

Vibrational Signals and Mating Behavior of Japanese Beetles (Coleoptera: Scarabaeidae)

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ABSTRACT We report that male Japanese beetles produce substrate-borne vibrational signals during mating. Males produced these signals while mounted on the female, mainly before genital intromission but also during intromission and afterwards during mate guarding. Females thus likely perceive male signals as contact courtship. We also describe new details of male mating behavior, including stroking the female with the forelegs. We discuss the likely function of male signaling and stroking behavior as courtship in the context of pre- and postcopulatory sexual selection.

KEY WORDS copulatory courtship, cryptic female choice, introduced species, seismic communication, *Popillia japonica*

Japanese beetles, *Popillia japonica* (Newman), are an important introduced pest species in eastern North America (Fleming 1972, Potter and Held 2002). Their reproductive biology has been studied in terms of their polygamous mating system (Fleming 1972, Tigreros and Switzer 2009), which is characterized by pair formation involving pheromones (Fleming 1972, Potter and Held 2002), costly mate guarding by males (Saeki et al. 2005b,c; Kruse and Switzer 2007; Tigreros et al. 2010), and male mate preferences for large, high-fecundity females (Saeki et al. 2005a,b; Tigreros and Switzer 2009; Tigreros et al. 2010).

The mating behavior of *P. japonica* was described by Barrows and Gordh (1978). Briefly, males mount females, grasping the female elytra with their forelegs and the female hind legs with their mid and hind legs. A single brief genital intromission is followed by prolonged mate guarding during which the male remains on the female without genital contact (Barrows and Gordh 1978, Tigreros and Switzer 2009). Rivals may displace the mating male during intromission or mate guarding (Kruse and Switzer 2007, Potter and Held 2002). Males have been described as courting the female while they are mounted, rubbing them with their antennae and hind legs (Barrows and Gordh 1978).

Here we report the use of substrate-borne vibrational signals in courtship by male *P. japonica*, with additional details on their courtship behavior. Vibrational signals are widespread among insects, and have been reported for *Aphodius* scarabs (Hirschberger and Rohrseitz 1995, Hirschberger 2001, Kasper and Hirschberger 2006) and various other beetles (Cocroft and Rodríguez 2005). We discuss some differences

between our observations and prior descriptions of *P. japonica* mating behavior (Barrows and Gordh 1978) in terms of potential geographic variation and behavioral plasticity arising from the social context.

Materials and Methods

To describe the beetles' mating behavior, we collected them by hand from vegetation (mainly wild grape vine, *Vitis riparia*) along or near the Milwaukee River in the vicinity of the UWM campus, during July–September 2012. We identified the beetles as *P. japonica* by their five distinctive white tufts on the sides of their abdomen (Evans 2014). We separated the beetles by sex (according to sex differences in their forelegs; Fleming 1972) and kept them in containers with vine trimmings until we used them for behavioral observations 1–84 d after capture.

We observed pairings in the lab, placing one male and one female on a vine trimming with several leaves, with the base of the trimming placed in a water tube. We used a new trimming for each pair. We observed $n = 10$ pairs, using each individual only once. We observed one pairing in full, from first approach until the couple separated at the end of mate guarding; we observed seven pairings from first approach until shortly after the end of intromission (i.e., we ended observations during mate guarding); and we observed two pairings that did not result in intromission. The temperature during these trials was 22°C.

We recorded the beetles' behavior with a Sony HDR–SR10 camera, and we recorded substrate vibrations with the beam of a laser vibrometer (Polytec CLV 2534; Polytec Inc., Auburn, MA) focused on a small piece of reflective tape attached to the vine. We recorded the laser vibrometer output on an iMac computer at a sampling rate of 44.1 kHz with the sound

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analysis program AUDACITY (version 1.2.5; <http://audacity.sourceforge.net>).

We described mating behavior and signals from the video and laser recordings. We used the criteria established by Eberhard (1994) to consider that a behavior performed during mating constituted courtship: the behavior was repeated and rhythmic, appropriate to stimulate the female, mechanically irrelevant for the male to remain on the female, and performed only in the context of mating. By these criteria, one male behavior performed with the front legs qualifies as copulatory courtship, and so does male vibrational signaling (see Results).

We described the temporal and spectral features of the beetles' signals with AUDACITY. For each male, we selected three 15-s-long segments of the recording representing intervals of high signaling rates (in order to avoid introducing variation due to the irregular nature of signaling; see below). From each segment we measured the following: signaling rate (across the whole segment); signal length (for every high-amplitude signal); and the fundamental, second harmonic, and dominant frequency from an FFT analysis (fft size = 4096 points) of clusters of ca. five high-amplitude signals; we took spectral measurements from signal clusters rather than individual signals to improve their accuracy, which increases for longer time windows (Fletcher 1992, Gerhardt and Huber 2002).

The description of mating behavior that we obtained according to the above procedure suggested that only males produce the vibrational signals that we observed (see below). We tested this hypothesis with a separate experiment using beetles collected during August of 2015 at the same sites as above, and also at Havenwoods State Forest (Milwaukee, WI). In the lab, we created experimental treatments consisting of live and frozen male/female pairings on vine trimmings, as follows: 1) live male/live female ($n = 9$ pairings); 2) live male/frozen female ($n = 8$ pairings); 3) frozen male/live female, placing the male on top of the female for 1 min to simulate a mount ($n = 11$ pairings); and 4) two live females, placing them within 3 cm of each other ($n = 10$ females). For the trials with frozen individuals, the beetles were placed in a freezer (at -5°C) for 30–45 min before use in the trials. We used a fresh vine trimming for each trial. Temperatures during these trials ranged from 21 – 23°C . We observed each pairing for 10 min or until intromission was attempted (in trials with live males). In the treatment with frozen males, we observed the female for 5 min after 1 min of simulated mount by the frozen male. With this experiment, the hypothesis that only males produce the vibrational signals that we describe below makes the prediction that signaling should only occur in treatments 1) and 2), but not in the other treatments. To detect signaling in treatments 3) or 4) would reject the hypothesis, and indicate either that females also produce vibrational signals, or that only females do so.

Results

Mating interactions began when a male and a female met on the vine trimming. As described by Barrows

and Gordh (1978), the male mounted the female grasping the front edges of her elytra with his fore tarsi and claws, and grasping her second legs with his mid and hind tarsi and claws. The male's hind legs were not permanently on the female; males often trailed their hind legs in the air next to the tip of the females' abdomen, and occasionally raised them upwards in the air, or rested them on the substrate (Fig. 1).

The stages of mating that we observed also agree with the description by Barrows and Gordh (1978). In most successful pairings, the male extended his aedeagus and achieved genital intromission (Fig. 1A) within a few minutes of mounting the female. Intromissions lasted ca. 3 min (Fig. 2). Upon withdrawing the aedeagus (Fig. 1B), males remained on the female. We ended observations before most males dismounted, as mate guarding can be prolonged in this species (Barrows and Gordh 1978, Saeki et al. 2005c). We continued observations for 1–143 min after the end of intromission, except for the pairing observed in full, which lasted 11 min (seven of which were after the end of intromission), and for the two pairings that did not result in intromission, which we observed for 82 and 118 min.

Japanese Beetle Males Produce Vibrational Signals and Stroke the Female. Our main novel observation is that males produced substrate-borne vibrational signals, beginning when they were attempting to mount females and continuing through to mate guarding. We observed signaling in all pairings involving a live male and a live female ($n = 9$ in 2012, and $n = 9$ in 2015). Note that we recorded these signals with the laser beam focused on the vine trimming on which the beetles stood, but females would perceive the signals directly through the male's legs. The experiment that we ran in 2015 confirmed that only males produce these vibrational signals; signaling only occurred with live males and never with frozen males or in interactions between females (Table 1)—these results were so clear-cut that no significance testing is required (Cohen 1994). Because in this experiment we only observed pairings until intromission was attempted, the following description relies on the observations made in 2012 (but all the 2015 observations agree with it).

Male signaling began as soon as males contacted the female. It occurred most often prior to genitalic intromission, but also during and after intromission (Fig. 3). In some cases, the whole body of the male could be seen rocking back and forth slightly (or sometimes the abdomen moving up and down slightly) as he signaled. No such movements were evident in females.

Another novel observation is that males stroked the edge of the females' elytra with their fore tarsi, using either one leg or both legs. We observed this copulatory courtship in five of the nine pairings. Stroking occurred in irregular clusters or bouts that varied in rate along the course of pairing interactions. Stroking was most common when males were mounted on the female prior to genitalic intromission (Fig. 3).

We also observed a gentle rocking of the male body that slightly thrust the aedeagus in and out of the female.

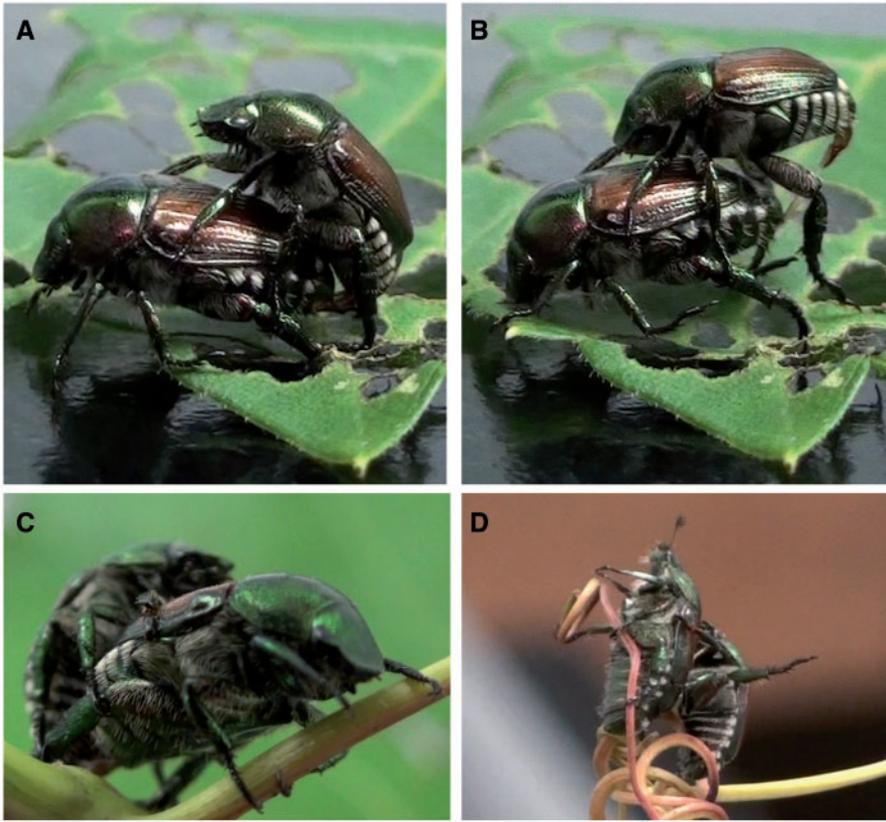


Fig. 1. Mating positions of *P. japonica* beetles. (A) Couple in intromission. The male was performing the abdominal thrusting described in the text. (B) The same couple, as the male withdrew his aedeagus from the female. Note the distended endophallus between the tip of the female's abdomen and the male's aedeagus. (C) Detail of the male's grasp on the female's elytra and legs, from a different couple. (D) The same couple as in (C) during mate guarding, with the male's hind legs in an elevated position.

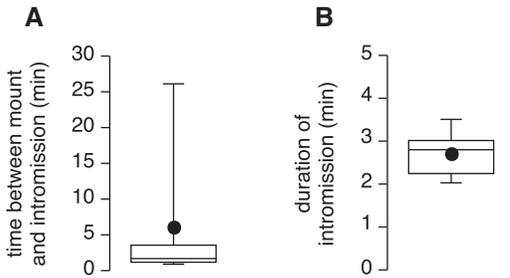


Fig. 2. Variation in the length of the mount and genital intromission phases of mating in *P. japonica* beetles. (A) Time elapsed between the male mounting the female and genital intromission, for those pairings that resulted in intromission ($n = 8$). (B) Duration of genital intromission ($n = 8$). Box lines show the 75th, 50th, and 25th percentiles; whiskers show the 90th and 10th percentiles; the circle shows the mean.

We failed to observe some behaviors mentioned by Barrows and Gordh (1978). We did not see males rub the female abdomen with their hind legs, nor thrust their hind legs toward the apex of the female abdomen.

Table 1. Test of the hypothesis that only males produce vibrational signals in *P. japonica* beetles

Treatment	Vibrational signals produced	No vibrational signals observed
1) live male / live female	9 ^a	0
2) live male / frozen female	5 ^b	3 ^c
3) frozen male / live female	0	11
4) two live females	0	10 ^d

We report the number of trials (pairings) in which vibrational signals were produced or not.

^aThese males attempted intromission within 5 min ($n = 7$) or climbed off the female right after signaling ($n = 2$).

^bThese males attempted intromission within 5 min ($n = 4$) or climbed off the female right after signaling ($n = 1$).

^cThese males climbed off the female within a few seconds.

^dThe females contacted each other ($n = 8$) or walked closely by each other ($n = 2$).

Movements of the male head and antennae seemed incidental with regards to a potential function of stimulating the female, mainly because they were not repeated rhythmically (re: criteria in Eberhard 1994; see above).

Description of Japanese Beetle Male Vibrational Signals. Male signals were short pulses repeated at irregular intervals (Fig. 4), each consisting of a

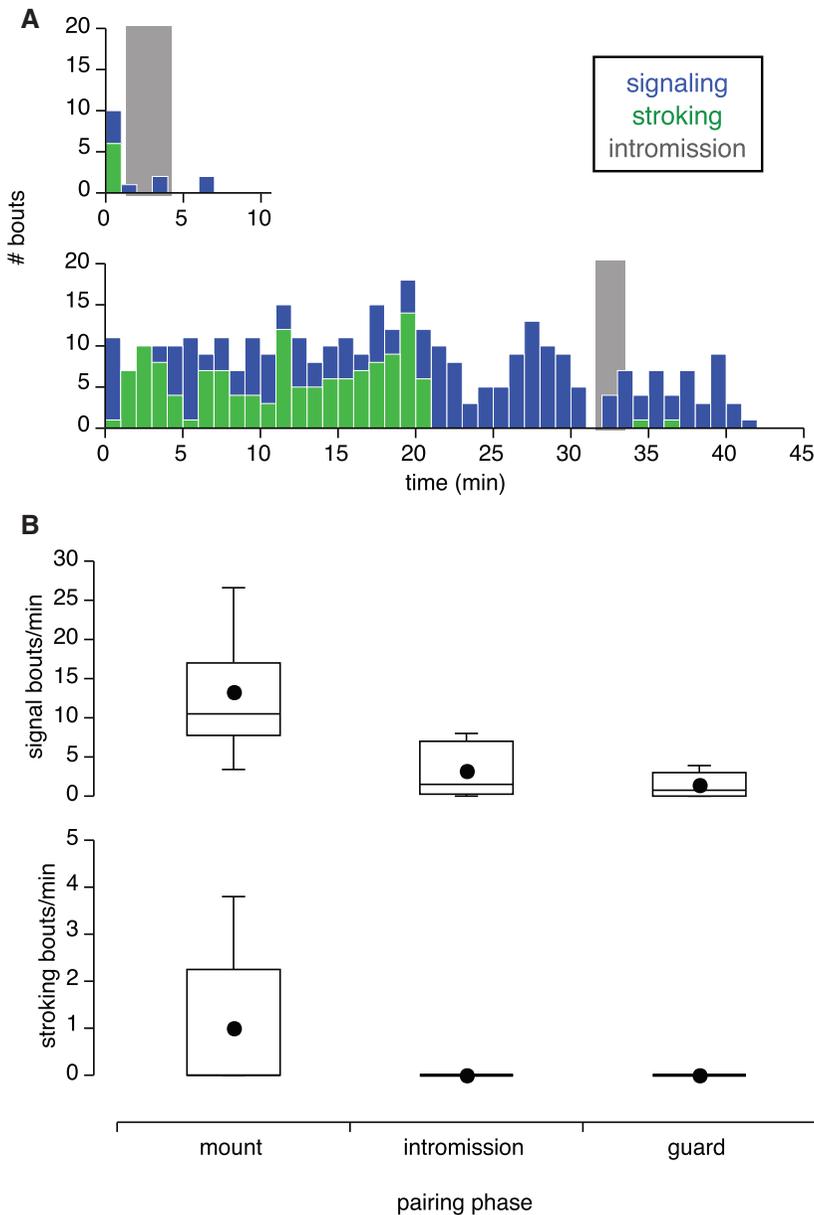


Fig. 3. Variation in the behavior of *P. japonica* males of producing vibrational signals and stroking the female with their forelegs during mating. (A) Two different pairings, showing the occurrence of each behavior in 1-min bins. Each blue or green bar shows the number of bouts of stroking or signaling, so that the height of each bar offers a rough indication of the rate for each behavior. Gray bars indicate the span of genitalic intromission. (B) Box plots for signaling and stroking bouts that occurred, in 1-min bins along the phases of mount, genitalic intromission, and mate guarding ($n = 8$ pairings). Box lines show the 75th, 50th, and 25th percentiles; whiskers show the 90th and 10th percentiles; the circles show the mean.

near sinusoidal waveform with two main frequency components (Figs. 4B, C, and 5), although some signals had more complex waveforms indicative of richer frequency structure (e.g., Fig. 4D). In most signals the fundamental frequency was dominant (Fig. 5), but for some the second harmonic was dominant (Fig. 5B). Which frequency had higher amplitude may be influenced by filtering through the female's body and the vine (Cocroft and Rodríguez 2005), so that our

measure of the fundamental frequency is likely the most consistent of the descriptors of signal spectral features.

Each signal lasted just over 100 ms, and during periods of high signaling rates males produced signals at just over 1 per second (Fig. 6A, B). The fundamental frequency and second harmonic were ~ 80 Hz and 180 Hz, respectively, with the second harmonic being about 4 dB lower in amplitude (Fig. 6C). Because the

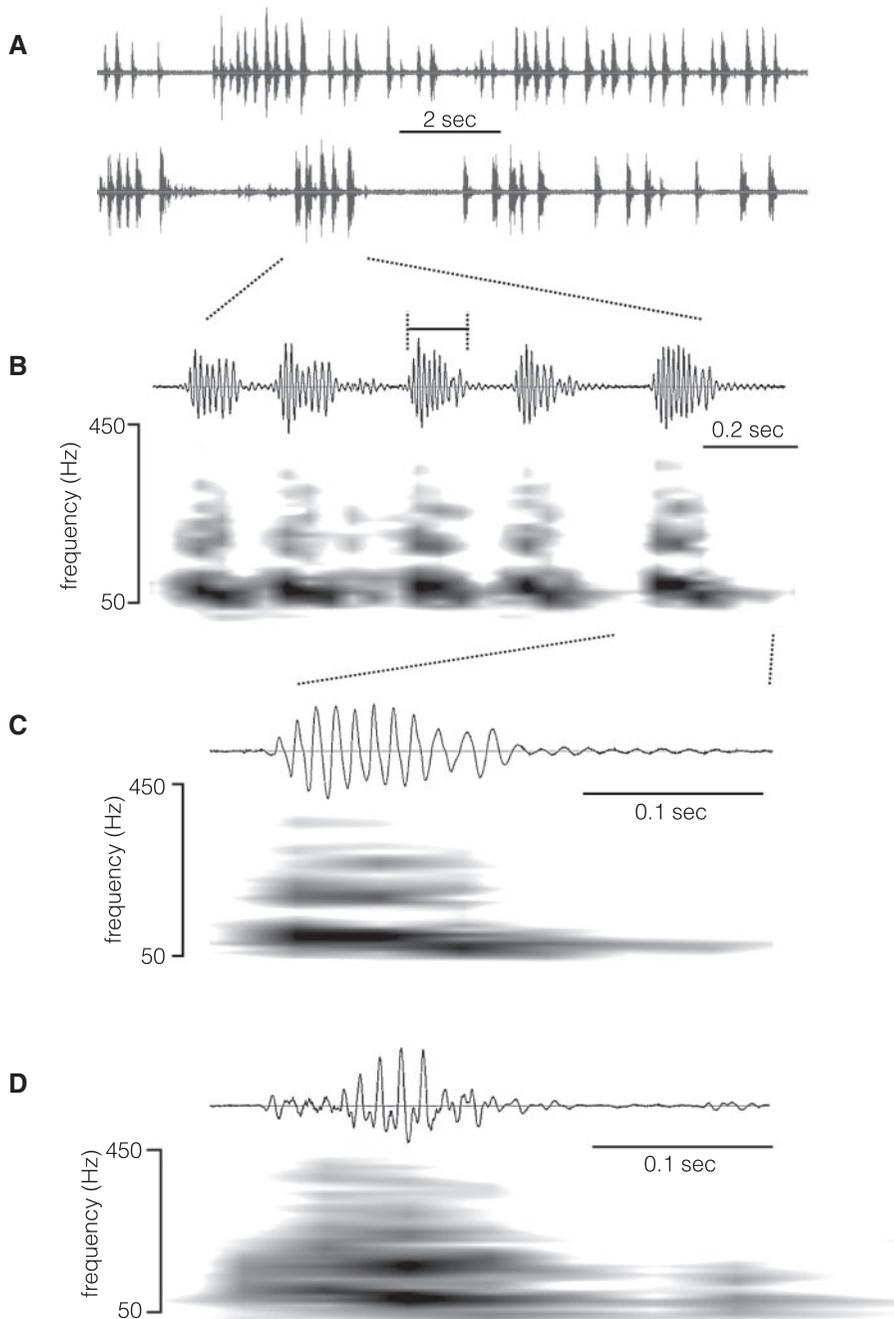


Fig. 4. Examples of *P. japonica* male vibrational signals. (A) Two 15-s recordings of the signals produced by one male, showing the waveform. (B) Detail of a cluster of those signals, showing the waveform and sonogram. The brackets indicate signal length. (C) Detail of a single pulse, showing the waveform and sonogram. (D) Another pulse produced by a different male. Note the more complex structure of the waveform and the corresponding difference in the frequency content of the sonogram. Note that we use sonograms to illustrate the frequency content of the signals, but we took measurements from the spectrograms that resulted from the FFT analysis (see Fig. 5).

dominant frequency was sometimes the second harmonic (right arrow in Fig. 5B), the dominant frequency was shifted up from the fundamental to ca. 90–120 Hz and showed a broader range (Fig. 6D).

Discussion

We make a contribution toward the study of the reproductive biology of *P. japonica* Japanese beetles. New in this report are the observations of the following

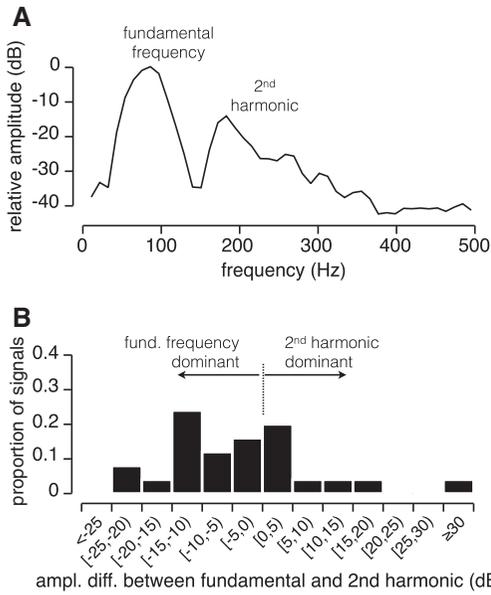


Fig. 5. Example of the FFT analysis of *P. japonica* male vibrational signals. (A) Spectrogram of the cluster of signals in Fig. 4B, showing the fundamental and second harmonic. (B) Histogram of the distribution of the amplitude difference between the fundamental and second harmonic, pooling across all signals for all males. In most cases, the dominant frequency was the fundamental as shown in (A), but sometimes the dominant frequency was the second harmonic.

behaviors: 1) male contact copulatory courtship with vibrational signals; 2) male contact copulatory courtship with the forelegs; and 3) male genitalic thrusting. There sometimes was a back-and-forth rocking of the male's whole body during signaling, suggesting that this body movement may be involved in the production of the vibrational signals.

Because most signaling and nearly all foreleg stroking occurred prior to genital intromission, these forms of courtship may function to induce the female to accept the male's attempts at intromission. There are, however, many additional possible functions of copulatory courtship, especially for the vibrational signals, which often continued during and after intromission. Such functions may include increasing the likelihood of the female accepting the male's sperm or refusing or delaying remating, and a wide range of additional possibilities (Eberhard 1994, 1996, 2009). In *P. japonica* there is evidence of last-male sperm precedence (Ladd 1966), suggesting that further research on the role of male copulatory courtship on inducing females to decline or postpone re-mating or to choose between the sperm of different males may be profitable.

The near sinusoidal waveform of some male signals (e.g., Fig. 4C) suggests that they may be produced by direct muscle contractions (Cocroft and Rodríguez 2005), although additional structures may be involved in the production of other frequency components (Fig. 4D; Wessel 2006). Signal production, which continues at some rate after intromission (Fig. 3), offers a

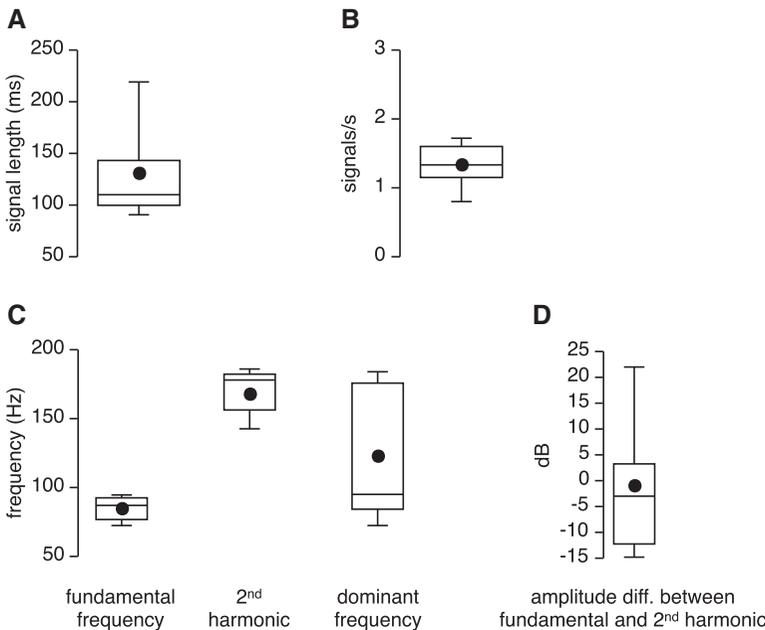


Fig. 6. Description of the features of *P. japonica* male vibrational signals, obtained from detailed analysis of three 15-s segments sampled from intervals of high signal rates for each pairing ($n = 8$ pairings). Box lines show the 75th, 50th, and 25th percentiles; whiskers show the 90th and 10th percentiles; the circles show the mean. (A) Signal length. Parameters calculated by pooling within each of the three 15-s recordings, then across the three recordings for each male, then across males. (For all other features, parameters calculated by pooling across the three recordings for each male, then across males.) (B) Signal rate. Note that this was obtained from segments of high signaling rates. (C) Fundamental frequency, second harmonic, and dominant frequency (see Fig. 5). (D) Difference in amplitude between the fundamental and the second harmonic.

potential explanation for the observation of higher thorax temperatures in mate-guarding males (Saeki et al. 2005b). Thus, signaling may influence the high physiological costs of mate guarding (Saeki et al. 2005b, Tigreros et al. 2010).

Some elements of our account of *P. japonica* mating behavior differ from previous descriptions (Barrows and Gordh 1978). It seemed to us that male head and antennal movements lacked a stimulatory function, as they failed to meet the criteria outlined by Eberhard (1994). And we did not observe males stroking females with their hind legs, nor appearing to attempt to block the female tip of the abdomen. This difference may reflect geographic variation, as beetles in the present study were from Wisconsin, whereas beetles in the prior study were from Virginia and Washington, D.C. (Barrows and Gordh 1978). Alternatively, the hind leg movements may represent rare behaviors that our relatively small sample size may have failed to detect (although it would seem unlikely that we would miss them altogether without some element of geographic variation in the frequency of the behaviors). Another, more likely, possibility is that the difference may reflect the social context of behavioral observations (Saeki et al. 2005c). By observing only one male and one female at a time (rather than in groups as in Barrows and Gordh 1978), our trials may have lacked a prompt for additional male efforts. Signaling and courtship may vary in rate with among social contexts, and experiments varying such contexts may help illuminate their function.

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