), before satisfactorily stimulating the female. Additionally, sexual selection can be a context-dependent process (Jennions and Petrie 1997; David et al. 2000; Jia et al. 2000; Qvarnström 2001), and our study did not control for female variation. Arguably, standards should be lowered from the most preferred mate in a less fit female in order to ensure she mates with any male (Milinski and Bakker 1992; Jennions and Petrie 2000). Accordingly, Reid and Baruch (2010) presented evidence that larger female *Dendroctonus ponderosae* are choosier than smaller females. Because the females in this study were captured from the wild, age, stress, and prior mating status of females

was not controlled for. While re-emergence rates of *D. valens* have never been reported, other *Dendroctonus* species have been found to successfully re-emerge and establish multiple broods (e.g. Coulson et al. 1978; Gagne et al. 1982; Anderbrant 1989). This phenomenon has largely been looked at only for predatory species, of which *D. valens* is not in its native range. Consequently it is possible that rejection rates in this study are attributable to uncontrolled female variability in choosiness. Understanding the tactics females employ when making mate choice decisions will be important to gain a full understanding of the significance of male acoustic signals during mating and the extent of their role in female mate choice decisions.

Why have two types of chirps?

We propose two hypotheses as to the advantage for males to switch from simple to interrupted chirps, and these two hypotheses need not be mutually exclusive. First, by inserting interruptions, a male can lengthen the duration of a chirp, and longer chirps could provide honest information to the female about body size. Body size in bark beetles is directly linked to fitness: larger bark beetles are more likely to fly, and can fly longer and farther (e.g. in *D. ponderosae*, Evenden et al. 2014); the number of eggs laid and egg hatchability is higher in larger females (e.g. in *D. ponderosae*, McGhehey 1971); larger males tend to produce more and larger offspring (e.g. in *Ips pini*, Reid and Roitberg 1995); and larger individuals are increasingly successful at re-emergence and establishment of a second brood (e.g. in *Ips* spp., Botterweg 1982; Anderbrant 1989). Yet, chirp duration alone may not sufficiently explain why males switch to interrupted

chirps, because, conceivably, a male could just increase the duration of his simple chirps owing to the fact that there are more teeth on the file than are used during a chirp. This suggests that there is something about interrupted chirps, in addition to their duration, that is attractive to a female. We propose that a male's acoustic performance may provide the female with information on his vigour. Like body size, vigour is coupled to fitness (Byers at al. 2010). We speculate that the gaps in interrupted chirps are produced by a more complex motor performance than required for simple chirps, and skill in the performance of a challenging action may also be a reliable indicator of fitness and developmental stability because the skill necessarily reflects musculoskeletal, nervous and sensory system function (Byers et al. 2010). Future research should characterize the sound production mechanism, focusing on describing the mechanical skill required to produce the interruptions and the energy requirements of signal production.

A possible alternative function for male chirps during courtship

An intrasexual selection function could be a potential alternative explanation for the presence of male acoustic signals during courtship encounters. Interrupted chirps in particular have previously been speculated to signal aggression in *Dendroctonus* spp. (Ryker and Rudinsky 1976a), and it could be that males' chirps are targeted to potential rival males. Rivalry sounds are considered to be signals that play a role in establishing dominance (Alexander 1967). Males of several *Dendroctonus* species have been found to produce acoustic signals classified as "rivalry signals" following this definition. These

signals have been observed in both unnatural conditions, such as by confining at least two males in close proximity (e.g. Rudinsky and Michael 1974; Ryker and Rudinksy 1976b; Fleming et al. 2013), as well as during more natural circumstances where either two males were released to a female's gallery entrance simultaneously, or a second "intruder" male was added to a gallery containing an already established mated pair (e.g Rudinsky and Michael 1974; Rudinsky and Ryker 1976; Ryker and Rudinsky 1976a). These earlier studies have been in agreement that putative rivalry chirps are qualitatively different from chirps that have a putative male-female function (e.g. Rudinsky and Michael 1974; Ryker and Rudinsky 1976a). More significantly, it has been shown that *Dendroctonus* males will produce signals when alone in response to chemostimulus by conspecific female attractive pheromones (e.g. Michael and Rudinsky 1972) as well as by synthetic attractants (e.g. Rudinsky 1973), indicating that acoustic signalling in males can be provoked by females alone. That these signals are intended for females has been supported by further studies demonstrating that *Dendroctonus* females respond to conspecific male acoustic signals by the release of anti-aggregation pheromones (e.g. Rudinsky et al. 1973; Rudinsky et al. 1976) and by differential acceptance of males as mates based on the presence or absence of male sonic signalling (Ryker and Rudinsky 1976b). While it is possible that male chirping can have a rivalry function under certain conditions, in this study the absence of any potentially confounding intruder males or male pheromones limited the findings to male signalling occurring in a purely male-female context.

Conclusion

The acoustic courtship signals of *D. valens* males are complex, with two distinct types of chirps present. These chirps in general, and interrupted chirps in particular, function as passwords encouraging a female to accept a male into her gallery. Future studies on bark beetle acoustics should test hypotheses explaining how and why males produce these diverse and complex signal patterns, and what sensory mechanisms females employ to detect and process signals. How females discern between signal characteristics should be investigated to elucidate the role of courtship signals in female mate choice decisions.

2.6 Acknowledgements

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CHAPTER 3: Repeated energy loading during sound production leads to a variable acoustic repertoire in a spring stridulating bark beetle (*Dendroctonus valens*)

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3.1 Abstract

In many insects, the function of acoustic signal variability has been well studied but the proximate mechanism underlying such variation is less understood. This is the case for the most common mechanism of sound production in one of the largest animal families (Curculionidae, Coleoptera): elytro-tergal stridulation, where an abdominal scraper (the plectrum) is run along an elytral file to produce sound. This study investigates the mechanics of signal production experimentally for the first time in an insect that uses elytro-tergal stridulation, and tests hypotheses on how signal variation is achieved. *Dendroctonus valens* is a bark beetle that produces two chirp types with distinct pulse repetition patterns: simple and interrupted. Using ablation experiments coupled with high speed video and audio recordings, it was determined that the mechanism of sound production is 'spring stridulation'. In spring stridulation, elastic potential energy is stored by deformation of the angle between the plectrum and abdomen; once the deforming force is removed, stored energy drives the plectrum along the file to produce sound. The changes to pulsation pattern which generate variable chirp types are achieved by altering the number of energy storing events; interrupted chirps result from recharging the energy store repeatedly over the course of a chirp. This is a novel method of employing elastic energy to incorporate variability into an individual's acoustic signal repertoire.

3.2 Introduction

Variation in the acoustic repertoires of animals is widespread, and has important functional implications including conveying different messages, enhancing the efficacy of transmission in different environments (Hebets and Papaj 2005) and affecting reproductive isolation and mate choice decisions (Gerhardt and Huber 2002). Thus, determining the mechanism of variable sound production has great value in elucidating the evolution of species. Within insect species, behaviourally significant variations in acoustic repertoires exist as well (Alexander 1957). However, the proximate mechanisms that underlie this variation in insects are little understood, and the focus of study of acoustic communication has largely been on the functions of signals (Podos and Patek 2014). The purpose of this study is to explore the proximate mechanisms underlying signal production and variability in a bark beetle (Scolytinae).

Bark beetles are a large subfamily that exhibit extensive variation in their acoustic repertoires both within and between species (eg. Ryker 1988). Between species, there exist three distinct structures for sound production - the elytro-abdominal tergite type, the gula-prosternal type, and the vertex-pronotal type - each so named for their anatomical location (Barr 1969). Within species, individuals use only one type of sound-producing structure to generate distinct chirps with variable temporal patterns. For instance, within the destructive genus *Dendroctonus*, males produce sound by an elytro-tergal stridulatory mechanism with an apparent pars stridens (file) located near the distal margin on the underside of the elytra, and an abdominal plectrum (scraper) located medially on the seventh abdominal tergite (Barr 1969). Using this singular

mechanism, an individual male can produce two distinct types of chirps - simple and interrupted - which are so named because of their respective continuous or interrupted sound pulse patterns (e.g. Lindeman and Yack 2015; see Chapter 2 of this thesis). These variable signals purportedly convey different information (territoriality, attraction, courtship, aggression and stress; Ryker 1988).

Little is known of how *Dendroctonus* spp. bark beetles use the elytro-tergal stridulatory mechanism to produce their diverse chirps. It has been proposed that males generate chirps by scraping the abdominal plectrum against the elytral file (e.g. Hopkins 1909; Barr 1969) and subsequently that each pulse contained in the chirp results from the impact of one tooth on the file being struck by the plectrum (in that pulses are referred to as a "tooth strikes", e.g. Michael and Rudinsky 1972; Ryker and Rudinsky 1976a). More generally, there is a dearth of information on the mechanics of elytro-tergal stridulation as a whole, despite the fact that it is the most common means of sound production in one of the largest animal families, Curculionidae (Lyal and King 1996). *Dendroctonus valens* is an ideal animal to test hypotheses on the mechanics of elytro-tergal stridulation and variable sound production because it produces both simple and interrupted chirps (Lindeman and Yack 2015), giving it the interesting variable signalling repertoire typical of bark beetles, and because it is one of the largest bark beetles, which facilitates examination of the sound producing structures.

The focus of this study was to elucidate the mechanical events contributing to sound production and sound variation in the elytro-tergal stridulator *Dendroctonus valens*. Four goals were identified. The first two goals were to build upon what has been

proposed but not confirmed of *D. valens* stridulation. The first goal was to identify whether both distinct chirp types are produced in a disturbance context and to describe their characteristics. The second goal was to confirm the anatomical structures proposed to be involved in sound production and to resolve whether each pulse in a chirp is the product of one tooth of the file being struck.

The third goal was to elucidate the mechanism of generating sound pulses and how variation is incorporated. First, two alternative hypotheses regarding how the plectrum strikes the file were considered: either the movement of the plectrum - and thus sound production - occurs in tandem with the body structure the plectrum is affixed to, as occurs for sound production and wing movements in crickets and some katydids (e.g. Montealegre-Z and Mason 2005; Prestwich and O'Sullivan 2005), or the plectrum can store elastic energy enabling an uncoupled movement between the plectrum and its associated body structure, as is the case in ultrasonic katydids (Morris and Pipher 1972; Montealegre-Z et al. 2006). For *D. valens*, in the case of the former hypothesis one would predict that the rate at which the plectrum strikes the file would coincide roughly with the velocity of movement of the abdomen. Conversely, the elastic energy hypothesis would require that the plectrum be highly flexible, enabling the storage of elastic energy through deformation (Montealegre-Z et al. 2006). Upon release from the deforming force, the rate at which the plectrum travels along the file is predicted to be independent of the velocity of movement of the abdomen. Regarding the incorporation of interruptions to produce variable chirps, three, non-mutually exclusive hypotheses were tested. The first hypothesis was that interruptions would be

introduced by rapid repetition of the sound production cycle, such as is seen during pulse production in cricket chirps, where each pulse and its subsequent brief period of silence are produced by one wing closing cycle (e.g. Otte 1992). The second hypothesis was that the plectrum would pause its movements while passing down the file, subsequently resulting in the sound pulse interruptions, as seen in katydid spring stridulators (e.g. Montealegre-Z et al. 2006). A third hypothesis was that interruptions would occur from skipped teeth as the plectrum moves along the file, as seen in experimental manipulations where teeth were removed from the file in katydids (Montealegre-Z and Mason 2005), and reported for "anomalous" interruptions in the sounds of *Gryllus campestrus* (Bennett-Clark and Bailey 2002).

The fourth goal was to test how the sound emitter vibrates to generate the spectral properties of the sound. Two hypotheses were tested: that the emitter might be sent into free vibration at its natural frequency following being struck, or it may be forced into vibration at the frequency of the strike rate (following Bailey and Broughton 1970). These hypotheses are not mutually exclusive, and are often seen to co-occur in stridulating insects so that the rate of stimulation (in other words, the strike rate of teeth on the file) matches the sound emitter's natural frequency, resulting in resonance. Resonant vibration in the tegmina for example has been reported for all cricket species studied to date (Koche et al. 1988; Bennet-Clark 1989, 2003). If forced vibration is occurring, whether in conjunction with resonance or not, then it is expected that the vibration of the sound emitter will be at a frequency equal to the rate at which the file is struck. Conversely, if the sound emitter is in free vibration there would be no such

relationship between the rate of stimulation and the frequency of vibration. Additionally, if a structure is in free vibration, then manipulating its mass will affect its frequency of vibration because the natural frequency of a system is equal to its stiffness (N/m) divided by its mass (kg). Alternatively, in a structure being forced to vibrate, the frequency of vibration is determined by the forcing, not by the properties of the system, and manipulating the mass will not alter the frequency of vibration and hence the spectral characteristics of the resultant sound. The manner of vibration of the *D. valens* file was examined by testing the relationship between the spectral characteristics of the sound produced, and the file's stimulation rate, as well as by testing the effect that changes in the mass of the file had on the sound frequency.

Using a bark beetle to answer questions concerning the mechanics of sound production will delineate how elytro-tergal stridulation differs from commonly described systems. Furthermore, it will generate a more comprehensive understanding of the motor performance involved in the incorporation of signal variability and as such lend perspective to the ultimate function of variability in acoustic signals.

3.3 Methods

Animals

Adult *Dendroctonus valens* (Curculionidae: Scolytinae) were collected from May-September of 2013-14 at several locations near Ottawa, Ontario, Canada (the arboretum at the Ottawa Central Experimental Farm, 45.391021,-75.70489; Carleton

Lands, Manotick, 45.183882,-75.604673; and outside Petawawa, 45.853530, -77.536156). Collection was done using Lindgren funnel traps baited with *D. valens* lure (Contech, British Columbia, Canada). Animals were kept at Carleton University and paired off into seven bolts of red pine (*Pinus resinosa*; one pair per bolt, rearing methods following Fleming et al., 2013; Appendix A of this thesis). Male offspring from these pairings were collected as adults upon emergence and used within one week. Voucher specimens are held at Carleton University.

Goal 1: Sound recording and analysis of chirp characteristics

Disturbance sounds were evoked for 30 sec recordings in 63 males by grasping their pronotum and head gently while avoiding the elytra (Ryker and Rudinsky 1976b). Individuals were held at 2 cm from a microphone (40 kHz omnidirectional, QTC40, Earthworks, Milford, USA) connected to a data recorder (sampling rate of 192 kHz, FR-2, Fostex, Los Angeles, USA). Using Raven Pro 1.5 (Raven Bioacoustics Research Program, Cornell Laboratory of Ornithology, Ithaca, NY, USA), an initial sample of 376 chirps from the 63 males were divided into simple (n=320) or interrupted (n=56) chirp types as based on their clear presence or absence of interruptions, as done previously (i.e. simple chirps identified as having approximately regularly spaced pulse intervals, while interrupted chirps identified as having obvious interruptions to the pulse repetition pattern, based on the appearance of the waveform, e.g. Ryker and Rudinsky 1976b). This sample was then used to quantitatively characterize the maximum pulse interval for a simple chirp; all chirps with pulse intervals greater than the cut off could then be

identified as interrupted (methods as for Chapter 2; Lindeman and Yack 2015). Following this analysis, all of the chirps from each of the 63 disturbance recordings were identified as simple or interrupted based on their calculated maximum pulse intervals. The percent simple and interrupted chirps over the disturbance trial was then calculated for each individual. Once all chirps were identified by type, ten simple and when possible 10 interrupted chirps were sampled randomly (every fifth chirp) per individual. Not all males produced interrupted chirps, however, and some produced fewer than 10 chirps of this type over the recording, in which case all interrupted chirps from that recording were used. The temporal, spectral and amplitude domains were characterized and compared for simple and interrupted chirp types. Temporal characteristics – chirp duration, number of pulses and pulse interval – were analyzed using Avisoft SAS Lab Pro (Avisoft Bioacoustics, Berlin, Germany). Spectral and amplitude envelope characteristics were analyzed using Raven Pro 1.5 (Raven Bioacoustics Research Program, Cornell Laboratory of Ornithology, Ithaca, NY, USA) (Hamming FFT window, 512 samples). In the spectral domain, dominant frequency and bandwidth at -6 dB were recorded. In the amplitude domain, chirp envelopes were characterized by the shape of the waveform. Furthermore, sound level measurement (dB SPL) for peak amplitude analysis was done separately for simple chirps only from five individuals at distances of 1, 2, 4 and 8 cm by analyzing the peak-to-peak voltage (mV) of signals recorded using a 1/4" condenser microphone (model 4939, Brüel & Kjær (B&K), Nærum, Denmark) amplified with a B&K Nexus conditioning amplifier (model 2690) connected to an oscilloscope (THS720A,

Tektronix, Richardson, USA). All recordings were performed in a walk-in type acoustic chamber maintained at 22 ± 2^{o}C.

Goal 2: Anatomical structures of the sound producing organ

Ablation trials

A file (pars stridens) occurs at the posterior margin of the underside of the elytra directly under the wing lock region, occurring mainly on the left elytron but extending slightly onto the right elytron in what appears as an unbroken, continuous file across both elytra when they are in the locked position (Michael and Rudinsky 1972). However, it has been proposed that to produce sound the file is struck mainly along the median line, and because the portion of the file on the right elytron is a great distance from the median line it would seldom be used in sound production (Michael and Rudinsky 1972). To test the hypothesis that only the portion of the file on the left elytron is used in sound production, disturbance sounds were evoked in 30 sec trials as above and chirp characteristics were compared between intact males and when either their left elytron was ablated (n=5) or their right elytron was ablated (n=6).

To test the hypothesis that the protrusion located medially on the seventh abdominal tergite functions as a plectrum in sound production, ablation trials were required to visualize the movement of the structure and its interaction with the file during sound production since both the file and abdominal tergites are normally obscured by the elytra when stridulation is occurring. The right elytra of six males were

removed and disturbance sounds were evoked as above. Male stridulation was filmed in high speed using a camera (frame rate: 240 fps, GoPro Hero 3, GoPro Inc., USA) connected to an external microphone (ECM-MS908C, Sony, via a GoPro 3.5mm microphone adapter) and mounted to a two-head observation light microscope (Leica, Wild M3Z, Wetzlar, Germany). Sounds were simultaneously recorded by an Earthworks microphone to a data recorder as described above. Audio was matched to the high speed video using Raven Pro 1.5 by aligning several pure tone test sounds made throughout the recording. The video was analyzed to assess the position of the seventh abdominal protrusion during sound production and determine whether the protrusion served the role of the plectrum by striking the file to produce sound.

Pulse Production

For bark beetle chirps the pervading assumption is that one pulse results from one tooth being struck by the plectrum, unlike the pulses production by crickets which incorporate many tooth strikes. This assumption was tested by comparing the number of sound pulses per chirp to the estimated number of teeth traversed. Analysis was done using simple chirps recorded during the six high speed trials above in conjunction with measurements of individual male file morphology. The distance travelled by the plectrum along the file during chirp production was estimated by calculating the distance between the position of the plectrum on the file at the first pulse and last pulse of a chirp. The distance estimates were based on the size of the animal calculated by Zeiss AxioVision digital image processing software (Oberkochen, Germany) from images

taken with a camera (Zeiss AxioCam MRc5, 1.4 megapixels, 1388 x 1040, Oberkochen, Germany) mounted on a light microscope (Olympus SZX12, Tokyo, Japan). The number of teeth and length of the file were determined using scanning electron micrographs (see below). Teeth were always counted along the medial line of the file to account for inconsistent tooth morphology along the edges where individual teeth occasionally disappeared or bifurcated (see Fig. 3.3A). The same distance that the plectrum was estimated to have travelled over the course of a chirp was measured along the individual's file and the number of teeth over that distance of file was calculated. The number of teeth over that distance was then compared to the number of pulses in the chirp. Tooth morphology analysis revealed successive increases in inter-tooth spacing over the length of the file (see pulse production section in results). This change in tooth spacing affects the number of teeth found over a given distance of the file and thus can influence the number of teeth the plectrum can strike over a given distance depending on the region of the file it travels over. There was no way to visually identify the exact start point of the plectrum on the file; consequently, tooth counts were made beginning at two alternative positions: either at the anterior most tooth, or at a position 1/3 of the way along the file from the anterior end (see Fig. 3.3A).

Goal 3: The mechanics of producing simple and interrupted chirps

The two alternative hypotheses regarding the interaction between the plectrum and file during sound production - that the plectrum either moves with the abdomen to produce sound or that their movements are uncoupled and the plectrum moves via

independently stored energy - were tested by determining whether plectrum movement and resulting sound production occurred in tandem with abdominal movement. High speed trials (from above) were analyzed to establish the location, direction and duration of stimulation by the plectrum during sound production. Furthermore, the angle of the plectrum relative to the abdomen was calculated during sound production, to determine whether deformation of the plectrum occurred. The three hypotheses for generating interruptions (i.e. by repeated sound production cycles, paused motion or skipped teeth) were then tested by comparing the plectrum-file interaction during simple and interrupted chirps.

Goal 4: Determining how the sound emitting structure vibrates to generate the dominant frequency

The sound emitting structure may vibrate to generate the dominant frequency in two general ways: the vibration frequency may be forced by the rate at which it is struck, or once struck it may be free to vibrate at its natural frequency. If it is in forced vibration, it is predicted that (1) the dominant frequency will be dependent on the rate at which the structure was struck, and (2) the energy in the system will be continuous (i.e. will not dissipate between strikes). These predictions were tested by comparing the tooth strike rate to the sound dominant frequency, and by determining whether or not the sound energy dissipated completely between tooth strikes. Conversely, if the sound emitting structure is in free vibration, it is predicted that (1) the dominant frequency will not equal the rate at which the structure is struck, and (2) the dominant frequency will

change if the natural frequency of the structure is manipulated, such as by adding or removing mass. To test the second prediction, the sound emitting structure was manipulated by adding and removing mass to and from the elytra. To add mass to the structure, one or two glass beads was added to two alternate locations along the elytra (the posterior margin – directly on top of the file – or medially along the longitudinal plane; n=5 for each). Weight was measured using a Sartorius CP224S Analytical Balance (Data Weighing Systems, Inc. Illinois, USA). Beads (average weight ± SE of one bead 2.1 ± 0.1 mg) were light relative to the entire beetle (average live body weight of whole beetle ± SE: 29.3 ± 1.7 mg), but were approximately double the weight of the elytra (average weight of both left and right elytra together: 1.3 ± 0.1 mg), making the elytra significantly heavier when the beads were affixed. To remove mass from the structure, a portion of the elytra was ablated. When *D. valens* males are at rest, their elytra are locked with the right elytron sitting atop the left at the location of the file, adding weight to the file. Consequently, the right elytron was removed and comparison of the dominant frequency of intact to ablated males was measured (n=6).

Scanning electron microscopy

Scanning electron micrographs of the seventh abdominal tergite (the plectrum) and the underside of the medial sutures of the left and right elytra (the file) were obtained from the six males used for the high speed video analysis. Elytra were cleaned using warm water (30^{o}C) in an ultrasonic vibrator (Magnasonic) and placed on aluminum stubs. The abdomens were prepared for SEM by dehydration with hexamethyldisilazane

following the procedure outlined by Rumph and Turner (1998) and placed on separate aluminum stubs. Specimens were sputter coated with gold-palladium and examined with a JOEL JSM-6400 scanning electron microscope.

Statistical analysis

Student's t tests assuming equal variance were done in all cases of comparing temporal characteristics of simple and interrupted chirps and interrupted chirps and trailing pulses, and to compare spectral characteristics of simple and interrupted chirps. In all other cases paired sample t tests were performed. A linear correlation using Pearson's r determined the relationship between the number of sound pulses and teeth on the file, as well as for that of stimulation rate and dominant frequency. Finally, analysis of variance tests were done to compare dominant frequencies of weight loaded, weight reduced and non-manipulated males. All probability tests were two tailed and had α set at 0.05 and were done using SPSS (SPSS Inc., Version 19, Chicago IL, USA).

3.4 Results

Goal 1: Signal structure of the two chirp types

The temporal, amplitude and spectral domains of simple and interrupted chirps were analyzed to determine any differences between chirp types. In the temporal domain, the maximum pulse intervals of 320 clearly simple chirps and 56 clearly

interrupted chirps from 63 males were analyzed. Almost all simple chirps had a maximum pulse interval of less than 5 ms (99.7%) while all interrupted chirps (100%) had maximum pulse intervals greater than 5 ms (consistent with the findings for simple and interrupted chirps produced during courtship, Lindeman and Yack, 2015) (Fig. 3.1A,B). Consequently, all chirps from the entire recording of the 63 males (n= 6,161 chirps) were categorized into simple and interrupted chirp type based on the 5 ms maximum pulse interval cut off; chirps with pulse intervals less than 5 ms were categorized as being simple while chirps with pulse intervals greater than or equal to 5 ms were categorized as being interrupted. While both simple and interrupted chirps were found, interrupted chirps were far less common with all males producing simple chirps and only 26.6% of males producing any interrupted chirps. Within the recordings containing interrupted chirps, this chirp type accounted for only on average 9.2 ± 3.4% of the total chirps produced by that individual (average ± SE number of simple chirps per individual 95.8 ± 4.5; number of interrupted chirps per individual 2.0 ± 0.9).

Simple chirps were significantly shorter in duration than interrupted chirps (average ± SE: simple 13.6 ± 0.6ms, interrupted 49.7 ± 5.4ms, $t_{81,2}$=-11.6, p<0.0001), had significantly fewer pulses per chirp (average number of pulses ± SE: simple 9.1 ± 0.4, interrupted 14.4 ± 1.1, $t_{81,2}$=-5.5, p<0.0001), and had significantly higher pulse rates (average ± SE: simple 695.1 ± 15.1 pulses/s, interrupted 342.2 ± 29.0 pulses/s, $t_{81,2}$=11.0, p<0.0001) (Fig. 3.1A,B). The difference in pulse rate was due to the interruptions; when pulse rate was measured for each component of an interrupted chirp there was no difference from the pulse rate of simple chirps (average ± SE of interrupted chirp

component pulse rate: 701.4 ± 36.0, $t_{78,2}$=-0.2, p=0.86). Apart from the main simple and interrupted chirps, low-amplitude pulses that trailed after the chirp were discovered for approximately one quarter of individuals (23.8%) following a small subset of their chirps (14.2 ± 4.6%) (Fig. 3.1C). These 'trailing pulses' are here identified as a novel component of *Dendroctonus* sound production. Trailing pulses began 141.4 ± 10.9 ms following the chirp (either simple or interrupted). This interval was significantly longer than the maximum pulse interval for any chirp, including interrupted chirps (average maximum pulse interval for interrupted chirps ± SE: 22.4 ± 2.1 ms, $t_{30,2}$=-12.7, p<0.0001). The number of trailing pulses per chirp ranged from one single pulse to up to 10 pulses (average number of pulses ± SE: 4.0 ± 0.4). How trailing pulses were produced was examined and discussed in the "production of trailing pulses" section below.

In the spectral domain, both simple and interrupted chirps were broadband (bandwidth at - 6 dB, average ± SE: simple 8.7 ± 0.3 kHz, interrupted 8.9 ± 0.9 kHz, $t_{80,2}$=-0.2, p=0.83) (Fig. 3.1A,B) and there was no difference between chirp type in their spectral energy distribution ($t_{80,2}$=0.7, p=0.52). For both types, energy was found to be distributed around three frequency peaks (average ± SE for simple chirps: peak one at 6.8 ± 0.2 kHz, peak two at 19.8 ± 0.4 kHz and peak three at 31.2 ± 0.5 kHz; for interrupted chirps: peak one at 6.4 ± 0.4 kHz, peak two at 19.1 ± 0.7 kHz and peak three at 29.2 ± 1.1 kHz; Fig. 3.1A,B). There was an overall bimodal energy distribution in both chirp types: peaks one and three had the most energy in the chirp and, because they tended to be approximately the same intensity, they had equal occurrences of being the dominant frequency. Finally, within interrupted chirps the spectral energy distribution

for each component was not different than that of the chirp as a whole (average dominant frequency ± SE of interrupted chirp components: 21.4 ± 3.2 kHz, $t_{10,2}$=0.1, p=0.91) (Fig. 3.1B).

In the amplitude domain, the amplitude envelopes of simple and interrupted chirps had clear differences. Simple chirps had overall descending amplitude envelopes (Fig. 3.1A,D). Interrupted chirps had irregular envelopes overall when considering the entire chirp (Fig. 3.1B,E); however, individual components of interrupted chirps had descending envelopes, similar to simple chirps (Fig. 3.1B). Regarding sound intensity, chirps were loud enough to be heard by the unaided human ear at distances of a few feet away, with simple chirps having measured intensities of 72.9, 68.2, 64.7 and 60.8 dB SPL at 1, 2, 4 and 8 cm, respectively. Simple and interrupted chirps had similar intensities, with no difference between their peak amplitudes (calculated as relative intensities of chirps produced at equal distances from the microphone; $t_{12,2}$=0.98, p=0.35). Conversely, trailing pulses had small amplitudes relative to the main chirp that they followed (average ± SE: chirp pulse intensity 0.13 ± 0.03 arbitrary units, trailing pulse intensity 0.04 ± 0.01 arbitrary units; $t_{12,2}$=4.3; p<0.001). Each trailing pulse was similar in amplitude resulting in an overall rectangular-shaped envelope for this group of pulses. The amplitude envelopes of simple chirps, interrupted chirp components and trailing pulses are a product of their mechanism of production, discussed below.

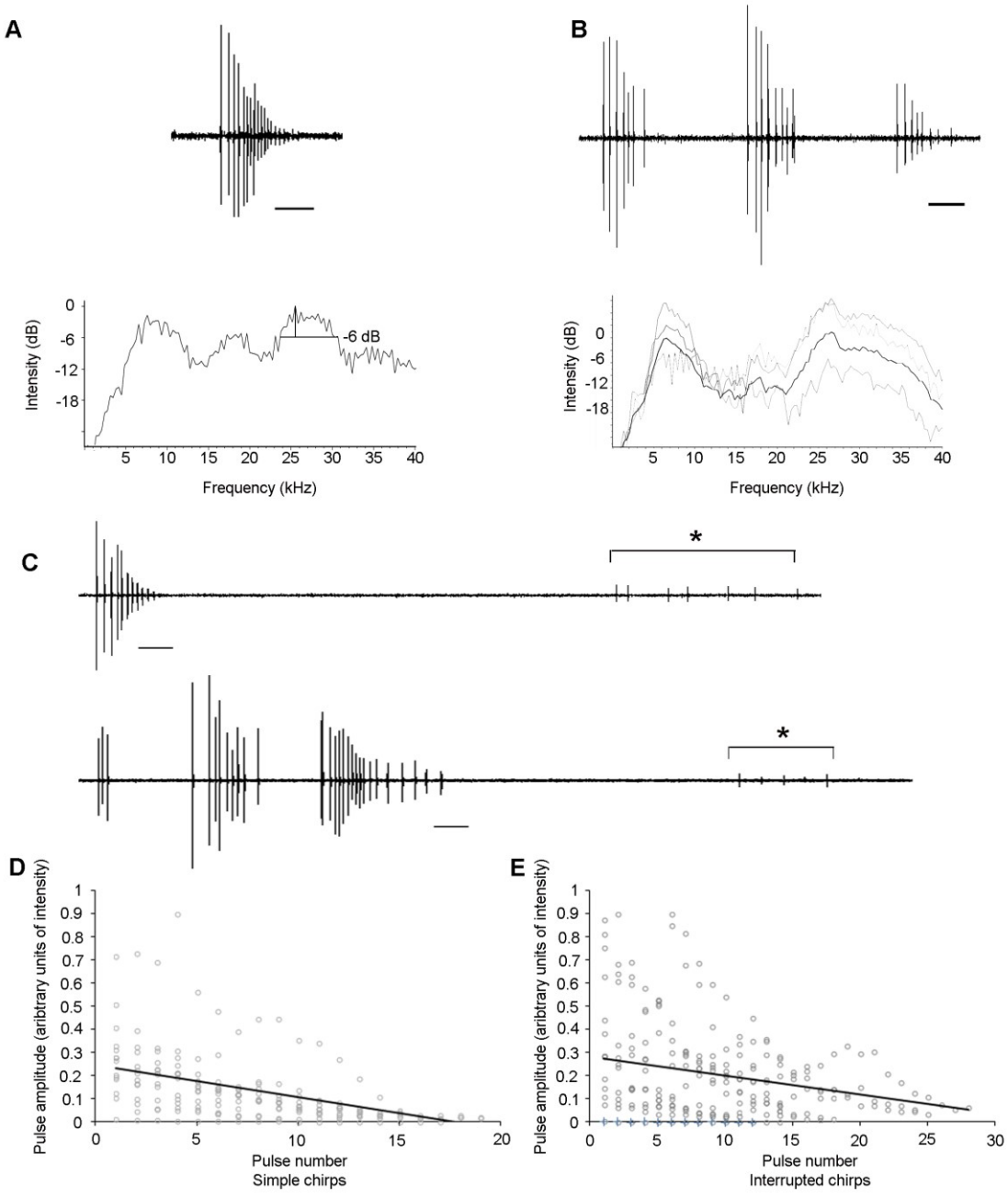

Figure 3.1. Chirp production in male *D. valens*. The waveform (top) and frequency spectrum (bottom) of (A) one simple chirp, with line indicating bandwidth measured at -6 dB, and (B) one interrupted chirp with three components, frequency spectrum depicts the spectral energy distribution on the whole chirp (in black) and each component (in grey). (C) Top: one simple chirp followed by seven trailing pulses; bottom: one interrupted chirp with three components followed by five trailing pulses. Trailing pulses identified by asterisks . (D-E) Descending amplitude of sequential pulses over the span of a chirp for simple chirps (D) and interrupted chirps (E), corresponding to an overall descending amplitude envelope for both chirp types. Scale bars (A-C) = 10ms.

Goal 2: Anatomical structures of the sound producing organ

Ablation trials

Consistent with the hypothesis that only some of the teeth on the left elytron are used in sound production, ablation of the left elytron (Fig. 3.2B) completely eliminated sound (Fig. 3.2E), while ablation of the right elytron (Fig. 3.2D) resulted in no change to the chirp characteristics (average number of pulses per chirp when intact ± SE: 13.0 ± 2.6, with right elytron removed: 13.6 ± 2.9; $t_{5,2}$=-0.43, p=0.68, Fig. 3.2E). Thus, this analysis resolved that the left elytron alone is both necessary and sufficient for chirp production. Regarding the plectrum, removal of the right elytron enabled visibility of the abdomen during sound production. Once visible, the protruding structure on each male's seventh abdominal tergite (Fig. 3.2C) was observed to strike the file during sound production, confirming its role as plectrum (described in detail under General mechanism).

Pulse production

The hypothesis that one sound pulse is created by one tooth being struck was investigated by comparing the number of tooth strikes to the number of sound pulses in the chirp. Since the number of teeth struck could not be directly counted because the file was not visible within the video frame, an estimate of the number of tooth strikes had to be calculated based on the distance along the file that the plectrum travelled during chirp production. Thus, first characterizing tooth morphology and spacing was

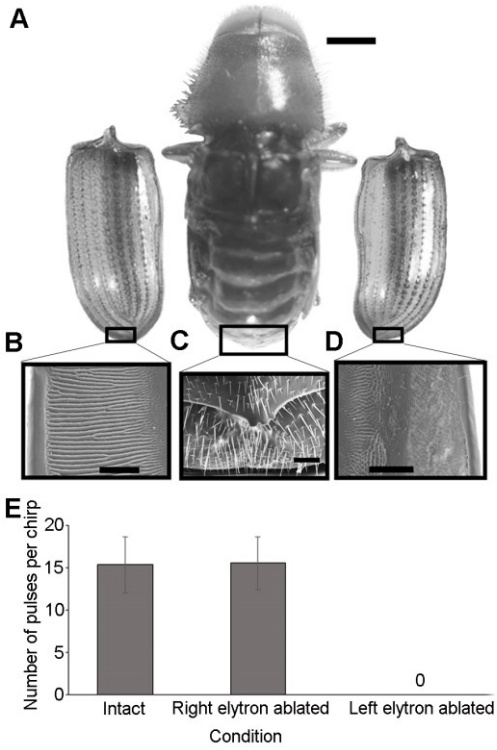

Figure 3.2. The sound producing organ of *D. valens.* (A) Light microscope image of a dorsal view of a male with his elytra removed, elytra placed upside down next to him; scale bar = 1000 µm. Boxes are associated with enlargements of regions in (B-D). SEMs of (B) the file of the left elytron, (C) the 7th abdominal tergite and plectrum, and (D) the wing lock region of the right elytron and its continuing portion of the file; scale bars = 100 µm. (E) Chirps contained the same number of pulses when the male was intact and following the removal of his right elytron; sound was absent, however, upon removal of the left elytron.

essential to obtaining an accurate estimate. It was first determined that there were more teeth along the file than there were pulses in the chirp; additionally the interval between teeth increased posteriorly along the file (Fig. 3.3B). Combined, these two findings indicated that: first, the starting position of the plectrum need not be at the anterior end of the file because not all teeth were involved in producing the chirp; and second, the starting position of the plectrum would affect the number of teeth it contacts over a given distance as teeth become increasingly spaced towards the posterior end of the file. Therefore, when estimating the number of teeth over the distance travelled by the plectrum two starting positions were considered: at the anterior most edge of the file, and at 1/3 of the way along the file. In both cases, the relationship between the number of teeth and number of pulses was very strong with an almost 1:1 relationship between teeth and pulses (for anterior estimates: $r=0.89$; $p<0.001$, slope = 0.93, Fig. 3.3C; for medial estimates $r=0.84$; $p<0.001$, slope=1.07, Fig. 3.3D). When chirps were estimated to begin anteriorly, there was a slight overestimation of the number of tooth strikes per chirp (approximately 3 teeth more, average number of teeth ± SE: 25.5 ± 1.0, pulses: 22.6 ± 1.1); conversely, when the plectrum was estimated to begin more medially, there was a slight underestimation of the number of tooth strikes per chirp (approximately 4 teeth less, average number of teeth ± SE: 18.3 ± 0.8, pulses: 22.6 ± 1.1). It can thus be concluded that each pulse is produced by one tooth on the file being struck by the plectrum and that the plectrum begins the chirp close to, but not at, the anterior most edge of the file.

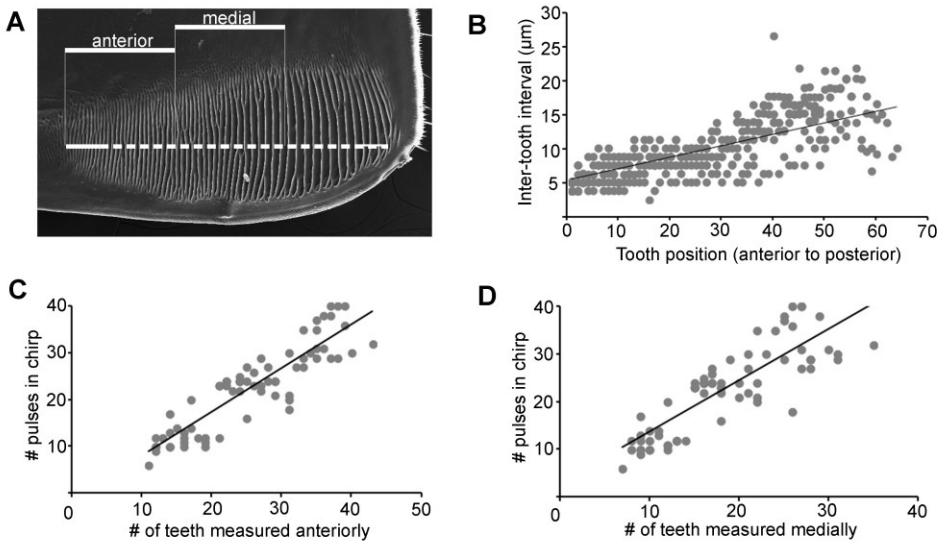

Figure 3.3. Correlation between the number of pulses observed in a chirp and the number of teeth passed over by the plectrum during chirp production. (A) SEM of male file illustrating a hypothetical sampling of teeth covering 200 µm from anterior versus medial locations. Dashed line indicates the medial line along which teeth were counted. (B) Correlation between the location of the tooth on the file (numerically, identified from anterior to posterior) and the inter-tooth distance. The distance between teeth increased at progressing locations along the file. This is also seen in (A) where 27 teeth are found over the anterior half of the file but only 18 teeth are found over the posterior half of the file. (C) Comparison of pulse to tooth number for chirps when teeth were measured anteriorly. (D) Comparison of pulse to tooth number for chirps when teeth were measured medially. In both cases (C and D) the pulse to tooth ratio is very close to 1:1, with the anterior measurements overestimating the number of tooth strikes and more medial measurements underestimated the number of tooth strikes per chirp.

Goal 3: The mechanics of producing simple and interrupted chirps

General mechanism of chirp production

The basic mechanism for chirp production (both simple and interrupted) was found to be by an initial deformation of the plectrum, which built a store of elastic potential energy that, when released, allowed for uncoupled movement of the plectrum and abdomen. Deformation of the plectrum was seen at the start of the stridulation behaviour: the plectrum locked onto the file and remained stationary while the abdomen moved posteriorly, resulting in an increased angle between the plectrum and abdomen (a small but significant increase in angle; average angle ± SE: at rest 29.7 ± 1.6 degrees, at "locked position" just prior to start of chirp 35.6 ± 2.2 degrees, $t_{5,1}$=-2.5, p=0.029) (Fig. 3.4B). When a deformation force acts on an elastic object an equal and opposite restoration force is created to return the object to its resting state; this is the stored potential energy in the object. Once the plectrum was released from where it was locked on the file, it was driven by stored potential energy and moved independently from the abdomen along the file, striking teeth along its length of travel. Because they were no longer coupled, there was a disparity in velocity of movement between the plectrum and abdomen as evidenced by the changing angle between the two over the course of the chirp (average angle ± SE: at the start of the chirp 35.6 ± 2.2 degrees, at the end of the chirp 16.6 ± 1.5 degrees. Average rate of decline: 0.7 ± 0.07 degrees/ms) (Fig. 3.4A). The decrease in angle signified that the plectrum was moving faster than the abdomen.

Production of interruptions

Of the three possible mechanisms from which interrupted chirps may arise - i.e. repeated sound production cycles, skipped teeth or paused motion - only the latter hypothesis was supported. As was seen in simple chirps, during interrupted chirps the movement of the plectrum was uncoupled from that of the abdomen, and sound production began with the plectrum locking onto the file causing its deformation as the abdomen advanced, and once released the stored elastic potential energy was used to drive the plectrum along the file. Over the course of the interrupted chirp, the plectrum moved discontinuously as it traversed the file with pauses in plectrum motion corresponding to the interval between chirp components (Fig. 3.4C). This was contrary to the movement of the abdomen which was continuous throughout the chirp. At each point when the plectrum paused it would once again lock onto the file, and the continuous movement of the abdomen would cause the plectrum to deform as at the start of the chirp. Thus, each interval between chirp components was associated with a deformation event, and each interrupted chirp component would begin with a new store of elastic potential energy to drive the plectrum further along the file. In conclusion, interruptions resulted from pauses of the plectrum's motion, and the interruptions functioned to reload the elastic potential energy stored in the plectrum.

Production of trailing pulses

Trailing pulses were produced by the plectrum striking the file as it returned to a resting position following either a simple or interrupted chirp. During this movement,

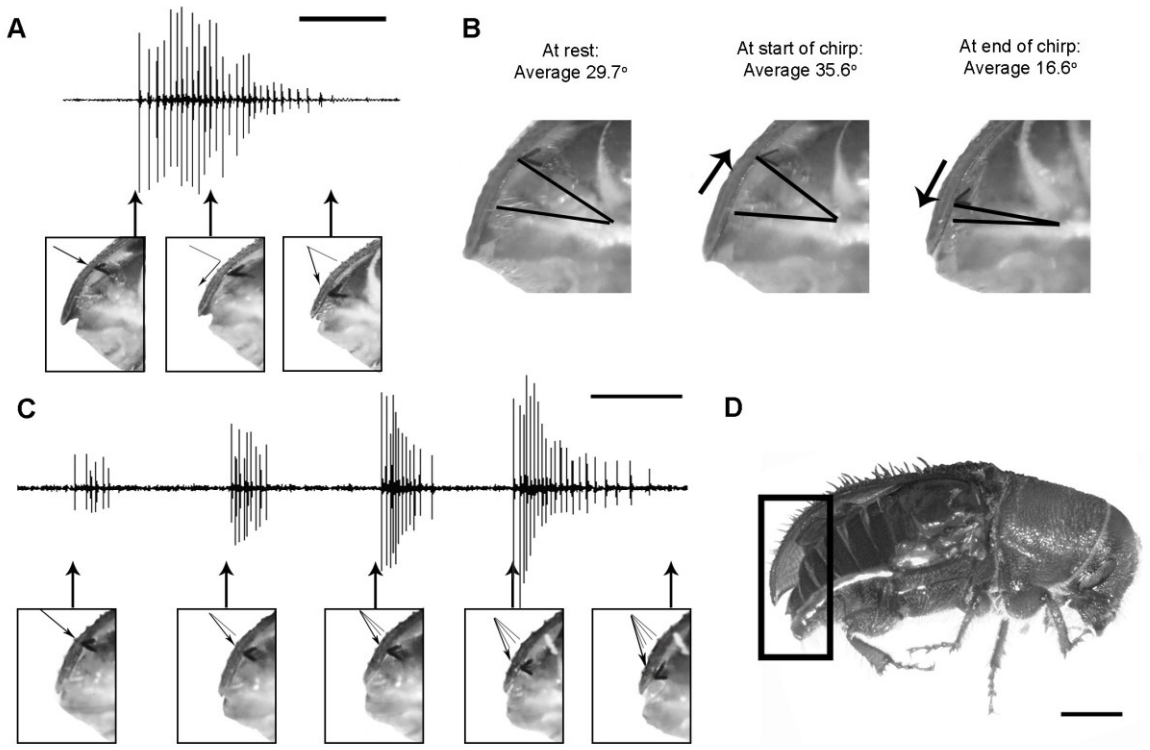

Figure 3.4. High speed analysis of *D. valens* male sound production. (A) One simple chirp where panels show a lateral view of the male's stridulatory organ (as outlined in D) corresponding to a time in the chirp. Arrows on top of panels illustrate the time in the chirp that the image still corresponds to, while arrows inside the panel point to the plectrum (outlined in black) at that time and lines point to the location the plectrum was in the previous panels. During simple chirps the plectrum moves as one smooth sweep. Scale bar = 20 ms. (B) Lateral view of a male's stridulatory organ (as outline in D) at strategic times in a chirp: at rest the plectrum has locked onto the file but has not yet been deformed; at the start of the chirp (just prior to plectrum's release from where it is locked onto the file) the angle of the plectrum has been deformed by the posterior movement of the abdomen; and at the end of the chirp (the location of the plectrum at the final sound pulse). Lines are overlaying images to illustrate the changing angle the plectrum makes with the abdominal sternites (slightly increasing before the start of the chirp and then decreasing over the course of the chirp). (C) One interrupted chirp with four components, where panels illustrate the plectrum's position corresponding to a time in the chirp, as in A). Scale bar = 20 ms. (D) Illustration of the beetle preparation. To see the plectrum, the right elytron was removed and the camera was focused on the abdomen (region highlighted by the box); scale bar = 1000 µm.

only a few of the possible teeth were struck, and stimulation was inconsistent (with not all individuals producing trailing chirps, and trailing chirps being relatively rare, see *pulse production*, above). This action was very different from the locking on and deforming action of the plectrum against the file during production of the main chirp. The movement of the plectrum in the production of these trailing pulses was in tandem with the movement of the abdomen, and it was in the anteriad direction as the abdomen returned to its original state following the chirp.

Goal 4: Vibration of the sound emitting structure

The sound emitting structure could either be forced to vibrate at the rate at which it is struck, or it could be sent into free vibration once struck. In the case of forced vibration the dominant frequency would depend on the tooth strike rate, whereas in free vibration a structure vibrates at its natural frequency, regardless of the rate at which it is struck. The average sound pulse rate (equal to the rate of stimulation, see pulse production section above) for simple and interrupted chirps (614.3 ± 21.1 s^{-1}) was too slow to produce any of the spectral energy peaks seen (~6.6 kHz for peak 1; ~19.5 kHz for peak 2; ~30.2 kHz for peak 3). Additionally, if the structure was in forced vibration there should have been a continual input of energy to maintain the vibration frequency. However, in both cases of simple and interrupted chirps each pulse was distinct, where the energy from the stimulation attenuated before the next pulse was produced, a property characteristic of a structure in free vibration. Consequently, these results support the hypothesis that the elytra were vibrating at their natural frequency.

The structure of the elytra were manipulated to further test the free vibration hypothesis. Increasing or decreasing the weight loaded on a structure will respectively decrease or increase the structure's frequency of vibration upon stimulation. In *D. valens*, because the right elytron sits atop the left at the wing lock region it is loading weight directly on top of the file. Removing the right elytron increased the average peak frequency of the chirp ($t_{5,2}$=-2.3, p=0.07) (Fig. 3.5B). Conversely, adding one or two weights directly over the file significantly decreased the dominant frequency (F=6.4, p<0.01) (Fig. 3.5C). Loading weight on the elytra at a location away from the file had no effect on frequency (F=0.05, p=0.98) (Fig. 3.5D), suggesting that only the file portion of the left elytron vibrates in sound production. Using change in sound dominant frequency as a proxy of sound emitter vibration, finding a changed vibration frequency in response to an altered amount of weight on the structure further supports that the structure is in free rather than forced vibration.

3.5 Discussion

This was the first study to take a detailed look at the mechanism of sound production in an elytro-tergal stridulating insect. Further, it determined how an insect with only one mechanism of sound production can introduce variability in its acoustic repertoire. *Dendroctonus valens* was found to employ elastic potential energy, in a mechanism hereby termed 'spring stridulation' (G. Morris, personal communication), in a novel way to produce complex sounds. By incorporating one or more events of storing

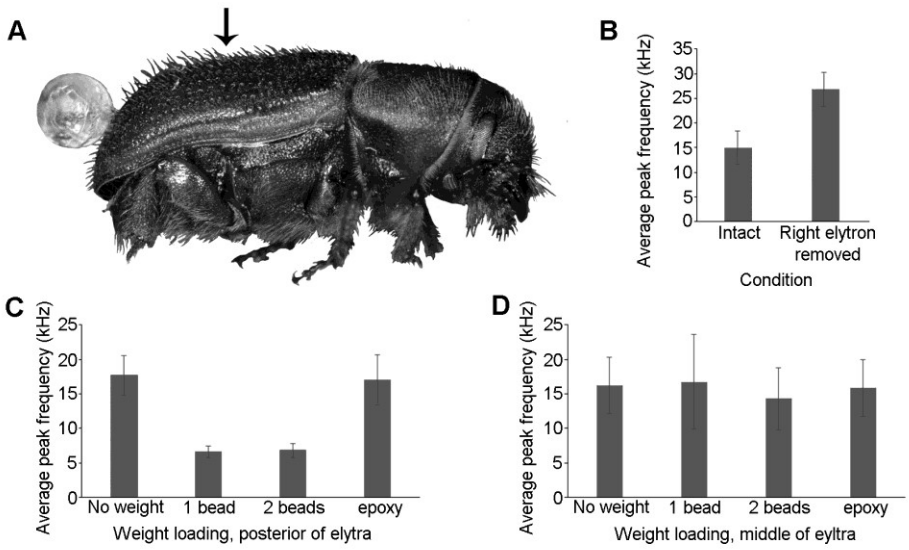

Figure 3.5. Changes to the frequency of chirps by alterations to elytral weight. (A) To increase weight, glass beads were adhered to the elytra at two locations. Pictured here is a lateral view of a male *D. valens* with the glass bead adhered to the posterior end of the elytra, over top of file location, while the arrow indicates the second location for weight located towards the middle of the elytra. (B) Removal of the right elytron caused an increase in dominant frequency. (C-D) Dominant frequency in intact males, with one or two beads or epoxy loaded (C) at the location of the file or (D) when weight was loaded medially (corresponding to the arrow in A).

energy to the sound producing organ, *D. valens* was capable of altering the temporal pattern of its chirps.

Goal 1: Characterizing simple and interrupted chirps

Dendroctonus valens males generated two chirp types - simple and interrupted - each with a characteristic temporal pattern. Simple chirps were shorter, contained fewer pulses and had smaller maximum pulse intervals. These findings, for chirps recorded during a disturbance setting, were consistent with those reported recently for *D. valens* signalling in a courtship setting (Lindeman and Yack 2015, Chapter 2 of this thesis). Thus, the temporal structure of signals is consistent across contexts. Additionally, there were differences in sound pulse rate and amplitude envelope for the two chirp types when interrupted chirps were considered overall, but these features were not found to differ when comparing the individual components of interrupted chirps to simple chirps. There were no other differences between chirps in the spectral domain.

The differences between chirp types may have evolved to exploit the sensory bias of the intended recipient (Searcy 1992; Hebets and Papaj 2005), and are likely functionally significant to *D. valens*. Interrupted chirps have been found to be common during courtship and to play an important role in this context, with successful and larger males producing a higher proportion of interrupted chirps than their counterparts during their courtship performance (Lindeman and Yack 2015). In the current study it was found that interrupted chirps were relatively rare during disturbance, with roughly

only a quarter of the 63 males sampled producing any at all. It was proposed that interrupted chirps are selected to function in female choice decisions because their increased length would be more stimulating to the female, or else their more variable temporal pattern demonstrates skillful movements (Lindeman and Yack 2015). In contrast, simple chirps have many of the characteristics in common with the general disturbance chirps reported for many stridulating insects (short duration, a simple temporal pattern and high repetition rate) that are reported to have an anti-predatory function (Masters 1979; Masters 1980; Conner 2014).

Goal 2: The structures of the sound producing organ

Elytro-tergal stridulation is the most common mechanism of sound production of one of the largest insect families, Curculionidae (Lyal and King 1996). In general, the study of this mechanism has been complicated by several factors outlined by Lyal and King (1996), including the diversity across taxa of structures which can function as a plectrum and the variability in the location of the file and plectrum across groups. The most common type of elyto-tergal organ contains a plectrum on the seventh abdominal tergite and a file on the underside of the elytra (Lyal and King 1996). However, in much of the literature reporting elytro-tergal stridulation, there has been no observation or experimental examination of the function of purported structures (reviewed in Lyal and King 1996). In many elytro-tergal stridulators, there is asymmetry in the file, with the portion of file on the right elytron often reduced as compared to the left, or absent altogether (Lyal and King 1996). This asymmetry was found in *D. valens* here, and

elsewhere (Ryker and Rudinsky 1976a). This asymmetry is likely owing to the orientation of the elytra while in the wing-lock position. The wing-lock location is situated over the median line with the right elytron sitting atop the left. The file is found at this location along the left elytron, and extends beyond the wing-lock region onto the right elytron. Thus, the portion of the file on the left elytron sits directly atop the processes of the seventh abdominal tergite, while the portion of file on the right elytron is far from the median line limiting its accessibility to the processes and thus its functionality. Similar arguments have made against the functionality of the right elytron in sound production for *Dendroctonus pseudotsugae* (Michael and Rudinsky 1972). By combining ablation experiments, high speed video and sound recordings, this study confirmed that the processes of the seventh abdominal tergite do strike the file during sound production in *D. valens* and only the portion of file along the left elytron is involved in sound production. This is consistent with the few empirical studies of the seventh abdominal processes of other curculionids, including the genus *Rhynchaenus* (Claridge 1968). Finally, high speed video combined with sound recordings and morphological analysis were used to show for the first time in any elytro-tergal stridulator (to the extent of our knowledge) that each sound pulse is produced by one tooth on the file being struck by the plectrum.

Goal 3: The mechanism for producing simple and interrupted chirps

The plectrum of *D. valens* might be driven to strike the file either by movement of the abdomen or by a localized store of potential energy. The former mechanism is

seen for instance in crickets and some katydids, tegmino-tegminal stridulators whose plectrum movement is by and large coupled to the movement of the tegmen it is attached to (Montealegre-Z and Mason 2005; Prestwich and O'Sullivan 2005). The latter mechanism has been found as a specialization of ultrasonic katydids, where elastic potential energy stored in the plectrum can propel the plectrum along the file independently from tegminal movement, and at high enough velocities to produce ultrasonic frequencies (Morris and Pipher 1972; Montealegre-Z et al. 2006). In the case of stored energy, the plectrum must have a flexible joint around which it can be deformed in order to load the structure with potential energy. This is analogous to the potential energy in a stretched spring; consequently, this method of sound production has been termed spring stridulation (G. Morris, personal communication). In *D. valens*, the plectrum was found to become deformed by locking onto the file and bending against it. Upon release at the start of each chirp the plectrum travelled along the file more rapidly than the movement of the abdomen, as evidenced by the rate of change in the angle between the plectrum and abdomen over the course of the chirp. This supports the hypothesis that *D. valens* sound production is driven by a spring stridulatory mechanism. Future studies will need to investigate the resilin content of the cuticle of the plectrum and abdomen to confirm that it has elastic properties. Resilin is a rubber-like protein found in arthropod cuticle (Andersen and Weis-Fogh 1964), and its presence in the plectrum and the plectrum's associated abdominal tergite could explain how energy is stored for a spring stridulatory mechanism.

The interruptions in the pulse pattern of interrupted chirps may hypothetically be generated in one of three ways: by repeated sound production cycles, skipped teeth or paused motion. It can be seen by observations of intact males that both simple and interrupted chirps are produced by a single posterior abdominal movement. This has been reported previously for other species as well (including *Dendroctonus ponderosae*, Ryker and Rudinsky 1976b). Thus, interrupted chirps are not repeated sound production cycles akin to a series of simple chirps in rapid succession. Rather, the results support the paused motion hypothesis. When the right elytron was ablated for a clear view of the sound producing organ, high speed video showed that males performing interrupted chirps repeatedly paused in motion, locked onto the file and were deformed. This indicates that each time elastic potential energy was restored before being released and recommencing motion. Each deformation event also corresponded with the interruption in sound pulses. Thus, unlike ultrasonic katydids, who use spring stridulation to produce high frequencies, *D. valens* performed single or repeated potential energy storing events to produce their diverse simple and interrupted chirp types, respectively. This is a distinct use of the spring stridulation mechanism to incorporate variability into the acoustic signalling repertoire in *D. valens*.

Vibration of the sound emitter

Two alternative hypotheses were considered for how *D. valens'* file vibrates upon being struck. First, with each strike by the plectrum, the bark beetle file may be sent into free vibration at its natural frequency (e.g. Bailey and Broughton 1970). This is,

for example, what occurs when a guitar string is plucked; the input of energy by plucking the string disturbs the string and forces it into motion, but then if the string is subsequently left undisturbed to vibrate freely it will do so at its natural frequency. The alternative is that *D. valens'* file is not left to vibrate freely, but rather is struck continuously forcing the vibration of the file to match the frequency of the strikes (e.g. Bailey and Broughton 1970). This, for example, is what happens to the bridge and soundboard of the guitar the string is mounted to; the continuous stimulation from the vibrating string forces the soundboard into vibration at that frequency, despite the fact that this is not the natural frequency of vibration of the soundboard. No relationship was found in *D. valens* between the tooth strike rate by the plectrum against the file and the resultant vibration frequency of the file (as determined by the sound frequency). Rather, during chirp production teeth were struck at a rate that was much too slow to produce the spectral energy at the high frequencies seen. It was also found that each tooth strike resulted in a discrete sound pulse, with the energy in the system dissipating before the next tooth strike. These findings suggest that *D. valens*' file is free to vibrate at its natural frequency following stimulation. In further support of the free vibration hypothesis, adding weight to the system significantly altered the generated sound frequency. If tension is held constant, changing the mass of any structure will affect its natural frequency. Returning to the guitar string analogy, the lower frequency producing strings on a guitar are wrapped in wire, to increase their mass as a way of lowering their natural frequency of vibration. This change in natural frequency only affects the resulting sound if the system is in free vibration; in forced vibration the rate of forcing

determines the frequency of vibration. Adding mass to the file of male *D. valens* significantly lowered the frequency of the sound they produced. Also, removing the mass from the file by ablating the right elytron, which sits directly atop the file, caused an increase in sound frequency. In all, these findings support the hypothesis that during sound production in *D. valens*, the file is in free vibration.

The lack of a relationship between the tooth strike rate and the file's natural frequency of vibration signifies that the vibratory oscillations of *D. valens*' sound emitter are not being reinforced by the plectrum strike rate, i.e. the sound emitter is not resonating. Unlike the calling sounds of crickets and katydids, which must be loud to communicate at far distances, bark beetles only use acoustic communication on a small spatial scale, with the intended recipient within several centimeters of the signaller. Thus resonance may not be important for the reliable reception of the signal. This is in contrast to ultrasonic katydids, which adopt this specialized sound producing mechanism (i.e. spring stridulaiton) to achieve high frequency sound (Montealegre-Z et al. 2006). Rather than forcing a particular sound frequency, the main benefit provided to *D. valens* by employing spring stridulation seems to be the enablement of signal variability.

Trailing pulses

Trailing pulses are described here for the first time for *Dendroctonus* sound production. These pulses were confirmed to result from strikes to the file as the plectrum and abdomen returned to a resting position following a chirp. It is proposed

that these are not 'intended' signals, but rather artifacts of the sound producing mechanism. This conclusion is owing to the rarity of these pulses, their low signal-to-noise ratio and their high variability. The finding of trailing pulses may have implications for interpreting the results of previous studies. Similar pulse trains have been reported previously within a courtship context, and have been identified as putative female chirps (Allen et al. 1958; Fleming et al. 2013). There is an ongoing debate as to whether *Dendroctonus* females produce any acoustic signals (e.g. Barr 1969). Although *Dendroctonus* females do not possess the seventh abdominal tergite processes that functions as a plectrum in males (Lyon 1958), some studies have suggested that they can and do produce acoustic signals in varied contexts (e.g. Rudinsky and Michael 1973; Ryker and Rudinsky 1976a; Fleming et al. 2013). The discovery of trailing pulses produced by males in the current study suggests that at least some earlier studies may have misinterpreted this component of male sound and incorrectly attributed it to a female source.

Conclusion and future directions

While insects are well known to generate different song types either between or within a species, the proximate mechanisms for generating variability are poorly understood. This study demonstrated how an elytro-tergal stridulator employed spring stridulation to generate and vary the temporal patterns of its chirps. Future studies should focus on the selection pressures that may have led to the divergence in signal

types, and the neuro-motor mechanisms that underlie such precision in pulsation pattern.

3.6 Acknowledgements

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CHAPTER 4: Acoustic sexual dimorphism in tree killing *Dendroctonus* bark beetles: Do females signal?

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4.1 Abstract

The idea of whether or not females of certain *Dendroctonus* species signal acoustically has been debated. Although these females lack the specialized sound producing organ of males, there have been reports that females can produce signals by way of an alternative stridulatory structure. To date, the evidence in support of female signals is equivocal. This study used two such contended species, *Dendroctonus ponderosae* and *Dendroctonus valens*, to test whether these *Dendroctonus* females signal acoustically. First, female sound emissions were characterized in contexts of disturbance, gallery construction, and female-female and male-female interactions within the gallery. Rarely, and only during disturbance and female-female interactions, irregular groupings of sound pulses were observed that were inconsistent with noise and thus candidates for female signals. Second, the presupposed female stridulatory structure was examined both morphologically and using high speed video paired with sound recordings for evidence of function in sound production. Although no evidence of elytro-tergal stridulation was found, there was some evidence to support the production of sound by striking the last sternite against the elytra. Third, the influence of putative resident female signals on the outcome of interactions with intruder females and potential mates was investigated. Although resident sound production did not alter the overall outcome of encounters, it was observed that intruding females tended to exit the gallery more quickly when residents produced sound. In conclusion, while the observations of female sound production in some contexts support the conclusion that *D. valens* females are capable of producing sound, sound function in communication is

as of yet unclear, given the rarity and irregularity of such sounds, the inconsistency in the observed mechanics of their production, and the lack of a clear influence on the outcome of interactions with conspecifics.

4.2 Introduction

Diversity in communicative signals exists between the sexes in many species. This sexual dimorphism is driven by opposing selective forces, with conspicuous and costly signals shaped by mate choice and intrasexual selection, while predation pressure and energetic costs push signals to be less conspicuous and less expensive (Moodie 1972; Ryan et al. 1982; Searcy and Andersson 1986; Pomiankowski 1987; Ryan 1988; Andersson 1994; Prestwich 1994). Sexual dimorphism in the acoustic signalling behaviour of insects is commonly seen. In some species both sexes produce sound, while in many others only one sex is capable of sound production and the other is mute (e.g. Ewing 1989; Gerhardt and Huber 2002; Sueur 2006). A thorough understanding of the sexes' behavioural differences is paramount to studying their behavioural ecology, and is necessary to fully understand their mating system and life history.

Many bark beetle species (Curculionidae: Scolytinae) exhibit sexual dimorphism in acoustic signalling behaviour (Barr 1969; Lyal and King 1996). This dimorphism is linked to the sex roles of the particular species; the sex that targets the host tree for gallery construction is mute while the attracted sex is typically capable of sound production (Barr 1969; Kirkendall 1983). However, there may be some species where

both sexes produce sound, as has been proposed for several species of the tree killing genus *Dendroctonus*.

Within the genus *Dendroctonus*, males are the "attracted" sex that joins a female once she has established a gallery (Kirkendall 1983; Six and Bracewell 2015) and all males produce sound with an elytro-tergal stridulatory mechanism (e.g. Ryker 1988). The sound producing organ is comprised of a plectrum on the seventh abdominal tergite and a pars stridens (i.e. the file) on the underside of the left elytron (Hopkins 1909; Barr 1969; also, see Chapter 3). Conversely, *Dendroctonus* females are the gallery initiators, and are anatomically sexually dimorphic in that they lack the structure on the seventh tergite that functions as the plectrum in males (Lyon 1958; Jantz and Johnsey 1964; Tate and Bedard 1967; Michael and Rudinsky 1972; Rudinsky and Michael 1973; Pajares and Lanier 1990). Despite the dimorphism in the sound producing organ, the controversy of *Dendroctonus* female acoustic signalling arises from reports of sound production in female *D. ponderosae* (Allen et al. 1958; Rudinsky and Michael 1973; Fleming et al. 2013), *D. pseudotsugae*, *D. rufipennis*, *D. brevicomis* (Rudinsky and Michael 1973; Rudinsky et al. 1976), *D. frontalis* (Rudinsky and Michael 1973) and *D. valens* (Ryker and Rudinsky 1976a). Rudinsky and Michael (1973) suggested that the chitinized posterior tip of the eighth abdominal tergite substitutes for the role of the plectrum in *Dendroctonus* females and strikes a file to produce communicative sound. The elytral file - i.e the same region that functions as the pars stridens in males (Chapter 3) - is expected to also function as a pars stridens in females in four out of the six species of interest (*D. pseudotsugae, D. ponderosae, D. valens* and *D. rufipennis*; Rudinsky and

Michael 1973). However, *D. brevicomis* and *D. frontalis* females, who do not have an elytral file, are speculated to produce sound using an alternative file located on the last sternite (Rudinsky and Michael 1973).

To date, the evidence for *Dendroctonus* female sound production has been equivocal. Often there has been an omission of the methods for sexing live individuals (Rudinsky and Michael 1973; Rudinsky et al. 1976; Ryker and Rudinsky 1976a), a lack of established controls to differentiate putative female signals from noise (such as that of struggle, gallery construction, territorial encounters and interactions with males: Rudinsky and Michael 1973; Rudinsky et al. 1976; Ryker and Rudinsky 1976a) or a lack of established controls to differentiate putative female signals from the sounds of simultaneously signalling males (Allen et al. 1958; Fleming et al. 2013). Furthermore, in the only tests of the behavioural response of conspecifics to female sounds, the resulting data was either conflicting or there was a finding of no change to behaviour (Rudinsky et al. 1976). Consequently, there is the need to revisit the question of female sound production within the genus *Dendroctonus* with a controlled, empirical study.

This study tested whether *Dendroctonus* spp. females signal acoustically using *D. valens* and *D. ponderosae* as models. There were three main goals of the study: (1) To examine the characteristics of sound emissions made by females during the context of disturbance and made by resident females while she is within her gallery alone or interacting with another female or male. Within the disturbance context putative signals were isolated from noise by comparing female sounds to the sounds of struggle recorded from mute controls, while for contexts within the gallery, resident female

sounds were compared against chewing and digging sounds recorded during gallery initiation. It was predicted that female communicative signals would be recognizably distinct from sounds identified as noise. (2) To examine the putative sound producing organ in two ways. First, the elytral file's morphology was examined in female *D. valens* and *D. ponderosae.* The homogeneity of the file's structure was compared both within females, and between females and males; the comparison to males was done with *D. valens* males owing to previous confirmation of the sound producing function of the file in these males (Chapter 3). Second, high speed video paired with sound recordings of *D. valens* females were used to examine the potential role of the eighth abdominal tergite as the putative plectrum. If females have an elytro-tergal stridulatory organ, then movements of the abdomen, or specifically of the eighth abdominal tergite, were predicted to be observed in conjunction with sound production. (3) For contexts during which females were interacting with other females or males within the gallery, the responses of conspecifics to resident female sound emissions were observed. If the presence or absence of female sounds affected the outcome of the encounter, it would be strong evidence for a communicative function of sounds (Busnel 1963). This study is the first controlled, empirical study to test the question of acoustic sexual dimorphism in *Dendroctonus* spp. and as such contributes to a better understanding of the acoustic ecology of the genus.

4.3 Methods

Animals

Dendroctonus valens

Adult *Dendroctonus valens* (Curculionidae: Scolytinae) were collected from May-September of 2014 at several locations near Ottawa, Ontario, Canada (the arboretum at the Ottawa Central Experimental Farm, 45.391021,-75.70489; Carleton Lands, Manotick, 45.183882,-75.604673; and outside Petawawa, 45.853530, -77.536156). Collection was done using Lindgren funnel traps baited with *D. valens* lure (Contech, British Columbia, Canada). Animals were kept at Carleton University and paired off into seven bolts of red pine (*Pinus resinosa*; one pair per bolt). Male and female offspring from these pairings were collected as adults upon emergence from bolts and used in data collection. Sex was identified by observing each individual under a light microscope (Olympus SZX12, Tokyo, Japan) and using a minuten pin to gently press down on the abdomen and examine the sexual dimorphism of the seventh abdominal tergite for the presence or absence of processes located medially at the posterior edge, which are found exclusively on males (e.g. Lyon 1958; Pajares and Lanier 1990). Voucher specimens are held at Carleton University.

Dendroctonus ponderosae

Adult *Dendroctonus ponderosae* were obtained from Baldy Mountain, British Columbia, (N49.110^{o}, W119.177^{o}) and stored at a secure insect holding facility at 8^{o}C at Carleton University until use. Sex was identified as for *D. valens*, above. Voucher specimens are held at Carleton University.

Ips pini

Adult *Ips pini* were collected in June 2015 at the arboretum at the Ottawa Central Experimental Farm, 45.391021,-75.70489. Collection was done using Lindgren funnel traps baited with two *I. pini* attractant lures, ipsdienol and lanierone (Contech, British Columbia, Canada). Animals were kept at Carleton University, where they were immediately sexed by examining the dimorphism in the declivity under a light microscope (Olympus SZX12, Tokyo, Japan). Males have an enlarged third declivity spine while in females spines two and three are joined at the base (Wood 1982). Only males were used in this study. Voucher specimens are held at Carleton University.

Sound characteristics during various contexts

Acoustic behaviour during disturbance

Two predictions were put forward to test the hypothesis that females produce acoustic signals during disturbance: (1) assuming that both males and females produce similar amounts of incidental sound due to struggling, the acoustic energy present

during female disturbance trials was predicted to be closer to that of likewise signalling males than to mute male controls, and (2) female signals were predicted to have characteristics that would be recognizably distinct from sounds identified as noise (with struggling noise characterized through an examination of the waveforms of mute male disturbance trial recordings).

To test the first prediction, the amount of acoustic energy above background in disturbance recordings for female *D. valens* (n=46) and *D. ponderosae* (n=8) was compared against two conditions: signalling or mute males. The signalling male condition had two samples: disturbance trial recordings of male *D. valens* (n=52) and *D. ponderosae* (n=15). The mute male condition had four samples. The first two samples were the recordings from male *D. valens* and *D. ponderosae* trials adjusted to exclude the acoustic signals (chirps) so that only the background and noise from struggling remained (all *D. ponderosae* recordings were used, n=15, while only a subset of *D. valens* recordings were used, n=10). Chirps were identified for exclusion based on their pulse arrangement into the stereotypical amplitude envelopes (e.g. Fleming et al. 2013; also, see Chapter 3). The other two samples in the mute male condition were disturbance recordings from naturally silent male *I. pini* (n= 11) and surgically muted male *D. valens* (n=5). Surgical muting was done by removing the file region of the elytra by surgical cutting using extra fine point dissection scissors (e.g. Lindeman and Yack 2015; Chapter 2 of this thesis).

To test the second prediction, that female signals have characteristics recognizably distinct from sounds identified as noise, the temporal domain

characteristics of the female recordings from above were examined and compared with that of male recordings from the control condition (see above). Emphasis was placed on the characteristics of sound pulses. This included documenting the presence of sound pulses and their abundance, and whether they were isolated in space or had clustered appearances (the latter was termed "groups" of pulses). If pulses were grouped, the number of pulses per group and the inter pulse interval was calculated, and observations were made of the overall regularity of spacing between pulses. The amplitude envelope of the groups of pulses was also considered.

Acoustic behaviour while inside the gallery

Similar to the predictions for disturbance signals, if females produce acoustic signals while inside the gallery then signals would be expected to have characteristics that are recognizably distinct from sounds identified as noises from gallery construction (i.e. scraping, digging and chewing). To test this prediction, female *D. valens* were first observed in a control condition where noise could be identified. Sounds were recorded from females (n=8) initiating gallery construction upon placement on a fresh pine bolt (*Pinus resinosa*). Females did not stridulate during this time - this was determined by visually monitoring each female as she began to chew and dig into the bark, and confirming the absence of abdominal movements that could indicate stridulation. The sounds of gallery construction noise were then compared to sounds made by resident females in three test conditions to isolate putative signals. The three test conditions were: (1) each resident female alone inside her gallery at 24 hours post introduction. For

this condition, the same females were used as from the control condition (n=8) now at 24 hours following their introduction to the bolt. In each case, the gallery had been established by this time, as evidenced by the buildup of frass around the gallery entrance. For each female a 2.5 min sound recording was conducted. (2) Resident females inside the gallery during interactions with a second, introduced female. The same resident females (n=8) were used as from the control condition. Each trial occurred at 48 hours post introduction. The trial began when a second *D. valens* female, designated the "intruder", was placed at a resident's gallery entrance. In all eight trials the intruder entered the gallery. Sound recordings were taken from the time the intruder entered the gallery until the time that she left. Intruders were never reused. (3) Resident females inside the gallery during interactions with males (potential mates). This trial was conducted using five resident females (not previously used) and five muted males (procedure for muting as above). Muted males were used in order to isolate putative signals as coming from females. Males were introduced to a female's gallery entrance (as in Chapter 2 of this thesis), and recordings lasted from the time the male entered the gallery until either the male left or for a maximum of 30 min.

The characteristics of the temporal domain of the recordings of females inside the galleries was examined and compared with that of digging and chewing noise from the control condition (gallery initiation) following the same method as for characterizing the putative female disturbance signals. Again, the presence of sound pulses and their abundance was documented, as was the presence of pulse groups.

Sound recording and analysis

Disturbance trials were conducted by grasping the pronotum and head of each bark beetle gently while avoiding the elytra to avoid interfering with the elytro-tergal stridulatory organ. The within gallery trials were conducted on bolts of pine that were cut from a freshly felled tree from Carleton Lands, Manotick, 45.183882,-75.604673. Bolts measured 36.3 ± 1.1 cm in length and 22.2 ± 0.4 cm in diameter (average ± SE). They were waxed at either end to reduce desiccation and mould infestation and stored at 5^{o}C for approximately three months. Prior to female introduction, small holes were cut to the cambium layer using a knife to stimulate gallery construction by the females. Only one female was placed on each log. A microphone was placed at 2cm from a beetle during disturbance, or at 3 cm above the gallery entrance for within gallery conditions (40 kHz omnidirectional microphone, QTC40, Earthworks, Milford, USA). The microphone was connected to a data recorder (sampling rate of 192 kHz, FR-2, Fostex, Los Angeles, USA). All recordings were performed in a walk-in type acoustic chamber maintained at 22 ± 2^{o}C. Sound recordings were analyzed using Raven Pro 1.5 (Raven Bioacoustics Research Program, Cornell Laboratory of Ornithology, Ithaca, NY, USA). For the quantification of acoustic energy above background for disturbance trials, in all individuals samples of the background noise were taken from the beginning of the trial before individuals were moved in front of the microphone. Root Mean Square (RMS) amplitude was first sampled for the background noise, and then for the disturbance trial starting from the time the individual was placed in front of the microphone and for the following 30 sec of recording. Percent acoustic energy above background was then

measured across each trial and compared between sexes and species. Because distance from the microphone was impossible to assess for females while inside the gallery such an analysis could not be done for recordings of within gallery contexts.

Putative sound production organ morphology and behaviour

Female and male elytral file morphology

Because it was hypothesized that the elytral file forms part of the sound producing organ of both sexes of *D. valens* and *D. ponderosae* (Rudinsky and Michael 1973) it was predicted that the anatomical structure of the elytral file would be well-developed and in both sexes as well. Scanning electron micrographs of females (*D. valens* n=4; *D. ponderosa* n=3) and male *D. valens* (n=15) elytral files were obtained by dissecting the animals' left elytra, placing them on aluminum stubs, sputter coating with gold-palladium and examining them with a JOEL JSM-6400 scanning electron microscope. Within each group, the file structure was compared among individuals to determine whether characteristics were common or variable. The characteristics examined were: the file outline, the topography of the file's cuticle, and the prominence of file teeth. The number of teeth was also counted (along the midline, as in Chapter 3). These characteristics were also compared between groups, to explore whether any features were common within species, within sex, or among all groups.

Associating body movements with sounds produced in females

This set of observations was meant to assess whether any female sounds were emitted from a dedicated sound producing organ, with particular emphasis on examining the putative elytro-tergal mechanism. Abdominal movements of female *D. valens* were observed during the same 30 sec disturbance trials (n=46) as above to determine whether any abdominal movements occurred. Then, to further investigate whether abdominal movement was correlated with sound production during disturbance, one female that displayed such movement was examined using a high video recording taken with a camera (frame rate: 240 fps, GoPro Hero 3, GoPro Inc., USA) connected to an external microphone (ECM-MS908C, Sony) and mounted to a two-head observation light microscope (Leica, Wild M3Z, Wetzlar, Germany). Sounds were simultaneously recorded by an Earthworks microphone on a data recorder as described above. Audio was matched to the high speed video using Raven Pro 1.5 by aligning several pure toned test sounds made throughout the recording. The female was surgically manipulated by removing the right elytron to expose a lateral view of the abdomen so that in addition to viewing large movements of the entire abdomen, small movements of the abdominal tergites could also be seen. This was important as the eighth tergite was speculated to function as a plectrum. The female was held at 1 cm from the earthworks microphone, either by grasping her between two fingers or with forceps. Observations on abdominal movements and corresponding sounds were made.

Conspecific responses to female sounds in different contexts

During resident - intruder female encounters (from above, n=8) and resident - muted male encounters (from above, n=5), the gallery resident was interacting with a potential acoustic "recipient". If *Dendroctonus* spp. females produce acoustic signals during these two contexts, it was predicted that the presence or absence of such signals may alter the outcome of the interaction. Consequently, during both female-female and male-female encounters, it was simultaneously observed whether putative signals were produced, and what the outcome was (i.e. whether the intruder female or potential mate remained in the gallery or left). A further comparison was done for the female-female trials examining the influence of putative resident signals on time for an intruder to exit the gallery. The identity of intruders and residents was distinguishable by a white dot placed on the elytra of intruders (using liquid paper).

Statistical analysis

To explore the difference in the amount of acoustic energy above background for the disturbance recordings of females and signalling males an analysis of variance (ANOVA) was performed. For all significant ANOVAs a Games Howell post hoc test was used, because in all cases the assumption of homogeneity of variance was not satisfied owing to the large variation in sample size (as determined by a test of homogeneity). As part of the exploration of the elytral file morphology, the number of teeth on male and female *D. valens* files (counted along the file midline, see Chapter 3) was compared by performing a Student's t-test assuming equal variance. Finally, in female-female trials, it

was determined whether the presence of putative resident signals influenced the amount of time the intruder spent in the gallery by performing a Student's t-test assuming unequal variance (significant difference in variance determined by an F-test for equality of two variances). In all cases an alpha value of 0.05 was used; IBM SPSS Statistics 22 was used for all statistical analysis.

4.4 Results

Female and male acoustic energy during disturbance

Comparing the acoustic energy during disturbance for females, males and controls

During disturbance, both *D. ponderosae* and *D. valens* males had significantly more acoustic energy above background than did females (Games Howell post hoc tests; *D. ponderosae*, $p = 0.04$; *D. valens*, $p < 0.001$) (Fig. 4.1A). Males produced many sequences of sound pulses that were grouped together into clear chirps (Fig. 4.1B). Furthermore, for the first time in male *D. ponderosae* we observed low amplitude groups of pulses that trailed after the main chirp - termed "trailing pulses" (Fig. 4.1C) which have previously been reported only for *D. valens* (Chapter 3) and have important implications to previous suggestions of female sound production (discussed below).

Following the exclusion of chirps from the males' waveforms there remained many "stray" pulses of sound that had no organized pattern (Fig. 4.1B). These adjusted waveforms were similar in appearance and acoustic energy to the disturbance trial

waveforms of the mute *I. pini* males and the surgically muted male *D. valens*. Accordingly, there was no difference found in the acoustic energy above background between female and adjusted male waveforms (Games Howell post hoc tests; *D. ponderosae*, p = 0.388; *D. valens*, p = 0.981) (Fig. 4.1A), or between female *D. ponderosae* (average ± SE: 4.4 ± 2.0%), female *D. valens* (3.7 ± 0.6%), surgically muted male *D. valens* (0.6 ± 0.2%), or male *I. pini* (1.1 ± 0.4%) (F=2.2; p=0.1). However, although not significant, females - in particular *D. valens* females - tended to have more acoustic energy on average than any mute control group. This increased energy is reflective of "pulse groupings" that were identified once waveforms were characterized.

Characterization of female disturbance trial waveforms

The majority of female disturbance waveforms (both *D. ponderosae* and *D. valens*) had pulses of sound that were not grouped together and had no pattern (Fig. 4.1E,F), thus resembling control mute male waveforms. However, a subset of *D. valens* females (13 out of 46) did display some pulses which were grouped by the regularity of their pulse pattern (Fig. 4.1D-G). In two exceptional cases, pulse groups appeared to be part of long pulse trains (Fig. 4.1H,I). Even so, pulse groupings were rare (only 46 observed groupings in total in all females), and there was no consistency either between or within individuals in terms of inter pulse interval (ranging from 2 - 84 ms, average ± SE: 24.3 ± 3.1 ms), amplitude envelope (envelopes of descending (Fig. 4.1D), ascending (Fig. 4.1E), convex (Fig. 4.1F), concave (Fig. 4.1G) and flat (Fig. 4.1H,I) shapes were seen), number of pulses (ranging from 2 - 22 pulses, average ± SE: 6.8 ± 0.6 pulses) or group

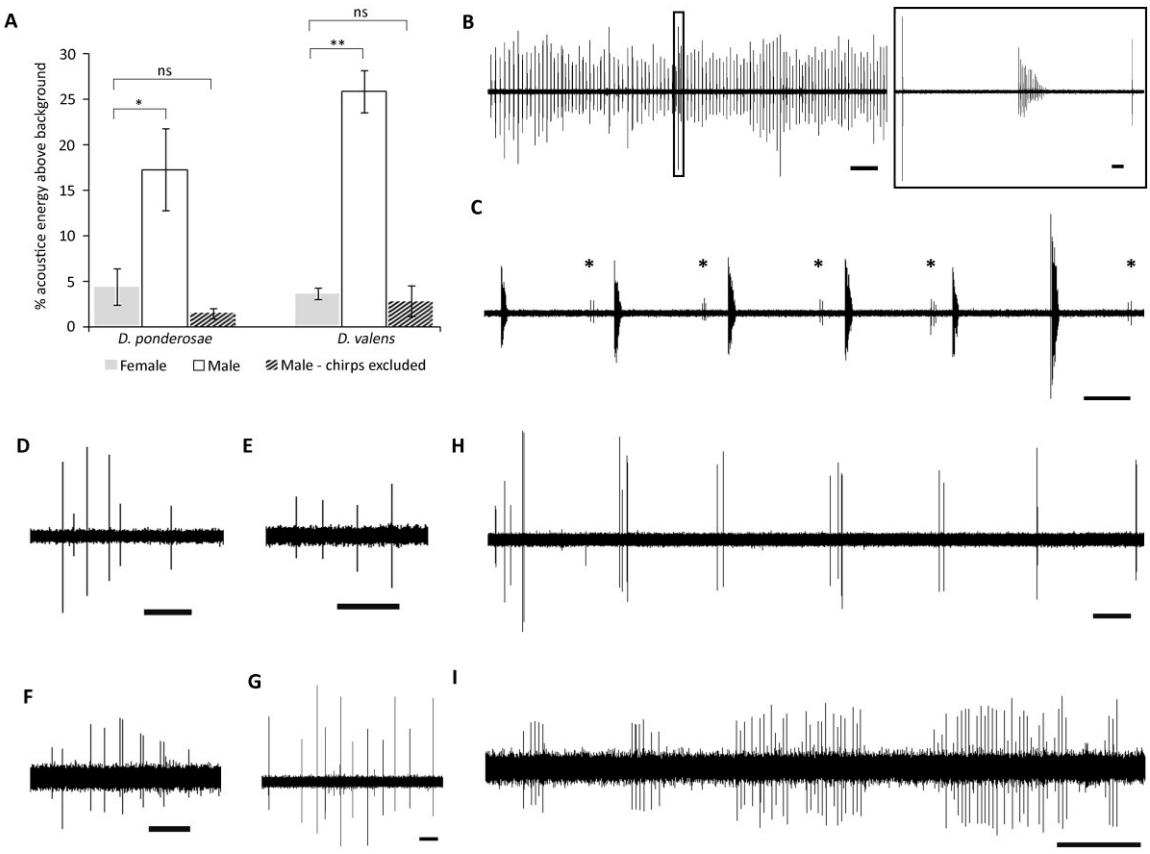

Figure 4.1. Comparisons of male, female and mute control sound production during disturbance. (A) Both *D. ponderosae* and *D. valens* male disturbance recordings had significantly more acoustic energy present than female recordings. This increase in acoustic energy was due to the male production of chirps, because upon their exclusion the level of acoustic energy during disturbance between the sexes was comparable. (B) 30 sec disturbance recording waveform for male *D. valens* with box enclosing a section that is expanded on the right. In the expansion, one chirp can be seen flanked on either side by a stray pulse; scale bars = 2 sec and 0.1 sec for sound train and expanded segment, respectively. (C) Six male *D. ponderosae* chirps; trailing pulses seen following five out of the six chirps (indicated by asterisks); scale bar = 0.1 sec. (D-G) Examples of groupings of pulses seen in *D. valens* females with diverse amplitude envelopes, number of pulses and durations; scale bars = 0.05 sec. (H-I) The two train-like assemblies of groupings seen in *D. valens* females; scale bars = 0.5 sec and 0.1 sec, for (H) and (I), respectively.

duration (ranging from 4.8 - 476.4 ms, average ± SE: 123.0 ± 16.0 ms). Many of these putative groups were seen among a noisy array of stray pulses. By comparison, males produced chirps with regularly spaced pulses and descending amplitude envelopes (Fig. 4.1B,C).

Female sound production within the gallery

Females (n=8) were observed at gallery initiation to begin actively digging into the log. Although sounds were produced, no abdominal movements indicative of stridulation were seen, thus sound was assumed to be noise arising from gallery construction activities such as that produced by the legs and mandibles during scraping, digging and chewing. Consistent with this assumption, investigation of the waveforms found randomly distributed sound pulses, with no pulses grouped or arranged in an organized pattern (Fig 4.2A,B). The sounds from the gallery initiation recordings were used as a template for comparison in other contexts.

When the females were alone in their respective galleries at 24 hours post introduction, waveforms were similar to those seen during gallery initiation; i.e. all sound pulses were randomly distributed. After approximately 48 hours, a second, "intruder" female was introduced to each resident's gallery. Territoriality was observed in all cases in that intruders that entered a gallery eventually left while the resident remained. Furthermore, in one case the resident was visibly observed to physically expel an intruder by pushing her out of the gallery. Regarding sound production, three out of eight trials had sound similar to the digging and chewing noise during gallery

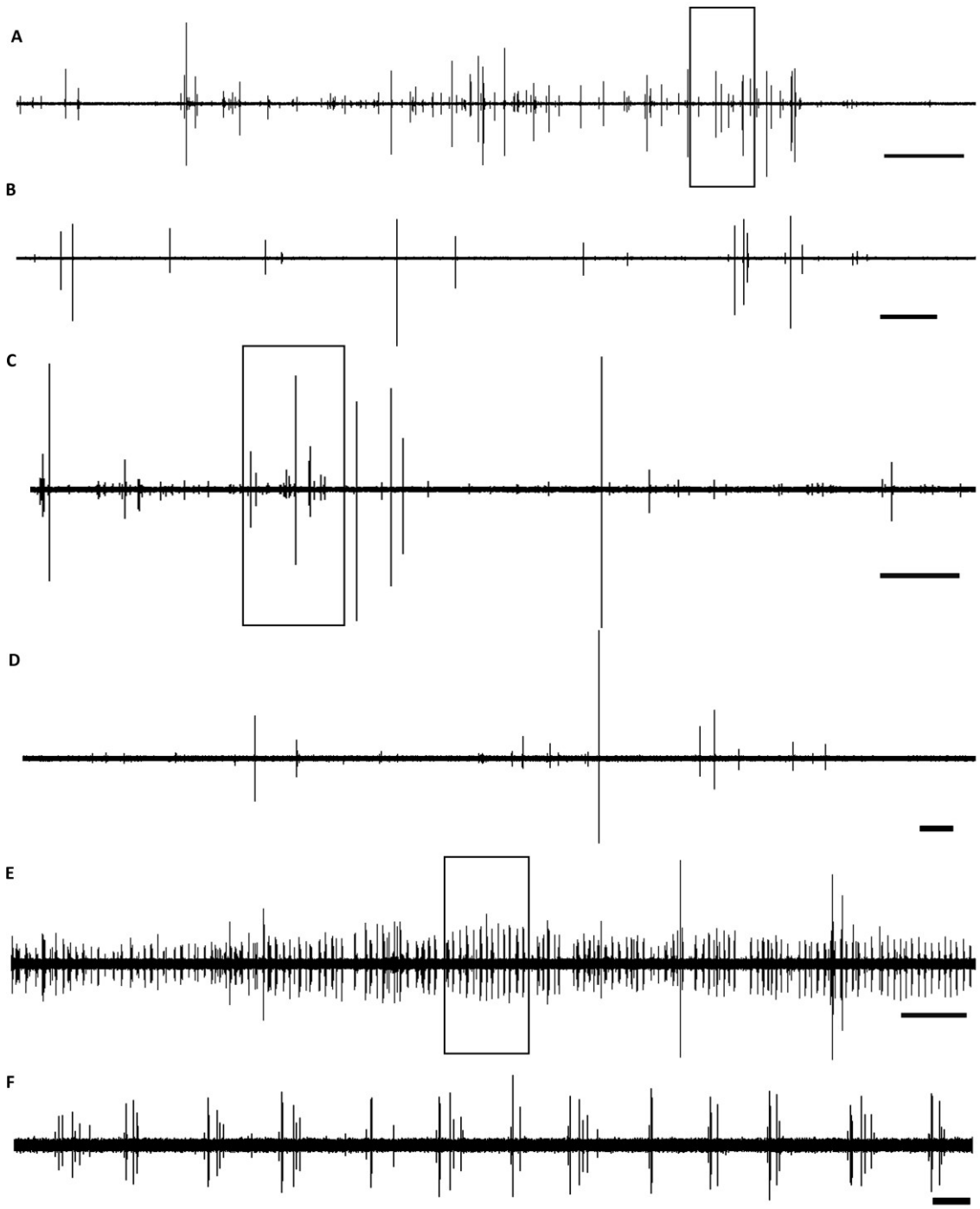

Figure 4.2. Comparison of waveforms during gallery construction and territoriality. (A) Two minute recording of a female *D. valens* initiating gallery construction. Sounds seen are due to the noise of digging and chewing, with no observed stridulatory actions throughout the recording; scale bar = 10 sec. (B) Expansion of outlined section from (A) showing randomly occurring pulses of sound; scale bar = 0.5 sec. (C) A two minute clipping of a territorial trial where the waveform was indistinct from noise of gallery construction; scale bar = 10 sec. (D) Expansion of outlined section from (C) showing randomly occurring pulses of sound; scale bar = 0.5 sec. (E) Entire territorial trial for a putatively signalling resident female from the time an intruder entered her gallery until the time the intruder left (2.75 min); scale bar = 10 sec. (F) Expansion of outlined section from (E) showing pulses of sound resembling signals based on their regularity and organization; scale bar = 0.5 sec.

construction (Fig. 4.2C,D). However, in five out of eight trials, there were regular and clearly grouped sound pulses that were continuously produced and persisted for minutes (Fig. 4.2E,F). In one of the three cases, the sound persisted for over a minute after the intruder was expelled, verifying that in at least that case, it was the resident producing the sound and not the intruder.

Male-female interaction trials (n=5) were also conducted by introducing a silenced male to the gallery 48 hours after the female began gallery construction. The waveforms resulting from the courtship trials were similar to those during gallery initiation and construction, i.e. unlike the organized pulses seen during the intruder trials, sound pulses seen during courtship with silenced males appeared random.

In conclusion, females during gallery initiation, construction and courtship produced only random sound pulses that through visual observation could be linked to chewing and digging activities. Conversely, in three out of eight "territorial" interactions there were grouped pulses with an organization that was unlike any seen from noise.

Putative sound production organ

Female and male elytral file morphology

Male *D. valens* and female *D. valens* and *D. ponderosae* each had files in the same location at the posterior tip of the underside of the left elytron (Fig. 4.3A-C). Furthermore, male and female *D. valens* were found to have approximately the same number of teeth on the file, with males tending on average to have slightly more

(average number of teeth on the file ± SE: females 59.0 ± 0.9 teeth; males 62.9 ± 1.8 teeth; $t_{17,2}$=-1.1, p=0.28). Female *D. ponderosae* had on average 45.7 ± 2.8 teeth along the file, which is less than has been previously reported for *D. ponderosae* males (an average of 71 teeth with a range of 63-76 teeth; Michael and Rudinsky 1972). From a top-down perspective, the files of females had appeared to have less defined teeth compared to those of males (Fig. 4.3A-C). Examination of these files from a lateral perspective further resolved the variation in tooth height/groove depth between the different groups. Male teeth were prominent owing to the deep grooves separating each one. Conversely, females of both species had only shallow grooves separating the teeth reducing their prominence (Fig. 4.3D-F).

Clear differences were found between the examined groups in terms of file shape. Across all male *D. valens* (n=15), the file shape was highly conserved and roughly ovular, varying only in size (Fig. 4.4A,B). Conversely, there was large variability in the appearance of female files in both species, with two kinds of file malformations commonly seen. First, in five out of seven females (all three *D. ponderosae* females and two of the *D. valens* females) tooth prominence became so reduced in some regions that teeth could no longer be observed (Fig. 4.4C,D). Second, wrinkling was commonly seen in the topography of the file, either as subtle recesses or bulges (Fig. 4.4E) or even as a large folding of cuticle in the file region (Fig. 4.4F) (both types of wrinkling found each in one *D. valens* and one *D. ponderosae* female).

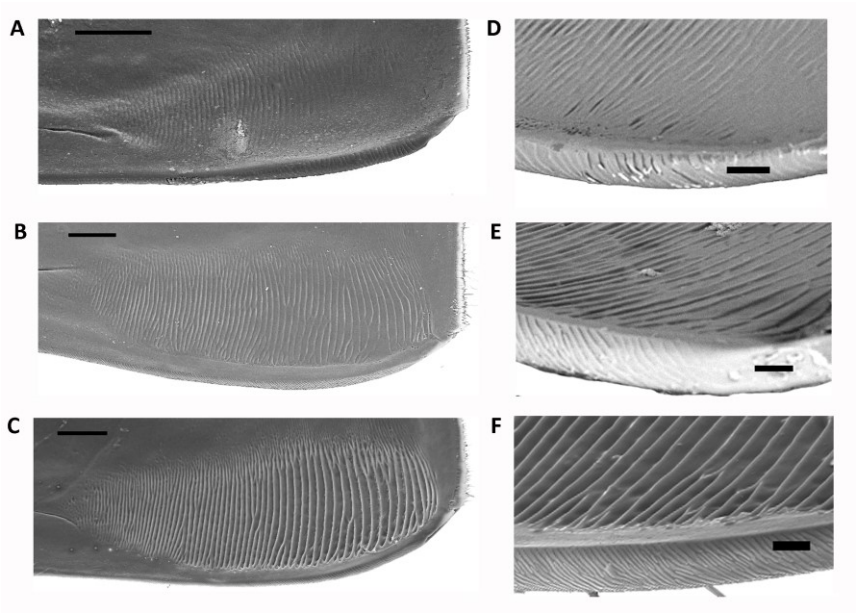

Figure 4.3. Scanning electron micrographs of the file region, located on the underside of the posterior end of the left elytron, showing tooth prominence. When considering the posterior tip of the sutural margin of the left elytron (from a top-down perspective) it can be seen that the file is located at the same position for (A) female *D. ponderosae*, (B) female *D. valens*, and (C) male *D. valens*; scale bars A-C = 100 µm. (D-F) Enlarged views of files from a lateral perspective. (D) Female *D. ponderosae* and (E) female *D. valens* images show that females have shallow grooves separating each tooth. (F) Male *D. valens*, by comparison, have more prominent teeth separated by deeper grooves; scale bars D-F = 25 µm.

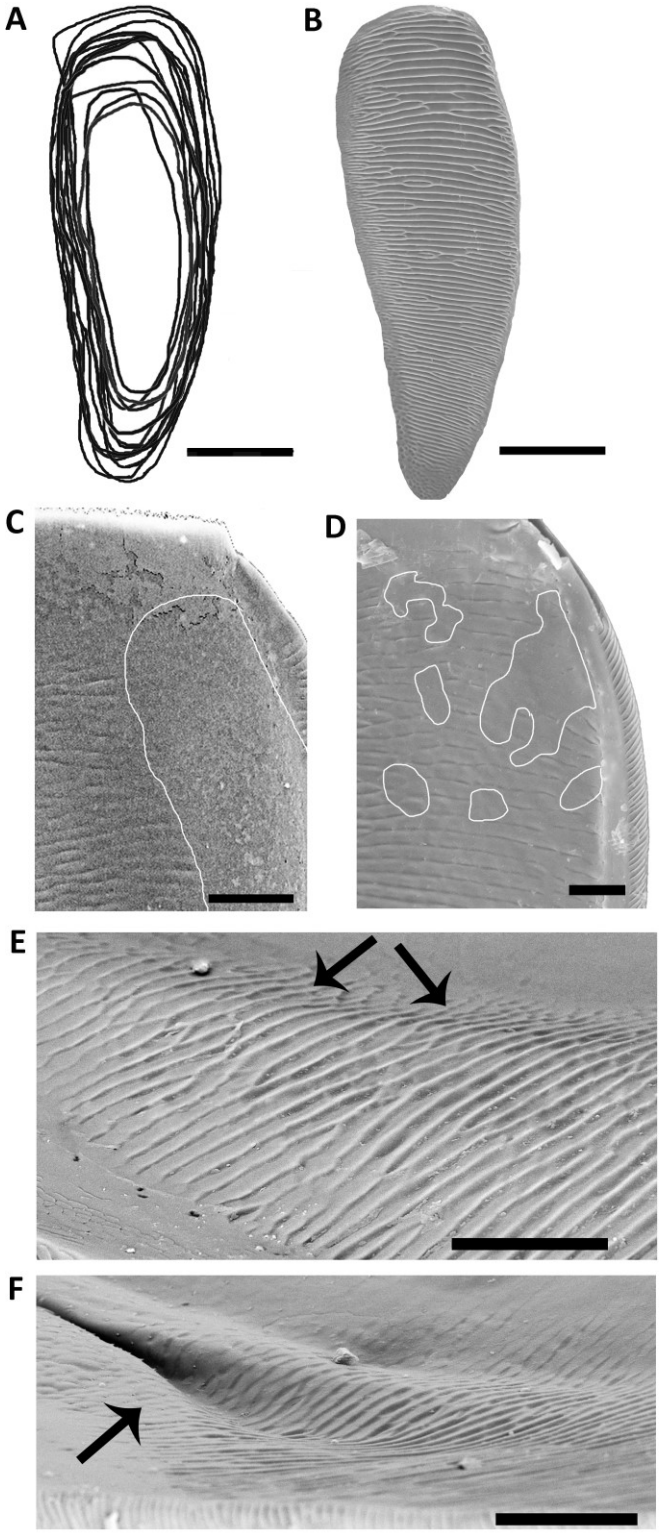

Figure 4.4. Comparison of male and female file shape. (A) Outlines of all male files superimposed depict a highly conserved, ovular shape among males, exemplified by (B) a cut out from the SEM of a typical male file; scale bars A-B = 200 µm. (C) SEM of a typical female *D. ponderosae* file irregularity where a large, continuous region of the file is absent and replaced by smooth cuticle and (D) a female *D. valens* file showing smaller, random sections of the file where teeth disappear and cuticle is smooth; regions indicated by white borders. (E) SEM of a second type of irregularity in the file of a *D. valens* female where a bulge in the cuticle can be seen causing wrinkling in the file and (F) a region of significant folding in the file of another *D. valens* female; scale bars C-F = 50 µm.

Female body movements and sound production

The presence of a dedicated sound producing organ in the production of sound pulses was investigated next. Particular attention was given to observing the putative female plectrum (the eight abdominal tergite) and pars stridens (the elytral file). While observing the abdominal movements of the 46 females during disturbance, it was noted that a quarter of females (n=11) made large abdominal movements where the entire abdomen, which usually was flush with the elytra, would be lowered and raised. On most occasions when this movement was observed there was no resulting sound (Fig. 4.5A) while on other occasions this movement was closely correlated with sound pulses (Fig. 4.5B). It seems possible that some individual clicks could be produced by the final sternite (sternite 7) hitting the elytron upon closing, but there was not enough resolution in the video to determine if sound occurs immediately upon elytra-sternite contact, or why in some cases multiple pulses of sound were observed following the abdominal movement (e.g. Fig. 4.5B).

Conspecific responses to female sounds in different contexts

During territorial encounters between females, five out of eight resident females produced putative signals, yet all intruders eventually left the resident's gallery. There was, however, a correlation between presence/absence of putative resident signals and time to exit the gallery. The intruders took less time to exit the gallery on average when resident sound was present (11.0 ± 6.1 min) as compared to intruders in trials where the sound was absent, which typically lasted longer than an hour ($t_{2,2}$=-2.16, p=0.16).

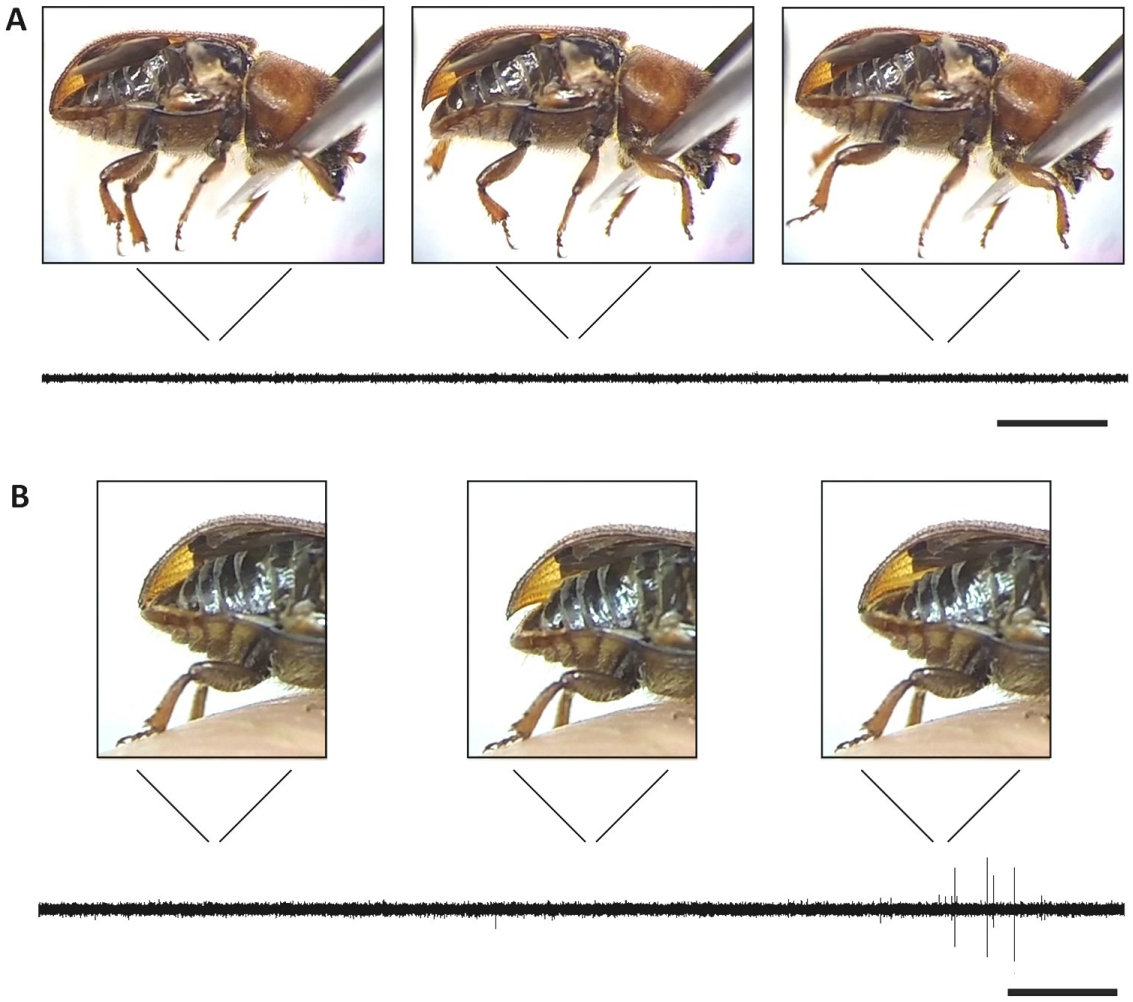

Figure 4.5: Video stills and waveforms of female abdominal movement and associated sounds. (A) Video stills are of lateral views of one female recording during disturbance - the female's right elytron has been removed to expose her abdomen - depicting the large abdominal movements that were frequently found in females. In the first panel (top left) it can be seen that the abdomen normally rests flush against the elytron, while the second panel shows the entire abdomen being lowered. In the last panel, the abdomen has returned to rest against the elytron. In this case no sound resulted from the action; scale bar = 0.1 s. (B) Video stills are of a lateral view of the abdomen and elytra only, and once again the second panel shows the large abdominal movement, but in this case the timing of the last panel, when the abdomen returned to its initial resting place against the elytron, corresponded with the emission of several sound pulses; scale bar = 0.1 s.

Regarding courtship, during each encounter the muted male initially entered the female gallery. However, in two out of five trials the male was rejected from the gallery and the encounter was deemed unsuccessful. No female sounds were observed, and there was no difference between sound recordings regardless of a female's eventual acceptance or rejection of the male.

4.5 Discussion

The purpose of this study was to investigate female *Dendroctonus* spp. acoustic signalling using two species, *D. ponderosae* and *D. valens*. Sound pulse production was observed in several female *D. valens* during disturbance and female-female interactions, supporting the idea that, at least for *D. valens*, females are capable of sound production. Despite this observation, the support for the hypothesis that the sounds function as signals remains inconclusive. Many individuals were not observed to produce such sound pulses and sound production was rare even within the individuals that were found to produce sound. Sounds were also highly irregular and their production was only inconsistently linked to a sternite-elytra clicking mechanism.

Problems with earlier studies reporting female acoustic signals

The speculation that certain *Dendroctonus* spp. females signal acoustically has arisen from reports of sound production in females of six species: *D. ponderosae* (Allen et al. 1958; Rudinsky and Michael 1973; Fleming et al. 2013), *D. pseudotsugae*, *D. rufipennis*, *D. brevicomis* (Rudinsky and Michael 1973; Rudinsky et al. 1976), *D. frontalis*

(Rudinsky and Michael 1973) and *D. valens* (Ryker and Rudinsky 1976a). However, the evidence to date has been equivocal. In perhaps the most alluring report of female acoustic signalling, Rudinsky and colleagues (1976) stated that *D. pseudotsugae* and *D. brevicomis* males released a higher concentration of pheromones in response to a playback of putative female stridulation than when in silence. However, while a difference was found in pheromone release for *D. pseudotsugae* males the test was only performed once, whereas for *D. brevicomis*, where two replicates were done, a difference in pheromone release was only found in one replicate while no difference was found in the other leading to conflicting results (Rudinsky et al. 1976). Also, with between 60 and 100 males in the pheromone recording chamber simultaneously, the identification of the individuals as male was a key variable in that study - the presence of even one female could have altered the responses of all males. However, no method of sexing was given (Rudinsky et al. 1976).

Several earlier studies reporting female signals have omitted to report on how individuals were sexed (Rudinsky and Michael 1973; Ryker and Rudinsky 1976a). Without knowing how live sexing was done, it is uncertain whether sound observations were definitively linked to a female producer. A similar concern is with reports of putative female sound production when signalling males were present during the recording as well (Allen et al. 1958; Fleming et al. 2013). In those cases, acoustic signals were distinguished as female produced because of their distinctiveness from "typical" male signals. Yet the concept of what constitutes a typical male signal is undergoing changes. Recent identification of low amplitude male trailing pulses in *D. valens*

(Chapter 3) calls into question whether these male sounds were the ones observed in earlier studies, which were then misinterpreted as coming from females.

In addition to reporting observations of female sound production, some studies have gone so far as to link female sounds to the context they were observed in to surmise a signalling function for communication within that context. In one such experiment, single pulses of sound termed "clicks" were reported as signalling displays of female territoriality (Rudinsky and Michael 1973); but in that study females were tested within minutes of being confined under mesh on a log, calling into question the presence of territorial motivation within such a context. Furthermore, no strong controls were ever present to disentangle putative signals from noises such as the digging, scraping and chewing noises that arise from gallery construction (Rudinsky and Michael 1973; Ryker and Rudinsky 1976a). In one study the authors listed only their own experience in observation as a control to distinguish female signals from this type of noise, a dubious claim given that they also reported that sounds were almost inaudible (Rudinsky and Michael 1973). Consequently, there was the need to revisit the question of female sound production within the genus *Dendroctonus* with a controlled, empirical study, which we did using *D. valens* and *D. ponderosae* as models.

Female sound production in various contexts

Many discrete sound pulses were seen for *D. ponderosae* (during disturbance) and *D. valens* (during disturbance and within gallery activities). It was determined that, in the majority of cases, these sound pulses were no different from noise. Notable

exceptions were found in a minority of *D. valens* females during disturbance and female-female interactions. In these cases, sound pulses were observed to be grouped together in either small groups or long trains, and their pattern was distinct from noise. These groupings were rare, found in only 13 out of 46 *D. valens* females during disturbance and five out of eight females during female-female interactions. They also had highly irregular structures both between individuals and between groupings within individuals, and differed from grouping to grouping in terms of their inter-pulse intervals, amplitude envelopes, number of pulses per group and group duration. This is consistent with some reports of the groupings of sound pulses of *D. pseudotsugae, D. brevicomis, D. ponderosae, D. rufipennis* and *D. valens* females, which were also characterized as being highly variable (ranging from 5 msec to in excess of 100 msec, and containing a variable number of irregularly spaced pulses) (Rudinsky and Michael 1973; Ryker and Rudinsky 1976a). In those studies, discrete pulses were labelled clicks while pulse groupings were labelled chirps, following Broughton (1963).

In contrast, Allen and colleagues (1958) and Fleming and colleagues (2013) reported observing female sounds that had more homogenous characteristics, albeit they were still rare. In those studies, however, there was no direct evidence linking sounds to females as opposed to males, since signalling males were also present during the recordings. The characteristics of the sounds in question in those studies - groups of low amplitude pulses which had regularly spaced intervals following the main male chirp - were consistent with a newly observed component to male chirps, termed trailing pulses (Chapter 3). Here, we observed trailing pulses following male *D. ponderosae*

disturbance chirps as well. This finding of trailing pulses now in males of not one but two species of *Dendroctonus* suggests their occurence may be widespread in males across the genus. These are likely not a part of the males' signal but rather a by-product of the sound production mechanism (Chapter 3). Consequently, it seems plausible that the speculated female chirps of those two earlier studies were produced by males.

Lack of evidence for a specialized female sound producing organ

Both *D. valens* and *D. ponderosae* females had a reduced elytral file as compared to male *D. valens*, and malformations of the female file were common. The file of male *D. valens* has been shown to be involved in acoustic signalling, and since signalling characteristics have been linked to successful courtship, the file thus plays an important role in male fitness (Lindeman and Yack 2015; Chapter 2). Therefore, the highly regulated structure of the males' files here was expected as a requirement for males to produce behaviourally significant sound. Although only *D. valens* males were examined here, the file shape found was consistent with previous reports of the file for males of other *Dendroctonus* species (e.g. Michael and Rudinsky 1972; Lyal and King 1996). This homogeneity of file structure likely extends to the males of many *Dendroctonous* species. Conversely, the reduction of the file in female *D. valens* and *D. ponderosae*, and its heterogeneous structure in individuals within and between species, may indicate that its potential use in sound production is not under strong selection.

Males and females do not necessarily need to have the same file structure in order for both sexes to produce sound; sound production may have evolved via

different mechanisms in both sexes. Consistent with this argument, females do not have the processes on the seventh abdominal tergite that function as the male plectrum (e.g. Lyon 1958; Jantz and Johnsey 1964; Tate and Bedard 1967; Michael and Rudinsky 1972; Rudinsky and Michael 1973; Pajares and Lanier 1990). Rather, females have been speculated to use a completely different structure as a plectrum: the chitinized tip of the eighth abdominal tergite (Rudinsky and Michael 1973). Thus females may still produce sound by an elytro-tergal stridulatory mechanism that is independent from the males. However, two out of the six *Dendroctonus* species for whom female sound production has been reported have also been found to not have an elytral file at all (Rudinsky and Michael 1973). In those species, females were suggested to produce sound by using a file on the sternite instead (see Rudinsky and Michael 1973 for sternite file details). If Rudinsky and Michael's (1973) speculations of separate elytro-tergal and tergal-sternite sound producing organs for various female *Dendroctonus* species is correct, then *Dendroctonus* females would have had to have developed a sound producing organ independently from males, not just once, but twice. A much more likely explanation is that the elytral file is not involved in any female *Dendroctonus* spp. sound production, and there is an alternative sound production mechanism common to all sound producing *Dendroctonus* females.

Using high speed video and simultaneous sound recording, we observed that sound pulses are produced by an interaction between the last abdominal sternite and the elytron. The abdomen, which usually sits flush against the elytra, would be lowered and raised, and upon raising would "click" back into place against the elytron. This

would produce pulses of sound, although not in all cases. Occasionally, multiple sound pulses were found to arise from a single abdominal movement event, although there was not enough resolution to determine how multiple pulses occurred. Such a mechanism is consistent with the qualitative characteristics of the sound. For example, Rudinksy and Michael (1973) described female sounds as what would be expected to emanate from the abrupt contact of two chitinous surfaces.

Although female *D. ponderosae* and *D. valens* both had elytral files, it does not necessitate that the files function in sound production. As Darwin (1859) observed: "Rudimentary, atrophied, or aborted organs. Organs or parts in this strange condition, bearing the stamp of inutility, are extremely common throughout nature." Here, we suggest that the reduced elytral files of female *Dendroctonus* spp. are not used in sound production. Rather, we postulate that sound is produced by a striking movement of the last abdominal sternite against the elytra.

Reactions by conspecifics encountering female sounds

Rare and irregular groups of sound pulses found in some *D. valens* females does not necessarily indicate that the sounds function as signals in communication. The best evidence found here in support of a signalling function for female sounds was the finding that during territorial encounters intruders tended to exit the gallery faster when the resident produced organized trains of sound pulses. However, because the interactions between the intruders and residents occurred within the gallery, it was impossible to isolate an intruder's reaction to the sound emissions alone. It is possible

for instance that increased aggression in a resident correlates with both increased sound emissions caused by aggressive activities, as well as a decreased time before the intruder is expelled. In this scenario, it is unclear whether the sounds are provoking the intruder to leave, or are by-products of the struggle of the resident against the intruder. In one of the encounters where putative signals were observed, the resident was seen to forcibly eject the intruder at the end of the trial, supporting the link to heightened aggression. However, in that same trial, the resident continued producing the sounds for over a minute following the departure of the intruder, indicating that sounds are not exclusively noise from resident-intruder struggle. Consequently, the meaning of this result is unclear. A straightforward finding, however, was that intruders always eventually left the resident's gallery, regardless of whether the resident signalled.

During encounters with males at the gallery entrance in this study, female *D. valens* were never found to produce putative signals. This is in contrast to previous reports of female-produced sounds during interactions with males for *D. valens* (Ryker and Rudinsky 1976a), *D. pseudotsugae* and *D. brevicomis* (Rudinsky and Michael 1973; Rudinsky et al. 1976). However, earlier studies also found no change in males' behaviour at the gallery entrance when in the presence of playbacks of female sounds or in silence (Rudinsky et al. 1976). This was consistent with the finding in the present study that variability in male behaviour could be observed independently of the presence of female sound production. These results taken together with those from previous reports suggest that there is variability in a male's behaviour at the gallery entrance regardless of whether the female produces sound.

Conclusions and future directions

The genus *Dendroctonus* has many destructive, tree-killing members, and as such research on this group is of ecological and economical value. Determining how acoustic signals come into play in important life history events and economically important behaviours such as female gallery spacing (territoriality) could help inform acoustic technology strategies as a targeted approach to managing this serious problem. Furthermore, there are practical reasons for studying the question of female acoustic signalling. Many researchers have reported sexing individuals of this genus by the presence or absence of disturbance signals and/or stridulatory movement without a clear picture of whether females also signal (e.g. Chapman 1955; McCambridge 1962; Tate and Bedard 1967; Godbee and Franklin 1978; White and Hobson 1993; Fleming et al. 2013; Shi and Sun 2010). Acoustic sexing would be a quick and useful sexing method if it is accurate, but using this method without a comprehensive understanding of female sound production may lead to misidentification of sex. In this study, females were found have measureable amounts of acoustic energy above the background, and in some contexts, including during territorial encounters, some individuals produced interesting groups of sound pulses. Whether these sounds are used in communication, however, remains unclear.

4.6 Acknowledgements

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CHAPTER 5: General discussion and conclusion

5.1 In brief

Bark beetle ecology has received intense research interest (see Rudinsky 1962 and references therein), and particular attention has been paid to destructive species, such as those of the genus *Dendroctonus* (Byers 1995). However, there remain certain research areas that have been previously underrepresented and for which little is known. The acoustic ecology of bark beetles is one of these lesser studied fields, despite the fact that the majority of species employ acoustic communication in many contexts. This doctoral thesis forms the most comprehensive story to date of the ultimate and proximate nature of sound production in both sexes in *Dendroctonus valens*, used as a representative of the genus *Dendroctonus*.

5.2 The ultimate function of male acoustic signals during courtship

Acoustic signals are commonly used by insects in the context of mating, and close-range courtship signals may be the most influential type of acoustic signal in mating decisions in insects (Fitzpatrick and Gray 2001; Rebar et al. 2009). It has been firmly established in the literature that for many bark beetle species the attracted sex stridulates during male-female interactions. The outcome of muting experiments for several species also suggested that sound production is important to courtship success (Wilkinson et al. 1967; Barr 1969; Ryker and Rudinsky 1976b). Yet, there remained many diverse opinions as to the function of signals and their role in courtship (e.g. Barr 1969; Ryker and Rudinsky 1976b; Yandell 1984; Raffa and Dahlsten 1995).

In Chapter 2, I tested the hypotheses that male acoustic signals during interactions with females are honest indicators of male condition and that females make mate choice decisions based on signal characteristics. It was observed that the abundance of interrupted chirps during a male's courtship performance correlated with an indicator of his quality, body size, with larger males producing significantly more interrupted chirps. The average number of interruptions per chirp was also related to male body size. These interrupted chirps were important to females, who only rejected males that did not include any in their performances. Based on these findings, I concluded that the females in our study distinguished between males on the basis of their chirp performances.

Why would interrupted chirps be attractive to females? Simple and interrupted chirps had very similar characteristics, except for two key features. First, interrupted chirps were significantly longer than simple ones, and thus may be more efficient at surpassing a female's threshold of stimulation before she elicits the desired behavioural response of accepting the male into the gallery (e.g. Marsat and Pollack 2010). However, it was observed that there were more teeth on the file than pulses in either simple or interrupted chirps. Without yet knowing the mechanism of sound production, it was assumed that each sound pulse was caused by a tooth being struck, a common assumption in bark beetle literature (e.g. Michael and Rudinsky 1972; Ryker and Rudinsky 1976a). To make chirps longer, why wouldn't males simply incorporate more tooth strikes into their chirp? The second key feature that separates simple from interrupted chirps is the difference in the temporal pattern of pulses between the two

chirp types. I speculated that the gaps in interrupted chirps were produced by a more complex motor performance than required for simple chirps, and skill in the performance of a challenging action may also be a reliable indicator of fitness and developmental stability because the skill necessarily reflects musculoskeletal, nervous and sensory system function (Byers et al. 2010). Consequently, by the conclusion of Chapter 2, questions remained regarding the proximate mechanisms of simple and interrupted chirp production, and how incorporating interruptions in particular might stimulate a response in females for successful mating.

5.3 The proximate mechanism of sound production

I addressed those unanswered questions relating to the proximate mechanisms of sound production in the project reported in Chapter 3: What are the mechanics of *D. valens* sound production? And how are interruptions incorporated to produce the distinct temporal pulse patterns for simple and interrupted chirp types? Proximate mechanisms underlying variable signal production are in general understudied in the literature (Podos and Patek 2014), but regarding elytro-tergal stridulators in particular almost nothing was known.

Using ablation experiments coupled with high speed video and audio recordings, the mechanism of sound production was observed to be consistent with 'spring stridulation'. The changes to the temporal pulse patterns, which generate the variable chirp types, were found to be achieved through the same mechanism of spring stridulation, but by altering the number of energy storing events between the chirp

types. In simple chirps, elastic potential energy was stored once by deforming the plectrum and then releasing it so that the plectrum sprang forward, interacting with each tooth on the file along its path. Conversely, interrupted chirps resulted from recharging the energy store repeatedly over the course of a chirp. Using spring stridulation to adjust the pattern of pulse production in this way is, to the best of my knowledge, a newly found method of employing elastic energy to incorporate variability into an individual's acoustic signalling repertoire.

Complexity in signalling in a species may have originated and been maintained because females prefer males who produce complex signals (Darwin 1871; Ryan and Keddy-Hector 1992; Andersson and Simons 2006; Elias et al. 2006). The ultimate reason for this may be because such signals predict male quality (Spencer et al. 2003; Hebets and Papaj 2005; Searcy and Nowicki 2005). In *D. valens,* the precision involved in adjusting the sound production mechanism to produce interrupted chirps likely reflects neuro-muscular control that could be associated with skill, and skillful motor actions are coupled to fitness (Byers et al. 2010). Consistent with this notion, the experiments of Chapter 2 linked chirp type production with signaller fitness. An alternative, but not mutually exclusive hypothesis is that the complexity of the signal exploits the females' sensory bias leading to its use by males (Searcy 1992; Stripling et al. 1997; Hebets and Papaj 2005). Because nothing is known of how bark beetles sense acoustic stimuli, this possibility remains unexplored.

5.4 Sound production in *Dendroctonus* females

> "The behaviour of an animal of a given species must be considered as forming a whole and, although it is convenient to split it up to study this or that particular point, the results obtained should always be fitted into the general framework and the different parts should be connected together in their natural sequence"
>
> - Busnel, 1963

The acoustic signals of *Dendroctonus* spp. males have been described many times over the past several decades (e.g. Ryker 1988). Contrarily, *Dendroctonus* spp. females have been a point of contention. They have been assumed by most to be mute (e.g. Barr 1969), and sexual dimorphism in sound production is the rule rather than the exception in bark beetles (Barr 1969; Lyal and King 1996). However, there have been reports over the years of purported female signalling in several *Dendroctonus* species (Allen et al. 1958; Rudinsky and Michael 1973; Ryker and Rudinsky 1976a; Fleming et al. 2013). In Chapter 4, I explored whether females signal acoustically as part of a comprehensive picture of sound production in *D. valens* and *D. ponderosae*.

The earlier reports of female acoustic signalling have tended to pick out characteristics of female sound and attribute communicative functions to them to fit the general idea of female signalling. However, if earlier reports of female acoustic behaviour are considered within a larger framework, as advised in the quotation above by Busnel (1963), concerns arise as to the strength of the conclusions drawn. Female sound production was rare, and in some cases sounds were so irregular that Rudinsky

and Michael (1973) noted the sounds were not amenable to quantitative analysis. Sounds were also never clearly linked to female stridulation or separated from noise. And finally, although in all studies females were interacting with conspecific males or females at the time of sound production, only inconclusive evidence was gathered to suggest that the sound influenced the behaviour of the "receiving" individual.

Consistent with earlier studies, my project in Chapter 4 identified groups of sound pulses in female *D. valens* which were rare and irregular, but which I also determined were distinct from noise. These sounds were not produced by a specialized elytro-tergal stridulatory organ but rather there appeared to be mechanism of striking the last abdominal sternite against the elytra to produce sound, a behaviour not seen in males. These findings do suggest that female *D. valens* are capable of producing sound. However there was little evidence to suggest that these sounds were used in communication, and their presence or absence did not conclusively alter the outcome of territorial or courtship encounters. Given the large number of females who were not observed to produce any sound, the irregularity of the sounds when they were present, and the indifferent response of interacting individuals, it can be concluded that this study did not find strong support that *Dendroctonus* spp. females signal acoustically.

5.5 Future directions

There remains much that can be done in the area of bark beetle acoustic ecology. Below, I discuss the areas where I see future research on this subject being the most valuable.

Male signal function in a disturbance context

It has been firmly established that one of the contexts in which sound producing bark beetles produce sound is during disturbance (Ryker 1988). These sounds have been proposed to have an anti-predation function (e.g. Ryker 1988). In Chapters 2 and 3 of this thesis, I found that *D. valens* males vary the proportion of chirp types produced in courtship and disturbance contexts, with simple chirps being heavily favoured over interrupted chirps during disturbance. Because simple chirps are short, have a simple temporal pattern and a high repetition rate, they are consistent with sounds shown to have an anti-predatory function in other insects (Masters 1979; Masters 1980; Conner 2014). Thus one future direction of study is to test whether *Dendroctonus* spp. simple chirps function in anti-predation.

Lewis and Cane (1990) tested the acoustic anti-predation hypothesis using *Ips calligraphus*, an acoustically sexually dimorphic bark beetle species where females stridulate and males are silent. In that study, it was determined that a predator, *Thanasimus dubius* (Coleoptera: Cleridae), released signalling females significantly more often than the naturally mute males during predation encounters. These findings support the hypothesis that disturbance stridulation is an anti-predatory behaviour; on a vertical surface like a tree dropped individuals may fall out of harm's way, thus increasing their chance for survival (Lewis and Cane 1990).

There has been one other study on deimatic stridulation in bark beetles, which found opposing results to Lewis and Cane (1990). Sivalinghem (2011) tested the bark beetle *Ips pini* against *T. dubius* and found limited evidence of deimatic stridulation in

that bark beetle species. Even though all females were capable of sound production, as determined prior to the experiment, not all females produced sound when attacked and those that did began sound production several seconds after the onset of an attack. Furthermore, none of the predators released their prey, and there was no increased latency in handling time for signalling females as compared to mute females or males (Sivalinghem 2011). The contrast between the two studies may be owing to the low signalling rate found in *I. pini*; high signal repetition rate is likely an important part to the anti-predatory function of signals (Masters 1979; Masters 1980; Conner 2014).

Lewis and Cane's (1990) findings may be generalizable beyond *I. calligraphus.* *Thanasimus dubius* is an oligophagous predator of *Ips* and *Dendroctonus* bark beetles (Vité et al. 1964; Frazier et al. 1981), thus it is possible that disturbance stridulation would have a similar anti-predatory function in any robustly signalling species, including *Dendroctonus* species. Consistent with this prediction, a study investigating the predator-prey interactions between *T. dubius* and *Dendroctonus frontalis* has shown that handling time was significantly higher for male *D. frontalis* than for females (Frazier et al. 1981). In that study, handling time was never linked to sound production, but male *D. frontalis* are known to produce robust chirps consistent with other *Dendroctonus* spp. males (Ryker 1988) while females are silent, and thus sound production may have been a key variable contributing to the handling time result.

A future study to test the role of male *Dendroctonus* spp. disturbance stridualtion during predator-prey interactions might go about first testing the latency during which a predator handles either a signalling prey or a surgically muted prey.

Surgical muting of *Dendroctonus* spp. males is simple and has virtually no recovery time owing to the location of the file (see Chapter 2). Despite the sexual dimorphism in sound production in *Dendroctonus* spp. (e.g. Chapter 4 and Appendix 1 of this thesis), using experimentally muted males as a control would be recommended against the use of males versus females as done in Lewis and Cane (1990). Females may be capable of emitting sound and there may also exist the potential of sex differences of prey in size or struggling ability. The acoustic predatory defence hypothesis would predict that intact males will begin signalling upon attack, and that a predator will be more likely to drop and have a longer handling time of signalling males over muted males.

Transmission of vibrations

Communication through substrate vibrations is pervasive in plant-dwelling insects (Virant-Doberlet and Čokl 2004; Cocroft and Rodriguez 2005). Up until recently, however, this transmission channel was ignored in bark beetle research. Over the last five years several studies have shown that there is a substrate-borne component to bark beetle acoustic signals (Sivalinghem 2011; Fleming et al. 2013; Goulding 2013). Goulding (2013) observed that when bark beetles are interacting within the gallery at unknown distances from a microphone, a vibration sensor is actually better than the microphone at receiving *I. pini* chirp signals above noise. In Appendix 2, I present a similar finding: although overall the microphone was better than the vibration sensor at detecting male *D. valens* chirps above noise, there were long train pulses of unknown function that were almost exclusively received over the substrate borne channel. Given the

environment in which bark beetles live and communicate acoustically - i.e. in galleries constructed along the phloem layer - substrate borne vibrations may be an important component of the acoustic signal, one which should be considered in more detail.

Now that the vibratory component to bark beetle acoustic signals is known, future studies should attempt to better characterize this component of the signal. This would include examining the type of vibration transmitting the signal. Vibrations are defined by particle motion (e.g. Hill 2008, 2009). Within the context of vibrational communication, the term "vibration" tends to refer to substrate-borne boundary waves (Hill 2008). The type of boundary waves used for communication are almost exclusively restricted to Rayleigh waves (combined longitudinal and transverse waves) or bending waves (Hill 2008), although there are examples of information being received through compressional waves (Brownell 1984) or transverse waves (Aicher and Tautz 1990). Identifying the type of vibration is an important step, as it can provide information on how a given signal would be transmitted through the environment (Hill 2008).

A second question for future research in this area to address is regarding the transmission properties of the substrate - the phloem - of which to the extent of my knowledge little is known. The question may be complex with the phloem's transmission properties potentially influenced by its density, which can vary both between and within trees, and its water content, which can vary within trees with time. However, this is an important area, because the characteristics of a substrate can affect the vibrations they transmit, either by acting as a filter or by attenuating frequencies differently (Hebets et al. 2008), and the acoustic behaviour of animals using these transmission channels are

often tuned to accommodate the substrate they are communicating on. For example, green plant tissues are effective low-pass filters (e.g. Čokl et al. 2007). In the case of species living on green plant tissues, communication over long distances may be achieved by using low-frequency signals with narrow frequency peaks that are attenuated less (e.g. Čokl et al. 2007), or else animals without finely tuned signals can use broader band spectra and restrict communication to a close range (e.g. Čokl et al. 2004). Nothing is known of the extent to which bark beetles might make use of the phloem as a transmission channel for their communicative signals, and if they do so, then how?

Identifying acoustic sensory receptors

In my studies on acoustic signalling during courtship in *D. valens*, it was observed that sounds produced by males function in female mate choice decisions. Other species from the genera *Dendroctonus* and *Ips* have also shown that females and males, respectively, respond to acoustic signals, because muted individuals are less likely to be successful during courtship (Wilkinson et al. 1967; Barr 1969; Ryker and Rudinsky 1976b). Nevertheless, no research has of yet addressed the question of what sensory organs are employed in detecting these signals. It is not known where on the body hearing organs may be located, or if they function in detecting the airborne or substrate-borne (vibratory) component of the sound.

Part of the trouble in identifying acoustic sensory organs in bark beetles is the lack of knowledge of such organs in Coleoptera in general. In 1798, Hermann Samuel

Reimarus first remarked that hearing organs necessarily exist for coleopterans, because sound production is so widespread in the order (Wessel 2006). Since Reimarus' remarks, Johnston's organs have been identified in Gyrinidae (Wilde 1941) and Dytiscidae (Lehr 1914; evidence of vibration sensitive mechanoreceptors has been found in this family as well, e.g. Hughes 1952). More recently, tympanal ears functioning in predator avoidance have been identified in two families: Cicindelidae (Spangler 1988; Yager and Spangler 1995) and Scarabaeidae (Forrest et al. 1997; Yager 1999). Finally, evidence for the detection of substrate-borne vibrations has been presented for Tenebrionidae (Slobodchikoff and Spangler 1976) and Geotrupidae (reviewed in Wessel 2006). Consequently, for an order representing almost a quarter of all animal life (Hunt et al. 2007) acoustic receptor organs have been severely under reported. This may be in part owing to the fact that hearing organs in insects can occur on almost every part of the body (Yack 2004), varying greatly in structure, from external hairs and sensillae to internal chordotonal organs (Hoy and Robert 1996; Yager 1999; Virant- Doberlet and Čokl 2004; Yack 2004).

In bark beetles, some features of their acoustic behaviour and life history may lend clues as to which type of acoustic receptor they possess. For example, there is a large component of energy present at high frequencies in the airborne sounds (Fleming et al. 2013, Chapter 3 this thesis), which may indicate the presence of a tympanal ear (e.g. Yack 2004). However, the bulk of evidence points to a vibration sensor. First, bark beetles signal acoustically only within very close distances (~1cm, Barr 1969). Second, the environment in which bark beetles spend most of their lives is under the bark of

trees in galleries constructed along the phloem layer. Many other species living in similarly confined environments (e.g. termites, social wasps, ants and social bees), or species who spend the bulk of their lives interacting on surfaces similar to the phloem (continuous and capable of transmitting vibrations; e.g. whirligig beetles or leaf hoppers) possess sensitive vibration detectors. Indeed, the vibratory channel is in general far more prevalent than the use of airborne sound for communication in insects (Michelsen et al. 1982; Vibrant-Doberlet and Čokl 2004). And, as mentioned above, recent evidence shows that bark beetle acoustic signals are not just airborne but can be transmitted through the substrate as well (Sivalinghem 2011; Fleming et al. 2013; Goulding 2013; Appendix 2).

Hearing/vibration detection in bark beetles is a wide field of research where many questions remain and thus it is a great avenue for future study. Based on the confined environment bark beetles communicate acoustically in and the higher frequency characteristics of the signal (i.e. greater than predicted for a near-field receptor), perhaps a leg or antennal vibration receptor would be the most compelling region upon which to focus. Future studies could employ a histological approach to examining the tibia and femur of sound producing bark beetle species to investigate the presence of chordotonal organs or campaniform sensilla.

5.6 General conclusion

The genus *Dendroctonus* has been hailed by forest entomologists as the "greatest threat to North American forests" (Hopkins 1909) and the "greatest tree killers

known" (Wood 1963). As managers attempt to decrease pesticide use, control through behavioural manipulation becomes an important management strategy (e.g. Cardé 1990; Wall 1990). To date, behaviour-modifying chemical compounds have been used against bark beetles as a form of integrated pest management, for example in fighting the outbreak of *Dendroctonus ponderosae* in Western Canada over the past several decades (Borden 1990). However, all sensory systems are intricately involved in an insect's behaviour, and so behavioural manipulation is not just limited to the use of chemical compounds. A number of management programs exist for pest insects in general which use acoustic technology. Knowledge of pest insect acoustic behaviour has been applied to monitoring species presence (for example in grain samples: Shuman et al. 1993; Mankin et al. 1997; grain bins: Hagstrum et al. 1996; and wood structures: Lemaster et al. 1997; Scheffrahn et al. 1997) and abundance (e.g. Fuji et al. 1990; Lewis and Lemaster 1991; Scheffrahn et al. 1993). Also, the development of acoustic baits (e.g. Walker 1988) has led to successful trapping of many insect pests, including mosquitoes (Kahn and Offenhauser 1949; Offenhauser and Kahn 1949), midges (Hirabayashi and Ogawa 2000; Hirabayashi and Nakamoto 2001), mole crickets (Ulagaraj and Walker 1973; Walker 1982), field crickets (Campbell and Shipp 1974; Walker 1986), moths (Spangler 1984, 1987) and fruit flies (Mankin et al. 2004). Finally, acoustic technology has also been applied to manipulating pest insect behaviour (e.g. Čokl and Millar 2009; Samarra et al. 2009). Since so little was previously known of bark beetle acoustic behaviour, these kinds of management programs have generally not yet been developed, although the first steps have been taken (e.g. Hofstetter et al. 2010; Aflitto

and Hofstetter 2014). To date, my research has added to our understanding of how crucial the acoustic sensory system is in the life history of *Dendroctonus* species. An understanding of the bark beetle acoustic sensory system will help inform new management programs, and enable the application of acoustic technologies as a targeted solution for this serious problem.

Appendix 1: A study on variables influencing rearing success, emergence patterns and acoustic sexing in *Dendroctonus valens*

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A1.1 Abstract

The purpose of this study was to develop a laboratory colony of *Dendroctonus valens* in order to assess which variables influence brood success, the patterns of emergence, and whether acoustic signalling can be used as a reliable method for sexing. The development of the colony began with an efficient, low-maintenance rearing method where sixty-four percent of matings successfully resulted in a second generation of emerging beetles. It was found that the size of parents had little effect on the success of broods. Conversely, the size of the host bolt had a close relationship to brood success, with successful broods emerging on average from bolts larger than bolts from which no offspring emerged. The emergence patterns for second generation females and males was similar, with the majority emerging between 141 and 150 days post introduction and an overall sex ratio of 0.91:1 (F:M). Finally, stridulatory sound evoked by handling disturbance was found to be an efficient and reliable mechanism for quick sexing of *D. valens.* While this method had 100% accuracy if testing was done on the day of emergence, food deprivation trials found an increased risk for males to cease chirping behaviour over time. This error was small, however, and on any given day over the trial 98% of males produced clear chirps. Together, these findings contribute to general *D. valens* life history knowledge as well as inform future methods for the study and rearing of this species.

A1.2 Introduction

Bark beetles (Coleoptera: Scolytinae) can be economically important pests which cause massive destruction in both their native ranges and in locations of accidental introduction. For example, the genus *Dendroctonus* contains many species which are aggressive within their native ranges in North America (e.g. Six and Bracewell 2015). Some *Dendroctonus* species have also become established and invasive in non-native ranges, such as *Dendroctonus valens* in China (Yan et al. 2005). The purpose of this study was to examine several life history traits of *D. valens* by producing a laboratory-reared colony which could be followed from mated pair to emerging offspring.

There are many challenges that researchers potentially face when studying bark beetles. One challenge is the difficulty with which some species are collected. In general, a bark beetle will remain within its host plant for the vast majority of its life cycle, emerging only briefly to search for new hosts or mates (Raffa et al. 2015). In seasonal climates, this life history trait may translate into short windows of dispersal during which individuals can be trapped in flight, as is the case for *D. valens* in the northern reaches of its range (Owen et al. 2010). For research purposes this often means many individuals might be collected within a short period of time, with a scarcity of beetles throughout the intermitting periods. A simple solution to this problem is rearing a laboratory colony to provide a controlled setting where a large number of individuals could potentially be available throughout the year. Such a laboratory-rearing method may also be an asset to working with destructive species, as it has proven to be a useful tool for the contained study of other pest insects (e.g. the Asian longhorned beetle,

Dubois et al. 2002). Previous rearing methods for *Dendroctonus* spp. have included collecting bolts from beetle infested trees and placing them in temperature-controlled settings until brood emergence (e.g. Atkins 1967) as well as artificially introducing mating pairs to bolts (e.g. Godbee and Franklin 1978). The present study was modelled after the latter approach so that broods could be followed from the initial mating pair through to emergence of offspring. One advantage of this method was that the size of parents and host bolts could be investigated to determine the extent to which such variables might influence brood success and offspring characteristics. A second advantage was that the emergence patterns of offspring, including total time spent in the log, could be monitored for each brood.

A second research challenge which is more specific to *D. valens* is in the difficulty of determining the sex of live individuals. In the genus *Dendroctonus* as a whole, there is a common morphological sexual dimorphism on the seventh abdominal tergite, where males have sclerotized processes located medially along the posterior ridge of the tergite and females do not (Lyon 1958; Rudinsky and Michael 1973; Godbee and Franklin 1978). In the case of *D. valens*, this is the only clear morphological sexual dimorphism (Fig. A1.1). However, owing to its small size and location beneath the elytra, this is an unpractical characteristic for quick sexing or when in the field. An easy solution to the sexing problem could be devised if the sexual dimorphism on the seventh abdominal tergite translates into a dimorphism in acoustic signalling ability. The

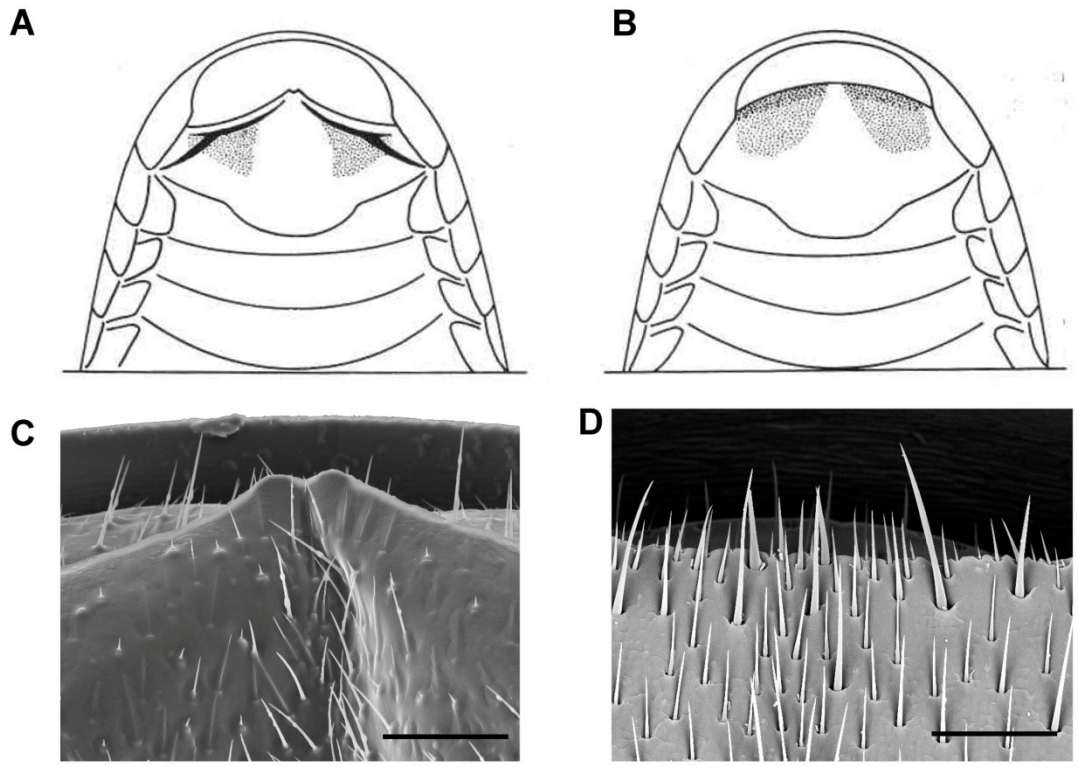

Figure A1.1. Sexual dimorphism of the seventh abdominal tergite. (A-B) Schematic drawings of *Dendroctonus brevicomis* abdomen illustrating the dimorphism in the presence or absence of processes on the seventh abdominal tergite in males and females, respectively (Sketch reproduced from Lyon 1958). (C-D) SEMs of the medial posterior margin of the seventh abdominal tergite in a male and female *D. valens*, respectively; scale bars = 100 µm.

structure in question plays the role of plectrum (an excitatory structure) in the stridulation of male *Dendroctonus* beetles (Barr 1969; see also Chapter 3), and thus its altered morphology in females may correspond to an inability for them to produce the stereotypical chirps seen in males (for male chirp characteristics, see Chapters 2 and 3). There are two key requirements for determining whether sound production can be used as an acceptable sexing method for *D. valens.* First, it must be established that males are dependable acoustic signallers. Acoustic signalling may be an energetically expensive behaviour for *D. valens*, as it has been found to be for other insect species (e.g. Mowles 2014). Thus, energetically costly behaviours like flight activity during dispersal might negatively affect a male's ability to signal, making this an unreliable trait. Second, it must be established that females are either mute, or that the sounds they produce are qualitatively different from males such that they can be easily discriminated between. Neither of these requirements has been investigated; nothing is known of how the depletion of energy stores may affect the reliability of male signalling behaviour, and female sound production is ambiguous (e.g. Ryker and Rudinsky 1976a; Chapter 4). Yet, some previous research has reported sexing *D. valens* by the presence or absence of stridulating sounds during disturbance (by assuming males do and females do not stridulate, e.g. White and Hobson 1993; Shi and Sun 2010). Before sound production can be used as an acceptable sexing method for *D. valens*, the present study sought to establish whether males are dependable acoustic signallers during disturbance - tested using food deprivation as a proxy for energy depletion during flight (e.g. Gries et al.

1990; Kinn et al. 1994) - and to confirm that female sounds, if present, are reliably distinguishable from males.

In this study, a low-maintenance laboratory rearing method is described for *D. valens*, with the emerging broods then used to test important life history questions: how do parent and bolt sizes influence brood success? What are the patterns of emergence for second generation males and females? These questions can help direct future breeding programs. Finally, we also tested the question of whether individuals can be sexed acoustically. If there is indeed a robust sexual dimorphism in acoustic signalling then this may be a quick and efficient method that could be used in any context without mechanical equipment.

A1.3 Methods

Animals

Adult *Dendroctonus valens* (Curculionidae: Scolytinae) were collected in August and September of 2013-14 at several locations near Ottawa, Ontario, Canada (the arboretum at the Ottawa Central Experimental Farm, 45.391021,-75.70489; Carleton Lands, Manotick, 45.183882,-75.604673; and outside Petawawa, 45.853530, -77.536156). Collection of live beetles was conducted using Lindgren funnel traps baited with *D. valens* lure (Contech, British Columbia, Canada). Individuals were stored for up to two weeks in individual vials at 10^{o}C with a moistened kimwipe and bark cuttings (red pine, *Pinus resinosa*) until use.

Rearing conditions

The first variable which was considered for its potential influence of brood success and offspring characteristics was the diameter of their host bolt. In May of 2013 and 2014, two red pine trees from Carleton Lands were selected based on their size (trees with an approximate average diameter between 15 and 30 cm) appearance of general health (assessed by colour of pine needles) and a lack of visible signs of insect infestation. Trees were cut down and divided into 1 – 1.5 foot long bolts (average bolt length ± SE: 36.3 ± 1.1 cm). Mating pairs were introduced to 14 bolts, seven during 2013 and seven during 2014, and bolt diameter ranged from 18.2 to 24.0 cm (average ± SE: 21.3 ± 0.5 cm). Within one day of cutting, both ends and any branch stumps on the bolts were sealed with wax (tissue embedding medium, Paraplast) to inhibit desiccation and fungal infestation. Bolts were stored in a walk-in cold room at 5^{o}C for three months before use (all introductions were done in August and September, 2013 and 2014).

The influence of the size of parents on brood success and characteristics was also an important variable considered. Male and female *D. valens* used as the mating pair were first measured for width (taken at the anterior end of the pronotum) using Zeiss AxioVision digital image processing software (Oberkochen, Germany) and a Zeiss AxioCam MRc5, 1.4 megapixel (1388 x 1040) camera (Oberkochen, Germany) mounted on a light microscope (Olympus SZX12, Tokyo, Japan).

The procedure for introducing mating pairs to the bolt was as follows. First, each bolt received only one mating pair of *D. valens*. Using a knife, small holes (~1cm wide) were dug through the bark to the cambium to encourage the females to initiate gallery

construction. One female was placed inside the hole and was sealed in by an Eppendorf tube that had its tip cut off and was affixed to the bolt using plasticine (Fig. A1.2). Females were left to begin gallery construction, as confirmed by a buildup of frass within the Eppendorf tube, which occurred within 24 to 48 hours. After this time, the Eppendorf tube was removed and one male was placed at the entrance to the gallery. Males were observed to interact with females at the gallery entrance and their entry into the gallery was confirmed by visual observation. Bolts with introduced mating pairs were then placed individually into separate enclosures and maintained on a 12 hour light-dark cycle for the months of brood development. Room temperature was kept consistent (21^{o}C; average temperature ± SE during 2013: 19.0 ± 0.2^{o}C; during 2014: 21.3 ± 0.3^{o}C). Enclosures were monitored daily for signs of emerging beetles.

Emergence monitoring

Once emergence began, the patterns of emergence were recorded. Enclosures were monitored twice daily and emerging offspring were collected. For each bolt, the date of emergence, number of offspring and offspring size (measured as for the parents, above) was recorded. The majority of individuals was then sexed by lifting the elytra using a minuten pin while gently pushing down on the abdomen and examining the dimorphism of the seventh abdominal tergite under a light microscope (Olympus SZX12, Tokyo, Japan) (refer to Fig. A1.1). A sample of individuals was not initially sexed (n=66), and instead were sorted by the presence/absence of chirp production during

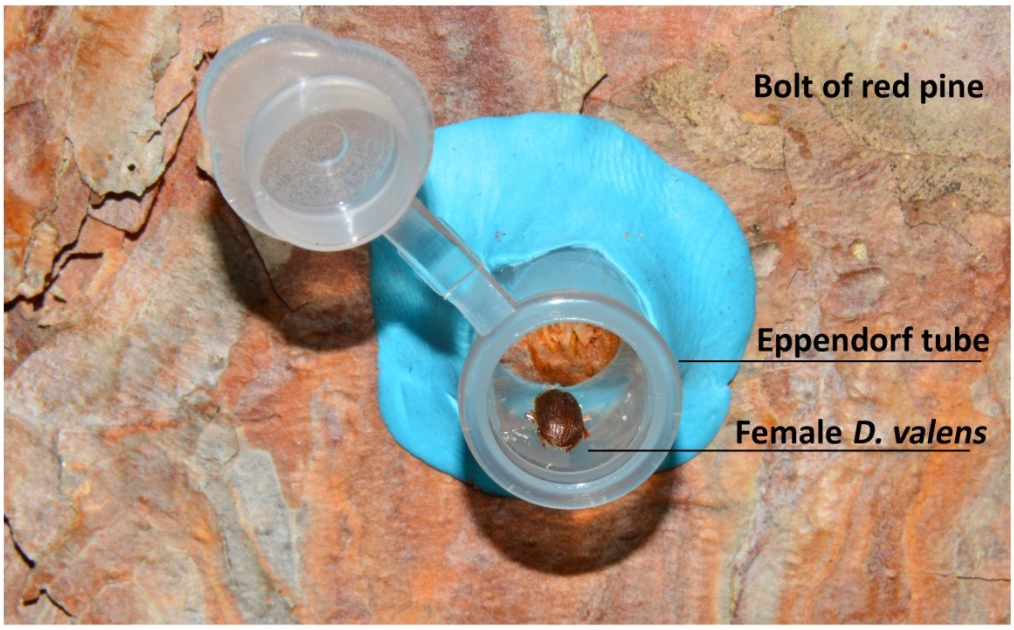

Figure A1.2. Image of an Eppendorf tube affixed to a bolt of red pine. Within the tube a female can be seen advancing towards a precut hole. The lid to the Eppendorf tube would then be closed to contain her. Scale: outer diameter of Eppendorf tube measures 10mm.

disturbance (see below) while blind to their sex, for use in the acoustic sexing study. These individuals eventually had their sex confirmed by checking the seventh abdominal tergite sexual dimorphism for inclusion of their data in the emergence study as well.

Sexing by presence/absence of chirps

The ease and accuracy of using chirp production for sexing *D. valens* was tested in three ways: (1) by determining the certainty with which males and females can be divided based on sound production using only the unaided ear of an observer on their day of emergence. This experiment incorporated the aforementioned individuals (n=66) who had been sorted blindly by an observer by the presence or absence of disturbance sounds. Disturbance sounds were evoked by holding individuals up to an observer's unaided ear while gently handling them by the head and pronotum for five seconds (e.g. Ryker and Rudinsky 1976b; Fleming et al. 2013; see also Chapter 3). Each individual was housed separately in a labelled vial. Vials were stored at 10^{o}C. The sex of each individual was then confirmed using the sexual dimorphism on the seventh abdominal tergite. (2) By confirming the reliability of an observer's unaided ear in sound detection by examining the recorded disturbance trial waveforms. This was done to assess the possibility that females may be chirping, but that the observer failed to detect the chirps. For this experiment, individuals (n=52 males and n=46 females) were first identified as male or female by examining the seventh abdominal tergite as above. Individuals were then subjected to 30 second long disturbance sound recordings (disturbance conditions as above). During disturbance, an Earthworks microphone

(model QTC40, Milford, USA) was held at 4 cm from each animal's abdomen, and was connected to a data recorder (FR-2, Fostex, Los Angeles, USA) set at a sampling rate of 96 kHz. Recordings were analyzed using Raven Pro 1.5 (Raven Bioacoustics Research Program, Cornell Laboratory of Ornithology, Ithaca, NY, USA). Files were examined for the presence of either stereotypical chirps (simple or interrupted; e.g. Lindeman and Yack, 2015; Chapters 2 and 3 of this thesis) or any other sounds present. Finally, (3) the reliability of the acoustic sexing method was determined over time by examining the consistency in male signalling behaviour with time. For this experiment, the same 52 males with disturbance trial recordings at emergence had repeated disturbance trials every day for the 10 days following emergence. Over this trial period, males were kept in separate vials and stored at 10^{o}C with moisture but no food. The rationale for food deprivation was as a means of energy depletion in order to observe how such a condition might impact signalling behaviour. Male sound files were analyzed for presence/absence of chirps and chirp rate (measured as number of chirps per second), and analysis was done to investigate changes to chirp presence and rate over time.

Statistical analysis

A linear correlation analysis was conducted using Pearson's r to test the relationship between the size of the parents and the size, number and emergence date of offspring, the relationship between the bolt diameter and the size, number and emergence date of offspring, the relationship between emergence time and offspring size, and the relationship between days since emergence and male chirp rate. Student’s

t-tests were also conducted to examine to relationship between the average bolt diameter for successful versus failed broods, and to explore the relationship between the size of males who were robust signallers over the course of the food deprivation study and those males who ceased signalling behaviour.

A1.4 Results

General emergence patterns

A total of nine out of 14 bolts had successful broods, with a total of 281 individuals emerged. There was an observed female:male ratio of 0.91:1, with 134 females and 147 males. There was a similar emergence pattern for males and females (Fig. A1.3A). Approximately half of the individuals emerged between days 141-150 post-introduction, with few individuals emerging very early, and the remaining individuals emerging between days 151 and 200 post-introduction (Fig. A1.3A). For males, the day of emergence had a significant effect on the size of the individual at emergence – the longer a male stayed within the log, the larger he was at emergence ($r=0.55$, $p<0.001$; Fig. A1.3B). However, this same pattern was not true for females, who showed no relationship between the day of emergence and size ($r=0.1$, $p=0.29$; Fig. A1.3B).

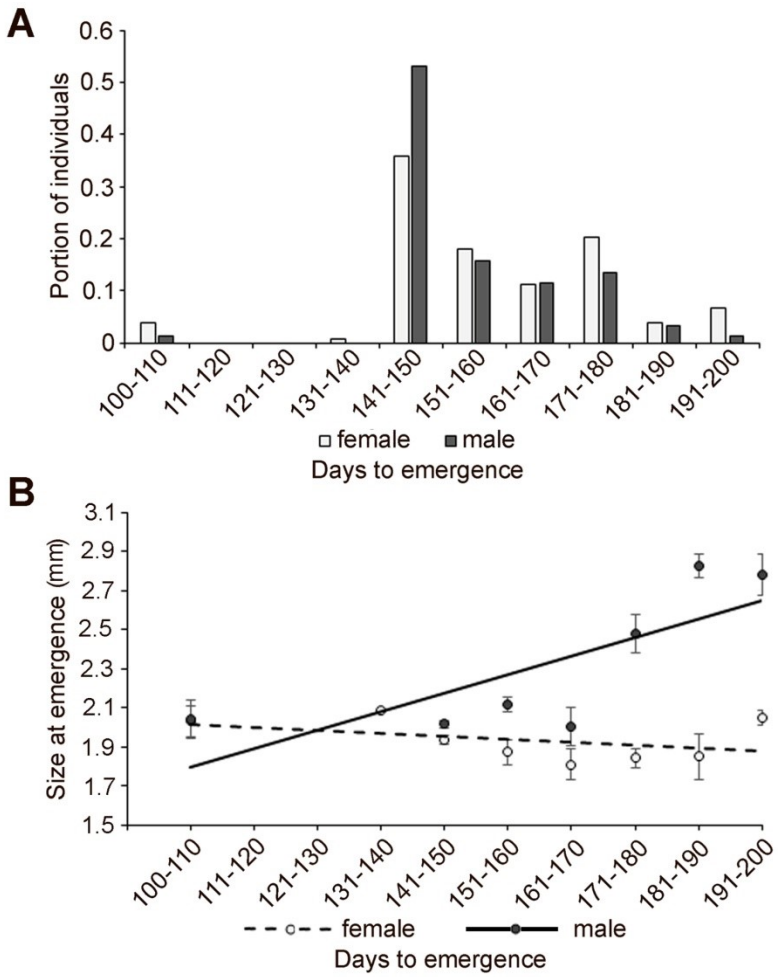

Figure A1.3. Emergence patterns of male and female *D. valens* reared on bolts of red pine. (A) Males and females followed similar emergence patterns, with the majority of offspring emerging 141- 150 days after the mating pair was introduced to the bolt. (B) There was an interaction effect between an individual's time to emerge and its size at emergence, where a significant positive relationship was found for males but no relationship was found for females.

Rearing success

Effect of parent size on brood characteristics

The characteristics of the emerging broods were examined to determine the effect of parent size on rearing success. Average parent size had no significant effect on the average size of the offspring (r=0.07, p=0.85; Fig. A1.4A), the number of offspring (r=0.28, p=0.33; Fig. A.4B), or the time until offspring first began emerging (r=0.41, p=0.27; Fig. A1.4C). The effect of each individual parent's size was also considered. The mother's size was not found to correlate with the number of offspring that emerged (r=0.22, p=0.6), the size of the offspring in general (r=0.002, p=0.99) or with the size of the daughters (r=0.33, p=0.52) (data not shown). Likewise, the father's size did not correlate with the number of offspring (r=0.26, p=0.53), the size of the offspring in general (r=0.15, p=0.73) or with the size of the sons (r=0.27, p=0.56) (data not shown). In all, there was little evidence that parent size had any effect on offspring characteristics.

Effect of bolt diameter on brood characteristics

The width of the bolt appeared to be of more importance to rearing success than parent size, with successful broods emerging from logs that were, on average, larger than logs with no emergence ($t_{2,8}$=2.1, p=0.07; Fig. A1.5A). Beside overall brood success, there was little relationship between bolt size and offspring characteristics. There was a weak trend for larger diameter logs to yield broods with a higher number of

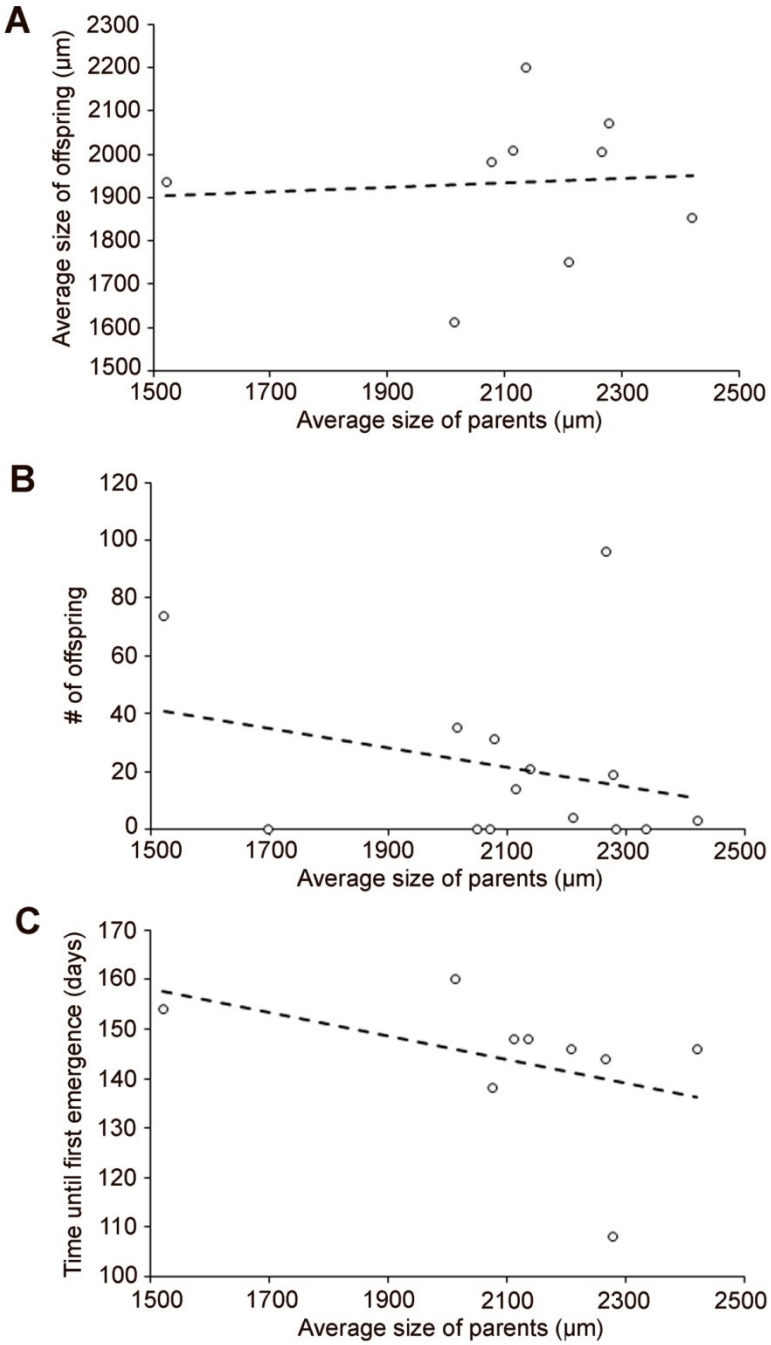

Figure A1.4. Relationship between the average size of a male-female pair and their offspring. There was no relationship between parent size and (A) the size of the offspring measured by the width of the pronotum; (B) number of offspring; or (C) time until offspring first began emerging from the bolt.

offspring (r=0.20, p=0.6; Fig. A1.5B) and an earlier emergence date (r=0.39, p=0.3; Fig. A1.5C). Also, individuals emerging from larger logs tended to be larger themselves (r=0.32, p=0.4; Fig. A1.5D). In general, brood and individual offspring characteristics were independent from parent size, while log diameter tended to correlate with variables related to rearing success.

Sexing by presence/absence of chirps

To test the accuracy of acoustic sexing of *D. valens*, the first test examined whether sex could be accurately determined by the presence/absence of chirps using only an observer's unaided ear on the day of a beetle's emergence. Sixty-six individuals were sorted in this way (35 identified as putatively female and 31 identified as putatively male) and it was found that 100% of the chirp-producing individuals were male, while individuals that were never observed to produce chirps were always female (based on an examination of the seventh abdominal tergite; Fig. A1.6A). Thus, on the day of emergence sexing by presence/absence of sound was found to be highly accurate.

As a further test of the observer's finding that females do not chirp, recordings were made for additional 30 sec disturbance trials of 56 confirmed males and 46 confirmed females on their day of emergence. Recorded waveforms were examined for the presence/absence of stereotypical chirps, and other putative sound production. Female waveforms had rare and irregular sound pulses (Fig. A1.6B). These pulses were

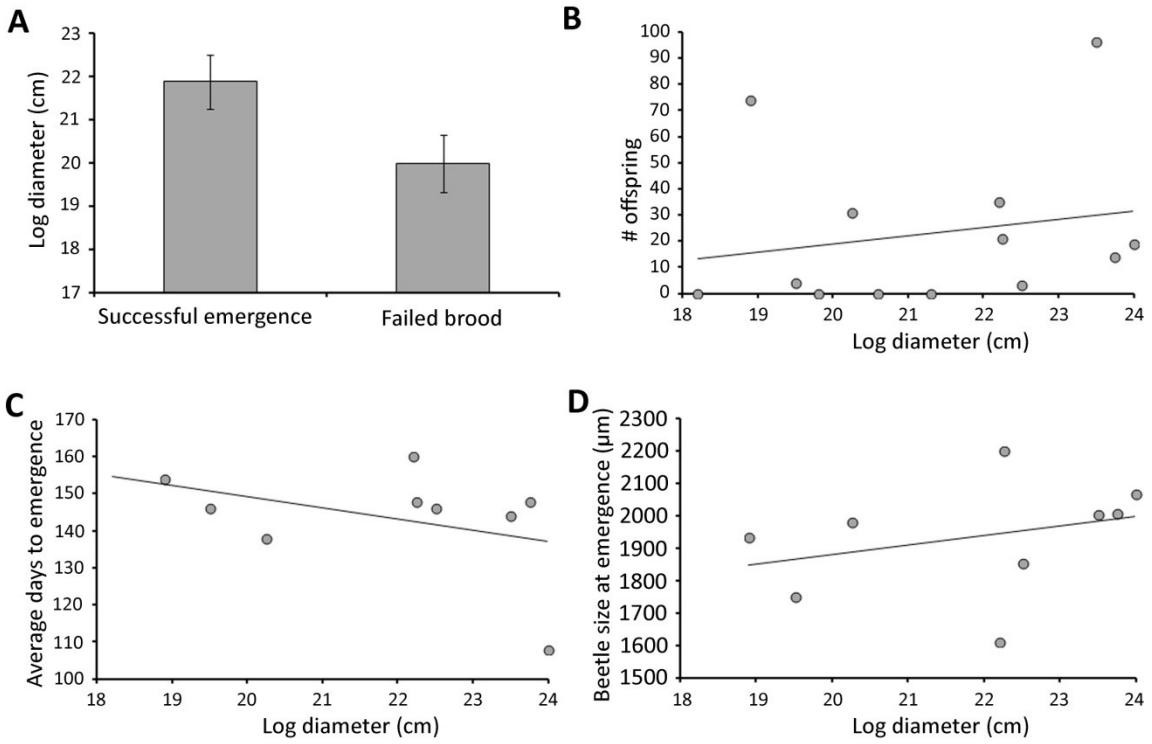

Figure A1.5. Relationship between bolt size and the brood reared within. (A) Successful broods emerged from bolts that were significantly wider than bolts with failed broods. (B-D) Broods reared on wide bolts tended to (B) contain a greater number of individuals (C) emerge earlier and (D) have larger individuals (as measured by pronotum width).

extremely different from the male recordings, both in terms of their qualitative sound and their temporal structure, as all male recordings contained grouped sound pulses that formed stereotypical simple and/or interrupted chirps, with patterns of regular spacing both between chirps and between pulses within chirps (Fig. A1.6C). Although male waveforms would occasionally contain irregular sound pulses similar to those seen in female waveforms, no females ever had the regularly spaced chirp pulses seen for males (further characterization of sound pulses in male and female waveforms described in Chapters 3 and 4). In conclusion, the waveforms of the different sexes had the quality of being clearly distinguishable.

To determine whether the presence of stereotypical chirping behaviour in males was reliable over time, males underwent repeated disturbance trial tests for 10 days following emergence. Chirping behaviour for males was robust, with the average chirp rate remaining constant over time up to 10 days post-emergence (no relationship between chirp rate and time, $r<0.01$, $p>0.99$, Fig. A1.6D). However, although there was no change for the average male chirp rate over time and the presence of chirps was a robust behaviour, there was a decline in the number of pulses per chirp over time ($r=0.44$, $p=0.2$; Fig. A1.6E). Over the course of the 11 days, seven males died and it was observed that the reduced fitness of these males played a role in their signalling behaviour. The chirp rates of the seven males dropped off steeply over time, with six of the seven individuals becoming mute from one to three days prior to death (relationship between day since emergence and chirp rate decline in the seven males: $r=0.57$, $p=0.09$;

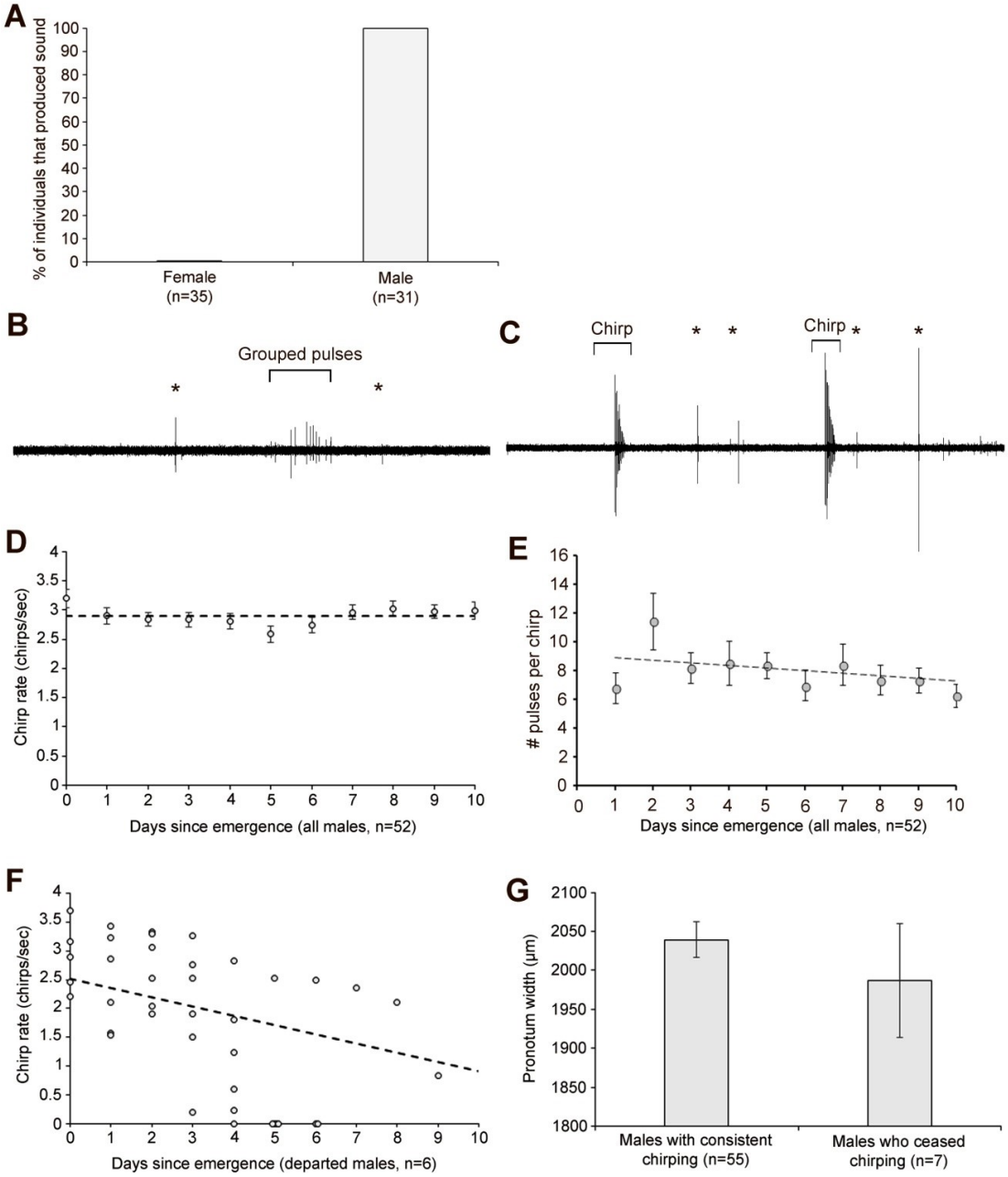

Figure A1.6. Chirping behaviour between the sexes, and in males over time. (A) No newly emerged females (n=35) were found to produce chirps during handling; conversely all newly emerged males (n=31) produced chirps. (B) 1 sec waveform of a female disturbance trial; asterisks identify isolated sound pulses commonly seen, and a rare grouping of sound pulses is labelled. Comparatively; (C) 1 sec waveform of a male disturbance trial; asterisks identify isolated sound pulses that are also occasionally seen in male recordings (though over-represented in this example), also two chirps are labelled. (D) The average chirp rate for all males (n=52) was steady and rapid up to 10 days post-emergence. However; (E) the number of pulses per chirp, which was very high on the day following emergence, declined over time. (F) Chirp rates of seven males who died over the starvation trial. Initially, and for the first few days following emergence, those males produced chirps at a standard rate, however the chirp rate rapidly dropped off in the days preceding death with 5 males producing 0 chirps from 1-3 days before dying. (G) There was a trend for males who produced chirps throughout the entire 10 day post-emergence trial (n=55) to be larger than males who ceased chirping at some point during the trial (n=7).

Fig. 6F). Therefore, six out of 52 males were mute over one to three days of the trial (followed by death, at which point they were removed from the sample population), which resulted in 98.7 ± 0.8 % of males tested on any given day producing robust chirps. Those males that did cease chirping were slightly, but not significantly, smaller than average ($t_{2,6}$=-0.53, p=0.62; Fig. A1.6G). Consequently, it was found that chirping behaviour is in general robust for a significant period of time following emergence, but that it can be negatively impacted by the fitness of the individual male.

A1.5 Discussion

General rearing method

Laboratory-rearing of *D. valens* in this study demonstrated how this species can be successfully reared indoors and yield large numbers of offspring. In this case we obtained 281 individuals from nine out of the 14 bolts. The number of individuals obtained and reasonable success rate of emergence per bolt (64%) make this an acceptable method for laboratory rearing of *D. valens*. Although in this study rearing always occurred in the fall, a potential benefit of such a method is that likely individuals can be bred over various times of the year, an asset to research on animals with seasonal emergence windows. Furthermore, the rearing procedure employed here was low-maintenance in that it did not require large amounts of time or space to implement, and could follow entire broods from the time of mating pair introduction, so that

investigations could be made on the influence of several variables - in this case parent and bolt size - on brood success.

General emergence

Emergence patterns of *D. valens* from logs was investigated to provide insight into the natural emergence patterns of males and females. Studies on emergence in the laboratory may be informative for emergence patterns in bark beetles under natural conditions, as previous studies have found consistency in various emergence traits occurring naturally and in the lab (Billings and Gara 1975). It has been suggested that there may exist a differential emergence pattern for male and female *Dendroctonus* spp. because of the role that females play in host selection. Males only join females once an attack on a tree has been initiated and gallery construction has begun, therefore an early emergence and high proportion of females may be a predicted adaptation to mortality in females associated with the initial attempts to colonize resistant hosts (Billings and Gara 1975). Consistent with this, some studies have found female bias in the sex ratio of emerging *Dendroctonus* spp. (e.g. Reid 1958; Billings and Gara 1975; Pureswaran and Borden 2003). However, another study on *Dendroctonus frontalis* found no difference between the emergence patterns or numbers of males and females (Coulson et al. 1979). Here, it was observed that the cumulative sex ratio of emerging individuals was close to 1:1. Moreover, there was no difference in the emergence times of each sex, which occurred consistently between 141 and 150 days post-introduction. This is a similar result as seen in *D. frontalis* (Coulson et al. 1979), but the inconsistency

found here between *D. valens* and other *Dendroctonus* spp. (e.g. Reid 1958; Billings and Gara 1975; Pureswaran and Borden 2003) may be due to the less aggressive nature of *D. valens* in its native range where it targets only weakened trees (Owen et al. 2010). The emergence pattern adaptation predicted for those species attacking resistant hosts would not be required by *D. valens* in its native range, and consistent with this both males and females were found to have the same emergence numbers and pattern.

Another pattern found here was that in males a later emergence time significantly correlated with increased size at emergence. This is similar to the findings of Atkins (1967) that, in general, individuals that emerged later weighed more and had a higher fat content at emergence. In that study, it was speculated that the size-emergence date relationship was caused by the longer feeding period for the individuals that emerged later. Conversely, other studies have found the opposite relationship, where later emerging individuals tended to be smaller than their earlier emerging counterparts (Safranyik and Jahren 1970; Reid and Roitberg 1995). Reid and Roitberg (1995) speculated that this reduced body size at later emergence was owing to the natural deterioration of phloem experienced by the later emerging individuals (Safranyik and Jahren 1970; Anderbrant and Schlyter 1989). It is interesting that here the positive size-emergence date relationship was found only in males. *Dendroctonus valens* larva feed in a communal larval chamber, and this result may suggest that females are in general better competitors than males, and while females can maintain a consistent feeding rate regardless of intraspecific competition, males who emerge later have

better feeding periods later on and grow to be larger owing to the reduced competition as their siblings leave the gallery ahead of them.

Rearing success

Effect of parent size

It was expected that the size of parents would play a significant role in brood success. In insects, female size is often positively correlated with the number of eggs laid, and this has been found previously within *Dendroctonus* spp. (e.g. McGhehy 1971). Similarly, in other bark beetles larger males have been found to produce more and larger offspring (Reid and Roitberg 1995). Here, the size of the parents together, or either individually, had no effect on the size, number or time of emergence of their offspring. One important consideration is that in these artificial circumstances there was no competition from outside the brood. Phloem is a limited resource, and competition for it can be detrimental to the brood (e.g. Berryman et al. 1985; Davis and Hofstetter 2009). It cannot be concluded whether being the offspring of a larger parent would have advantages in terms of size, number and emergence in a more competitive environment.

Effect of host log diameter

It was expected that bolt size would play a significant role in brood success. In nature, the importance of tree width is seen in that male bark beetles are biased

towards selecting female galleries in wider trees (Reid and Baruch, 2010). This may be an attempt by the beetle to select the mate with more resources, as competition for phloem can negatively impact a brood (Berryman et al. 1985) and thus increasing conspecific density can limit reproductive rates (Davis and Hofstetter 2009). Consequently, from a rearing perspective, increasing the size of the bolt should reduce within brood competition by providing an increased amount of phloem, and may be beneficial to brood success. In support of this, it was found that the logs that had successful emergence were significantly larger than the logs that did not. Also, there were trends for larger diameter logs to support more offspring, which in turn tended to emerge earlier and be larger. These trends suggest that bolt diameter should be an important concern in laboratory rearing attempts.

Other potential effects

Temperature may also play an important role in brood development and success. Over both years we attempted to control temperature around 21^{o}C (i.e. room temperature). This is an intermediate temperature to the lower temperature limit which has been found to hinder emergence in various *Dendroctonus* spp. (Reid 1962; Billings and Gara 1975) and the upper temperature limit which likewise constrains emergence (Billings and Gara 1975). However, there was still slight variation in temperatures between years in this study, with temperatures on average 2.3^{o}C warmer during the 2014 trial than the 2013 trial. Temperature changes within this range can have an effect on *Dendroctonus* spp. brood development. For example, studies of *Dendroctonus*

terebrans (Godbee and Franklin, 1978) and *Dendroctonus pseudotsugae* (Atkins, 1967) have shown that emergence rates increased with consistently warmer temperatures. Consequently, the temperature variability may have had different effects on the broods between years.

Sexing by acoustic signal production

Accuracy of acoustic sexing on day of emergence

It was found that on the day of their emergence from the bolt males and females could be sexed with 100% accuracy by their acoustic emissions during disturbance. All males produced disturbance chirps that were identifiable both by the unaided ear of an observer in one test, and by examining the recorded waveform in a second test. Furthermore, females were distinguishable from males by their complete lack of chirps. They were not completely silent, and in the second test examination of the females' waveforms revealed that rare, irregular and widely-spaced sound pulses were present. However, these sounds were qualitatively different from male chirps and thus easily distinguishable by both the unaided ear and by examination of the waveform given that male chirps are characterized by consistent groupings of regularly and closely spaced sound pulses. Based on these results, it can be concluded that using acoustic emissions for sexing is an accurate method on the day of the individual's emergence.

Reliability over time

Although males reliably signalled during disturbance upon first emerging from the bolt, it needed to be verified that this signalling behaviour was robust over time and with energy depletion. This is because in a field setting, for example, the age and energy expenditure of the individual is likely unknown. It was speculated that stridulation may be an energetically costly behaviour, consistent with the findings of the energetic cost associated with stridulation in other insects, such as increases in aerobic respiration with increasing stridulation rates (Mowles 2014). The potential effect of reduced energy on acoustic signal reliability is an important concern for bark beetles, whose energy stores become depleted with dispersal in nature. It has been previously found that the reduction in energy stores in bark beetles due to flight can be simulated by food deprivation (Gries et al. 1990; Kinn et al. 1994). Using the food deprivation approach, it was found that the majority of males still produced robust disturbance signals throughout the 11 day starvation period. Only seven out of 56 males developed a low chirp rate over time or ceased chirping behaviour. In these seven males the decline in signalling behaviour mostly occurred from one to three days before the beetle expired, such that on any given day the majority of males sampled were signalling. Taken together with the findings of an absence of disturbance chirps in females, it can be concluded that sexing individuals by chirp production is a highly reliable method of sorting, where all of the individuals that produce chirps can be classified as males with 100% accuracy, and individuals that do not produce chirps can be classified as females with a < 2% chance of error within 10 days of emergence.

The observed decline in chirp rate in a small number of individuals is likely biologically significant even though it did not achieve statistical significance. These males expired over the course of the 11 day trial, and in each case the chirp rate was found to decline or cease in the days immediately preceding their death. The lack of statistical significance was likely owing to the variable survival period in the seven individuals; in six out of seven individuals there was a steep decline and then ceasing of chirping within the first six days of the trial, but the seventh individual chirped robustly until day seven and then had a shallow decline in chirp rate over the remainder of the trial, skewing the average chirp rate result. In general, males who ceased chirping tended to be smaller than the average, which may suggest a lower fitness level than their robustly signalling and surviving counterparts. Other potentially negative contributors to fitness (such as parasite load) were not measured here. Meanwhile, in all the remaining males who continued signalling throughout the trial, despite their consistent signalling rate there was evidence of declining signalling vigour - there was a relationship between the number of pulses per chirp and day since emergence, with much higher numbers of pulses per chirp on the first couple of days post emergence and then a decline in pulse number over time. Because each sound pulse is produced by one tooth being struck by the plectrum (Chapter 3), the decrease in pulses translates into the plectrum travelling a shorter distance over the file and striking less teeth. This decline in various characteristics of chirping behaviour likely has biological significance in terms of the energetic cost to sound production in bark beetles.

Conclusion

This study found that a low-maintenance method for rearing *D. valens* resulted in a high yield of offspring, and may be suitable for rearing animals indoors which offers the advantage of rearing beetles throughout the year. The findings suggest that when establishing an in-lab colony special attention should be paid to the diameter of host bolts selected. This study also provides a convenient method for sexing *D. valens.* Females never produced chirps, and on occasions where female sound was seen it was easily distinguishable from male disturbance chirping. Conversely, on the day of emergence all males produced stridulatory chirps and on any given day over the course of an 11 day food deprivation trial 98% of males were found to produce such sound. Sexing by stridulatory disturbance sound is thus an accurate method for *D. valens*, though not infallible and should be relied upon only as an initial means of sorting individuals when examination of the seventh abdominal tergite is inconvenient.

A1.6 Acknowledgements

We thank C. Wood and the experimental farm and arboretum in Ottawa and Carleton University for allowing us to hang collecting traps, and M.D. Connolly, L.E. McMillan, S. Sivalinghem, C. Denadai, M. Goulding, A. Mikhail, C. Nathan, C. O'Connor and C. Shaheen for various help procuring logs and checking traps. This research was funded by the Natural Sciences and Engineering Research Council of Canada (Discovery Grant to JEY, PGS-D to AAL), the Ontario Ministry of Training, Colleges and Universities (Ontario Graduate Scholarship to AAL), the Canadian Foundation for Innovation (JEY),

the Ontario Innovation Trust (JEY), and the Ontario Ministry of Economic Development and Innovation (Early Researcher Award to JEY).

Appendix 2: Acoustic automated species identification of sympatric bark beetle species

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A2.1 Abstract

Dendroctonus ponderosae, Dendroctonus valens and *Ips pini* are sympatric species which can be found sharing hosts in overlapping regions of their natural ranges. Correct identification of each species may have important management implications; *D. ponderosae* is an aggressive tree killer within its natural range and *D. valens* has demonstrated the potential to become invasive in regions of accidental introduction. Here we developed a pilot study for an acoustic automated species identification system as a non-invasive way to identify sympatric bark beetle species hidden inside their galleries. The first goal was to develop a preliminary identification system, where recordings of acoustic signals (chirps) from the three species were preprocessed and features extracted and fed to a classifier. Initial separation between species based on several features from the temporal domain of chirps and their amplitude envelopes was found. The preliminary success rate for identifying species based on one chirp was between 63% and 61%, depending on the classifier used. The second goal was to determine whether chirps could be detected from bark beetles signalling within their galleries. Airborne and substrate borne recordings of *D. valens* mated pairs interacting inside their galleries were obtained, and it was determined that beetles did produce chirps that could be detected in this context. These findings suggest that with further noise reduction and separation of the features, an acoustic species identification system could be used to detect bark beetle presence and species identity while beetles are out of sight under the bark of trees or logs. Such a tool would be useful for monitoring the presence and/or abundance of destructive species. Potential applications include

monitoring range expansions and providing an alert to the presence of invasive species while exporting/importing timber.

A2.2 Introduction

Acoustic communication in animals is widespread, and these acoustic signals can be used to detect the animal's presence and, in many cases, their species identity. Recent years have seen an upsurgence in acoustic techniques aimed at automated species recognition and/or monitoring for a variety of animals, including birds (e.g. Bardeli et al. 2010), bats (e.g. Vaughan et al. 1997; Parsons and Jones 2000; Obrist et al. 2004), marine mammals (e.g. Deecke et al. 1999; Brown and Miller 2007; Mellinger et al. 2007) and fallow deer (Reby et al. 1997). The availability of systems that can automatically identify species or taxonomic groups have great potential benefits, particularly if monitoring can be done on a continual basis (Gardiner et al. 2005; Chesmore 2007). One of the main advantages of such an automated bioacoustic approach lies in the potential for long-term recording in the absence of an observer. This method would also allow for estimates of species presence or abundance in ecologically sensitive areas or remote locations that are difficult to access. In insects, acoustic monitoring systems are becoming commonly applied for detection of pest species (e.g. Shuman et al. 1993, 1997; Hagstrum et al. 1990; Haack et al. 1997; Mankin et al. 2000; Mankin and Weaver 2000; Chesmore 2008). The purpose of this study was to establish an approach to species identification of destructive bark beetles using

pattern recognition algorithms developed for three species of pine-dwelling bark beetles.

Bark beetles (Curculionidae: Scolytinae) are a large group of insects found around the globe (Raffa et al. 2015). While the vast majority of individuals play a key role in supporting the ecosystems in which they live, there are a few species that can aggregate en masse and kill large numbers of trees (e.g. Raffa et al. 2015). In this regard, the genus *Dendroctonus* is of considerable note, as it contains several species capable of great destruction (e.g. Six and Bracewell 2015). In Canada, *D. ponderosae* has impacted over 18 million hectares of Western Canadian forest since the 1990s, causing considerable economic loss to the forestry industry (Natural Resources Canada 2015). The threat is ever growing - with climate warming *D. ponderosae* is expanding its geographic range, moving into the boreal forest and Canada's Northern and Eastern pine forests (Carroll et al. 2003). In addition to climate-induced range expansions, because bark beetles spend much of their life-history within plants they can also be accidentally transported by humans. For example, *D. valens* was accidentally introduced to the Shanxi Province in 1998, where it has since become a major pest (Yan et al. 2005). Developing a tool that could track the range expansion of species, and/or identify them within logs being exported or imported, could thus be useful to bark beetle management. Acoustic technologies may provide such a tool, since it is well established that many species of bark beetles are capable of acoustic signalling and do so in many contexts (e.g. Barr 1969; Lyal and King 1996). Thus, the first goal of this study was to establish a pilot program for the identification of bark beetle species by their acoustic

signals, using three sympatric species (two potentially primary pests: *D. ponderosae* and *D. valens*, and one secondary species: *Ips pini*).

For a bark beetle acoustic automated species identification system to be most useful in the field, ideally the acoustic signals of bark beetles need to be detectable while the animals interact inside their galleries. It has been previously reported that *Ips pini* chirped continuously while inside the gallery over a month-long recording period which began with the introduction of a mating pair (Goulding 2013). High rates of chirping were in particular observed during the first few days and the last week of the observation period. This kind of chirping behaviour while inside the gallery has yet to be shown for any *Dendroctonus* species. If this within gallery signalling behaviour is common to bark beetle species, it would give an identification system more utility to managers as a tool to identify species without disturbing the host tree or log. The second goal of this study was thus to determine whether *D. valens* also produces detectable chirps from inside the gallery.

By developing a preliminary test of an acoustic automated species identification system for three sympatric species of bark beetles - *D. ponderosae, D. valens,* and *I. pini* - and determining whether *D. valens* produces chirps that are detectable from inside the gallery, the findings of this study promote a program which could be applied to remote monitoring for species presence and abundance, or to detect which species may be present within a log before transporting it and accidentally contributing to human-facilitated dispersal.

A2.3 Background of *D. ponderosae, D. valens* and *I. pini* chirp characteristics

The development of an acoustic automated species identification system requires initial consideration of the characteristics of each species' acoustic signal. Three considerations were made based on previous work. First, an important consideration was the variability that can exist within individuals. *Dendroctonus* spp. have two chirp types: simple and interrupted (see Chapters 2 and 3 of this thesis). Simple chirps were selected as the focus for this pilot project because their production is common across contexts in which males signal acoustically.

The second consideration was regarding which domain (temporal, spectral or amplitude) to extract features from and which features to extract for use in the identification system. Frequency characteristics would not be useful for identifying individuals interacting under the bark, and thus the spectral domain was excluded. Instead, the temporal domain and amplitude envelope were selected as likely to provide the most robust information across contexts. Temporal domain characteristics and amplitude envelopes have been previously reported for each species. Fleming and colleagues (2013) reported the temporal characteristics of simple chirps of *D. ponderosae* for several contexts (Table A2.1). In that study, the authors also reported that the majority of *D. ponderosae* simple chirps were observed to have descending amplitude envelopes. *Dendroctonus valens* simple chirps have similar descending amplitude envelopes, but there appears to be a difference between the two species in the temporal domain, particularly in regards to chirp duration and number of pulses (Table A2.1; data from Chapter 3). *Ips pini* chirps have the most distinct chirps of the

three species, likely because *Ips* spp. have a different stridulatory mechanism than *Dendroctonus* spp. (Barr 1969). *Ips pini* had chirps which appear to be much longer and contain many more pulses than the chirps of either *Dendroctonus* species (Table A2.1; Sivalinghem 2011). The amplitude envelopes of *I. pini* are a different shape than those of the two *Dendroctonus* species with pulses containing a relatively consistent amplitude throughout the bulk of the chirp but tapering off at both ends (Sivalinghem et al., in prep). Consequently, chirp duration, pulse number and amplitude envelope were considered in this study as the putative species-discriminating features.

The final consideration was which channel of sound transmission supplies the best signal-to-noise ratio, which is important for obtaining the highest quality recordings to import into the identification system. Only recently has it been determined that there is a substrate borne component to bark beetle chirps (e.g. for *D. ponderosae*, Fleming et al. 2013; and *I. pini*, Sivalinghem 2001; Goulding 2013). Although recordings of beetles interacting on the bark surface seem to suggest that the airborne channel has better signal-to-noise ratio (Sivalinghem 2011; Fleming et al. 2013), Goulding (2013) concluded that the substrate borne channel may be the superior method to record from beetles signalling from inside the gallery. The present study used *D. valens* to further investigate the question of recording from the airborne or substrate borne channels.

A2.4 Acoustic automated species identification systems background

The work of ecologists can often be constrained by a taxonomic impediment - the limited ability to accurately identify species encountered (Wheeler 2003).

Table A2.1. Simple chirp characteristics for three bark beetle species (*Dendroctonus ponderosae, Dendroctonus valens* and *Ips pini*). *Dendroctonus ponderosae* data taken from Fleming et al. (2013), *D. valens* data taken from Chapter 3 (this thesis), and *I. pini* data taken from Sivalinghem (2011).

Species	Context	Chirp Duration (ms)	# pulses per chirp
D. Ponderosae	Disturbance	21.8 ± 1.8	17.4 ± 1.9
	Male-Female	30.0 ± 6.8	21.3 ± 3.5
D. valens	Disturbance	13.6 ± 0.6	9.1 ± 0.4
I. pini	Disturbance	105 ± 9	103.06 ± 7.2
(Albertan population)	Male-Female	120 ± 6	91.23 ± 5.9
I. pini	Disturbance	124 ± 8	136.85 ± 8.6
(Ontarian population)	Male-Female	158 ± 17	166.6 ± 13.7

Automated species or taxon identification systems are tools that can be used to overcome such limitations. These systems are comprised of four main components (illustrated in the schematic in figure A2.1): (1) The first component of the system is a sensor that can detect the features of specific interest upon which the species discrimination is based. For bark beetle acoustics, this sensor may either be a microphone, to detect the airborne component of sound, or a vibration detector. (2) The second component is a preprocessor. In the case of an identification system based on acoustic features, this component is often used as a way to amplify the sound and reduce unwanted noise from the signal. (3) The third component is a feature extractor. This is the essential step for the pattern recognition that powers the identification system. Features are measurements or categorical characteristics that describe variance between species. For each sample in the dataset, an n-dimensional feature vector is constructed, where n is the number of features identified as being relevant. The aim is to define a feature space in which the individual species are grouped into separable clusters. The more the features overlap between species, the greater the risk of species misidentification. (4) The final component is the classifier, into which the output from the feature extractor is fed. This is the program that, using classification algorithms, identifies the point at which each feature transitions from belonging to one species to another. This is accomplished by training the classifier with a set of labelled samples. Once trained, the classifier can predict the species of an unlabelled sample.

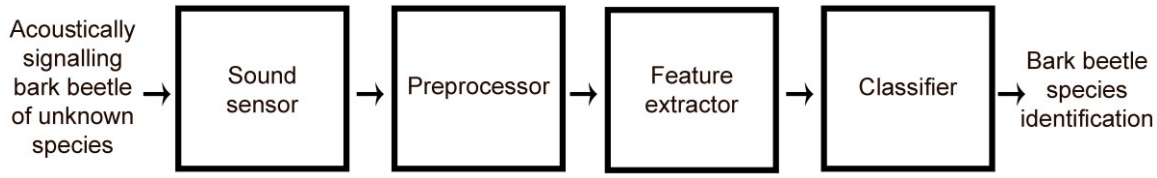

Figure A2.1. Example of an automated species identification system using bark beetle acoustic signals. The acoustic signal is first recorded, undergoes preprocessing to amplify the signal, and the relevant features of interest are extracted and then fed to a classifier, which then determines the species identity of the signaller (diagram modelled after Chesmore 2008).

A2. 5 Methods

Animals

Dendroctonus ponderosae

Fifteen male adult *Dendroctonus ponderosae* (Curculionidae: Scolytinae) were obtained from Baldy Mountain, British Columbia, (N49.110^{o}, W119.177^{o}) during the winter and spring of 2015. These animals were stored at a secure insect holding facility at 8^{o}C at Carleton University until use in sound recording, following which time they were fixed for use in other studies. Reference specimens are held at Carleton University.

Dendroctonus valens

Adult *Dendroctonus valens* were collected from May-September of 2014 at several locations near Ottawa, Ontario, Canada (the arboretum at the Ottawa Central Experimental Farm, 45.391021,-75.70489; Carleton Lands, Manotick, 45.183882,-75.604673; and outside Petawawa, 45.853530, -77.536156). Collection was done using Lindgren funnel traps (Contech Enterprises Inc., Victoria, British Columbia, Canada) baited with *D. valens* lure (Contech). These animals were mated within bolts of red pine (*Pinus resinosa*) and stored in mesh cages at room temperature (21^{o}C) for a period of six months (following methods reported in Appendix 1). Offspring were collected as adults and used in sound recordings. Reference specimens are held at Carleton University.

Ips pini

Adult *Ips pini* were collected from May-September of 2015 from Lindgren funnel traps (Contech) baited with two *I. pini* attractant lures, ipsdienol and lanierone (Contech) at the arboretum at the Ottawa Central Experimental Farm, 45.391021,-75.70489. Eight female *I. pini* were used for sound recordings. Reference specimens are held at Carleton University.

The acoustic automated species identification system

Chirp sampling

Recordings used for samples in the species identification system were collected by provoking chirps from disturbed beetles. To do so, *D. ponderosae* and *D. valens* were grasped by the head and pronotum and gently pinched to allow free movement of the elytro-tergal stridulatory mechanism of these beetles (e.g. Chapter 3). Conversely, *I. pini* was grasped by the abdomen and elytra to allow free movement of the vertex-pronotal stridulatory mechanism (Barr 1969). Beetles were held at a distance of 4 cm from the microphone (Earthworks, QTC40, Milford, USA) which was connected to a data recorder (sampling rate of 192 kHz, FR-2, Fostex, Los Angeles, USA).

Preprocessing samples

Samples were first converted to MP3 using an encoder (LAME, v3.99.5) which also applied a high pass filter (1 kHz). Following this, samples were converted to an array

of amplitude values using a Python package for audio analysis (LibROSA v0.4.0). Statistical analysis of the data (using SciPy v0.16.0) determined that amplitude values fit a normal distribution around zero (Fig. A2.2). Noise was estimated as occurring within 3 standard deviations from zero (the mean). As a first step in isolating sound pulses from noise, all values that fell within 3 standard deviations of the mean were excluded.

Feature extraction from samples

Following the preprocessing stage, each sample was processed as a stream, first to extract chirps and pulses, and then to extract specific features of interest from the chirps and pulses. The first step was the development of chirp extractor and pulse extractor programs. These programs were similar in nature. Chirps were extracted first; the start of a chirp was identified by a non-zero amplitude value, which commanded the chirp extraction program to begin collecting data, while the end of the chirp was identified by a determined threshold of a certain number of zero values. The threshold value for identifying the end of a chirp was determined by exploration of the samples and estimation of chirp duration based on previous data (e.g. Sivalinghem 2011; Fleming et al. 2013; Chapter 3 of this thesis). Once a chirp was extracted, within the chirp the array of zero and non-zero amplitude values was assessed by the pulse extraction program to detect the start and end of each pulse, as in the chirp extractor. As for chirps, the threshold value for the end of a pulse was determined by exploration of the samples and estimation of the pulse duration. Because inter chirp intervals are much

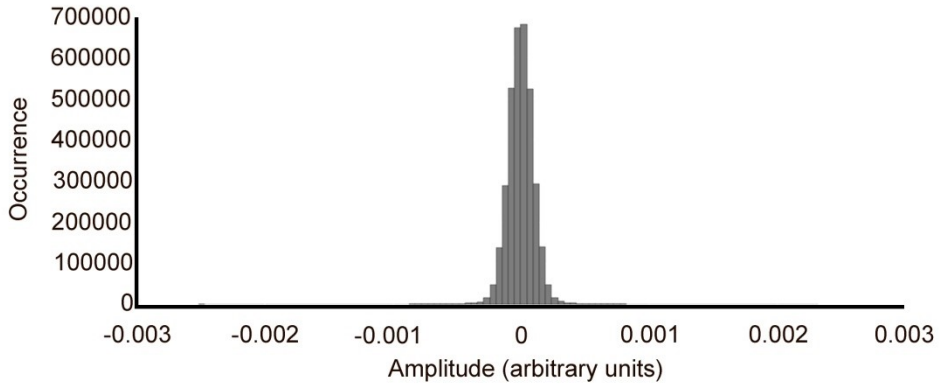

Figure A2.2. Histogram of instantaneous sound amplitude measurements. Once sound recordings were converted to an array of amplitudes during preprocessing, it was clear that amplitudes fit a normal distribution around zero, with most instantaneous sound measurements having an amplitude very close to zero.

larger than inter pulse intervals (e.g. Chapter 3), the threshold for determining the end of a chirp was much greater than that for a pulse.

Following the identification of chirps and pulses, specific chirp and pulse features could be extracted using iPython in conjunction with the Python data analysis library Pandas (v0.16.2) for data manipulation. The features selected as candidates for species separation were: (1) the duration of each chirp, (2) the number of pulses per chirp, (3) the average pulse rate, (4) the percent of non-zero amplitude values (as an estimate of average pulse duration), and (5) the percent of pulses that had a smaller amplitude than the pulse preceding them (as an estimate of fit to a descending amplitude envelope). The extracted features were then plotted using a Python plotting library (Matplotlib, v1.4.3) in conjunction with the visualization library Seaborn (v0.6.0).

Feeding features of interest to a classifier

The final step in an identification system is to feed the extracted features to a classifier and, once the classifier is trained, to test it using an independent sample not used for training. Using Scikit-learn (v0.16.1), two types of classifier methods were fed the training data from the extracted features. The first classifier used was "random forests". This is an ensemble learning method for classification where a number of decision tree classifiers were fit to the training dataset. Decision trees look at everything as a series of "if-then" analyses and use averaging to improve the predictive accuracy and control over-fitting. The second classifier used was "support vector machines".

These are supervised learning models with associated learning algorithms that analyze data and recognize patterns. Separate tests were run using each type of classifier.

Detecting chirps from bark beetles inside galleries

Eight bolts of red pine (*Pinus resinosa*) were introduced with one mating pair of *D. valens* each. Acoustic recordings were made for 15 minutes at the time of introduction, and for 15 minutes every 24 hours for 10 days (240 hours) post-introduction. Recordings were made of both the airborne transmission and the substrate borne transmission of chirps. To collect airborne sound, a microphone (as for sound recordings above) was positioned at 3 cm above the gallery entrance. To collect the substrate borne vibrations, an Acoustic Emission sensor (R3, MISTRAS, Cambridge, UK; peak frequency of 24.9 kHz and maximum value 82.35 dB) was placed against the bolt, at a distance of 4 cm from the center of the sensor to the gallery entrance. To maximize coupling of the sensor to the bolt, loose bark was removed and a small amount of high vacuum grease was applied (Dow Corning, Midland, USA). The sensor was held in place by an elastic band tied around the bolt. The sensor was connected to a hydrophone amplifier (Avisoft, Berlin, Germany; 200 Hz with +20 dB/100 pF,) which was then connected to a second channel on the same data recorder as the microphone. Files were analyzed using Raven Pro 1.5 (Raven Bioacoustics Research Program, Cornell Laboratory of Ornithology, Ithaca, NY, USA). A 500 Hz high pass filter was applied to each file (both microphone and sensor channels) in Raven. For each 15 minute recording for each bolt over the 10 days, it was noted whether chirps were detected, and if so

whether chirps occurred in isolation or as part of a chirp train. For each observation of chirping, it was also noted whether the microphone or sensor waveform had a better signal to noise ratio.

A2.6 Results

The acoustic automated species identification system

In the preprocessing stage, the goal was to remove as much noise as possible in order to increase the clarity of signals. Two steps were taken: first, a high pass filter was applied (1 kHz) and second, all values that fell within 3 standard deviations of the mean amplitude (zero) were excluded. Following this step, it was found that 99.7% of data was removed, leaving mainly the sound pulses of the chirps. This greatly improved the signal-to-noise ratio of the samples (Fig. A2.3A-C).

There were five chirp and pulse features examined: (1) the duration of each chirp, (2) the number of pulses per chirp, (3) the average pulse rate, (4) the percent of non-zero amplitude values (as an estimate of average pulse duration), and (5) the percent of pulses that had a smaller amplitude than the pulse preceding them. Plotting these features against each other, the feature extractor was able to find preliminary separation between the species, although there was still a significant amount of overlap between species (Fig A2.4A-C). This overlap led to some misidentification of species in a preliminary test of the system, although species identification was still significantly better than chance even at this early stage, with an identification rate for members of

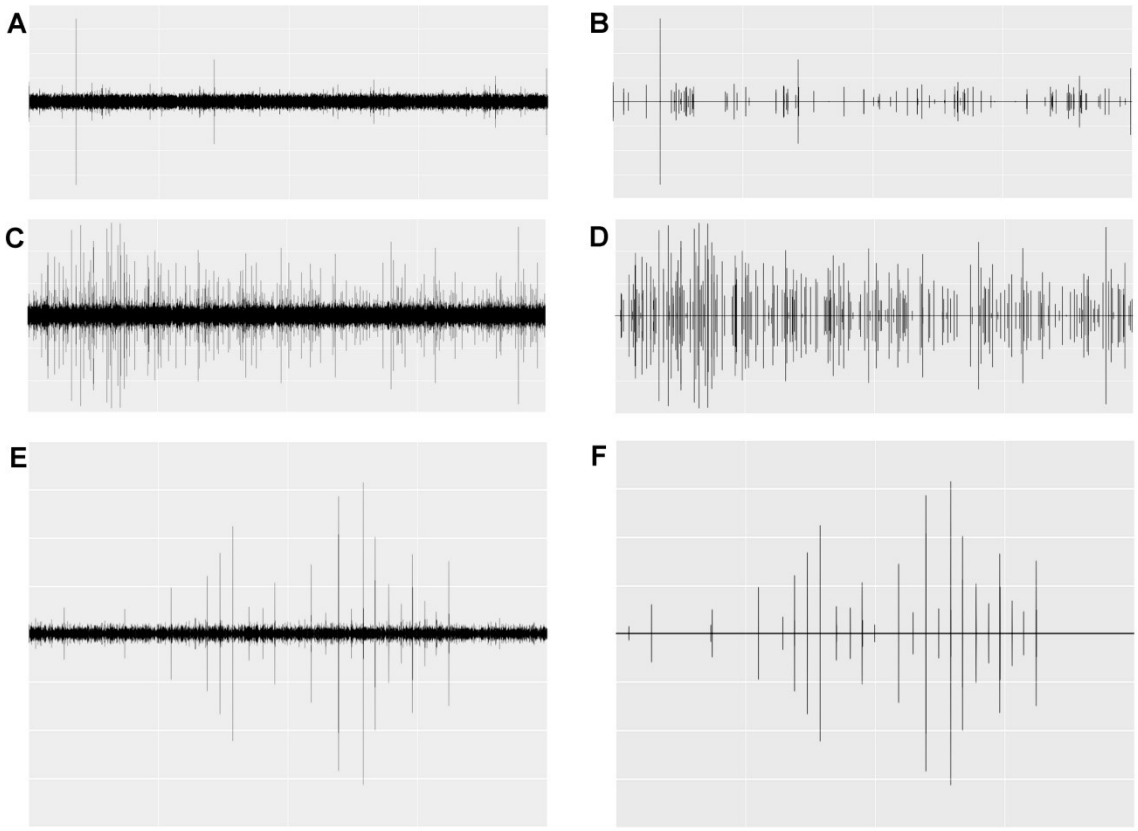

Figure A2.3. Waveforms of unfiltered and filtered sound following preprocessing for (A-B) *Ips pini*, (C-D) *Dendroctonus ponderosae* and (E-F) *Dendroctonus valens.* Chirps become more clearly visible upon noise reduction.

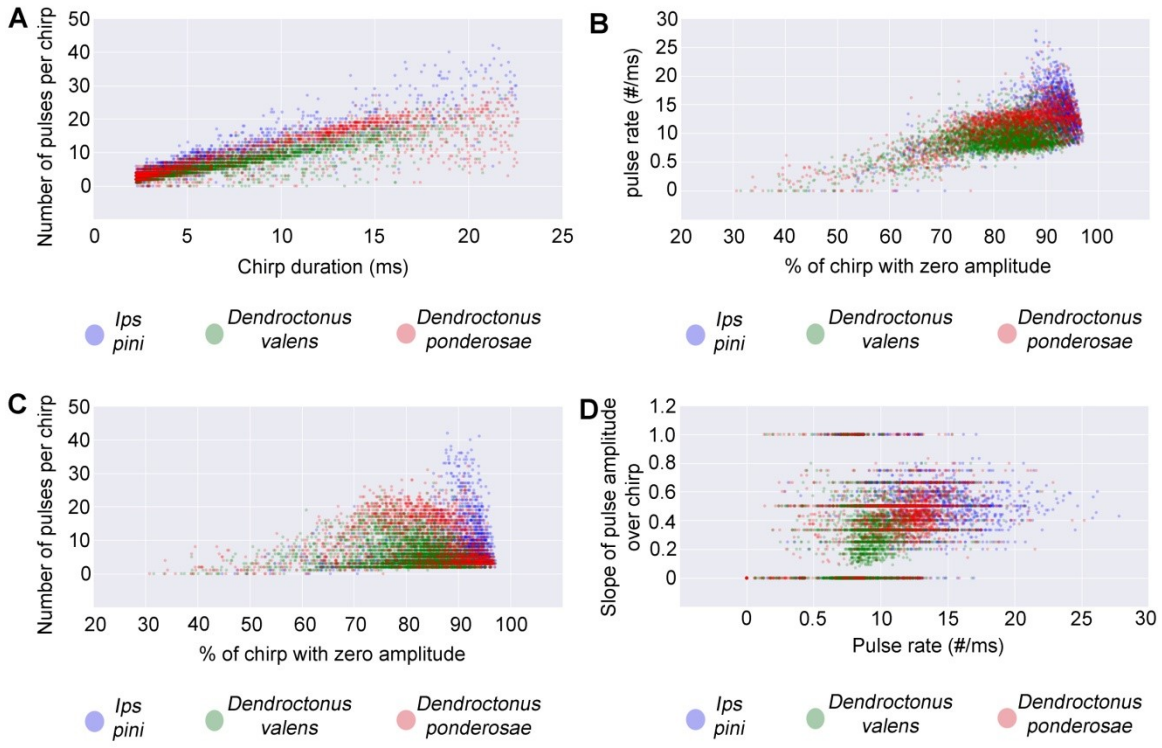

Fig. A2.4. Scatter plots depicting various extracted features for *Ips pini, Dendroctonus valens* and *Dendroctonus ponderosae*. (A) Number of pulses per chirp versus chirp duration. (B) Pulse rate versus the percent of the entire chirp with zero amplitude (as a measure of overall pulse duration). (C) Number of pulses per chirp versus the percent of the entire chirp with zero amplitude. And (D) the slope of pulse rate amplitude over the course of a chirp (as an indicator of amplitude envelope) versus pulse rate. Plots A-D depict preliminary separation between the species in terms of the features extracted.

the three species between 63% and 61%, depending on the classifier used (random forest versus support vector machine, respectively).

Detecting chirps from bark beetles inside galleries

Chirps were detected coming from bolts every day over the course of the 10 days; however, the percentage of bolts from which chirps were detected decreased over the course of the 10 day trial (Fig. A2.5A). In most recordings where chirps were present, chirps were found to be part of trains (i.e. 77.5% of recordings where chirps were present). Here, a chirp train is described as two or more chirps following in rapid succession; usually (in 61.3% of chirp trains) more than five chirps were seen in a train. However, it was not uncommon to observe isolated chirps (in 22.5% of recordings where chirps were present they were isolated). Furthermore, in several cases an entire 15 minute recording passed with only one isolated chirp observed. Thus, it is possible that in all bolts beetles produced chirps every day, but they were not present over the course of sampled time because of the low chirp rate.

Occasionally the acoustic emission sensor and microphone had similar signal-to-noise ratios in detecting male chirps (in 3.8% of recordings). However, most of the time the microphone was better (Fig. A2.5B). An interesting side observation was made as well. In two separate logs on two days long trains of pulses were observed that were almost exclusively detected by the sensor (Fig. A2.5C). These pulses are not of interest to the automated acoustic species identification system; however, they are a previously unreported part of acoustic behaviour of *D. valens* living inside galleries under the bark.

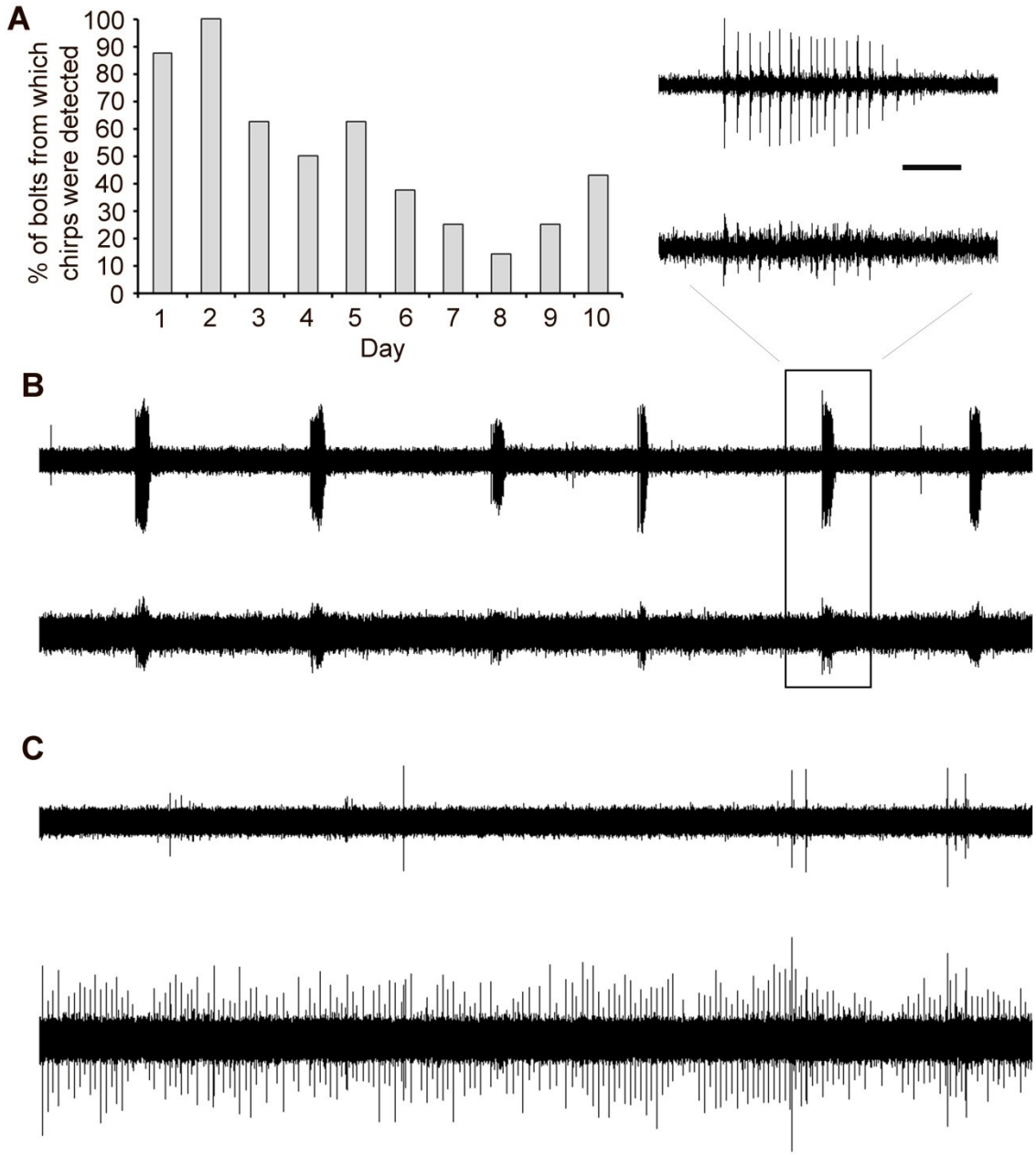

Fig. A2.5. Signalling characteristics for *D. valens* within galleries, over time. (A) Over the course of a ten day recording period chirps were observed from a subset of logs every day. (B) A 5 sec excerpt of the waveform from one log at 24 hours following introduction. Top trace depicts the recording of the airborne component of sound, while the bottom trace depicts the substrate borne component of sound. Outline and expansion show one male simple chirp; scale bar = 20 ms. (C) A 5 sec excerpt from a waveform showing the previously unreported pulse train, distinct from the male's chirps, which was picked up almost exclusively by the vibration sensor. The top trace depicts the recording of the airborne component of sound, while the bottom trace depicts the substrate borne component of sound.

A2.7 Discussion

The goals of this study were first to develop a preliminary test of an automated species identification system for three sympatric species of bark beetles - *D. ponderosae, D. valens,* and *I. pini* - and second to determine whether *D. valens* produce chirps from within their galleries over the days following courtship. In conclusion, the findings from these two goals tell us that these three species of bark beetles can be identified by their chirps as they signal within the gallery without disturbing them or their host tree. Although in only a preliminary stage, this identification system could identify species at a rate significantly higher than chance based on separation found between species' chirp features. Further, *D. valens* produced signals from within the gallery over the course of the entire observation period. Together, these findings suggest that such an identification system may be a useful tool for detecting the presence of bark beetles and identifying the species inside trees or logs without disturbing the bark.

Future steps for the automated species identification system

One of the immediate areas for improvement in the acoustic automated species identification system in its present form is to minimize the amount of overlap present between species. Much of this overlap is likely due to noise which is misidentified as a chirp or pulse before features are even extracted, or by single chirps being broken up by the program and misidentified as multiple chirps. For example, considering the chirp duration data that was extracted, it is clear that the majority of the sounds labelled as

chirps for *I. pini* are much shorter than expected (Fig. A2.4A). Moreover, there were several chirps identified as having almost zero pulses, with pulse rates of zero. Clearly, these values contain errors as well. The next step in addressing this problem will be to add additional logic to the chirp and pulse extractor programs. Once a putative chirp or pulse is extracted, there will be an additional algorithm to consider a minimum value (to discard the putative chirp/pulse if it is shorter in duration than a threshold minimum) and a maximum value (to discard the putative chirp/pulse if it is shorter in duration than a threshold maximum). This should help to eliminate further noise.

Conclusion

Continual monitoring of trees and logs to detect the presence of potentially aggressive bark beetles would be a useful tool for managers on the front lines of pest insect control. The ability to identify the bark beetle to the species level would make such a tool even more powerful. This pilot project shows that such a tool can be developed by applying technology to what is known of bark beetle acoustic ecology. Eventually, such a system can likely be implemented as a tool for tracking the spread of bark beetle species in remote or difficult to access regions, as well as to monitor imported/exported timber for destructive stowaways.

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