What is the password? Female bark beetles (Scolytinae) grant males access to their galleries based on courtship song

Amanda A. Lindeman*, Jayne E. Yack

Department of Biology, Carleton University, 1125 Colonel By Drive, Ottawa, ON K1S 5B6, Canada

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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 the 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.

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1. 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), or in species recognition (e.g. Yandell, 1984; 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), and in recent years have become significant economic pests in China (Yan et al., 2005).

* Corresponding author. Tel.: +6135202600.
E-mail address: amandalindeman@cmail.carleton.ca (A.A. Lindeman).
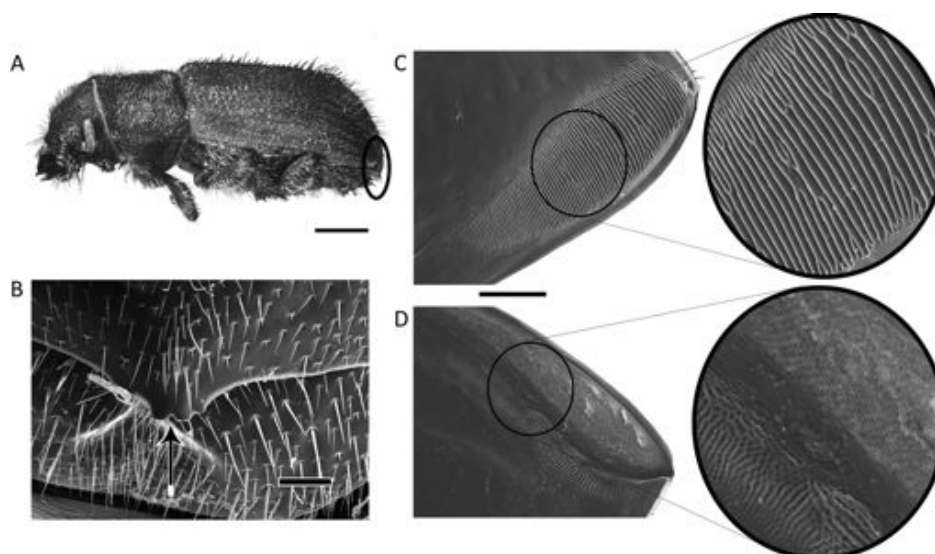
Fig. 1. Light, and scanning electron microscope (SEM) images of male *Dendroctonus valens*. (A) Light microscope image of a male, with circled area indicating location of stridulatory organ; Scale bar = 1000 μm. (B) SEM of the 7th and 8th abdominal tergites, showing the plectrum (arrow); Scale bar = 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.

This species employs a mating strategy prevalent among bark beetles – serial monogamy – where 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 during courtship (Ryker and Rudinsky, 1976a). Bark beetles produce sound in general 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. 1). Their chirps have been distinguished 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. The meaning 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 presence or absence of signals 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. Methods

2.1. Animals

Adult *D. valens* (Curculionidae: Scolytinae) were collected from May to September of 2011–2013 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 °C until use. Bolts of red pine (*Pinus resinosa*) were obtained by cutting fresh trees taken from Carleton lands into bolts (~60 cm long, ~15 cm 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.

2.2. 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. 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.

2.3. 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 additional trials were done 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-capsule. Gallery construction could be observed by the accumulation of frass surrounding the entrance hole, and after at least 24 h 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 min, 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). A simultaneous video recording was 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 $22 \pm 2\,^{\circ}$C.

2.4. Acoustic signal measurements and analyses

Temporal characteristics were analysed over the entire time spent signalling (performance) using Avisoft SAS Lab Pro (Avisoft Bioacoustics, Berlin, Germany). 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 min, 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 our method of calculating chirp rate. The inverse of the intervals between chirps was 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 plucked by the plectrum (Michael and Rudinsky, 1972). Inter-tooth strike intervals were individually measured using Avisoft.

2.5. 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 is classified as simple, and any chirp that is subjectively assessed to contain gaps is 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 analysed 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 ($n = 13$).

### 2.6. 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 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.

2.7. 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 MP, 1388×1040, 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.

2.8. Female preference

We calculated the percentage of intact males ($n = 25$) 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 hidden beneath the bark, and then 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
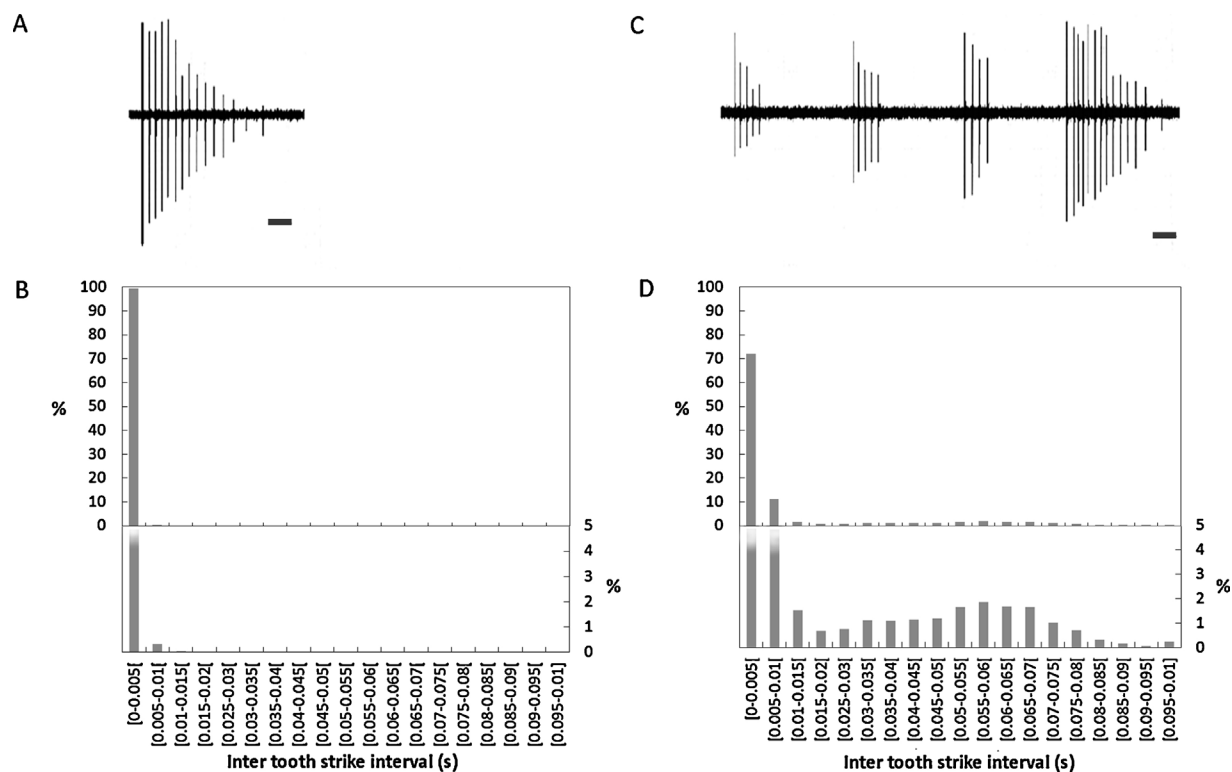
Fig. 2. Comparison of simple and interrupted chirps. (A) Example of a typical simple chirp. Scale bar = 10 ms. (B) Panel chart demonstrating the frequency of inter-tooth strike intervals in simple chirps sampled from 8 individuals during male-female interactions. The top panel summarizes the overall 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 = 10 ms. (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.

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 elytron and the wing-lock area of the 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 stress sounds when disturbed by pinching the head and pronotum; the absence of 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.

2.9. Statistical analyses

Paired sample *t* tests for unequal variances were used to test for differences between characteristics of interrupted and simple chirps. A linear regression analysis tested for 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).

3. Results

3.1. Differentiating simple and interrupted chirps

Simple and interrupted chirps were distinct from one another in a number of respects (Fig. 2). Simple chirps contained almost exclusively inter-tooth strike intervals less than 5 ms (99.6%) (Fig. 2A and 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. 2C and 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. 3A) and were significantly longer than simple chirps ($t_{12} = -8.27$, $p < 0.001$; Fig. 3B). The duration of interrupted chirps was strongly correlated with the number of components it contained ($r^2 = 0.93$, $p < 0.001$; Fig. 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 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 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.
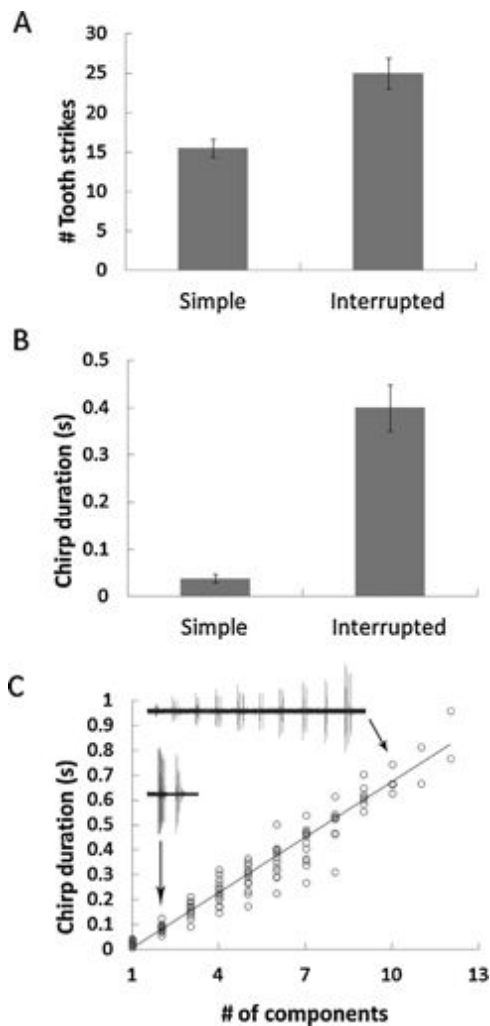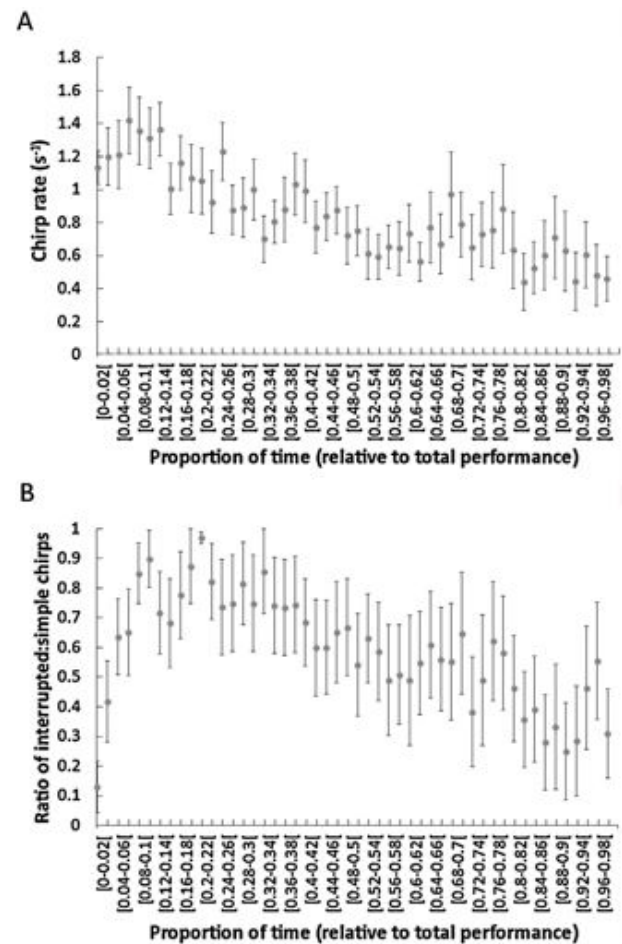
Fig. 3. Comparison of simple and interrupted chirp properties including (A) the average number of tooth strikes per chirp and (B) the average chirp duration. (C) There is a strong positive correlation between the number of components contained in the chirp and its duration (1 component = simple chirp, multi-components = interrupted). Insets illustrate examples of chirps with 2 and 10 components.


Fig. 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 decrease their chirp rates. (B) During the initial stages of an encounter (the first 5% of a performance), males produce predominantly simple chirps, but switch to mainly producing interrupted chirps throughout the first half of the performance. By roughly halfway through their performance, males tend to switch back to producing a majority of simple chirps.

3.2. 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: $1.03 \pm 0.1\,s^{-1}$; during the second half of an encounter: $0.60 \pm 0.1\,s^{-1}$; Fig. 4A), and more interrupted chirps (average $62.2 \pm 9.4\%$ of chirps during the first half of an encounter, as opposed to $43.7 \pm 14.4\%$ during the second half of an encounter; Fig. 4B) (for a demonstration of a male producing simple chirps during an encounter with a female, and then the same male producing interrupted chirps later in the encounter, see the video in supporting information).

3.3. 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. 5A). Larger males tended to have shorter performances before acceptance (i.e. they stopped signalling sooner, $r^2 = 0.46$, $p = 0.11$; Fig. 5B), but they produced more chirps overall ($r^2 = 0.53$, $p = 0.07$; Fig. 5C), although this trend was not significant. Larger males produced chirps at a significantly higher rate ($r^2 = 0.63$, $p = 0.02$; Fig. 5D), with a significantly larger portion of those chirps being interrupted ($r^2 = 0.60$, $p = 0.04$; Fig. 5E). Additionally, larger males tended to produce interrupted chirps with more components ($r^2 = 0.54$, $p = 0.06$; Fig. 5F).

3.4. 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. 6A). Moreover, females differed in their acceptance rate of
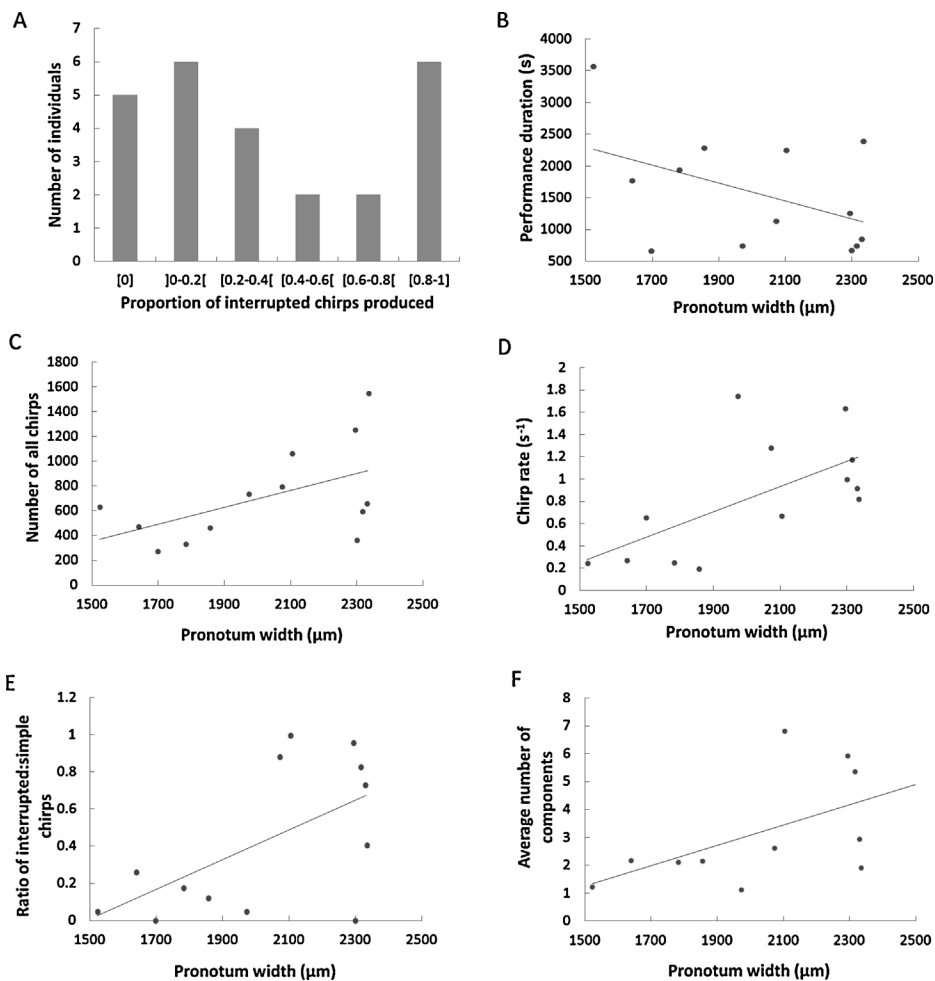
Fig. 5. Measurements of between-male variability. (A) Each male's chirp train varied in terms of the proportion of interrupted chirps performed. Although most of the males (total $n = 25$) produced at least some interrupted chirps, 5 males produced exclusively simple chirps. Correlation between body 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, thus $n = 11$.

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. 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. Fig. 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 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: $19.8 ± 7.2$ min; silenced males + those producing exclusively simple chirps: $82.7 ± 31.6$ min).

4. 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.

4.1. Male courtship signals vary between individuals

We demonstrate that male performance during courtship is not uniform. The production of interrupted chirps is the rule, rather than the exception, with 80% of individuals producing 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. 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
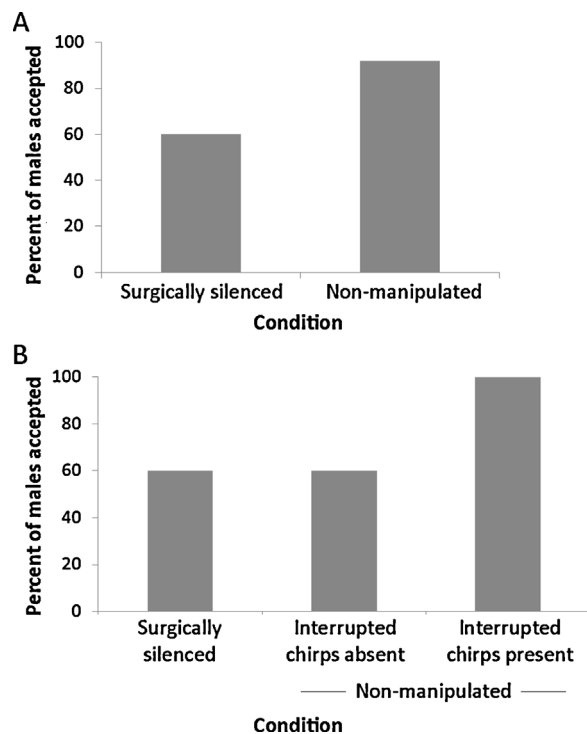
Fig. 6. Relationship between male success and signalling performance. (A) Males that were surgically silenced ($n = 5$) had less success at being admitted to galleries than their non-manipulated, signalling counterparts ($n = 25$). (B) Data for surgically silenced males ($n = 5$) is compared to data 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.

information present in the chirps relating to body size could be a valuable resource for the female.

4.2. 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 his 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. 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 female's response 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 exclu-
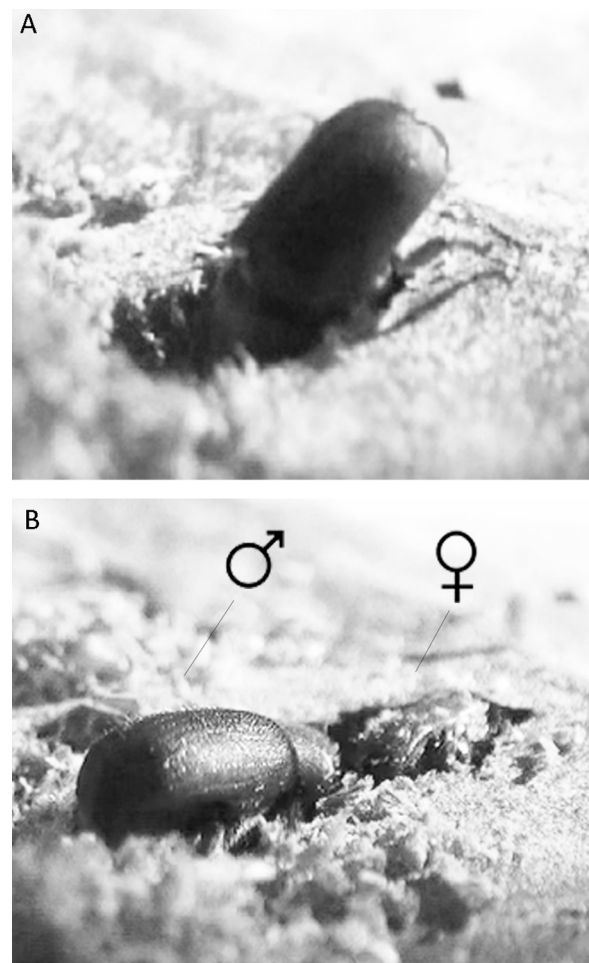
Fig. 7. Video frames of female blocking a silenced male. (A) The silenced male begins his initial attempt at entering the gallery. (B) At 2 min into the interaction, the female emerges and pushes the male backwards, completely blocking the entrance to the gallery.

sively 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 include interrupted chirps) were granted access into galleries sooner; likewise, larger males performed for shorter periods of time (Fig. 5B). It may be that males with less attractive performances (i.e. fewer to no interrupted chirps) need 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. 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.

4.3. 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 chirp production. 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.

5. 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.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.beproc.2015.03.009.

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