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RESEARCH ARTICLE

Motor and mechanical bases of the courtship call of the male treehopper *Umbonia crassicornis*

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ABSTRACT

This study is a physiological, anatomical and biophysical analysis of how plant-borne vibrational signals are produced by the treehopper *Umbonia crassicornis*. During courtship, males and females engage in a vibrational duet, with each producing a characteristic call. For males, this consists of a frequency-modulated tonal signal which is accompanied by rhythmic broad-band clicks. Although previous studies have described these complex signals in detail, little is known about how they are produced. By combining video recordings, electromyograms, dissections and mechanical modeling, we describe the mechanism by which the male produces his courtship signal. High-speed videos show that the tonal portion of the call is produced by periodic dorso-ventral movements of the abdomen, with a relatively large amplitude oscillation alternating with a smaller oscillation. Electromyograms from the muscles we identified that produce this motion reveal that they fire at half the frequency of the abdominal oscillation, throughout the frequency modulation of the tonal signal. Adding weight to the abdomen of a calling male reduces the frequency of motion, demonstrating that the abdominal motion is strongly influenced by its mechanical resonance. A mathematical model accounting for this resonance provides excellent qualitative agreement with measurements of both the muscle firing rate recorded electrophysiologically and the oscillatory motion of the abdomen as recorded in the high-speed video. The model, electromyograms and analysis of video recordings further suggest that the frequency modulation of the abdominal response is due to a simultaneous modulation in the muscle firing rate and a fluctuation in stiffness of the abdominal attachment.

KEY WORDS: Insect, Vibration, Courtship

INTRODUCTION

Substrate-borne vibration is a common form of communication in the insect world, and is used by an estimated 195,000 plant-dwelling insect species (Čokl and Virant-Doberlet, 2003; Cocroft and Rodríguez, 2005; Cocroft et al., 2006). These insects send and receive vibrational signals most commonly through the stems or leaves of the plant on which they reside (Čokl and Virant-Doberlet, 2003; Čokl et al., 2005; Čokl, 2008; McNett et al., 2006). Plant-dwelling insects are often able to create a range of differing vibrational disturbances in the stems depending on their intended purpose. Changes in the characteristics of the signal serve as cues to defend their territory from approaching predators, to develop mutualistic relationships and to inform other group members about available resources (Cocroft, 1999a,b, 2001; Hamel and Cocroft, 2012). Because low-frequency vibrations can be transmitted up to several meters through the plant stem, this can be an effective mechanism for small insects to use for relatively long-range communications (Michelsen et al., 1982; Bennet-Clark, 1998a; Čokl and Virant-Doberlet, 2003).

The treehopper *Umbonia crassicornis* (Amyot & Serville 1843) uses vibrations to locate mates and to signal danger (Hamel and Cocroft, 2012; Cocroft, 1999a,b). To locate mates, male *U. crassicornis* use a 'call-fly strategy', in which they fly to a tree and, upon landing on a branch, generate a vibrational calling signal. This signal consists of a frequency- and amplitude-modulated tonal component in the 100–200 Hz range lasting approximately 1 s that is accompanied by broadband higher-frequency clicks in the 400–2000 Hz range (Cocroft et al., 2006; Cocroft and McNett, 2006; Cocroft and De Luca, 2006; McNett et al., 2006). If a receptive female is in the area, she will respond with her characteristic vibrational signal (Cocroft and McNett, 2006). The insects continue this 'duet' as the male walks along the branch and locates the female. He will continue signaling during the ensuing courtship, which may include direct contact before mating (Cocroft and McNett, 2006; De Luca and Cocroft, 2009). While male *U. crassicornis* generate courtship calls throughout adulthood, females will make their characteristic duet call only when they are receptive to mating (C.I.M. and M.J.L., personal observations).

Insects use a variety of mechanisms to produce vibratory signals, including tapping or drumming directly on the substrate, tremulation of body parts, stridulation and movements of tymbal organs (Ewing, 1989; Virant-Doberlet and Čokl, 2004). Signals that are tonal are often associated with vibrations of the abdomen, while any other components of the signal, if present, may be generated by the other mechanisms. It is not unusual for an insect to use more than one signal generating method in its characteristic call (Cocroft and McNett, 2006; Virant-Doberlet and Čokl, 2004). For *U. crassicornis*, it has been hypothesized that the tonal portion of the signal is produced by abdominal vibrations, while the higher-frequency clicks are generated by a mechanical frequency multiplier system, such as a tymbal (De Luca and Cocroft, 2009). Little is known, however, of the methods used by this animal to create these complex signals.

Our present purpose is to explore the mechanisms underlying the generation of the male *U. crassicornis* calling and courtship signal. This is accomplished using a combination of video analysis, anatomical studies, electromyogram recordings and mathematical modeling. We identify muscles that generate both the tonal and click portions of the signal, and describe their motor patterns. We find that the natural resonance of the abdomen plays an important role in the generation of the tonal signal. Unlike other insects that utilize abdominal vibration to generate plant-borne communication

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List of abbreviations

A1	first abdominal segment
I a apod	apodeme in the first abdominal segment
I a dlm	dorsal longitudinal muscle attaching in the first abdominal segment
I a dvm1	first dorso-ventral muscle in the first abdominal segment
I a dvm2	second dorso-ventral muscle in the first abdominal segment
III vlm	ventral longitudinal muscle in the third thoracic segment
T3	third thoracic segment

signals, we find that the muscle contraction occurs only once for every two cycles of the abdominal oscillation. We also present evidence that suggests that the frequency modulation of the signal depends on a simultaneous modulation in muscle firing rate and a time-dependent change in the effective stiffness of the abdominal attachment to the thorax. We present a simple mathematical model of how the natural resonance can produce the type of abdominal motion that we record.

MATERIALS AND METHODS
Animals
Umbonia crassicornis (Hemiptera: Membracidae) males and females were obtained from a colony at the University of Missouri at Columbia and from individuals collected in southern Florida, USA. They were housed in a temperature-controlled (65–80°C) greenhouse facility at Binghamton University in Binghamton, New York, on *Albizia julibrissin*, *Calliandra emarginata* or *Calliandra surinamensis* trees. Once they reached sexual maturity, males and females were kept on separate plants, unless brought together for breeding purposes.

Video recordings
Sexually mature, naive males were used for video recordings. They were prepared for recording by removing the wings on one side so that the abdomen was clearly visible. Recordings were made either at 30 frames s^{-1} using a Panasonic camcorder (SDR-H60P/PC, Panasonic, Kadoma, Japan) or at 320 frames s^{-1} using a Fujifilm Finepix camera (model HS 25EXR, Fujifilm, Tokyo, Japan) fitted with +1, +2 and +4 macro lenses (Vivitar SERIES 1, Vivitar, Santa Monica, CA, USA). Males were stimulated to signal by placing a female within 5 cm, facing the male. Alternatively, the males were exposed to playback of a male–female duet. Videos were analyzed frame-by-frame after recording. The motion of the abdomen was described relative to the total height and length of the individual's abdomen before signaling (neutral position), to account for different body sizes. Movement of the abdomen was then measured at maximum positions from neutral.

High-speed video
Video recordings of abdomen motions were acquired at 10,000 frames s^{-1} using a high-speed camera (Phantom v2011, Vision Research, Wayne, NJ, USA). Time traces of point locations on the abdomen were extracted from the videos using processing software (Cv version 2.6.749.0, Vision Research). A Savitzky–Golay filter was also employed for data smoothing of time traces (MATLAB version 8.3.0.532, R2014a, The MathWorks, Natick, MA, USA).

Playback of signals
Recordings of female calls or male–female duets were used to stimulate males to call. These were either played through a nearby loudspeaker, which was sufficient to produce a vibrational signal on

the plant stem, or a miniature shaker was attached to the plant (Samsung Linear Vibrator, model DMJBRN0934AA, Ridgefield Park, NJ, USA).

Anatomical dissections
Adult males were euthanized by freezing. They were dissected in saline and later treated with a 70% ethanol solution. Still images were taken with a Polaroid camera (model DMC-2, Waltham, MA, USA) or by using a smartphone (Motorola DROID Turbo, 21 megapixel camera, Chicago, IL, USA). In some cases, a 10% solution of Methylene Blue (Fisher Scientific, Fair Lawn, NJ, USA) was applied to the dissected preparation and allowed to stain for approximately 2 min before it was rinsed with saline and observed.

Electromyogram recordings
Sexually mature adult males were selected from a colony of males on an *A. julibrissin* or *C. surinamensis* tree. Each male was anesthetized on ice for approximately 5–10 min and then positioned on his side in soft modeling clay under a dissecting microscope. For muscle recordings, an insect pin (size 000) was used to create a small hole in the exoskeleton. A 25 µm diameter platinum-iridium wire, insulated except at the tip (A-M Systems, Carlsborg, WA, USA), was inserted into the hole and held in place with a drop of cyanoacrylate glue. An indifferent electrode wire was inserted laterally into the metathorax and glued into place. The precise locations of the electrode tips were determined by dissection after the recordings were completed.

The insect was then placed in some cases on a freshly cut small branch of an *A. julibrissin* tree approximately 10 cm long and approximately 2 cm in diameter held in place on one end by a clamp. In other cases, a small (56 cm high, 2 cm circumference) potted plant of *Calliandra emarginata* was used. The electrodes were connected to an AC-coupled differential amplifier (A-M Systems), and the animal was free to move about the tree or branch to the extent that his wire tether allowed (a distance of around 15 cm). The muscle action potential signals were recorded digitally (Axon Instruments/Molecular Devices, Sunnyvale, CA, USA). An audio monitor (A-M Systems) was used to listen to the recorded muscle activity.

Muscle potentials during a vibrational call were recorded when the male was on the tree or stick. They were induced either by introducing a female or by playback of courtship calls. After successful recordings, the electrodes were cut at the point where they entered the cuticle, the insect was removed from the stick, weighed and placed into a freezer for later dissection to determine electrode placement. For some recordings, the videorecorder was set up adjacent to the stick and recorded the male's behavior while the muscles were recorded. The muscle recordings were synchronized with the video clips, thus relating body movement to audible output from the amplifier.

For most recordings, an accelerometer (model 352A24, PCB Piezotronics, Depew, NY, USA) was attached to the side of the branch directly opposite to the insect's position, to record its movement simultaneously with muscle activity. In this way, the muscle potentials could be correlated to the motion of the branch.

The effect of adding mass to the abdomen was tested by attaching a length of solder (0.5 or 1 cm) to the abdomen using low-melting point wax. The additional mass was weighed, along with the weight of the animal without the mass. The added masses ranged from 10 to 20 mg. The masses of the individual treehoppers ranged from 25 to 35 mg. Electromyograms from the animal and accelerometer recordings from the *C. emarginata* tree during courtship were made for each male with and without the added mass.

## RESULTS

Video recordings

When the male was stationary on the branch, there were no observable abdominal movements. During courtship, the male exhibited a rapid abdominal vibration that produced the characteristic low-frequency tonal portion of the signal. Over the course of the abdominal vibration, the male pulled the abdomen anteriorly until it met the posterior wall of the metathorax, then relaxed so that it then moved posteriorly (Movie 1). All the movement appeared to be taking place about the thoracic–abdominal junction; the abdomen itself did not change in length. The anterior motion is significant, as can be seen in Movie 1, but the shortening does not appear to involve a telescoping-type action in any other of the abdominal segments. The abdomen was the only part of the male's body that was consistently observed to be active through the courtship signal (Fig. 1).

Abdominal movements during courtship were quantified for four males based on each insect's image on a computer screen at 30 frames s^{-1}, and for two males at 10,000 frames s^{-1}. During the courtship signal, the abdomen moves dorso-ventrally, primarily as a single unit that pivots at the thoracic-abdominal junction. It does not show any bending or stretching along its length. The abdomen tip moves ventrally from its neutral position a mean (±s.e.m.) value of 11.9±1.11% of abdominal height (n=6; Fig. 1B,D). From the neutral position, the maximum dorsal deflection of the tip of the abdomen was 4.5±0.97% of abdominal height (n=6; Fig. 1C,D). Over the first half of this signal it also moved anteriorly towards the metathorax by roughly 2.9±0.2% (n=6) of abdominal length. The video recordings indicate that the structure producing the courtship signal is the abdomen. Its action is a dorso-ventral motion, without striking the substrate. This results in a fluctuating force that is transmitted to the branch through the legs. The metathoracic legs typically rest lightly on the surface of the branch, while the prothoracic and mesothoracic legs grip it tightly (Fig. 1A). It would appear likely that the prothoracic and mesothoracic legs are primarily responsible for the transmission of the signal to the substrate.

Anatomy

During courtship, the video recordings show that the motion of the abdomen is primarily driven from the junction between the metathorax (T3) and first abdominal segment (A1). There are four major sets of muscles that are located at the T3–A1 border and are in the position to be moving the abdomen during courtship. The nomenclature we propose for these muscles is based on that used by Wessel et al. (2014), and originated from the anatomical studies of Ossiannilsson (1949). There is a bilaterally paired dorsal longitudinal muscle (I a dlm) that has attachments to dorsal apodemes in A1 and T3 (Fig. 2A,B). Ventrally, all of the remaining three pairs of muscles attach at one end to a dorso-ventrally oriented cuticular plate that spans the midline (Fig. 2A,B,D). This plate is made of fairly flexible cuticle, and it is located on the anterior border of A1. In fact, the lateral wings of the plate make up the anterior face of A1. Medially, the plate is partially internal, and has two dorsally extending internal 'horns' that are separated ventrally by a triangular shaped gap through which the ventral nerve cord runs. The distal face of the ventral cuticle that forms the gap is external, and comprises a small portion of the medial ventral sternite. The dorsally projecting horns provide attachment sites for the muscles. Similar cuticular plates were described by Ossiannilsson (1949) for other Auchenorrhyncha. He referred to them as apodemes, and the cuticular plate we describe would be designated I a apod according
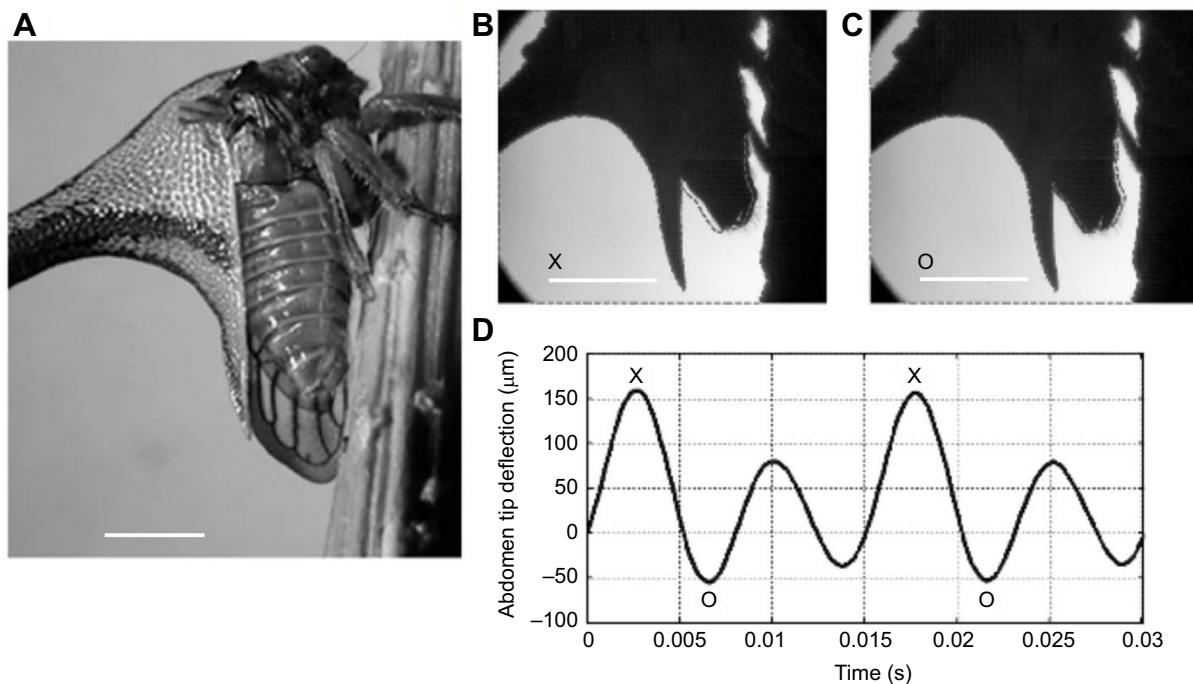
Fig. 1. The dorso-ventral abdominal motions alternate between larger amplitude and smaller amplitude, and ventral motion is larger than dorsal. (A) A male *Umbonia crassicornis* in position for courtship. One wing has been removed to allow for a clear view of the abdomen. (B) A different individual, single frame from a high-speed (10,000 frames s^{-1}) video, showing the extent of the abdomen's ventral motion. The dashed line outlines the neutral position of the abdomen. (C) Another single frame from the high-speed video, showing the extent of the abdomen's dorsal motion. The dashed line outlines the neutral position of the abdomen. Scale bars in A–C are 2 mm. (D) The dorsal-ventral motion of the tip of the abdomen, extracted from the high-speed video of the call. The ventral-most deflection is marked with 'x' and represented by B, while the dorsal-most deflection is marked with 'o' and represented by C. On the *y*-axis of the plot, 0 indicates the neutral position.
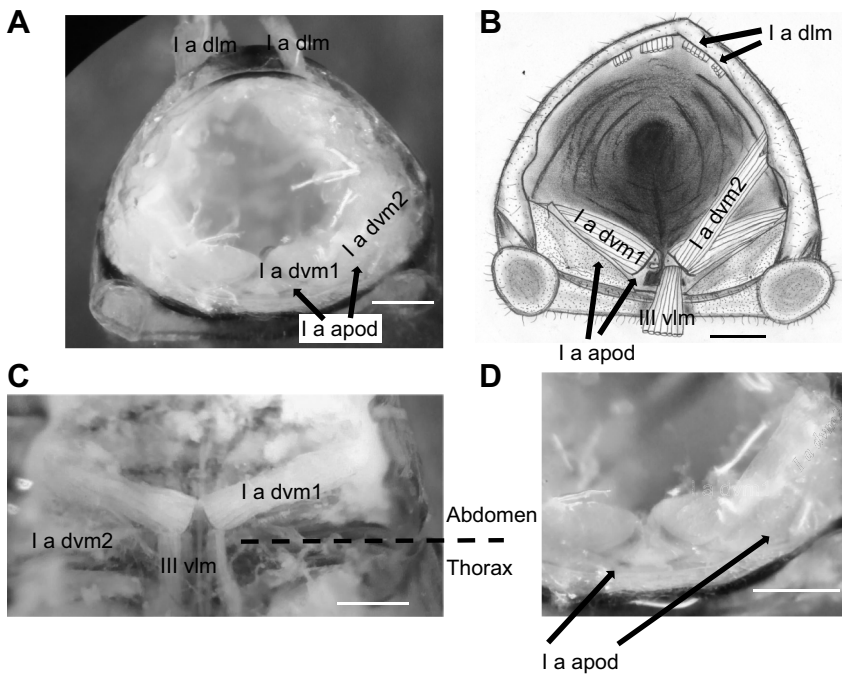
Fig. 2. Anatomy of musculature at the metathoracic–abdominal junction reveals four pairs of muscles.
(A) View of the interior of the abdomen looking posteriorly from its articulation with the thorax, which has been removed. The I a dlm muscles, since being disconnected from their thoracic attachments, are bent dorsally; in the intact preparation they would extend anteriorly toward the reader. Arrows point to the I a apod. The arrow on the right is on its external face; the arrow on the left indicates the internal portion with the triangular gap for the ventral nerve cord. The III vlm muscles are missing in this preparation. (B) Sketch of the same view as A. The III vlm muscles are shown only on one side so that more detail of the I a apod can be illustrated. (C) View from the dorsal side of the animal, to show the position of the III vlm muscles. The overlying gut has been removed. The ventral nerve cord can be seen running down the midline between the paired I a dvm1 and III vlm muscles. The boundary between the thorax and abdomen is indicated on one side with the dashed line. The I a dvm2 on the right side is missing in this preparation. (D) Closer view of the cuticular plate and the dorso-ventral muscles. Arrows are positioned to indicate the same features as in A and B. Scale bars, 1.5 mm.

to his nomenclature. We will use this terminology. A bilateral pair of ventral longitudinal muscles (III vlm, Fig. 2B,C) attach in T3 to a rigid apodeme on either side of the midline. In A1, they attach to an anteriorly projecting 'lip' on each of the two horns of the cuticular plate (Fig. 2B,C).

There are two pairs of muscles that are oriented in the dorso-ventral direction. The most medial of these, dorso-ventral 1 (I a dvm1) attaches distally to a rigid apodeme on the posterior margin of A1 and proximally to the posterior face of the horns of the cuticular plate. The more lateral dorso-ventral muscle (I a dvm2) attaches proximally to the slightly dished anterior face of the lateral horns of the cuticular plate, and distally, it splits into two heads that attach around the internal face of the pivot point of the T3–A1 junction.

Based on their attachment sites and the articulation of the abdomen to the thorax, the I a dlm and III vlm muscles should pull the abdomen toward the thorax, as occurs during the first half of the courtship call. The I a dvm2 should move the abdomen ventrally. I a dvm1 would pull on the horn of the I a apod. Because its attachment site on the abdominal wall is quite rigid, it is likely that any motion it creates would need to be of the more flexible I a apod horn.

Electromyogram recordings

As described in earlier studies (Cocroft and McNett, 2006; De Luca and Cocroft, 2009), accelerometer recordings of the courtship call show two major components: (1) a tonal portion with the greatest intensity in the 100–200 Hz range that is periodically superimposed with (2) high-frequency clicks (Fig. 3). Over the course of the signal, the tonal frequency declines gradually from approximately 200 Hz to approximately 140 Hz (Fig. 3C). The call lasts approximately 1.249 ± 0.031 s (mean$\pm$s.e.m., $n=35$). Over its time course, there is an increase in the amplitude followed by a decrease (Fig. 3A). In some cases, amplitude modulations are apparent, especially near the onset (Fig. 3A,B); however, their prominence varies between individuals.

Recordings from the III vlm and I a dlm muscles during courtship show activity during roughly the first third to half of the courtship

call. The III vlm muscle was recorded for three courtship calls in each of two individuals, and showed activity on average for the first $50\pm3\%$ (mean$\pm$s.e.m., $n=6$; Fig. 4) of the courtship call. For the I a dlm, the average amount of time the muscle was active relative to the duration of the call for three calls from each of three individuals was $32\pm2\%$ (mean$\pm$s.e.m., $n=9$).

The I a dvm1 muscle appears to be responsible for the high-frequency clicks (Fig. 5). Here, activity of the I a dvm1 muscle alone was recorded while the male was producing his courtship signal. In this figure, the muscle activity in A aligns with the clicks recorded by the accelerometer in B. The accelerometer recording in B shows the complete courtship signal the male was producing, including the tonal component of the courtship signal, which is not produced by the I a dvm1 muscle, but by the I a dvm2 muscle, as shown below. Activity of the I a dvm1 muscle is also observed whenever clicks are recorded between courtships (not shown). The I a dvm2 muscle is active during the tonal portion of the courtship call (Fig. 6A,B).

Interestingly, the activity of the I a dvm2 muscle is not 1:1 with the motion of the substrate as measured by the accelerometer (Fig. 6B). Throughout the call, the I a dvm2 muscle fires during every other cycle of the motion. The accelerometer trace (lower curve) in Fig. 6B shows two full cycles of oscillation for each muscle potential (upper curve). This is not because of alternating activity of the I a dvm2 muscles on the two sides of the body. The two I a dvm2 muscles fire synchronously, with both muscles showing a firing frequency that is half that of the signal produced (Fig. 6C).

There are at least two possible explanations for why the dominant frequency of the substrate motion is twice that of the muscle action potential. One is that the I a dvm2 works in conjunction with another muscle(s) to move the abdomen, but we did not find any clear candidates for this. Another possibility is that the abdomen is moving at its natural resonant frequency. It is the mechanical properties of the abdomen that cause it to oscillate at approximately 160 Hz, initiated and subsequently supplemented by activity of I a dvm2. To determine whether the resonance of the abdomen was at least partly responsible for its motion, we weighted the abdomen to
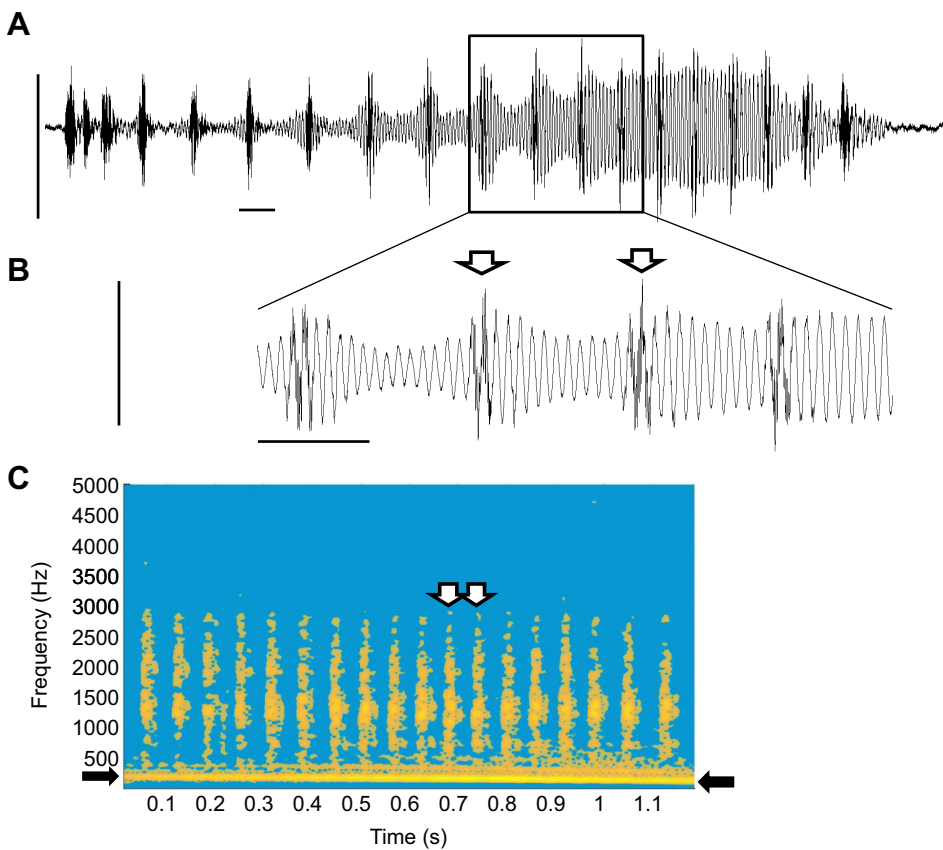
Fig. 3. The male courtship signal contains a frequency-modulated tonal signal with rhythmic broad-band clicks.
(A) Accelerometer recording of a courtship signal. (B) Reduced time scale of the region marked with a box in A to show clicks superimposed on the tonal oscillations. Two of the clicks are marked with arrows.
(C) Spectrogram of a male courtship signal. The intensity of the signal is indicated by its brightness. The signal shows a continuous tonal portion of ~200 Hz that declines to ~140 Hz by the end of the signal (black arrows). The periodic broad-band clicks of up to 1500 Hz are apparent, with the same two clicks as in B marked with white arrows. For A and B, the time scale bars are 50 ms; vertical scale bars are 0.4 m s^{-2}.

dramatically decrease its natural resonant frequency, and compared the accelerometer recordings before and after adding the weight. In some cases, we did this while recording from I a dvm2. If the abdominal motion was primarily driven by the mechanical properties of the abdomen, then we might expect that even if the I a dvm2 is firing at the same frequency during a courtship call with a weighted abdomen, the abdomen itself should oscillate at a lower frequency. This is illustrated in Fig. 7. Adding 10 to 20 mg of mass to the abdomen resulted in no difference in the firing frequency of the I a dvm2 muscle throughout the courtship call (Fig. 7A; $n=5$ weighted animals; $n=13$ unweighted animals; simple linear regression, $P>0.5$). In contrast, the signal produced by the animal shows a substantially lower frequency with the mass added to the abdomen, at all time points through the call (Fig. 7B; $n=6$ weighted, $n=18$ unweighted; simple linear regression, $P<0.0001$). The periodicity of the clicks is not affected by the additional weight (t-test, $P>0.3$). During the peak amplitude of the signal (which is generally much lower in the presence of added weight), the click

frequency was 14.60±0.43 Hz (mean±s.e.m.; $n=9$ animals) for weighted abdomens and 15.15±0.34 Hz (mean±s.e.m.; $n=10$ animals) for unweighted abdomens.

The results above show that: (1) the I a dvm2 muscles do not fire every cycle of the tonal vibration they produce and (2) weight added to the abdomen reduces the frequency of the signal produced but not the frequency of the muscle contraction. This is evidence that the abdominal vibrations may be a function of the mechanical resonance of the abdomen, initiated and periodically maintained by contraction of the I a dvm2 muscles.

Mathematical model

If the abdomen response is strongly influenced by a mechanical resonance, it should be possible to predict its motion using a simple mathematical model. The model examined here treats the abdomen as an equivalent spring/mass/damper that is driven by force impulses due to the muscles. The results presented in the following indicate that this model is consistent with two key
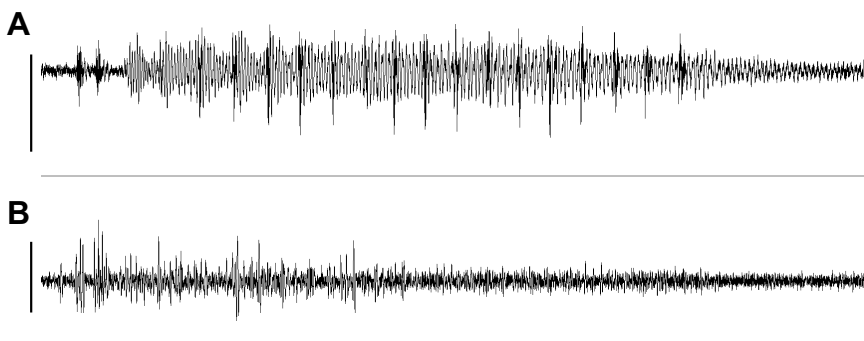
Fig. 4. The ventral longitudinal muscle is primarily active during the first half of the courtship call.
(A) Accelerometer recording. (B) Simultaneous electromyogram of the III vlm. Time scale bar is 100 ms. Vertical scale bars are 2 m s^{-2} (A) and 5 mV (B).

A

B
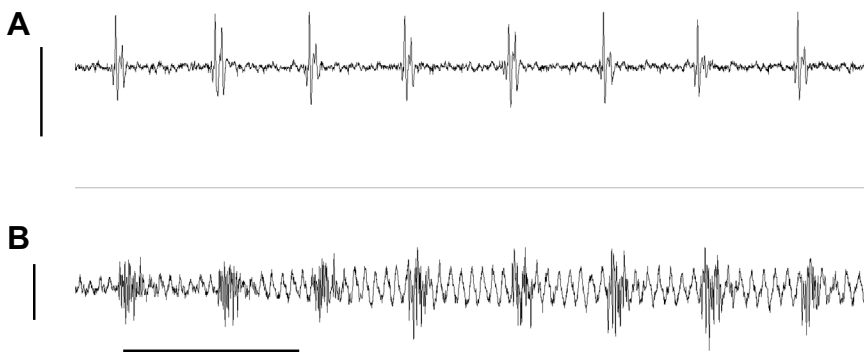
Fig. 5. Activity of the l a dvm1 muscle correlates 1:1 with the clicks recorded by the accelerometer. (A) l a dvm muscle activity. (B) Accelerometer recording. Time scale bar is 100 ms. Vertical scale bars are 8 mV (A) and 1.0 m s⁻² (B).

features of the signal structure. The first is that the abdomen motion consists of repeated pairs of oscillation cycles in which a peak of higher amplitude is followed by one of lower amplitude (as can be seen in Fig. 1D). This higher amplitude and lower amplitude sequence is repeated throughout the call. The second key feature is that the frequency of the oscillation cycles produced on the substrate decreases linearly through the call duration as shown in Figs 3C and 7. Qualitative agreement between results predicted by our mathematical model and experimental results would provide further evidence that a mechanical resonance in the abdomen plays a central role in determining its response. In the following, we describe a highly simplified mathematical model for abdominal vibration that would be produced by idealized repeated impulses provided by the muscles contracting every other cycle.

Let the vertical displacement of the abdomen toward the stem be x (t). We will represent the dynamic response of the abdomen using an equivalent spring/mass/damper having a response that is governed by the following second-order ordinary differential equation:

$$\ddot{x} + \omega_0^2(1 + \epsilon t)^2 x + 2\omega_0 \zeta \, \dot{x} = \frac{f(t)}{m}, \qquad (1)$$

where ω_0 is the initial natural frequency in rad s⁻¹, m is the equivalent mass in kg, ζ is the damping ratio and $f(t)$ is the equivalent force applied by the muscles in N. ϵ is a parameter that accounts for a linear change in the system's natural frequency over time.

We will assume that the applied force consists of a sequence of pulses due to the muscle contraction. The time dependence of each pulse is taken to have the shape of a Gaussian probability density function with variance chosen to be 1 ms, giving each pulse a nominal duration of 1 ms. Numerical experimentation has shown that the detailed shape of this pulse has very little influence on the character of the abdomen response; the only critical parameter is the frequency of repetition of these pulses in relation to the natural frequency of the abdomen. If $\omega(t)$ is the frequency of repetition of the muscle pulses, we then take the applied force to be:

$$f(t) = \frac{1}{\sigma\sqrt{2\pi}} \sum_{i=1}^{N} e^{-(t-T_i)^2/(2\sigma^2)}, \qquad (2)$$

where each pulse occurs at time T_i. These times are separated by intervals given by $\tau_i = 2\pi/\omega(t)$.

Our interest here is not on a quantitative agreement with our measurements, but to determine whether the important characteristics of the time-domain response are reasonably accounted for. We have not, for example, attempted to accurately estimate the magnitude of the force; we consider the force to be simply a periodic sequence of short pulses. The results presented here indicate that even this simplified representation leads to remarkable qualitative agreement with measurements of the abdomen vibration. The resonant nature of the abdomen causes its response and that of the plant substrate to not be strongly determined by the specific time dependence of the force (Miles, 2016).

A   **B**   **C**

Right side

Left side
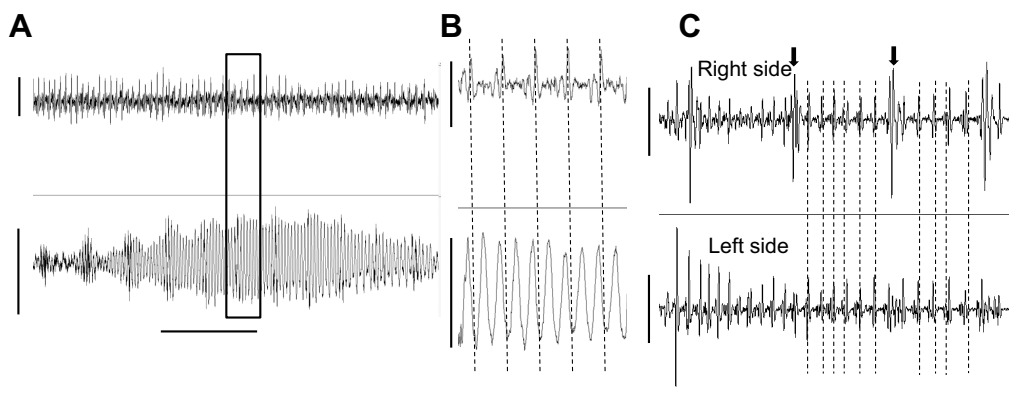
Fig. 6. Activity of l a dvm2 is not 1:1 with the tonal cycles. (A) Recording of l a dvm2 (top trace) and accelerometer (bottom trace) during courtship. Time scale bar is 200 ms. (B) Expanded time scale and gain, showing the details of activity of l a dvm2 (top) and acceleration (bottom trace) from the same recording as in A. Dashed lines are positioned over each muscle action potential to highlight the alternate cycle activity of the muscle relative to the vibration produced. Time scale bar is 25 ms. (C) Simultaneous recordings from both sides of an individual, showing the synchronous activities of the l a dvm2 muscles on the right and left sides. Because of slight differences in electrode placement on each side, the right side shows more of the l a dvm1 activity, marked in two cases with arrows. A number of the l a dvm2 action potentials are marked with dashed lines to highlight their synchronicity. Time scale bar is 100 ms. Vertical scale bars are 5 mV.
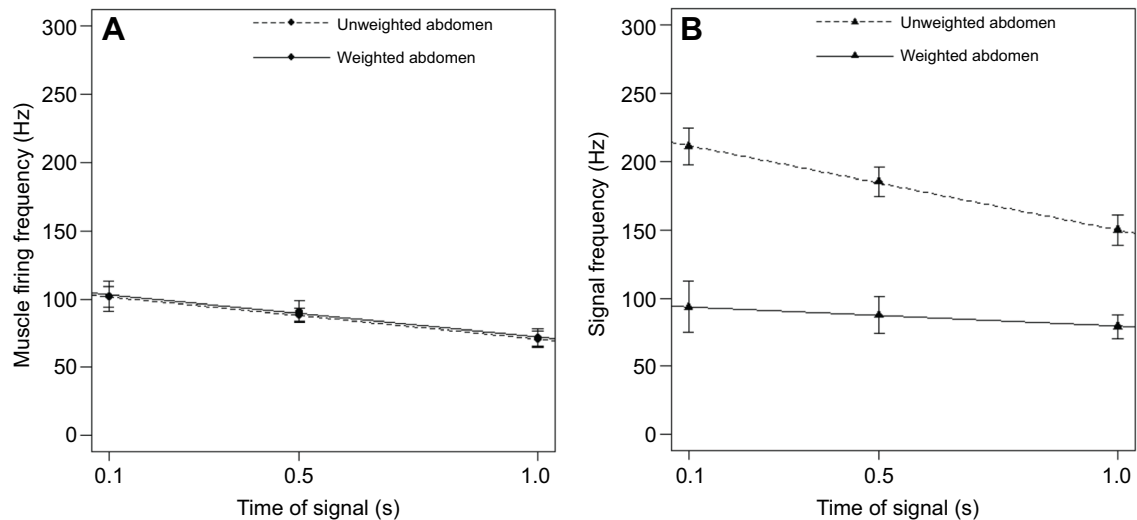
Fig. 7. Adding mass to the abdomen changes the frequency of the tonal signal but not the firing frequency of the muscle that produces it. (A) Firing frequencies of the l a dvm2 muscle at three time points during the courtship call (mean±s.d. at each time point). The data for individuals with a weight added to the abdomen (solid line; n=5 animals) can be compared with that of individuals that had no additional weight added (dashed line; n=13 animals). (B) Accelerometer-recorded frequencies of the signals produced by individuals with weight added to the abdomen (solid line; n=6 animals) and those without additional weight (dashed line; n=18 animals) at three time points during the courtship call (mean±s.d. at each time point).

Because the data shown in Fig. 6 (or compare the curves for unweighted abdomens in Fig. 7A,B) indicate that the muscle spikes occur at half the dominant frequency of the signal oscillation, we normally set the muscle spike frequency $\omega(t)$ equal to half the effective natural frequency of the abdomen, $\omega(t)=\omega_0(1+\varepsilon t)/2$. Again, we find that both the signal frequencies and the muscle pulse frequency vary in time together in this 2:1 ratio. Solutions to Eqn 1 may be obtained in a variety of ways. We have obtained solutions using a power series in t along with using a common numerical solver (ode45 in MATLAB). These two methods provided identical results.

The result of solving Eqn 1 near the beginning of a call is shown in Fig. 8. Fig. 8A shows abdomen displacement measured using our high-speed video camera. The displacement has the characteristic repeating pattern of a large peak followed by a smaller peak. Fig. 8B shows the result of the model where the force pulse frequency was taken to be half that of the abdomen's resonant frequency. Again, because no attempt has been made to determine the magnitude of the force, the results are shown with an arbitrary scale on the vertical axis. The results shown in Fig. 8C were obtained by setting the muscle pulse frequency equal to the natural frequency of the abdomen. As expected, each peak in the response in Fig. 8C has essentially identical amplitude, unlike the repeating pattern of the measured data shown in Fig. 8A. The fact that this dominant feature of the response seems to be predicted quite well by a model that
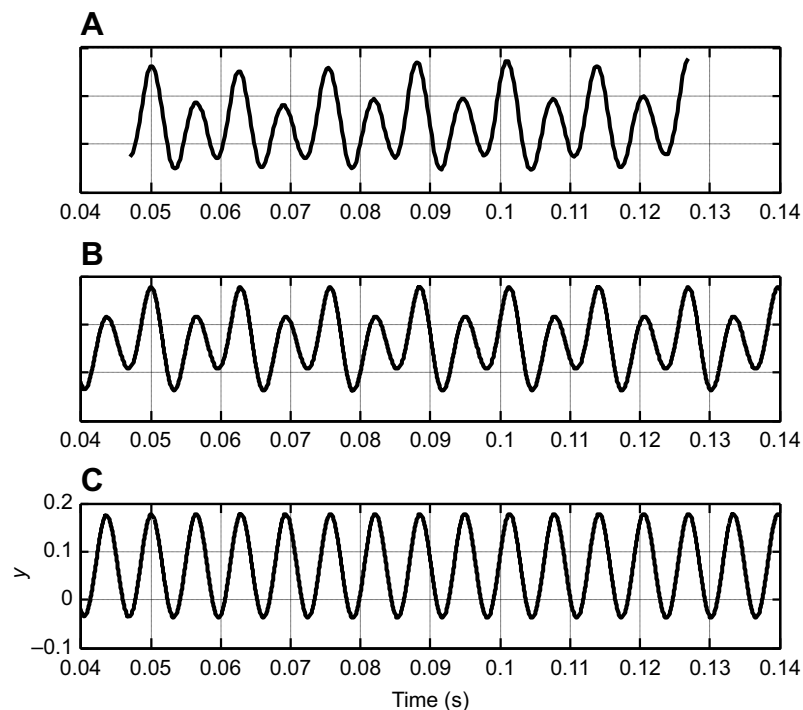
Fig. 8. Results obtained from our mathematical model have temporal characteristics that are very similar to those of our measured data. (A) Abdomen displacement measured using our high-speed video camera. The displacement has the characteristic repeating pattern of a large peak followed by a smaller peak. (B) The result of solving Eqn 1 where the force pulse frequency was taken to be half that of the abdomen's resonant frequency (156 Hz). Because the magnitude of the muscle force has not been estimated, the results are shown with an arbitrary scale on the vertical axis. (C) Results obtained by setting the muscle pulse frequency equal to the natural frequency of the abdomen. As expected, each peak in the response has essentially identical amplitude, unlike the repeating pattern of the measured data shown in A.

represents the abdomen as a simple single-degree-of-freedom harmonic oscillator driven by idealized pulses once every other cycle of oscillation strongly supports our observation that mechanical resonance plays a crucial role in the dynamics of this system.

In order to examine the influence of the abdomen resonance on the frequency modulation of the signal, the governing equation includes the term εt, which serves to modify the natural frequency ω_0 as a function of time. The parameter ε has been chosen so that the abdomen's resonant frequency varies with time, as does the signal frequency in Fig. 7. If ε is set to zero, the model will retain a natural resonance that is equal to that at the beginning of the call. As the muscle pulse rate slows down during the call, the mismatch of the abdomen resonance frequency will cause the pulses to not line up with one of the peaks in the signal. This is shown in Fig. 9. Fig. 9A shows the predicted response when the abdomen resonance frequency stays at twice the muscle pulse frequency throughout the call. The resulting signal is very similar to the measured result obtained with our high-speed video camera (see Fig. 8A). Fig. 9C shows the simulated Gaussian force pulses. Note that each force pulse corresponds to one of the larger response peaks. The results shown in Fig. 9B correspond to where ε is set to zero in Eqn 1. In this case, the response no longer has the repeating pattern observed in the high-speed video data shown in Fig. 8A. In this case, because ε is set to zero, the abdomen's resonant frequency remains at its initial value of ω_0. As the muscle pulse frequency is reduced at the end of the call, the force pulses no longer align with one of the oscillation peaks in the abdomen response. These results show that a model that represents the abdomen as a system having a time-
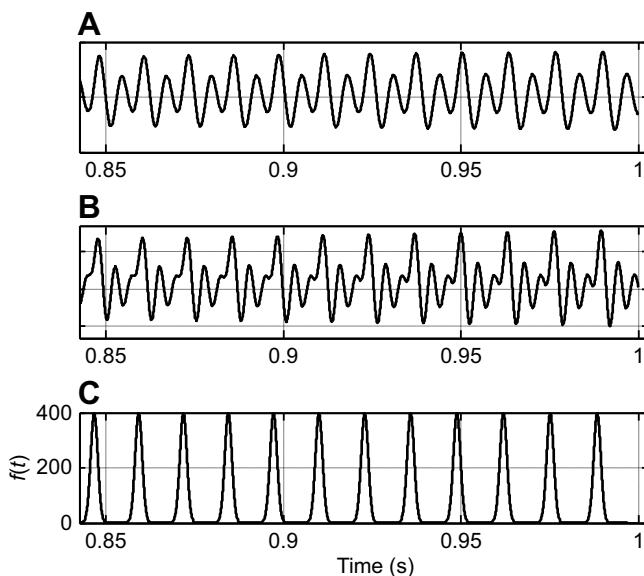
Fig. 9. Model results obtained by solving Eqn 1 suggest that the abdomen resonant frequency varies in proportion to the time-varying muscle pulse frequency. (A) The predicted response when the abdomen resonance frequency stays at twice the muscle pulse frequency throughout the call. (B) Results corresponding to where ε is set to zero in Eqn 1. In this case, the response no longer has the repeating pattern observed in the high-speed video data of Fig. 8. Because ε is set to zero, the abdomen's resonant frequency remains at its initial value of ω_0. As the muscle pulse frequency reduces at the end of the call (which is the time interval shown here), the force pulses no longer align with one of the oscillation peaks in the abdomen response. These predicted results strongly suggest that the abdomen's resonant frequency varies in concert with the muscle excitation frequency throughout the duration of the call.

varying resonant frequency (through the parameter ε) provides remarkable resemblance between predicted and measured results throughout the duration of the call.

DISCUSSION

Abdominal vibration for communication is seen in a number of insects (reviewed in Virant-Doberlet and Čokl, 2004) including *Drosophila* (Hoy et al., 1988), lacewing (Henry and Wells, 2015), planthoppers (Ichikawa and Ishii, 1974) and bugs (Žunič et al., 2008). In the present study, we have confirmed an earlier suggestion that this method of moving the substrate is also used by the treehopper *U. crassicornis* (De Luca and Cocroft, 2009). In addition, we have added to the information about this mode of communication by identifying the muscles involved in the movement and recording the pattern of their activities during courtship communication. We have found a mechanism for generating these vibrations that we do not believe has been previously described: utilizing the natural resonance of the abdomen to efficiently produce a robust vibrational signal without the need for a muscle contraction at every cycle of motion.

Umbonia crassicornus is in the suborder Auchenorrhyncha and the superfamily Cicadiae. The possibility of it having some sort of tymbal, like the male cicada, is therefore of great interest. However, we did not find any structures resembling a tymbal. Ossiannilsson (1949) found quite frequently in the Auchenorrhyncha he examined that the I a dvm 1 muscle of male insects attached distally to a flexible cuticle or to the abdominal wall, which could be seen vibrating when the male was generating a song. In *U. crassicornis*, the distal attachment site for this muscle is quite rigid. In addition, we did not find any sexual dimorphism in the muscular arrangement or cuticular structure (C.I.M., personal observation). Female treehoppers generate a tonal signal during courtship, and also generate clicks while brooding eggs or nymphs (Cocroft, 1999a,b; Cocroft and McNett, 2006; Hamel and Cocroft, 2012), so the lack of sexual dimorphism is not surprising. The position and orientation of the I a dvm1 muscles do bear a resemblance to the tymbal muscle of the cicada, but whether this is a homolog and whether its action on the I a apod could be considered a tymbal-like action need to be further studied. If so, this could be a unique way to construct a tymbal.

The set of muscles that we have found involved in the signaling of *U. crassicornis* fit with the set proposed by Wessel et al. (2014) as being components of the tymbalian tymbal organ. According to their definition of a tymbalian tymbal organ, the muscles III vlm, I a dvm1 and I a dvm2 are all members of this set that together produce movements that generate vibrations used for communication. Although the I a dvm 2 muscles are not myogenic like the tymbal muscles in the cicada, the motor pattern that drives them is similar to that of the cicada, in that the two I a dvm2 muscles contract synchronously (Pringle, 1954a,b). In addition, our experiments of adding mass to the abdomen suggest that because the motor pattern for the I a dvm 2 muscles appears to be unchanged by this manipulation, the motor pattern is produced by a central pattern generator (Wilson, 1960).

As has been reported in a number of other studies, the male *U. crassicornus* courtship call has two components: a continuous tonal call with frequency that declines slowly from approximately 200 to 140 Hz over the course of the call, and periodic high-frequency clicks that are superimposed on the tone at a frequency of approximately 15 Hz (Cocroft et al., 2006; Cocroft and McNett, 2006; Cocroft and De Luca, 2006; McNett et al., 2006). Earlier studies proposed that while the tonal portion was likely produced by

thoracic or abdominal muscles, the click should be the result of some frequency multiplier system such as a tymbal (Cocroft et al., 2006; De Luca and Cocroft, 2009). We have found that this does appear to be the case for the clicks. The I a dvm1 muscles attachment sites are a lateral rigid apodeme on the A1–A2 border, and a more flexible dorsally projecting horn from the I a apod that lies at and makes up part of the anterior face of the abdomen. We were unable to test the mechanical properties of this apodeme, but it would appear likely that a shortening of the I a dvm1 muscle would pull on the horn, and perhaps it would cause a snapping that could generate a high-frequency click by abdominal resonance. Our recordings from the I a dvm1 show that a single spike in the muscle leads to a series of high-frequency oscillations as recorded by the accelerometer. These outlast the duration of the action potential (see Fig. 5). Clearly, further work needs to be done to determine exactly how this system generates the click.

For the tonal portion of the signal, there is apparently only one pair of muscles responsible for the dorso-ventral vibration of the abdomen. This is the I a dvm2 muscle that attaches at the I a apod and to points around the pivot for the abdominal–thoracic junction. This muscle would be expected to pull the abdomen ventrally when it shortens. There does not appear to be a muscle that moves the abdomen back up again. The I a dvm2 muscle does not show an action potential with every 'down' cycle of the abdomen. These two observations underscore the importance of the mechanical resonance in the abdominal motion. Additional evidence that resonance may be important in this system is provided by the observation that adding weight to the abdomen can slow the tonal frequency of the signal created, even though it does not affect the firing frequency of the I a dvm2 muscle.

Our mathematical model of a resonating abdomen that is driven every alternate 'down' cycle, simulating the activity of the I a dvm2, predicts motion of the abdomen that corresponds closely to the actual motion that was measured in a high-speed video recording of the male courtship behavior. This result shows that the treehopper's abdomen behaves very much like a damped mass on a spring. In addition, the frequency modulation of the tonal portion of the call can be accurately modeled by including a time-dependent change in the natural frequency of the resonant system.

This raises the question of how such a change in natural resonance could come about over the course of the courtship signal. Although the abdomen moves anteriorly, the change in overall length produced by pulling forward is not nearly sufficient to explain the change in resonant frequency. To account for a decline from 200 to 140 Hz, the oscillating mass would have to shorten by 46% to obtain the necessary change in the mass moment of inertia; we measured a forward movement of only 2.9%. Because the mass of the abdomen is unlikely to be changing over this time, the most likely way this is done is by changing the stiffness of the oscillating abdomen. Contraction of the III vlm and I a dlm muscles, pulling the abdomen toward the thorax, could be the source of an early stiffening of the thoracic–abdominal junction. We do not predict that the stiffening is due to the muscles themselves, but to the way the abdomen is braced against the thorax. It is at its maximum when it is flush against the thorax, being stiffened by its contact with the large immobile thoracic box. The stiffness soon declines as the abdomen relaxes posteriorly. Movie 1 highlights this motion. As these muscles relax and ultimately cease firing after the first part of the courtship signal, the natural resonance of the abdomen would be expected to decline. One remaining question, however, is how the I a dvm2 remains phase-locked to the signal generated, even as its frequency declines. The firing frequency of this muscle remains at

half the frequency of the signal it generates, for the entire courtship call (compare muscle firing frequencies in Fig. 7A with the signal frequencies for unweighted abdomens in Fig. 7B). Such a coupling could come about if the motor pattern generator that produces the I a dvm2 activity were receiving sensory feedback about the stiffness at the thoracic–abdominal junction, perhaps by stretch receptors located here. This would be similar to the feedback to the flight oscillator by stretch receptors in the tegula of the grasshopper wing (Wilson and Gettrup, 1963; Pearson et al., 1983). At this point, we have not identified stretch receptors, and this remains to be determined.

Resonance of the exoskeleton is used by many insects to produce tonal signals (Bennet-Clark, 1999). In the bladder cicada, *Cysotsoma saundersii*, an acoustic resonance in the abdomen plays an important role in the radiation of air-borne sound (Bennet-Clark, 1998b). The resonance in this case involves an oscillation of energy between the compression of air within the abdomen (behaving like a spring) as the abdomen's internal volume fluctuates because of the in-and-out motion of the abdomen wall (which behaves as the mass). To our knowledge, however, the treehopper is the first reported example of a resonance potentially being used for abdominal motion that generates vibrational signals. In lacewings and bugs, which are known to produce dorso-ventral abdominal vibrations, the evidence is that muscles are used to move the abdomen with every cycle (Amon and Čokl, 1990; Čokl, et al., 2005; Gogala, 2006; Čokl, 2008; Henry and Wells, 2015). The advantage to the treehopper of making use of the natural resonance of the abdomen is that it is almost certainly metabolically less costly to have one pair of muscles contract once every other cycle than it is to have two pairs of muscles (one to raise the abdomen and one to lower it) contracting with each cycle.

The III vlm and I a dlm muscles could play a role in adjusting the stiffness and thus the natural frequency of the abdomen by bracing it against the thoracic box. The firing frequency of the I a dvm 2 muscle parallels this change in stiffness so that the animal can continue to enjoy the benefits of making use of natural resonance to keep its metabolic energy costs low.

Supplementary information
Supplementary information available online at
http://jeb.biologists.org/lookup/doi/10.1242/jeb.147819.supplemental

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