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RESEARCH ARTICLE

Sound radiation and wing mechanics in stridulating field crickets (Orthoptera: Gryllidae)

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SUMMARY

Male field crickets emit pure-tone mating calls by rubbing their wings together. Acoustic radiation is produced by rapid oscillations of the wings, as the right wing (RW), bearing a file, is swept across the plectrum borne on the left wing (LW). Earlier work found the natural resonant frequency (f_o) of individual wings to be different, but there is no consensus on the origin of these differences. Previous studies suggested that the frequency along the song pulse is controlled independently by each wing. It has also been argued that the stridulatory file has a variable f_o and that the frequency modulation observed in most species is associated with this variability. To test these two hypotheses, a method was developed for the non-contact measurement of wing vibrations during singing in actively stridulating *Gryllus bimaculatus*. Using focal microinjection of the neuroactivator eserine into the cricket's brain to elicit stridulation and micro-scanning laser Doppler vibrometry, we monitored wing vibration in actively singing insects. The results show significantly lower f_o in LWs compared with RWs, with the LW f_o being identical to the sound carrier frequency (N=44). But during stridulation, the two wings resonate at one identical frequency, the song carrier frequency, with the LW dominating in amplitude response. These measurements also demonstrate that the stridulatory file is a constant resonator, as no variation was observed in f_o along the file during sound radiation. Our findings show that, as they engage in stridulation, cricket wings work as coupled oscillators that together control the mechanical oscillations generating the remarkably pure species-specific song.

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Key words: neuroactive substances, microinjection, stridulation, resonance, acoustic radiation, laser vibrometry.

INTRODUCTION

Male field crickets (Orthoptera: Gryllidae) conventionally generate mating calls by rubbing together specialized regions of the forewings or tegmina (e.g. Ewing, 1989). A vein in both forewings is ventrally modified with a series of hard pegs that form a stridulatory file, while the anal wing region harbours a plectrum on its medial side. As the plectrum of one wing is swept across the file of the opposite wing, a series of impacts occur, generating vibrations of the surrounding wing membranes (Pierce, 1948).

During singing, the two forewings open and close simultaneously, yet most of the acoustic energy is produced during the closing stroke (Walker et al., 1970; Elliott and Koch, 1985; Koch et al., 1988; Bennet-Clark, 1989). Even though the file and the plectrum are featured on both wings, in crickets it is usually the animal's right wing (RW) that lies on top of the left wing (LW). Thus, during stridulation, the plectrum of the LW contacts teeth on the ventral side of the RW (Elliott and Koch, 1985) (see also supplementary material Movie 1). Sound production by tegminal stridulation is therefore functionally asymmetrical, with the two wings having different functions (Forrest, 1987).

The main sound radiator in the forewings of crickets is a specialized region known as the 'harp' (Fig. 1) (Bennet-Clark, 1970; Nocke, 1971; Michelsen and Nocke, 1974; Bennet-Clark, 1999a; Bennet-Clark, 2003), although other wing cells (i.e. wing regions) have also been attributed a role in the overall resonant behaviour of the wing (Bennet-Clark, 2003).

The carrier frequency (f_c) of the calling song of most cricket species is highly tonal (Leroy, 1966; Otte, 1992; Walker and Moore, 2002). This observed acoustic purity is explained by an escapement-like mechanism, analogous to the escapement of clocks (Elliott and Koch, 1985; Koch et al., 1988). In this model, the vibration of the wing cells (at their resonant frequency, f_o) controls the catch and release of the plectrum from tooth to tooth in the file (Elliott and Koch, 1985; Koch et al., 1988; Prestwich et al., 2000; Bennet-Clark and Bailey, 2002). In crickets and mole crickets, one file sweep creates a single sound pulse or syllable. A song pulse is thus made of sequential tooth strikes sustained at a nearly constant rate and the catch and release of the plectrum from every file–tooth pair is produced at a more or less constant rate by the up and down vibration of the right harp and file at their f_o (Bennet-Clark and Bailey, 2002).

It has been shown, however, that cricket wings do not operate as perfect clocks, as most species exhibit frequency modulation (FM) in the pulses of their calls, which is observed as a fall in frequency or 'glissando' within the song pulse of some 10–15% of the main carrier frequency (Leroy, 1966; Koch et al., 1988; Simmons and Ritchie, 1996; Prestwich et al., 2000; Bennet-Clark and Bailey, 2002; Bennet-Clark, 2003). This problem was first pointed out by Leroy (Leroy, 1966), but was revived by Simmons and Ritchie (Simmons and Ritchie, 1996), who were the first to try to find a morphological explanation for the glissando. Bennet-Clark (Bennet-Clark, 2003), working on *Teleogryllus oceanicus*, suggested that this drop in

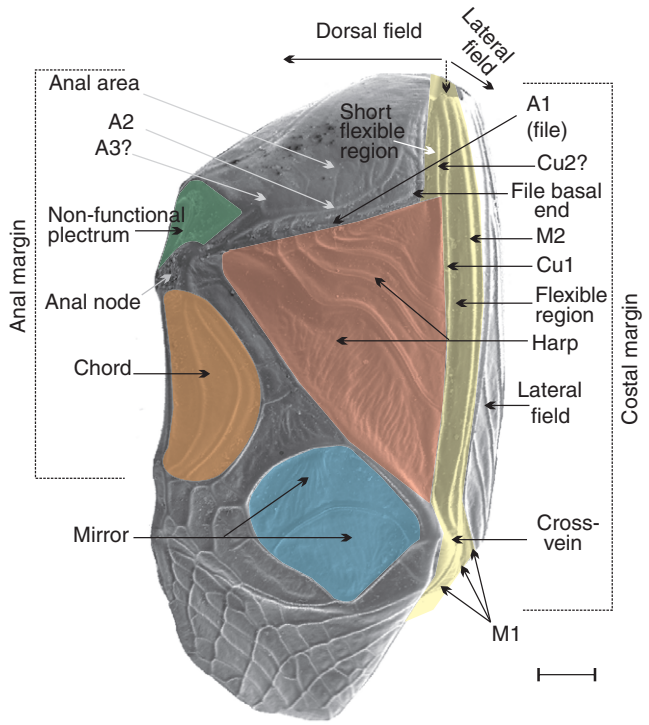

Fig. 1. Right tegmen of *Gryllus bimaculatus*, showing the main areas involved in sound production. Nomenclature of wing venation follows Desutter-Grandcolas (Desutter-Grandcolas, 2003), and wing cells follow Bennet-Clark (Bennet-Clark, 2003). M, medial veins; Cu, cubital veins; A, anal veins. Scale bar, 1 mm.

frequency might be associated with the vibrations at the basal quarter of the file, which is connected to the short flexible region (Fig. 1). At this end the file seems to exhibit lower resonances than in the rest of the file body; thus, when the plectrum traverses this critical region, its catch and release occurs at lower rate than in the anal or centre regions of the file (Bennet-Clark, 2003). But some other authors have attributed this modulation to the level of asymmetry between the two wings (Simmons and Ritchie, 1996).

Even though cricket tegmina look bilaterally symmetrical, they exhibit some degree of morphological asymmetry (Simmons and Ritchie, 1996; Bennet-Clark, 2003; Klingenberg et al., 2010), which seems to result in differences in their individual f_o (Nocke, 1971; Bennet-Clark, 2003) (this study). For example, Nocke (Nocke, 1971), working on *Acheta domesticus*, *Gryllus campestris* and *G. bimaculatus*, found the f_o of the RW to be lower than that of the LW. Bennet-Clark (Bennet-Clark, 2003), however, obtained two different results actuating wings isolated from the insect's body at two different wing areas: (1) from the lateral field [see table 4 in Bennet-Clark (Bennet-Clark, 2003)] and (2) from the plectrum [table 5 in Bennet-Clark (Bennet-Clark, 2003)]. Therefore, there seems to be no consensus on which wing exhibits a lower or higher f_o, and/or whether the observed asymmetry has an effect on the magnitude of the response. Establishing the nature of these differences is the first step for building a model. These differences in the individual resonances of the wings have been the subject of discussion (Bennet-Clark and Bailey, 2002; Bennet-Clark, 2003) because they are not manifest in the f_c of the call, which consists of a single sharp frequency peak. Although the effect of the escapement mechanism (Elliott and Koch, 1985) is to couple the wings together, and possibly smooth out differences in their f_o (see Bennet-Clark, 2003), the way left and right tegmina interact mechanically during stridulation to have such an effect and generate a tone with high spectral purity remains largely unknown. The present work addresses this problem by characterizing the resonant behaviour of two main sound-radiating regions (mirrors and harps) in both wings during stridulation.

Interestingly, in *G. campestris*, the harps of LWs are smaller than those of RWs (t=3.05, d.f.=116, P=0.003) (Simmons and Ritchie, 1996) (our unpublished data also confirm these results in *G. bimaculatus*, with LW harp surface area 5% smaller than that of the RW). On the basis of harp ablation experiments, the same authors concluded that the properties of the LW control the frequency of the first third or half of the song pulse and those of the RW control the later part of the pulse (Simmons and Ritchie, 1996). Bennet-Clark (Bennet-Clark, 2003) did not find any morphological differences in the forewings of *T. oceanicus*, although he did find differences in mass between the two wings (LW mass ~4.5% greater than that of the RW).

The mechanical interactions of the LW and RW in crickets have only been approached from an analytical perspective based on individual resonances measured from wings in isolation (e.g. Bennet-Clark, 2003). Here, using non-contact, non-invasive measurements on actively stridulating crickets (*G. bimaculatus*) and on freely vibrating wings, we document the resonance differences between LW and RW (measured from mirrors and harps), and among localized cells and regions of the RW, and determine whether the two wings differ in response amplitude. For this purpose, we first developed a new method to investigate wing vibrations from actively stridulating, intact crickets. We then tested the hypotheses: (1) that the stridulatory file (as potential regulator of the f_c) is a variable-frequency oscillator (*sensu* Bennet-Clark, 2003), and (2) that the frequency at different sections of the song pulse in crickets is controlled independently by the two wings, as suggested by Simmons and Ritchie (Simmons and Ritchie, 1996). The results presented here show that the LW exhibits a lower f_o than the RW, that the right file is a constant resonator, and that the spectral composition of the song is jointly controlled by the two wings, as they lock to generate the mechanical oscillations necessary for species-specific acoustic radiation.

MATERIALS AND METHODS

Animals

Adult male crickets (*G. bimaculatus*, de Geer), obtained from colonies maintained at the University of Bristol, were used. Teneral males were randomly chosen from the breeding colony and maintained in individual cages isolated from females. This ensured that the wings were preserved intact (the RW especially undergoes considerable damage in crowded situations, e.g. dense colonies). After a few days, males begin to stridulate, and those willing to sing for long periods were preferred for the experiments, because these animals usually responded better to pharmacological stimulation. The calling song of 65 males was recorded several times on different days for a period of 10 days. All males recorded were singing with the usual wing overlap (RW over LW). Some of these males were used in the pharmacological experiments. Spectral as well as zero-crossing (ZC) analysis was conducted on these recordings (for details on ZC see below). These analyses served to compare the song of intact animals with the song of those specimens whose calls were elicited using pharmacological brain stimulation (see below). Because crickets modulate the frequency of their calls (e.g. Leroy, 1966), we surmised that the level and form of this FM

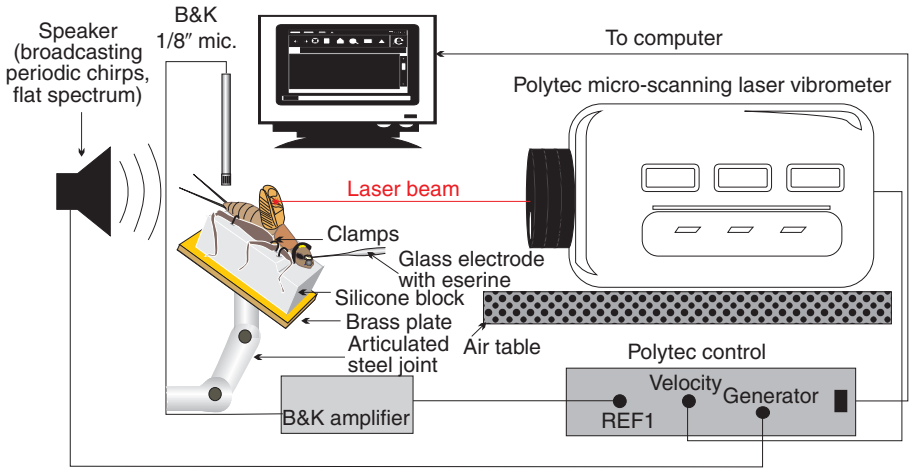

Fig. 2. Preparation used for recording wing vibrations in tethered singers (*G. bimaculatus*) stimulated with neuroactive substances. The specimen was mounted assuming a prognatous head orientation; this allowed full access to the brain, different degrees of rotation and alignment, and perpendicularity of the wing with respect to the laser beam. After the insect stopped singing, wings were extended and basally fixed with wax. The speaker was used to excite the wings with sound after the experiment and to obtain whole laser scans. Diagram not to scale. B&K, Brüel & Kjær; mic., microphone; for details, see Materials and methods.

within the pulse is constant for each individual and would therefore enable comparison of the quality of a natural call with that of a pharmacologically elicited call. Acoustic analysis was done in Matlab (version 7.8.0347, R2009a; The MathWorks Inc., Natick, MA, USA), and ZC analysis for sound recordings was done with the Zero-crossing module [Zero-crossing v.7 and detailed user manual provided by K. N. Prestwich (http://www.holycross.edu/departments/biology/kprestwi/ZC/) for Canary software (Cornell University, Laboratory of Ornithology, Ithaca, NY, USA).

Mounting and preparing the specimens for pharmacological stimulation

After the calling song of intact male *G. bimaculatus* was recorded, specimens were immobilized by cooling in a normal household fridge for 5–6 min at 6–7°C. Then their legs were gently fixed to a block of silicone or Blu-Tack using staple clamps, without causing injury (Fig. 2). The block surface was homogeneously flat except on one end, where the block became gradually thinner. The specimen's head was attached with wax to a metal clamp inserted into this end. This orientation also facilitates access to the frons, and forces the prothorax to bend downwards, giving the wing freedom to open and close in a stridulating position. The silicone block was fixed to a brass plate, which was screwed to an articulated rod that allowed rotation and tilting at different levels and angles (Fig. 2; see also supplementary material Movie 2).

Under a dissecting microscope, to expose the insect's brain, a small window was opened on the head frons by removing some of the cuticle, leaving the antennae intact. This was done using a fine sapphire scalpel (World Precision Instruments Inc., Sarasota, FL, USA). Cricket Ringer's solution [after Fielden (Fielden, 1960)] was used to avoid desiccation and to rinse excess clotting haemolymph. The holder (and the specimen) was then mounted on a micromanipulator (World Precision Instruments Inc.). Wing position and orientation with respect to the laser's optical field were adjusted, so that during stridulation the laser beam was approximately perpendicular to the wing surface.

Neuropharmacological stimulation

For the pharmacological stimulation of the neuropil in the cricket's anterior protocerebrum that harbours the dendritic arborizations of the command neuron responsible for eliciting singing behaviour (Wenzel et al., 1998), we mostly followed the preparation suggested by Wenzel and Hedwig (Wenzel and Hedwig, 1999). Here, we used borosilicate glass microcapillaries (1B120F-3; i.d. 0.68 mm; World Precision Instruments Inc.) pulled with a Sutter microelectrode puller (Sutter Instrument Company, Novato, CA, USA) to produce ~10 μm-wide tips. These microcapillaries were then filled with eserine salicylate/cricket Ringer's solution (10^{-2} mol l^{-1}, Sigma-Aldrich Company Ltd, Dorset, UK), and connected to a picospritzer (Picospritzer II, Parker Hannifin, Pneutronics Division, NJ, USA) *via* a custom-built electrode holder. This setup allowed us to administer small drops of eserine into the neuropil in the range 0.1–5 nl (depending on tip size and amount/duration of pressure applied with the picospritzer).

The electrode holder with the attached microcapillary was then mounted on another micromanipulator, allowing the experimenter to gradually move and insert the glass electrode into the protocerebrum following the locations and brain maps provided by Wenzel et al. (Wenzel et al., 1998), aiming for an area between the pedunculus and the α-lobe of the mushroom bodies. A successful procedure elicited stridulation a few seconds to minutes after injection; animals of unsuccessful trials were disposed of 1 h after the first injection. Recordings of the songs produced were obtained with the equipment described below.

We measured the quality of the calls produced with pharmacological brain stimulation by correlating the FM modulation pattern (obtained by ZC analysis) of these elicited calls with that of natural calls. As a convention, we considered elicited calls to be of sufficient quality if the correlation (Pearson's r) was higher than 0.85. We correlated the gradual frequency change (along the entire pulse) that occurred in natural and elicited calls. Following Bennet-Clark and Bailey's (Bennet-Clark and Bailey, 2002) superimposing method (see fig. 4 of their paper), we evaluated correlations between pulses of similar duration in the two treatments. As individuals modulate frequency in a fixed way (see Results), the FM pattern of longer pulses was cut to match the FM range of the shorter pulse. This procedure helped us to obtain vectors of the same length to facilitate the statistical procedure.

Recordings of wing resonances in stridulating males of *G. bimaculatus*

When a mounted specimen began to stridulate, different types of calls could be produced (Wenzel and Hedwig, 1999), so we waited a few minutes until the typical calling song pattern was totally adopted. Wing vibrations were measured with a laser Doppler vibrometer (Polytec PSV-300-F; Polytec GmBH, Waldbronn, Germany) with an OFV-056 scanning head fitted with a close-up attachment. The laser recordings were performed in the single-shot mode. With this method, a single vibration recording is obtained from a chosen location. The measuring event can be set to be a one

shot event, or the sequential and linear averaging of several measurements at one location. Acoustic and vibrational measurements were all recorded simultaneously with Polytec scanning vibrometer software (version 7.4, Polytec GmBH, Waldbronn, Germany). Sound recordings were obtained using a 1/8 inch condenser microphone Brüel & Kjaer Type 4138, connected to a Brüel & Kjaer 2633 preamplifier (Brüel & Kjaer, Nærum, Denmark). The microphone was positioned posterior to the specimen, 5 cm away from the wings, so that it would not interfere with the radiating sound field (see Fig. 2). Simultaneously, wing vibrations were recorded with the laser vibrometer focused on the harp and mirror, recording first the output of the wing on top (RW) and then that of the plectrum-bearing wing (LW). The LW is not exposed completely during stridulation, yet in *G. bimaculatus* the two wings are separated enough from each other to focus the laser beam on both harps. The laser spot position was controlled by galvo-actuators and monitored *via* a live video feed to the vibrometer's controlling computer. Thus, the laser beam could be positioned on any region of interest of either wing to capture its vibrations during sound radiation. Time data were acquired at a rate of 512,000 samples s^{-1}, with a time resolution of 1.953 µs. Fast Fourier transform (FFT) analysis was done simultaneously in other analyser windows.

To obtain precise simultaneous recordings of sound and wing vibration, the microphone signal was used as trigger, using the amplitude component of a pulse (Figs 2 and 3). This guarantees that only the wing vibrations involved in sound production were recorded. For the purpose of this paper, the system was programmed to record typically 2 ms of a song pulse during the maximum amplitude event, which corresponds to about 0.4 mm of wing displacement. Longer or shorter time events were also recorded by adjusting the magnitude of the trigger value. The laser beam was pointed to the harp and mirror regions that exhibit maximum deflection in response to sound (Nocke, 1971; Bennet-Clark, 2003).

Individual resonances and free vibration of unengaged wings measured from wings in motion

This procedure is crucial for studying the individual tuning of both wings when they are not in contact with each other. The results from these free vibration scans can be compared with the individual tuning of engaged wings during stridulatory acoustic radiation. Stridulation in Ensifera encompasses two events: an opening stroke, which in several species (including *G. bimaculatus*) is usually silent (see Fig. 3 and supplementary material Movie 1), and a closing stroke, where the main amplitude components of the sound are produced (Fig. 3). Instead of triggering the system to simultaneously record sound and wing vibrations produced during the closing stroke, data acquisition can be programmed to record the vibrations that occur during the silent phase (opening stroke) in response to external acoustic stimulation. Here we used the decaying part of the pulse as a trigger and recorded the subsequent silent part (Fig. 3). A loudspeaker (ESS AMT-1; ESS Laboratory Inc., Sacramento, CA, USA) mounted 10 cm behind the singing insect (Fig. 2) was used to broadcast periodic chirps (1–20 kHz, flat spectrum 55 dB SPL ±1.5 dB at the cricket's wing). Periodic chirps were generated by the PSV 300 internal data acquisition board (National Instruments PCI-4451; Austin, TX, USA), amplified with a Sony Amplifier (Model TAFE570; Tokyo, Japan) and passed on to the loudspeaker via a step attenuator. SPL was measured using the same 1/8 inch precision pressure microphone as in the previous experiment.

When the animal was singing, the loudspeaker continuously played chirps, and during the opening stroke (i.e. when the wings were not engaged) the wings were vibrating only in response to the sound played. These vibrations can be taken as a reliable free vibration measurement because the wings are in their natural singing position and they do not radiate sound during the silent opening stoke. This method yields reliable and realistic wing vibration data. Data were recorded in the single-shot mode, capturing 2 ms of vibration and sound during the silent interval that occurs ~10–12 ms before the first oscillations of the actual sound pulse (Fig. 3). From previous recordings of sound and wing motion using a sensitive motion detector (Hedwig, 2000) and high-speed video (see supplementary material Movie 1), we estimated with high accuracy a fragment of the silent interval that does not contain vibrations of the free decay of the previous pulse and which guarantees that the wings are uncoupled at maximum separation (Fig. 3).

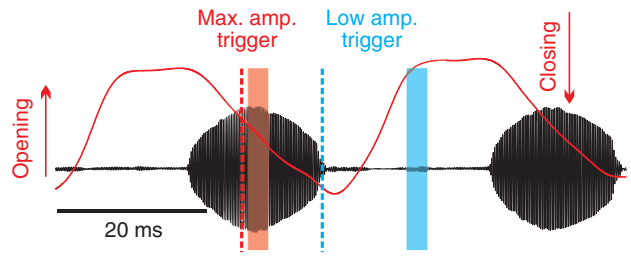

Fig. 3. Triggering system used for wing vibration recordings in actively stridulating insects. Two typical sound pulses of *G. bimaculatus*, and the associated wing movements (red outline), obtained with a motion detector, are shown to indicate the two trigger systems used. Microphone signals were used to trigger the recordings. In the first case (red rectangle) the maximum level of pulse amplitude (closing stroke) was used to trigger recordings of 2 ms (1 ms before and 1 ms after maximum amplitude of the pulse). In the second case (blue rectangle), the decaying amplitude of the last oscillations of a pulse were used to trigger a recording (same duration as previous) of the following silent opening stroke at maximum amplitude of the wings (usually 12 ms before the start of a pulse, at 23.5°C). This trigger was used to record free wing vibration in response to sound, while the animal had its wings in a singing position but disengaged.

Individual resonances of unengaged fixed wings (free vibration)

After concluding the previous experiments, the wings of each specimen were carefully extended and separated from each other by fixing their axillary sclerites with a mix (0.5:0.5) of bee's wax (W/0200/50; Fisher Scientific UK Ltd, Loughborough, Leics, UK) and Colophony (60895-250G; Sigma-Aldrich Co.). The wings were extended in such a way that they were out of contact from the pronotal lateral and posterior edges, for which a correct bending of the prothorax when mounting the specimen is crucial. The specimen and loudspeaker were maintained in the same position as in the previous experiment. The loudspeaker was used to broadcast periodic chirps in the same frequency range as before (1–20 kHz, flat spectrum, 55 dB SPL ±1.5 dB at the insect's wings). The microphone was placed dorsally in the middle of both extended wings (Fig. 4). The laser system was set to record in scan mode. A complete scan of the extended wings in response to the periodic chirps was performed with the micro-scanning laser vibrometer, using 250–300 scanning points, averaging 10 times each point. For each point a frequency spectrum was generated using a FFT with a rectangular window, at a sampling rate of 512,000 samples s^{-1}, 64 ms sampling time, and a frequency

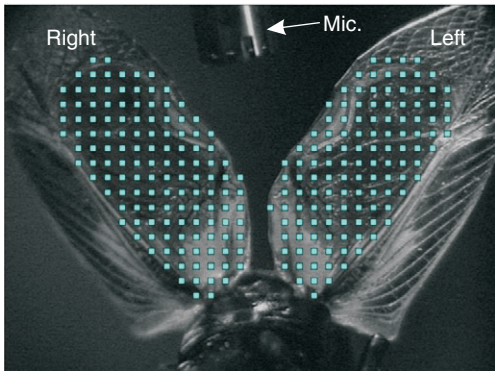

Fig. 4. Capture of the video image from the laser Doppler vibrometer illustrating the extended wings (axillary sclerites fixed with wax) in frontal view during measurements and the lattice of laser scanning points (*N*=260 points; mesh size, 170 μm; dot positioning accuracy, ~1 μm). The condenser microphone (Mic.) was positioned on top in the middle of the wings. This setup was used to record the vibration of extended wings in a fixed position, while stimulated with sound.

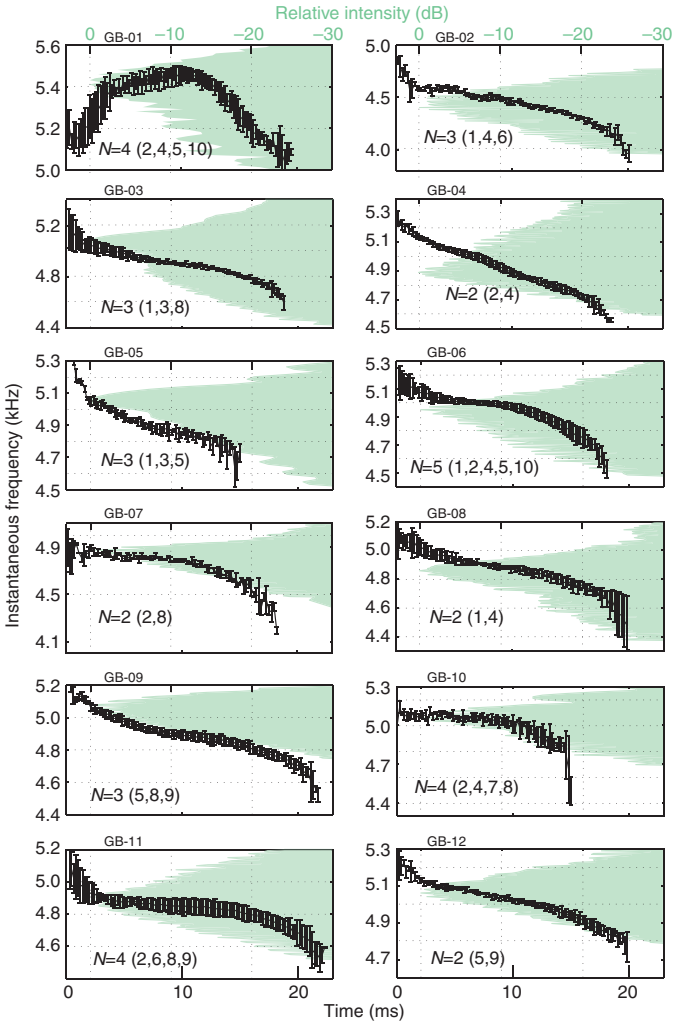

Fig. 5. Frequency modulation plots recorded from 12 specimens of *G. bimaculatus*. Each box represents a different specimen. Traces within a box are the mean instantaneous frequencies of single pulses picked randomly from recordings of the same specimen obtained on different days within a 10 day span. Error bars indicate standard deviation from the mean. Note that each specimen modulates its call in a particular way. Lateral ghost plots depict the spectrum of the call of each specimen, recorded on the last day. *N*=number of recordings, numbers in parentheses indicate the days on which recordings were made.

resolution of 15.63 Hz. This final experiment allowed comparison of the vibrations of the engaged wings with their free resonances. These recordings of 'free' vibration of extended wings were compared with those obtained from free vibration scans of unengaged stridulating wings to evaluate the validity of the two methods.

Wing vibration produced by stridulation (eserine-elicited stridulation) was successfully obtained in a total of 13 field crickets. Free wing vibration in response to sound stimulus during the opening stroke was successfully obtained in only five of these specimens. The wings of all 13 specimens were extended, fixed with wax, and successfully scanned in response to sound. To produce a more conservative analysis, the results of 'free' vibration of extended wings were reinforced with recordings obtained from 31 additional specimens, for which only fixed wing vibration was recorded. These specimens were not treated with eserine, but their natural calls and scans of fixed wings were recorded in the same way as for the 13 specimens mentioned above.

The quality factor Q is a dimensionless index that indicates the sharpness of the resonance: the higher the Q, the sharper the resonance [for details of calculation see Bennet-Clark (Bennet-Clark, 1999b)]. Here, Q was calculated as the ratio of the frequency of the peak response divided by the spectral width at the two points above and below f_o with amplitudes of 0.707 times the peak value (equalling 3 dB below peak amplitude) (Fletcher, 1992). We thus calculated Q for the wing resonances in response to acoustic stimulation (here termed Q_{free}) and also for the resonances resulting from active stridulation (Q_{locked}). Q was also measured for the calls of all specimens studied (Q_{call}).

All experiments were carried out at room temperature (23.8±0.7°C). Statistical analysis performed on engaged wings involved comparison of means with the Wilcoxon test, and data are presented as means ± s.d. or s.e. Mean wing f_o in unengaged wings (free vibration) was compared with a paired-sample *t*-test; data are presented as means ± s.d. or s.e. Means of local resonances in individual wings were compared using Kendall statistics for related samples. Interactions of wing resonances and other measured parameters were studied by classical linear regression. Statistics were done using SPSS 16.0.1 for Windows (IBM Corporation, New York, NY, USA) and Matlab (version 7.8.0347, R2009a; The MathWorks Inc.).

RESULTS

Calling songs of individual crickets recorded on different days

Sound recordings obtained on different days from 65 virgin *G. bimaculatus* showed that the pattern of FM is constant for every specimen (Fig. 5). Over a period of 10 days, the way an individual modulated the frequency of its pulses is highly constant. Although some specimens might show minor variation from call to call, there was always a typical pattern for every specimen. This suggests that FM is related to the intrinsic mechanical properties of the wings of each individual rather than being behaviourally controlled. These

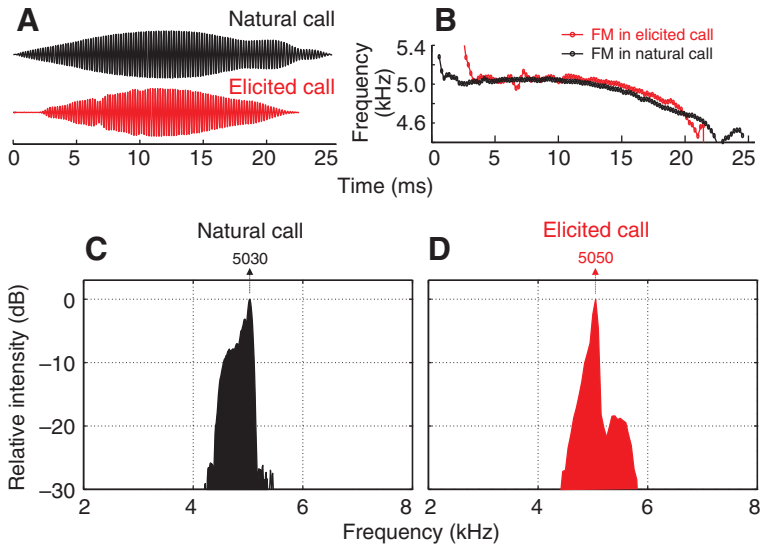

Fig. 6. Comparison of calls before and after using pharmacological brain stimulation in one male *G. bimaculatus*. (A) Oscillograms of two pulses randomly selected from calls recorded before and after eserine injection into the brain. (B) Zero-crossing (ZC) analysis of these pulses. (C,D) Fast Fourier transform (FFT) analysis of natural and elicited calls (two chirps in each case) recorded from the same insect.

results also imply that the FM pattern can be used to evaluate the call of pharmacologically stimulated males.

Pharmacologically elicited stridulation *vs* natural stridulation

Of major concern when using pharmacological stimulation to elicit singing behaviour is the question of whether elicited stridulation is different from that of naturally singing animals. One way to verify the validity of this method and its potential effect on the frequency and time components of single pulses is to compare natural pulses with pharmacologically elicited ones by ZC analysis. As a control and for investigation of the general features of the calling song, the calling song of several specimens was recorded several times before the experiments (as explained above, Fig. 5). From 33 trials, elicited stridulation took place in 13 males.

The parameters that helped identify calling song pulses in this study were duration, envelope and FM pattern. Pharmacological stimulation can elicit both calling and/or courtship songs (Wenzel and Hedwig, 1999), yet calling songs are longer and more stable, and therefore of more interest for the analysis of wing mechanics. In both cases, the syllable repetition rate might be altered in some individuals, but this potential variability of the inter-pulse interval, though important for species-specific recognition and courtship success, is not relevant to the present study.

Table 1. Correlation of instantaneous frequencies between natural and elicted calls in 13 specimens

	Spearman's *r* (2-tailed)	*P*
Specimen 1	0.962	<0.0001
Specimen 2	0.894	<0.0001
Specimen 3	0.972	<0.0001
Specimen 4	0.860	<0.0011
Specimen 5	0.956	<0.0001
Specimen 6	0.962	<0.0001
Specimen 7	0.935	<0.0001
Specimen 8	0.810	<0.0017
Specimen 9	0.908	<0.0001
Specimen 10	0.830	<0.0013
Specimen 11	0.879	<0.0001
Specimen 12	0.899	<0.0001
Specimen 13	0.889	<0.0001

Natural and elicited call variables were set to equal lengths for the purposes of analysis.

Conducting ZC analysis on both recordings (natural and elicited songs) showed in all cases that the instantaneous frequency pattern of all individuals was preserved during pharmacological stimulation. Both the temporal and instantaneous frequency components of individual pulses were unchanged after pharmacological stimulation (Fig. 6). Therefore, every single individual (N=13) delivered its call in the same way before and after the eserine treatment. The correlation of the instantaneous frequencies of both elicited and natural pulses was in all cases higher than 80% (Table 1).

Individual resonances and free vibration of unengaged wings measured from wings in motion

For five (of the 13) stridulating crickets, a loudspeaker playing broadband chirps was placed behind the wings (Fig. 2). The free vibration in response to this acoustic stimulus was recorded from the harps during the silent phase of the stridulatory movement (the opening stroke). These experiments show that the free f_o of each harp is different, being higher for the RW (Table 2A; Fig. 7C).

Recordings of mirror vibration in the same specimens, obtained in the same way, show that there is no significant difference in the mirror f_o. However, the left mirror f_o was, on average, higher than that of the right mirror. These results agree with those obtained from extended fixed wings, stimulated with sound (Table 2D; Fig. 7D, see below).

Individual resonances of unengaged fixed wings (free vibration)

The recordings of five *G. bimaculatus* with their wings fixed with wax (after elicited stridulation ceased) show that the harps differ in their free f_o, with f_o for the RW being significantly higher than for the LW (Table 2A). These results are not statistically different from those obtained from the free vibration of unengaged wings (Fig. 7C,D; Table 2A). When data from all 13 specimens used in the elicited stridulation experiments were pooled for the analysis of harp f_o with wings basally fixed, the results were consistent: the left harp exhibits a lower f_o (Table 2B). To produce a more powerful analysis, the wings of 31 additional males for which only the calling song was previously recorded were similarly extended by fixing the axillary sclerites with wax. The analysis of harp f_o on all 44 specimen showed results consistent with those obtained from the free vibration in the 13 specimens with extended fixed wings, and in the five specimens actively stridulating (Table 2B). The RW exhibited a

Table 2. Statistics for wing resonant vibration measured with laser vibrometry on coupled and uncoupled wings

	Mean f_o (kHz)	s.d.	s.e.	*t*-test	d.f.	*P*
A. Harp f_o, in response to sound						
Stridulating, wings uncoupled; *N*=5						
RW	5.189	0.238	0.107	–3.798	4	0.015
LW	4.900	0.298	0.133			
Wings fixed with wax; *N*=5						
RW	5.188	0.243	0.109	–3.053	4	0.038
LW	4.941	0.288	0.129			
Comparison of the two methods used for testing wing f_o						
RW_{fixed} *vs* RW_{free}				–0.994	4	0.376
LW_{fixed} *vs* LW_{free}				0.057	4	0.957
B. Harp f_o in response to sound						
Wings fixed; *N*=13						
RW	5.232	0.455	0.126	–2.733	12	0.018
LW	4.873	0.369	0.102			
Wings fixed; *N*=44						
RW	5.163	0.467	0.070	–2.897	43	0.006
LW	4.998	0.378	0.057			
C. Free harp f_o *vs* f_c; *N*=44						
RW_{f_o} *vs* f_c	5.163	0.467	0.126	3.261	43	0.002
Call f_c	4.995	0.238	0.036	–	–	–
LW_{f_o} *vs* f_c	4.998	0.378	0.102	–0.157	43	0.876
D. Free mirror f_o						
Stridulating, wings uncoupled; *N*=5						
RW_{mirror}	5.094	0.274	0.123	1.207	4	0.294
LW_{mirror}	5.550	0.701	0.131			
Wings fixed; *N*=44						
RW_{mirror}	5.761	0.955	0.265	1.351	43	0.202
LW_{mirror}	6.332	1.223	0.339			

Paired *t*-test was used where applicable.
RW, right wing; LW, left wing; f_o, resonant frequency; f_c, carrier frequency.

significantly higher f_o than the LW (Fig. 8A). Variance of wing resonance was higher for the RW (0.218) than for the LW (0.143). These data suggest that either method used to obtain free wing resonance under acoustic stimulation is reliable.

The left mirror f_o was found to be ~26% higher than the left harp f_o, while the right mirror f_o was only 10% higher than the f_o of the right harp (Table 2; Fig. 8B). The mirror resonates with lower amplitude when vibrating at the harp's f_o, and in this case the difference in resonance between left and right mirrors was usually similar to that between the harps (LW_{mirror}<RW_{mirror}). Mirror f_o measured from fixed wings in all 44 specimens did not show statistical differences between wings, but the f_o of the left mirror was usually higher than that of the right mirror. The left mirror exhibited a greater variance in f_o (1.495 kHz) than did the right mirror (0.912 kHz) (Table 2D).

From the free resonances measured in all 44 specimens, the *Q* factor was calculated (here termed Q_{free}). The *Q* values measured are within the range of those measured from isolated tegmina and from calls in other species of crickets (Bennet-Clark, 1970; Nocke, 1971; Bennet-Clark, 1989; Prestwich et al., 2000; Bennet-Clark and Bailey, 2002; Bennet-Clark, 2003). There were no significant differences in the Q_{free} between the LW and RW (Table 3). From 44 animals measured, 50% (22) exhibited higher Q_{free} in their LW than in their RW, the remaining half showed RWs with Q_{free} higher than that of the LW. From this sample, only 3 specimens exhibited similar Q_{free} values for the two wings (difference <1). The mean Q_{free} of individual wings was also compared with Q_{call}. The mean Q_{free} for the LW was significantly higher than Q_{call}, while mean Q_{free} of the RW was statistically similar to Q_{call}. Lower variance was observed in the Q_{call} compared with the Q_{free} of both wings (Table 3).

To evaluate whether the f_c of the calling song is associated with the f_o of a particular wing, the mean f_o values of the harps of both wings in all 44 specimens were compared with the mean f_c of the respective calls. This analysis showed that the f_c of the call is statistically identical to the LW harp f_o, but different to the RW harp f_o (Table 2C; Fig. 7). Nevertheless the f_o of both wings is associated with the call f_c (Fig. 9); this implies that f_c increases proportionally with f_o although the values are not necessarily identical.

These measurements in extended wings also suggest that the two wings respond with similar amplitudes at their respective f_o (LW=163.14±85.51 nm Pa^{-1}, RW=154.40±89.45 nm Pa^{-1}; *t*=0.887, d.f.=43, *P*=0.380), although there was a tendency for the left harp to vibrate with higher amplitude than the right harp (see Fig. 8A, Figs 10 and 11, and supplementary material Movies 3 and 4). From 44 study cases, 25 (~55%) animals had a louder LW, and the rest (19, 45%) exhibited a louder RW, at their respective f_o. Notably, at the f_o of the LW (the frequency that determines the call), the LW harp vibrated with higher amplitude than the RW harp by 1.6- to 2.0-fold in all cases (Fig. 10; see also supplementary material Movie 3). This implies that the LW plays a major role in the control of sound radiation and in the control of the call f_c, and that the acoustic inertance of the two wings is different.

In addition, it is apparent that the stridulatory file and other adjacent areas of the wings are resonators contributing mechanical oscillations at the f_o generated by the harp. Vibrations of the RW stridulatory file were measured in response to acoustic stimulation in four different locations (Fig. 12A). The resonant properties of the file did not in themselves support any frequency change or FM pattern. All regions measured resonated at one identical frequency. The file f_o was also identical to that of the harp and the adjacent

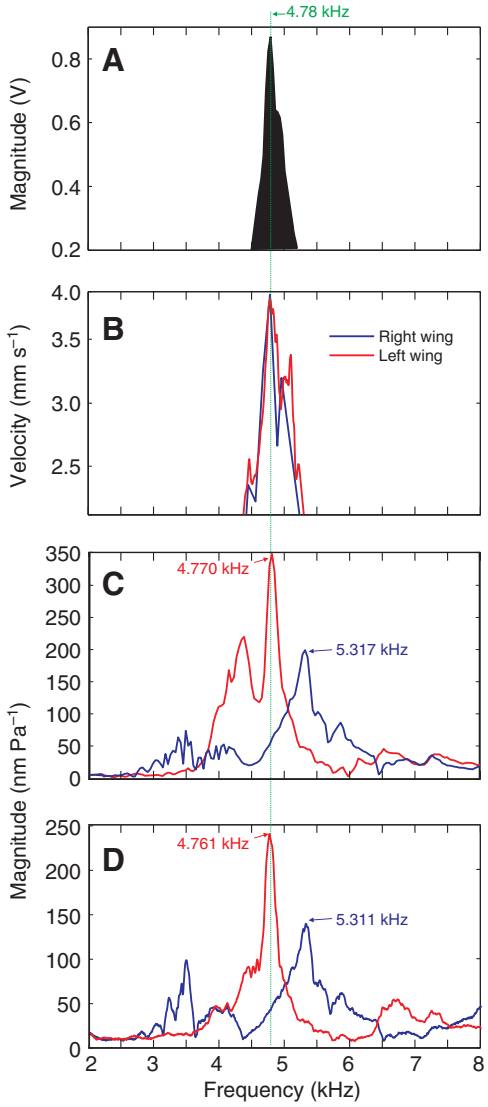

Fig. 7. Mechanically coupled resonance and free resonance of the forewings of *G. bimaculatus*. (A) Spectrum of the natural calling song recorded during the experiment. (B) Wing vibration measured from the harps of both wings during stridulation using micro-scanning laser vibrometry. Spectra were normalized to the maximum value of the left wing (LW), for comparative purposes. (C) Free resonance of both wings in response to acoustic stimulation during the opening phase of the wing during stridulation. (D) Resonance of the wings in response to acoustic stimulation measured with both wings extended with axillary sclerites fixed with bee's wax. Note that in C and D the response is similar; the LW resonates at lower frequency than the right wing (RW); the magnitude of the response in both recordings is higher in the LW.

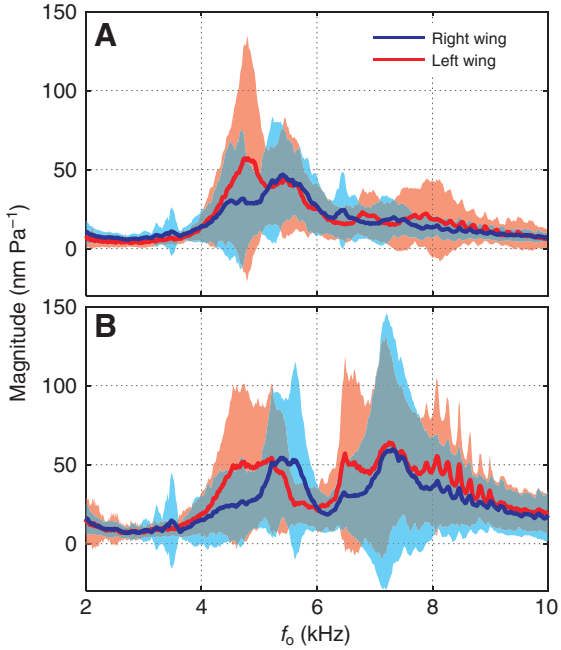

Fig. 8. Resonances of free wing vibration in response to acoustic stimulation. (A) Mean resonance frequency (f_o) of the harps of 44 specimens. (B) Mean f_o of the mirrors of the same 44 animals. Blue and red lines show the RW and LW mean, respectively. Shaded areas indicate standard deviation in both cases.

short flexible region (Fig. 12E). The maximum amplitude of deflection of the file was observed on the basal half and not at the centre (file area 3 in Fig. 12A,B,D). However, this asymmetrical deflection pattern does not completely correspond to the pattern observed in the sound envelope (Fig. 12B). We note here that the deflection shape observed in the left file was more symmetrical, reaching maximum amplitude towards the centre (see supplementary material Movie 2). The right file and harp vibrate in conjunction in a cantilever manner, with the pivot point located close to the plectrum (Fig. 12B; see also supplementary material Movie 2). Therefore, the right plectrum vibrates with opposite phase to that of the combination of file and harp in the LW (Montealegre-Z et al., 2009). These findings highlight the role of the right harp, file and anal veins in sound radiation from the RW, and support prior work by Bennet-Clark (Bennet-Clark, 2003) with regard to other wing areas involved in sound radiation, but not his findings of the variable file resonances observed in *T. oceanicus*.

Recordings of wing resonances in stridulating males of *G. bimaculatus* (wings coupled)

The f_o of the wings of 13 pharmacologically stimulated, singing animals were recorded during sound production (closing stroke) using laser vibrometry at the time of maximum pulse amplitude. This experiment consistently showed that there was no difference in the f_o of the left and right harps in stridulating animals (LW_{harp}=4.985±0.312 kHz; RW_{harp}=4.982±0.315 kHz; Wilcoxon, Z=–1.219, P=0.223; Fig. 7B, Fig. 11B). In addition, the f_o of the left and right mirrors were also not statistically different (LW_{mirror}=4.987±0.313 kHz; RW_{mirror}=4.989±0.311 kHz; Wilcoxon, Z=–0.298, P=0.765). The f_o of all four cells are thus undistinguishable from the carrier frequency of the calling song. Similarly, no apparent differences between the Q_{free} and Q_{locked} of the wings were found in the 13 specimens studied with this method (Table 3).

A further question is whether wings engaged in stridulation also display differences in the magnitude of their mechanical response, as seen for unengaged wings stimulated by sound. From 13 stridulating animals recorded, 11 showed a larger amplitude response in the LW than in the RW (Fig. 7C; Fig. 11B).

During these experiments, time domain recordings were also obtained from the last 4 ms of a pulse in 10 specimens (Fig. 13). This was done to evaluate the FM content in the vibration pattern of the mirrors and harps. In these last 4 ms of the pulse, the plectrum sweeps approximately 20 teeth, which are located in the basal region

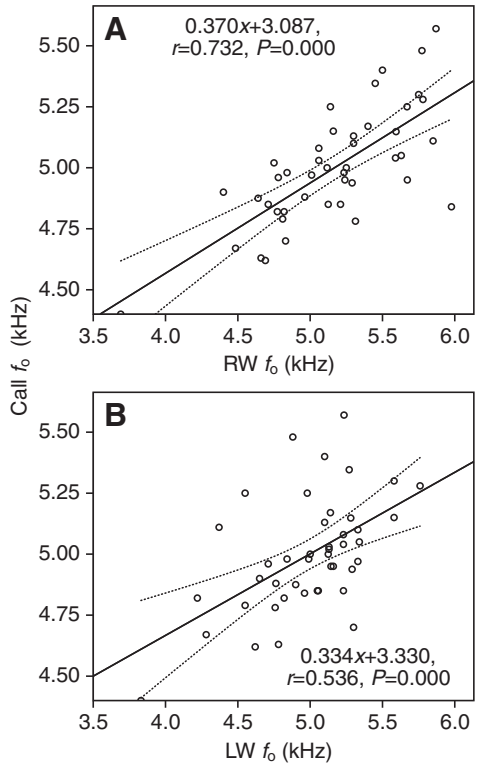

Fig. 9. Correlations of resonance in the forewings of *G. bimaculatus*. Carrier frequency of the call (f_c) as function of wing f_o for RW (A) and LW (B). Dotted lines represent 95% confidence intervals.

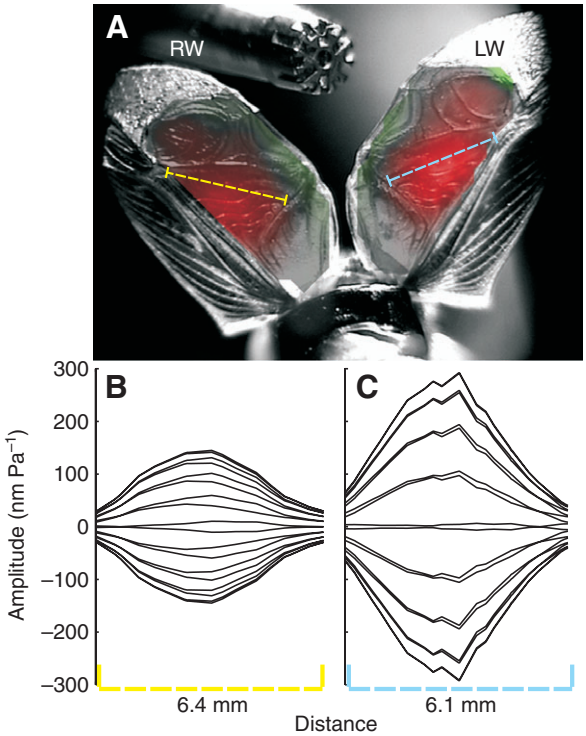

Fig. 10. Amplitude response of the wings to acoustic stimulation. (A) Picture of the wings extended, illustrating the sections through which the deflections were built. (B,C) Envelope of mechanical deflections along the transects shown in A for a series of phases in the full oscillation cycle (for this specimen, the resonance of the LW was 5.125 kHz,). B, RW; C, LW.

of the file (see supplementary material Movie 1). The distance travelled by the plectrum is ~0.6–0.7 mm, a short distance that guarantees the laser beam is scanning within the cell of interest. ZC analysis performed on these vibrations and on the corresponding microphone signal shows that the instantaneous frequency of the pulse is covariant with sound and wing vibration recorded from the mirrors and harps (Fig. 13). The difference in the instantaneous frequency between LW and RW was very small, within ±100 Hz (Fig. 13E,F).

DISCUSSION

We have developed a non-invasive method that allows the recording of wing f_o in actively stridulating, tethered crickets, from either coupled or uncoupled intact wings (Figs 2 and 4). This method enables the quantification of local f_o from different wing regions in a wide range of controllable mechanical contexts. For uncoupled wings, two methods were used: (1) free f_o in self-stridulating wings (uncoupled during the opening stroke, Fig. 2 and supplementary material Movie 2) and (2) f_o measured from extended wings with axillary sclerites fixed with bee's wax (Fig. 4). The results show that both methods are suitable and generate reproducible spectral and time domain data (Fig. 7C,D).

Nocke (Nocke, 1971) and Bennet-Clark (Bennet-Clark, 2003) measured wing resonances in cricket wings using different approaches. Nocke (Nocke, 1971), using sound stimulation and a

Table 3. Measurements of the quality factor *Q* in freely vibrating and engaged wings

	Mean *Q*	Variance	s.d.	s.e.	*t*-test	d.f.	*P*
Q_{free} calculations and comparisons across 44 specimens							
RW	18.4	72.6	8.5	1.3	1.445	43	0.156
LW	21.2	65.0	8.1	1.2			
RW *vs* call	–	–	–	–	–0.929	43	0.358
Call	17.1	12.3	3.5	0.53	–	–	–
LW *vs* call	–	–	–	–	–3050	43	0.004
Q measured from free and engaged wings in 13 specimens							
Q_{free} RW	16.3	78.2	8.8	2.5	1.459	12	0.170
Q_{locked} RW	11.8	38.6	6.2	1.7			
Q_{free} LW	21.7	46.8	6.8	1.9	1.465	12	0.169
Q_{locked} LW	17.9	87.2	9.3	2.6			

Q values are compared between LW and RW, and between wings and call, in all animals studied (*N*=44) using a paired *t*-test. *Q*-values measured from elicited calls in wings engaged and disengaged (*N*=13) are also compared.

capacitive electrode to determine resonances in isolated wings in three species of crickets, found the f_o of the RW to be lower than that of the LW. Bennet-Clark (Bennet-Clark, 2003), in contrast, working with *T. oceanicus* mechanically stimulated wings isolated from the insect's body from two different areas: (1) the lateral field and (2) the plectrum [see tables 4 and 5, respectively, in Bennet-Clark (Bennet-Clark, 2003)]. In the former case, the results agree with those of Nocke (Nocke, 1971) but in the latter case the results are similar to ours (RW f_o>LW f_o). However, in both papers, LW f_o was reported to be closer to the f_c of the calling song, as our results suggest. From data taken from table 3 in Nocke (Nocke, 1971), there were no statistical differences in f_o between the harps of the LW and RW, but the LW harp exhibited a slightly (1.5%) higher f_o than the RW. Only Bennet-Clark (Bennet-Clark, 2003) reported differences between the f_o of the two wings, with f_o being higher for the LW than for the RW. From the work of these two authors, it was already known that the mirror cell resonates with higher f_o than the rest of the wing (not harmonically related to the main wing f_o). We confirm these observations, and document the variation across a larger population (Fig. 8).

Bennet-Clark (Bennet-Clark, 2003) obtained different results by actuating the wings on the lateral field (LW f_o>RW f_o) and on the plectrum (as stated above). He attributed more relevance to the data derived from lateral field actuation as these showed f_o and Q values significantly different from the f_o of free resonances of the RW and from the f_c of song pulses. This procedure also implied that the lateral field is isolated from the dorsal field radiating the sound; thus, the actuation device is less likely to alter the effective mass and/or stiffness of the dorsal field. However, our findings derived from the free vibration of the wings only support Bennet-Clark's (Bennet-Clark, 2003) results obtained when actuating the wing from the plectrum.

The f_o of an ideal (undamped) resonant system (Bennet-Clark, 1989; Bennet-Clark, 1995; Bennet-Clark, 2003) in which a mass and a spring interact, is given, in its simplest form, by:

$$f_o = \frac{1}{2\pi}\sqrt{\frac{k}{m}}\ , \qquad (1)$$

where k is stiffness and m is mass. Therefore, a decrease in f_o implies that the mass of the system has increased and/or that its stiffness has decreased. If only one of these variables (mass or stiffness) has changed, the factor by which it has done so can be calculated from Eqn 1.

One of the major indicators of asymmetry found in *G. campestris* by Simmons and Ritche (Simmons and Ritche, 1996) was in the areas of the two harps, with the right harp (the wing on top) having a greater area than the left harp. (These differences have been corroborated by us in *G. bimaculatus* – material in preparation.) If the size of the radiator (the harp in crickets) is linked to its f_o (Nocke, 1971; Bennet-Clark, 1998; Montealegre-Z, 2009), one would expect the right harp with a larger surface area to exhibit lower f_o. But our measurements show the opposite; the right harp resonates at a higher f_o (see Table 2). According to Eqn 1, one would expect f_o to decrease with increasing mass/area. Therefore RWs should, on average, show lower f_o than LWs, but this is not the case here (see Table 2). Eqn 1 also shows that an increase in f_o can be related to a rise in stiffness. Stiffness, relating to Eqn 2 and assuming constant force (**F**), is inversely proportional to deflection (δ):

$$k = \frac{\mathbf{F}}{\delta}\ . \qquad (2)$$

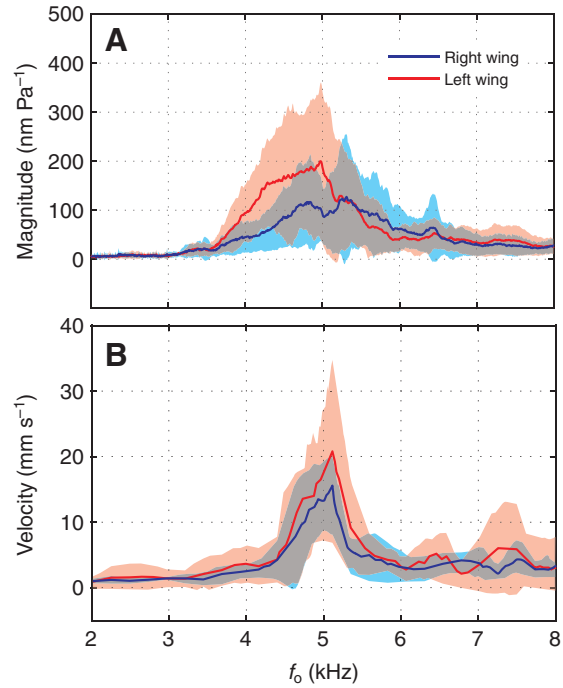

Fig. 11. Wing resonances measured from free and engaged wings. (A) Mean f_o of the harps of 13 specimens with wings extended, stimulated with sound. (B) Mean f_o measured from the harps of the same 13 stridulating specimens. Blue and red lines show the RW and LW mean, respectively. Shaded areas indicate standard deviation in both cases.

Hence, as our data consistently show lower values of deflection in the RW harps than in the LW harps while stimulated with the same force (Fig. 10), we assume the observed rise of f_o in the RW harp to be caused by stiffness.

We found that the LW exhibits the lowest f_o, which equals the f_c of the calling song, while the f_o of the RW is 5% greater than the f_c. The statistical analyses (Table 2) suggest that during engagement, f_o of the LW changes very little or not at all; therefore, most of the change in f_o seems to occur in the RW. From Eqn 1, it is conceivable that the effective f_o of the RW is achieved by adding load during plectrum and file engagement (most likely increasing the effective mass and stiffness), yet decreasing its f_o by nearly 5%. The statistical analysis of the wing resonances in response to sound suggests that the LW dictates the main components of the call f_c. In fact, the LW harp vibrates with higher amplitude in response to sound than does the RW harp (when both freely vibrate at the LW f_o, or during stridulation). Consequently, Eqn 1 is not sufficient to explain the immediate vanishing of the higher resonances of the mirror when stridulation takes place. This points to the necessity of describing the behaviour of such a resonant system beyond that of a simple spring and mass, including the dynamic visco-elastic properties of the respective coupled resonators.

Localized resonances around the stridulatory file

One of the main objectives of Bennet-Clark (Bennet-Clark, 2003) was to account for the FM or glissando effect that universally occurs within the cricket pulses. This FM has been the subject of interest because if the purity of the cricket song is explained by an escapement that regulates the catch and release of the plectrum–teeth interaction, at a specific frequency along the file,

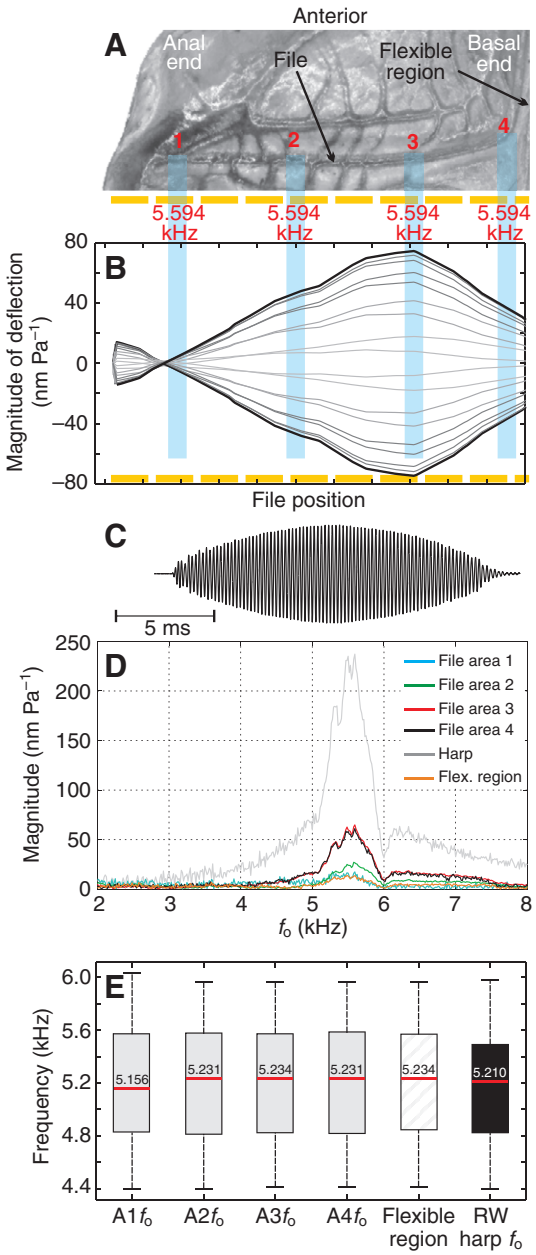

Fig. 12. Vibration of the stridulatory file. (A) Picture of a RW segment including the stridulatory file and plectrum. Numbers 1–4 indicate the different file locations monitored with the laser vibrometer during acoustic stimulation. The RWs of 44 specimens were stimulated with sound and the vibrations at file regions 1–4 recorded (values in red are measurements from one specimen). (B) Amplitude of vibration of the file in the same specimen (shown as the transfer function between file displacement and sound pressure) at the f_o of the RW of this specimen (~5.9 kHz). Yellow transect lines indicate the equivalent file position between picture and chart. Blue rectangles connect the file regions 1–4 in the picture with the respective envelope of deflection. (C) Envelope of a sound pulse produced by the same specimen for comparison with the file deflection shape. (D) Resonances recorded at the file regions, harp and short flexible region. (E) Mean resonances of 44 specimens of the same wing areas as depicted in D (Kendall's W for related samples: W=0.37, d.f.=5, χ^2=8.054, P=0.154). A, file area.

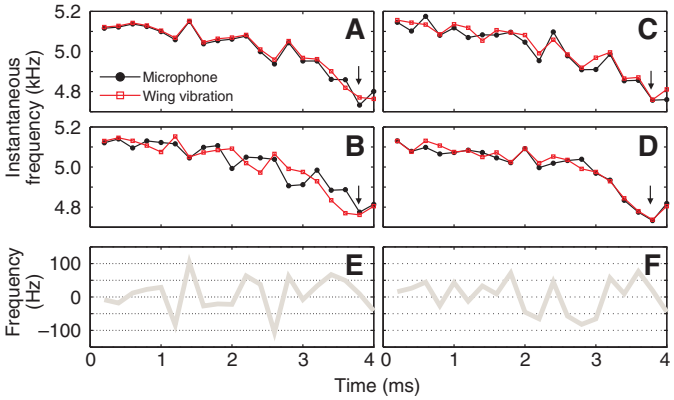

Fig. 13. Frequency modulation (FM) obtained by ZC analysis on sound (black circles) and wing vibration (red squares) recorded simultaneously from one specimen of *G. bimaculatus* (call elicited with eserine). (A,B) Cycle-by-cycle frequency analysis of recordings obtained from the left and right harps, respectively. (C,D) Cycle-by-cycle frequency analysis of recordings obtained from the left and right mirrors, respectively. (E) ZC difference between the instantaneous frequencies measured from the left and right harps. (F) ZC difference between the instantaneous frequencies measured from the left and right mirrors. In all four events shown in A–D, the data acquisition system was programmed to record the last 4 ms of wing closure. Every event was obtained from a different pulse. Note that FM occurs within the same levels in all wing regions recorded. Arrows in A–D point to a potential plectrum–file disengagement event.

why should this frequency begin to drop gradually during the pulse? This, of course, suggests that the escapement is not perfect, as pointed out by several authors (Koch et al., 1988; Prestwich et al., 2000; Bennet-Clark and Bailey, 2002). Bennet-Clark (Bennet-Clark, 2003) found lower resonances in the short flexible region connecting the file with the harp (see Fig. 1), and he postulated that in this region the escapement should operate at lower frequency than in the rest of the file. However, he stated that his analysis does not account for all the FM observed in the cricket pulses, which usually begins half-way (or even before, but see Fig. 5) through the sound pulse.

Bennet-Clark (Bennet-Clark, 2003) did not support Nocke's (Nocke, 1971) idea that the harp is the main resonator in the cricket wing. He suggested that the major elastic component of the wing resonant system was the file plus the first anal vein, and that the mass component is the combined mass of the file, anal area and harp. Our results support Bennet-Clark's (Bennet-Clark, 2003) conclusions, but differ from his findings that the basal file area, close to the flexible region of the wing, exhibits a lower f_o than the rest of the wing. At least in *G. bimaculatus*, there is but one resonance in the file and surrounding areas, and not a varying resonance as Bennet-Clark found in *T. oceanicus* (Bennet-Clark, 2003). However, in 2 (out of 44) specimens, we observed lower resonances in the flexible region, but these occurred towards the proximity of the cross-veins (Fig. 1); nonetheless, this lower resonance did not affect the vibration of the harp in this vicinity.

In the present work, measurement of resonances along the file and surrounding cells suggests that the short flexible region connecting the harp with the file (Fig. 1; Fig. 12A) generates substantial displacements while keeping f_o constant across the file. Therefore, the mechanism suggested by Bennet-Clark (Bennet-Clark, 2003) to account for the glissando in the cricket pulse, based on specific resonances of the file, does not gain support from the evidence provided here. The glissando observed in the pulse of almost all field crickets must be explained by a mechanism other than file resonances.

This study documents the mechanical behaviour of wings as they generate frequency modulation, providing insight into the complex mechanism of tegminal stridulation (e.g. Fig. 13), and adding a new perspective in this research area. Notably, we showed in Fig. 5 that the FM pattern is specific for every individual, which suggests that the glissando is caused by a combination of different factors particular to the wing anatomy of every individual. Several variables should be put in this pool; for example, (1) to what degree are the differences between harp and mirror f_o critical for maintaining song purity?; (2) the minor or large differences in Q_{free} between the two wings and their relation to f_o (Fig. 9).

In the 13 animals measured during elicited stridulation, we found 11 in which the LW vibrated with higher amplitude than the RW; in the remaining two the opposite occurred. The free resonances of the harps of these two insects exhibited the normal differences in amplitude response (LW_{harp}>RW_{harp}). Do these discrepancies account for any specific FM? If the observed differences in the magnitude of deflection between LW and RW during active stridulation apply to all field crickets, do they also have implications for the quantity and quality of sound radiation and its directional pattern? Nocke (Nocke, 1971) reported differences in the sound field of the wings, measured at the wing surface from singing insects. According to him, the SPL of the RW was 4.5 dB higher than that of the LW (in 12 of 15 crickets). Although in the present paper only wing vibration was measured, the observed differences in deflection wing pattern (LW ~2 times larger than RW) would suggest that such asymmetry is necessary for the LW to overcome the problem of being acoustically sheltered by the RW during stridulation.

Another issue related to the glissando is how much FM is manifest in the call of a cricket. Simmons and Ritchie (Simmons and Ritchie, 1996) found that the degree of morphological asymmetry between the two wings predicts the degree of FM. Morphological variables must be included in the pool.

Contributions of the left and right harps to the pulse

Simmons and Ritchie (Simmons and Ritchie, 1996) suggest that the LW makes a major contribution to the first third of the pulse, and the RW becomes more important in the later part of the pulse. They came to this conclusion by recording animals before and after harp ablations.

The presented measurements of wing resonance and the FM pattern recorded from coupled wings (Figs 11 and 13) show that the two wings are simultaneously involved in controlling the frequency of acoustic radiation during stridulation, but that the LW dominates particularly in the magnitude of the deflection. These findings do not support the results and conclusions of Simmons and Ritchie (Simmons and Ritchie, 1996), where the contributions of the two wings were deemed to take place at different times and at different frequencies. The conclusion obtained by Simmons and Ritchie (Simmons and Ritchie, 1996) might have been the direct result of the unknown effects of wing clipping. The evidence presented here supports the notion that, during natural stridulation, wings engage to work as coupled resonators (e.g. Fig. 13). As such, given the importance of harp resonance (hence its mechanical properties), substantial damage to either wing (i.e. harp ablation) is likely to strongly impair the quality and consistency of their acoustic radiation.

Our results concur in part with those of Bennet-Clark (Bennet-Clark, 2003), confirming that RW and LW differ in their intrinsic mechanical resonances. Our results, however, establish that these differences vanish when the wings are engaged in the process of tegminal stridulation (Fig. 11), as Bennet-Clark (Bennet-Clark, 2003) hypothesized.

Interestingly, it follows that neither wing is a perfect resonator that generates the exact carrier frequency that is the hallmark of the species' song (Figs 7 and 11). Rather, our results show that both the specific frequency and the spectral purity are emergent properties resulting from the coupling between the two wings as they radiate acoustic energy. Coupled wings during sound production are a larger effective sound source (when compared with individual wings) on which the acoustic damping will be greater (Bennet-Clark, 2003). However, this lack of significance might be the result of the relatively small sample of crickets studied under pharmacological stimulation and the high variation of the measurements. More measurements need to be taken with this approach.

The mechanisms supporting these emergent properties constitute a further point of interest; in the morphological construction of the cricket wings (see Klingenberg et al., 2010), which anatomical and mechanical characteristics are crucial to the emergence of pure tones? Also, what makes the cricket wings such accurate and resilient sound radiators?

Potential applications of the method

One of the major concerns in the study of wing mechanics in Ensifera using tegminal stridulation has been the use of invasive methods, mostly due to the techniques available at the time. Some studies involve analysing wings removed from the animal's body and stimulation of the wings is achieved by using small mechanical probes or sound (Nocke, 1971; Bennet-Clark, 2003; Montealegre-Z and Mason, 2005). Other workers have approached the same problem by studying intact wings still attached to the animal, but their methods are also invasive (e.g. touching the wings with transducers, loading them with microparticles, or removing wing parts) (Rakshpal, 1960; Bailey, 1970; Nocke, 1971; Sismondo, 1979). These studies have formed a solid basis for understanding that an elaborate resonant system supports the production of highly tonal songs.

To investigate the vibrational properties of both harps during stridulation, we used a system that involves simultaneous sound recording and laser vibrometry. Because a cricket sound pulse is only produced during the sweep of the plectrum across the stridulatory file from the anal to the basal file regions, and each tooth represents a sound oscillation, it is possible to associate file teeth with sound cycles (Koch et al., 1988; Bennet-Clark, 2003). In *G. bimaculatus* the pulse duration is usually around 20 ms and the f_c is ~4800 Hz; therefore, the period of each oscillation should be about 0.21 ms. Hence, there should be about 100 teeth involved in the production of a pulse. The first forced oscillations of a song pulse will result from the impact of the plectrum with the first few teeth of the anal region, and the last forced oscillations will correspond to the interaction of the plectrum and the last teeth in the basal file region. Using precision acoustic and substrate vibration recording technology and reliable trigger functions, the present experimental approach permits the quantification of simultaneous acoustic radiation from the entire wing and the mechanical vibration of any radiating structure (e.g. the chord) at different times during the stridulatory stroke in an intact animal. Such experimental control can now be used to investigate the mechanical and physiological basis of the frequency modulation observed in individual crickets, mole crickets and katydids. Deeper questions are now accessible, such as the role of file and plectrum morphologies in the FM profile of individual crickets and, ultimately, whether such individual profiles play any role in the design of songs and sexual selection.

It would also be interesting to further investigate whether FM is an unavoidable by-product of the escapement mechanism (Koch et al., 1988) at work during the impact of the plectrum on the file. In this respect, earlier experiments (Elliot and Koch, 1985; Bennet-Clark and Bailey, 2002; Montealegre-Z et al., 2009) indicate that the degree of spectral purity of the song depends on this impact rate, the mechanical basis of which could lie in the intrinsic resonant properties of the acoustic radiators, mainly the harp and associated veins, and the mirror. Finally, the information and methodology presented here are vital to refine the current models of power transfer to the tegminal oscillators in crickets (Prestwich and O'Sullivan, 2005), which is missing information on several variables (e.g. spring constant, elasticity).

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