

RESEARCH ARTICLE

Mechanisms of high-frequency song generation in brachypterous crickets and the role of ghost frequencies

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SUMMARY

Sound production in crickets relies on stridulation, the well-understood rubbing together of a pair of specialised wings. As the file of one wing slides over the scraper of the other, a series of rhythmic impacts causes harmonic oscillations, usually resulting in the radiation of pure tones delivered at low frequencies (2–8 kHz). In the short-winged crickets of the Lebinthini tribe, acoustic communication relies on signals with remarkably high frequencies (>8 kHz) and rich harmonic content. Using several species of the subfamily Eneopterinae, we characterised the morphological and mechanical specialisations supporting the production of high frequencies, and demonstrated that higher harmonics are exploited as dominant frequencies. These specialisations affect the structure of the stridulatory file, the motor control of stridulation and the resonance of the sound radiator. We placed these specialisations in a phylogenetic framework and show that they serve to exploit high-frequency vibrational modes pre-existing in the phylogenetic ancestor. In Eneopterinae, the lower frequency components are harmonically related to the dominant peak, suggesting they are relicts of ancestral carrier frequencies. Yet, such ghost frequencies still occur in the wings' free resonances, highlighting the fundamental mechanical constraints of sound radiation. These results support the hypothesis that such high-frequency songs evolved stepwise, by a form of punctuated evolution that could be related to functional constraints, rather than by only the progressive increase of the ancestral fundamental frequency.

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INTRODUCTION

Studies on mechanisms of sound production continuously contribute to a better understanding of animal communication, its role in life history and its evolutionary pathways (Stuart and Currie, 2002; Montealegre-Z, 2009; Leal and Losos, 2010). Whilst basic or generic mechanisms of communication can be investigated in model species, important comparative information on specialised, highly evolved mechanisms can be obtained from species diverging from the norm. As such, species-specific adaptations constitute evolutionary potentialities that came true, presenting us with diverse solutions to the common problems of animal communication.

In field crickets (subfamily Gryllinae), the mechanism of sound production is under precise mechanical constraints (Elliott and Koch, 1985; Koch et al., 1988; Bennet-Clark, 1999; Prestwich et al., 2000; Bennet-Clark and Bailey, 2002; Bennet-Clark, 2003; Montealegre-Z et al., 2009; Montealegre-Z et al., 2011). Given the constraints each species experiences, a diversity of forms and functions can be studied (Mhatre et al., 2012). Such variation is to be expected in the Eneopterinae clade, in which a diversity of sound production structures has been documented (Robillard and Desutter-Grandcolas, 2004a).

Cricket songs are produced in a two-step process (Bennet-Clark, 1989; Michelsen, 1998). The first step, called stridulation, is a mechanism of frequency multiplication that allows the slow muscle

contractions (7–30 Hz) to generate the 2–8 kHz (5 kHz in most field crickets) pure tone typical of cricket songs (Michelsen, 1998). During forewing closure, the plectrum of the left wing hits a series of cuticular teeth under the right wing (i.e. the stridulatory file). For the 5 kHz carrier frequency (f_c) in field crickets, a single strike between the plectrum and a tooth of the file (together known as the stridulum) produces one elementary oscillation with a decay lasting 3–6 ms. Such oscillation is sustained by the wing resonant properties only if the elementary vibration produced by the next tooth occurs before the decay of the previous oscillation and at the correct phase (Bennet-Clark and Bailey, 2002). In field crickets, singing at *ca.* 5 kHz, the subsequent stimulus following an elementary oscillation will occur at nearly 2 ms from the onset (Bennet-Clark, 2003). The song syllable thus corresponds to the sum of the elementary oscillations produced by all the teeth involved in a given plectrum-file sweep. Tooth strikes result in a sequence of sustained vibrations of similar but not identical frequencies, each vibration matching wing resonant frequency (f_o) (Elliott and Koch, 1985; Bennet-Clark, 2003). Therefore, modification in the multiplication process can be achieved by either increasing the density of file teeth (e.g. reduction of the inter-tooth spacing) or increasing the rate of tooth strikes (for instance by increasing the speed of wing closure), or both.

The second step in this process pertains to signal amplification and involves the action of a wing resonator, conventionally identified

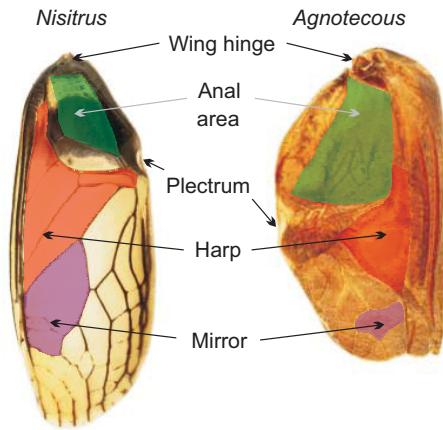


Fig. 1. Right tegmen of *Nisitrus vittatus* and *Agnotecous robustus*, two of the Eneopterinae cricket species studied in this article, showing the main wing cells involved in sound production. The wings are shown in opposite orientation (mirror image of the wing of *N. vittatus*) to facilitate comparison.

as the triangular wing cell called the harp. In this regard, initial studies were undertaken in *Gryllus campestris*, *Gryllus bimaculatus* and *Acheta domestica* (Nocke, 1971; Bennet-Clark, 1999). More recent studies in field crickets (*Teleogryllus oceanicus*) (Bennet-Clark, 2003), and *G. bimaculatus* (Montealegre-Z et al., 2011) have shown that other wing regions resonate and thus also contribute to sound production. The comparative morphology of major wing cells is illustrated in Fig. 1. Like frogs (Ryan and Keddy-Hector, 1992), crickets exploit resonant mechanical properties of their sound radiators to augment the amplitude of their songs and their acoustic range.

By exploiting resonance, crickets can produce signals with a high quality factor Q and finely tune their f_c to a species-specific frequency, usually in the range from 2 to 8 kHz (Otte, 1992; Michelsen, 1998). However, recent work has revealed that most cricket species from the Lebinthini tribe (subfamily Eneopterinae) are capable of producing signals at higher frequencies (Desutter-Grandcolas, 1997; Robillard and Desutter-Grandcolas, 2004b) from 12 to 26 kHz, a range distinctly above that usually reported for crickets.

The spectral composition of the Lebinthini calling songs is also distinctive. Notably, dominant energy is found at the second peak of the spectrum in the 'brachypterous' clade (*Lebinthus* – *Agnotecous*) or at the third peak in the 'long-winged' genus *Cardiodactylus*, these two clades being sister groups (Fig. 2) (Robillard et al., 2007) (see supplementary material Fig. S1 for an illustration of the song spectrum of 12 species showing this spectral composition where the second peak dominates over the first).

The present study focused on the unusually high-frequency monophyletic subclade including the brachypterous Lebinthini genera *Lebinthus* and *Agnotecous*. In order to investigate the mechanisms involved in the production of high-frequency signals and the origin of the particular spectral composition observed in this clade, we measured the fine-scale movements of the forewings during stridulation and the mechanical properties of the wings using a motion-sensitive photo-diode, high-speed video (HSV) and micro-scanning laser Doppler vibrometry (LDV).

In the general frame of cricket stridulation mechanism, the detailed arrangement of wing resonances is responsible for the harmonic-rich composition of the calling song. One syllable is produced per forewing closure, with tooth strikes matching wing

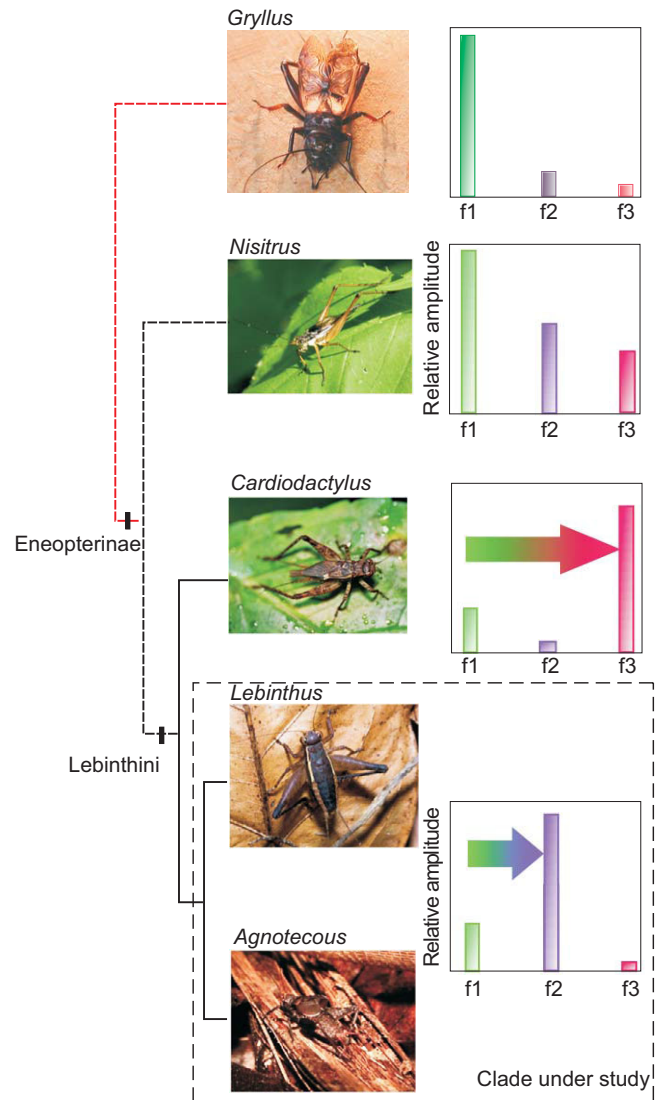


Fig. 2. The studied taxa and their phylogenetic relationships, illustrating the observed change in the spectrum of the call.

resonance. Two alternative hypotheses can thus explain the harmonic structure observed in the Lebinthini.

In the first hypothesis (evolution of the wing resonant properties), the observed harmonic structure is achieved by altering the sound radiation mechanism. Stridulation remains unchanged, with a low tooth strike rate (TSR) similar to that of field crickets (2000–8000 teeth s^{-1}). Instead, the resonant properties of the wing sound radiators generate a harmonic structure whereby the second harmonic is dominant, but the carrier frequency remains the low-frequency component.

In the second hypothesis (evolution of the stridulum), the observed harmonic structure is achieved by changing the frequency multiplication mechanism (file and plectrum) to match high-frequency resonances pre-existing in the radiating wing cells. Here, stridulation directly generates the high dominant frequency. Such mechanism requires the TSR to increase either by increasing the speed the wings' closing phase or by increasing tooth density, or both. A consequence of this scenario is that the song spectrum is modified; the plesiomorphic even harmonics (f_2, f_4, f_6 , etc.) remain present, while uneven harmonics [$f_1 (=f_c), f_3, f_5$] disappear altogether (Fig. 2).

This study investigated the mechanisms of sound generation in Lebinthini crickets and how these mechanisms determine the songs' spectral composition. Previous studies have shown that high frequencies are an evolutionary novelty in Eneopterinae crickets, which occurred in a plesiomorphic context of tonal low frequencies (Robillard and Desutter-Grandcolas, 2004b; Robillard and Desutter-Grandcolas, 2011b). The present analysis shows that the production of high frequencies in brachypterous Lebinthini is a result of specialisations of both file structure and wing resonances.

MATERIALS AND METHODS

Taxonomic sampling and studied specimens

The Lebinthini crickets consist of a monophyletic tribe that belongs to the subfamily Eneopterinae (Robillard et al., 2007; Nattier et al., 2011). We analysed two species of the subclade of brachypterous Lebinthini, *Agnotecous robustus* (Chopard 1915) and *Lebinthus* n. sp. aff. *bitaeniatus* Stål 1877 (hereafter *Lebinthus* n. sp.). One eneopterine species without high-frequency song, *Nisitrus vittatus* (Haan 1842) (Nisitriini), was also studied for outgroup comparison within Eneopterinae. To enrich the comparison, we also included previously published data on the model species *G. bimaculatus* De Geer 1773 (Montealegre-Z et al., 2011).

Specimens came from field collection in New Caledonia (*A. robustus*, F1 colony) and in Singapore (*Lebinthus* n. sp. and *N. vittatus*), wild specimens collected 2 weeks before the experiments began).

Acoustic analysis

The basic cricket song terminology follows that used elsewhere (Ragge and Reynolds, 1998). One song unit is called a syllable and corresponds to one opening–closure cycle of the male forewings. A group of syllables constitutes an echeme, which corresponds to a call unit in terms of communication.

Agnotecous robustus, *Lebinthus* n. sp. and *N. vittatus* were recorded in the field and in the laboratory from specimens collected as juveniles or as sub-adults. The recordings were made in the field with a modified condenser microphone capsule CM16 (Avisoft Bioacoustics, Berlin, Germany), with a frequency range of 5 kHz to 150 kHz (± 6 dB) (R. Specht, personal communication), connected to a Tascam HD-P2 digital recorder (96 k-samples s^{-1} , 16 bit), or using Avisoft Triggering Harddisk Recorder version 2.97 and an 8-Pre MOTU sound card at a sampling frequency of 96 k-samples s^{-1} (16 bit). Sound recordings of four males per species were used to measure the acoustic features of each species. Acoustic analyses were performed using the computer software Avisoft-SASLab Pro version 4.40 (Specht, 2009) and Matlab software (The MathWorks, Inc., Natick, MA, USA). Song features were measured using the automatic commands under Avisoft-SASLab Pro. All recorded files were deposited in the Sound Library of the Muséum national d'Histoire naturelle (MNHN), Paris.

Recordings of stridulatory movements

Stridulatory wing movements and associated sound production were recorded from two *A. robustus* males and two *Lebinthus* n. sp. males. Songs were recorded with a Brüel and Kjær (B&K) ¼ in microphone type 4939, a frequency range of 4 Hz to 100 kHz, and a lower limiting frequency of -3 dB at 3 Hz. Wing movements were simultaneously recorded using a highly sensitive optoelectronic device (Hedwig, 2000). The motion detector was used as described previously (Montealegre-Z and Mason, 2005). Sound and wing-movement signals were recorded on separate channels of a computer data acquisition board at 300 k-samples s^{-1} on each

channel, *via* a data acquisition board (USB-6251, National Instruments, Austin, TX, USA) using Labview Software (National Instruments). To avoid aliasing, signals were low-pass filtered at 100 kHz during recording using a custom-built electrical filter installed in the motion detector. The temperature in the room was $23.9 \pm 0.85^\circ\text{C}$.

Recording wing motion using the opto-motor detector is not a straightforward process for *N. vittatus* males, which possess good diurnal vision; they are easily disturbed by any movement in the surrounding environment and may cease singing for days. The motion detector camera must be repositioned as the insect changes position to guarantee that the reflecting light remains in the viewer field. These shy insects get disturbed when the piece of retro-reflective tape is fixed to the wings. Therefore, it was not possible to obtain recordings of the wing movements of *N. vittatus*. However, we recorded some insects in the laboratory using a HSV camera (AOS Technologies AG, Baden-Daettwil, Switzerland), which allowed the characterisation of the basic wing motion of this species. Video recordings were obtained at 1250 frames s^{-1} , while sound was recorded at 96 k-samples s^{-1} .

Wing velocities during the closing phase were estimated by calculating the derivative function of wing position in time as obtained using the calibrated motion detector and bespoke Matlab code. This procedure provides information on the instantaneous wing velocity of every closing and opening phase. For insects with a continuous wing closure, we estimated an average vector based on the cumulative instantaneous velocities. For *Lebinthus* n. sp., which exhibits a call with pulse trains (i.e. with a discontinuous closure), we measured the maximum wing velocity attained during the production of a discrete sound pulse. We present a mean velocity obtained across the pulses of five consecutive syllables. For *G. bimaculatus*, we used published wing velocity values (Montealegre-Z, 2005). In *N. vittatus*, wing velocity was measured from HSV recordings. A HSV does not provide data on wing instantaneous velocity of as high a quality as those from the motion detector, which means that wing velocity can only be determined at certain moments during wing closure.

Recordings of wing resonance

Specimens raised in Paris (MNHN) were transported to Bristol University, UK, for laser vibrometry experiments. Wing resonance was measured in four males of each species using a micro-scanning LDV (Polytec PSV-300-F; Waldbronn, Germany) with an OFV-056 scanning head, fitted with a close-up attachment. The laser spot location on the wing membrane was monitored by live video feed to the vibrometer controller and data management computer.

The entire stridulatory field in both tegmina was also measured using 250–300 measurement points, as described previously (Montealegre-Z and Postles, 2010). Tegminal vibrations were examined in the frequency domain in response to broadband acoustic stimulation (periodic chirp) in the range 1–50 kHz. The spectrum of the stimulus at 80 dB (re. 20 μPa SPL) was corrected and flattened with an error of ± 0.3 dB. The acoustic signals were generated by the PSV 300 internal data acquisition board (National Instruments PCI-4451), amplified using a Sony amplifier (TAFE570; Tokyo, Japan) and passed to a loudspeaker (ESS AMT-1; ESS Laboratory, Inc., Sacramento, CA, USA) located 11 cm below the specimen. For vibration recordings, an intact specimen was mounted on a silicone holder using soft metallic clamps to fix the legs. The wings were laterally extended by fixing the axillary sclerites with bees wax (see Montealegre-Z et al., 2011), and sound was passed through the loudspeaker. For comparative purposes, we used wing

resonances and call of the field cricket *G. bimaculatus* previously obtained using the same protocol (Montealegre-Z et al., 2011).

Morphology

To characterise the morphology of the stridulatory files, scanning electron microscopy (SEM) observations of cricket left (LW) and right (RW) forewings were performed at the Plateforme de Microscopie électronique of the MNHN, using a JEOL-JSM 840 electronic microscope (7kV), after a 60 s gold-coating. File measurements were obtained on digitised SEM images using the dimension tool of the program Corel Draw X4.

Forewing (FW) morphology was previously studied in the species considered here in a phylogenetic context, and homologies were established between veins and FW areas (Robillard and Desutter-Grandcolas, 2004b).

RESULTS

Morphology of the stridulatory file

We observed an apparent reduction in the length of the stridulatory file in the Eneopterinae studied here compared with that of field crickets (see Table 1). File length varied from 1.8 mm in *N. vittatus* to 2.7 mm in *A. robustus* (Fig. 3A, Fig. 4A, Fig. 5A). There was also a notable reduction of inter-tooth spacing with an associated increment in tooth density compared with *G. bimaculatus* (Table 1, Fig. 3B, Fig. 4B, Fig. 5B). Except for *N. vittatus*, the number of effective teeth used did not differ much from the number of teeth swept by the plectrum in *G. bimaculatus*. This suggests that tooth strike rate is increased in the brachypterous Lebinthini by increasing tooth density and the speed of wing closure.

Teeth of the stridulatory file in all species preserved the typical arrangement for pure-tone stridulation (Montealegre-Z, 2005; Montealegre-Z, 2012), i.e. inter-tooth spacing gradually and systematically increased in the direction of plectrum motion (Fig. 3B, Fig. 4B, Fig. 5B).

Acoustical analysis of the songs

The calling song of *N. vittatus* (Fig. 3C–E) is very tonal and consists of rapid triplets of syllables repeated at length (Fig. 3D). Each syllable has a duration of 6.9 ± 1.4 ms. The frequency spectrum shows a dominant peak at 7.3 ± 0.1 kHz and several peaks harmonically related; the magnitude of the first spectral peak slightly dominates the other peaks at 30°C (Fig. 3E).

A complete call of *Lebinthus* n. sp. (Fig. 4C–E) lasts 2.6 ± 1.6 s and consists of syllables made of pulse trains, whereby amplitude modulation results in distinct pauses within the syllable (Fig. 4C,D).

This call is organised into two sections, an initial part consisting of 11 ± 5 well-spaced syllables (longer period, not visible in Fig. 4) and a final part consisting of a short trill of 24 ± 3 syllables set closer together (shorter period, Fig. 4C). Each syllable contains discrete pulses, produced by regular plectrum pauses, which are caused by a discontinuous closing phase (Fig. 4D). This stridulation pattern produces a spectrum of ca. 18 kHz bandwidth, spanning 12–30 kHz, with the main energy peak at 16.7 ± 1.3 kHz, corresponding to the first and only peak of the spectrum (Fig. 4E).

A single call of *A. robustus* is a trill (echeme or group of syllables) lasting 1 s and consisting of 56 ± 5 syllables without amplitude modulation (syllable duration 10 ± 2.4 ms, syllable period 18 ± 2.4 ms). A short segment of the echeme is shown in Fig. 5C. A vanishing peak is present at ca. 5 kHz, but the dominant frequency occurs at 11 ± 0.8 kHz (Fig. 5D). Other peaks of low energy are detected at 15, 23 and 34 kHz. This spectral composition is typical of all the previously described songs of *Agnotecous* species (Robillard et al., 2007; Robillard et al., 2010) and of some *Lebinthus* species (Robillard, 2009) (supplementary material Fig. S1).

Wing motion

Wing motion was recorded using an opto-motion detector and HSV. Males of all species studied here produce calling songs in the typical way of Ensifera using wing stridulation, i.e. during the closing phase of the wings (Fig. 3C, Fig. 4C, Fig. 5C). The smooth wing closure of *G. bimaculatus* (and other Gryllinae, e.g. *G. campestris* and *Teleogryllus oceanicus*) (Koch et al., 1988; Bennet-Clark, 2003; Montealegre-Z, 2005) constitutes the external group for comparison. The species in this study have either (1) preserved this continuous closing pattern (e.g. *N. vittatus* and *A. robustus*; Fig. 3D, Fig. 5D) or (2) introduced short silent intervals produced by regular pauses during wing closure, which results in syllables with pulse trains (e.g. *Lebinthus* n. sp., Fig. 4D).

The estimated average velocity during wing closure for one complete syllable varies between ca. 100 and 270 mm s^{-1} in *N. vittatus* and the brachypterous Lebinthini (excluding plectrum pauses in the respective silent intervals in *Lebinthus* n. sp.). The average wing closure velocity of field crickets previously studied (Koch et al., 1988; Montealegre-Z et al., 2011) is within this average range (i.e. $<200 \text{ mm s}^{-1}$) (Table 1). However, in *Lebinthus* n. sp., the syllable is made of discrete pulses and the motion detector recordings reveal that each discrete pulse is produced after the scraper pauses, distorts and springs forwards, striking file teeth at higher velocities of $180\text{--}370 \text{ mm s}^{-1}$ (higher than the average of the syllable) (Fig. 4D).

Table 1. Morphological measurements of the stridulum in crickets associated with acoustic and dynamic variables

	Total file length (mm)	No. teeth used	Tooth density (no. mm ⁻¹)	Total distance used (mm)	Average tooth distance (µm)	TSR (strikes s ⁻¹)	Pulse duration (ms)	Carrier	Q-factor	Average speed (mm s ⁻¹)
<i>A. robustus</i> (N=4)	2.7±0.03	95	61.29	1.55	15.6±3.79	11,880	8	11.2	28.5±2.9	167.76
<i>Lebinthus</i> n. sp. (N=4)	2.13±0.06	85	70.25	1.21	15.3±3.2	10,630	8	16.7	3.6±1.1	270.0*
<i>N. vittatus</i> (N=4)	1.76±0.04	60	61.22	0.98	17.4±1.04	6000	10	7.3	19.8±2.2	98.3
<i>G. bimaculatus</i> (N=6)	4.7±0.21	90	35.71	2.52	30.11±1.18	4500	20	4.8	17.1±3.5	145

Data are for *Agnotecous robustus*, *Lebinthus* n. sp. aff. *bitaeniatus* (*Lebinthus* n. sp.), *Nisitrus vittatus* and *Gryllus bimaculatus*.

TSR, tooth strike rate; Q-factor, quality factor.

*Average speed measured during the production of discrete pulses only.

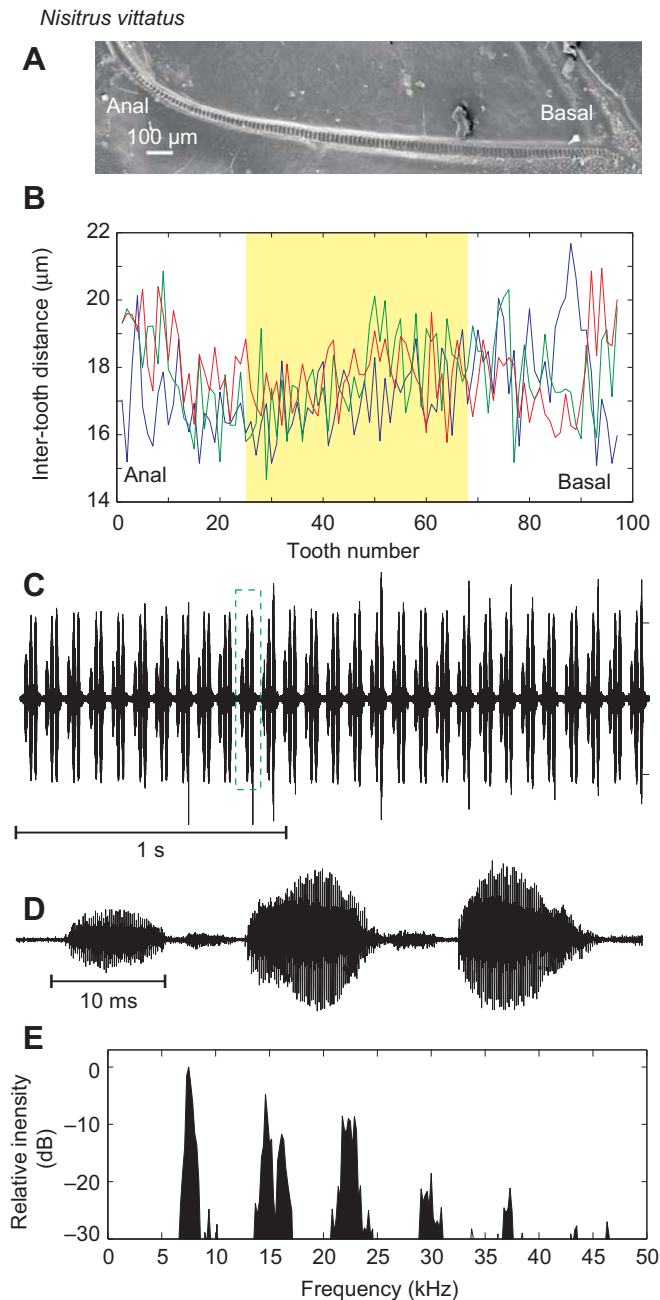


Fig. 3. Stridulation in *Nisitrus vittatus*. (A) SEM of the stridulatory file. (B) File inter-tooth spacing measured in three specimens (shown in different colours). (C) A 2.5 s sequence of calls. Wing motion was recorded using high-speed video (HSV). (D) Close-up view of the call enclosed in the dashed green box in C. (E) Power spectrum of the calls shown in D. The yellow area in B highlights the file region used effectively for sound production.

Altogether, these data establish that high frequencies in brachypterous Lebinthini are generated by a high tooth strike rate and that the dominant peak of the spectrum is the carrier frequency produced by tooth impact and not the second harmonic of a lower carrier frequency. Despite the harmonic relationship observed in the spectrum, the lower component in the song of *A. robustus* (and other Lebinthini species not analysed here; see supplementary material Fig. S1) is not part of the formal harmonic series of the current carrier frequency (harmonics are multiple integers of the carrier only), and is referred to hereafter as a ghost frequency.

More precisely, the data show that a high tooth strike rate in the Lebinthini is achieved mainly by increasing tooth density rather than the average wing closure velocity. However, *L. bitaeniatus* uses both an increment in tooth density and local increments in tooth strike rates derived from local increments in wing velocity.

Wing resonance

Fig. 6 depicts average resonant frequencies (f_0 values) obtained with LDV for LW and RW in eneoapterine species, as well as for *G. bimaculatus*. Field crickets are known to exhibit sharp f_0 near 5 kHz with Q values around 20 (Nocke, 1971; Bennet-Clark, 2003; Montealegre-Z et al., 2011) (see Table 1). In *G. bimaculatus*, the RW resonance is usually higher than that of the LW, but the LW resonance appears to dictate the f_c (see Montealegre-Z et al., 2011). In field crickets, little energy is radiated above 10 kHz (Fig. 6). The average resonance of the Lebinthini species differs from that of field crickets mostly in the fact that there is significant energy radiated at and above ca. 10 kHz, especially in the brachypterous Lebinthini species. Simple averaging of resonance, across both the whole wing and individuals, gives only general information as it can occlude and distort data. More accurate information can be obtained by analysing individual resonances, as shown in Fig. 7.

The wings of eneoapterine species show a more flexible resonant system than those of *Gryllus* spp. For instance, *N. vittatus* wings, except for their transparency and the shape of the mirror, are morphologically very similar to field cricket wings (Fig. 7). *Nisitrus vittatus*, however, uses a f_c of 7.3 kHz, ca. 2 kHz higher than that of field crickets (e.g. *G. bimaculatus*) and has relatively sharp average resonance in both wings at 6.9 ± 0.68 kHz for the LW and 7.0 ± 2.41 for the RW. Notably, average free wing f_0 is a few Hertz lower than the average f_c of the calling song, and in general the RW exhibits more variation in quality factor Q than the LW, which shows lower Q in all regions measured (Table 3). Other harmonically related frequency components are observed in the free vibration of both wings, but these do not exhibit high energy compared with the fundamental peak, as observed in the calling song (Figs 6, 7). However, in some *Nisitrus* males, the resonance of the RW mirror is broader and shows a high energy peak at ca. 12 kHz. As the calculated TSR matches the fundamental frequency of the call at 7.3 kHz (Table 2), the LDV recordings show that during engagement of the wings, both RW and LW resonance increases by nearly 300–400 Hz. This also implies that during wing engagement the energy of the higher modes of this fundamental resonant frequency becomes stronger than that of the equivalent modes in the free vibration. Such sharp wing resonance at ca. 7 kHz is observed in both wings, but harmonic components are more prevalent for the RW, especially in the mirror area (Fig. 7).

Lebinthus n. sp., in contrast, produces a broad f_c , with most of the acoustic energy spread between 12 and 40 kHz. A relatively broad peak (with low Q) of low intensity at nearly 15 kHz is found in all males (Fig. 4E, Table 3). The wings of *Lebinthus* n. sp. have an irregular resonant pattern. In the LW there is a low intensity resonant peak at nearly 4 kHz in all cells, which could potentially produce a ghost frequency in the song if the syllable was produced continuously; a peak of higher energy is located at 15 kHz, being more pronounced in the harp (Fig. 7). In the RW, all regions exhibit a sharp f_0 at nearly 12 kHz with higher Q (Table 3), but the mirror also shows a resonant peak at nearly 3 kHz; this peak is negligible in the other cells of the RW (Fig. 7). This variation in f_0 is considered further in the Discussion.

Agnotecous robustus produces a relatively narrow band call at nearly 11 kHz with a less intense ghost frequency at nearly 5 kHz. The ghost frequency can be seen in the wings' resonant spectrum

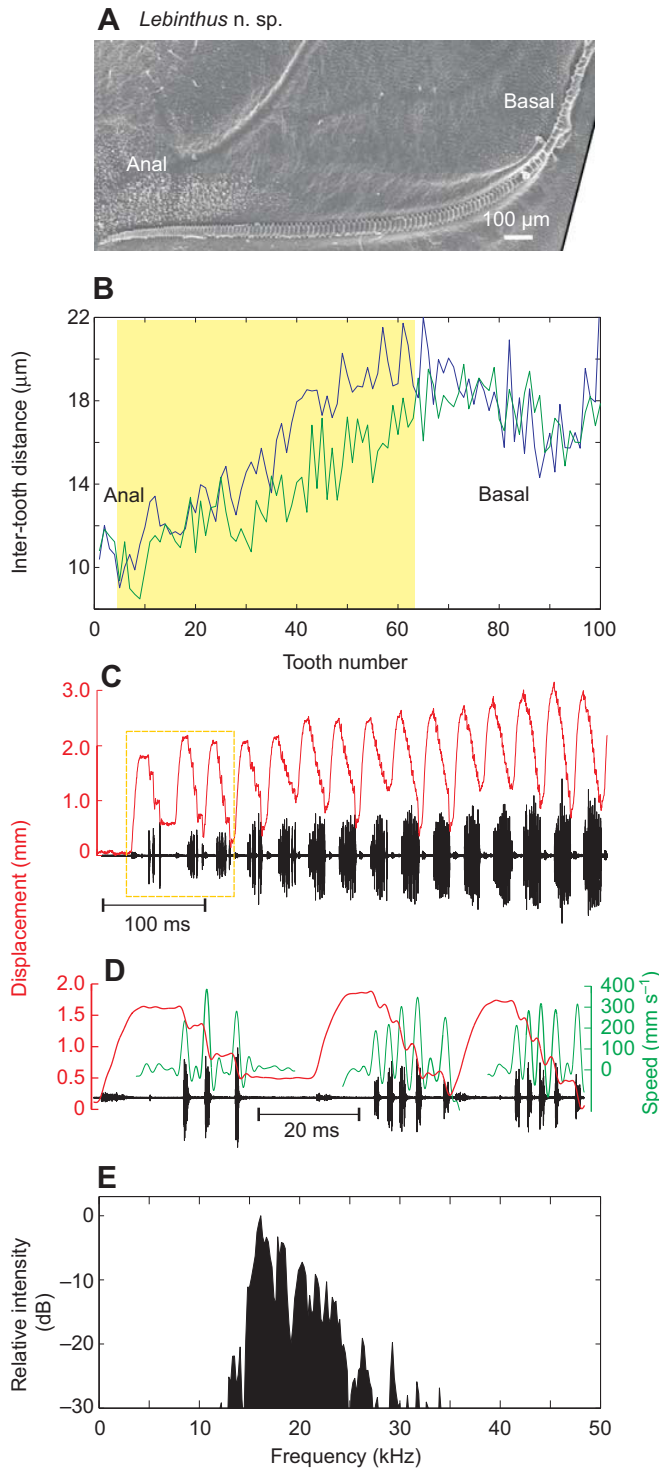


Fig. 4. Stridulation in *Lebinthus n. sp.* (A) SEM of the stridulatory file. (B) File inter-tooth spacing measured in two specimens (shown in different colours). (C) Sequence of 15 syllables (black) with associated wing movements (red). (D) Close-up view of the syllables enclosed by the dashed yellow box in C with associated wing motion (red) and derived wing velocity (green). (E) Power spectrum of the sequence shown in C. The yellow area in B highlights the file region used effectively for sound production.

at ca. 4 kHz, although it is more pronounced in some regions than in others. The dominant peak of the call seems to be related to the resonant spectrum with energy distributed between 10 and 25 kHz

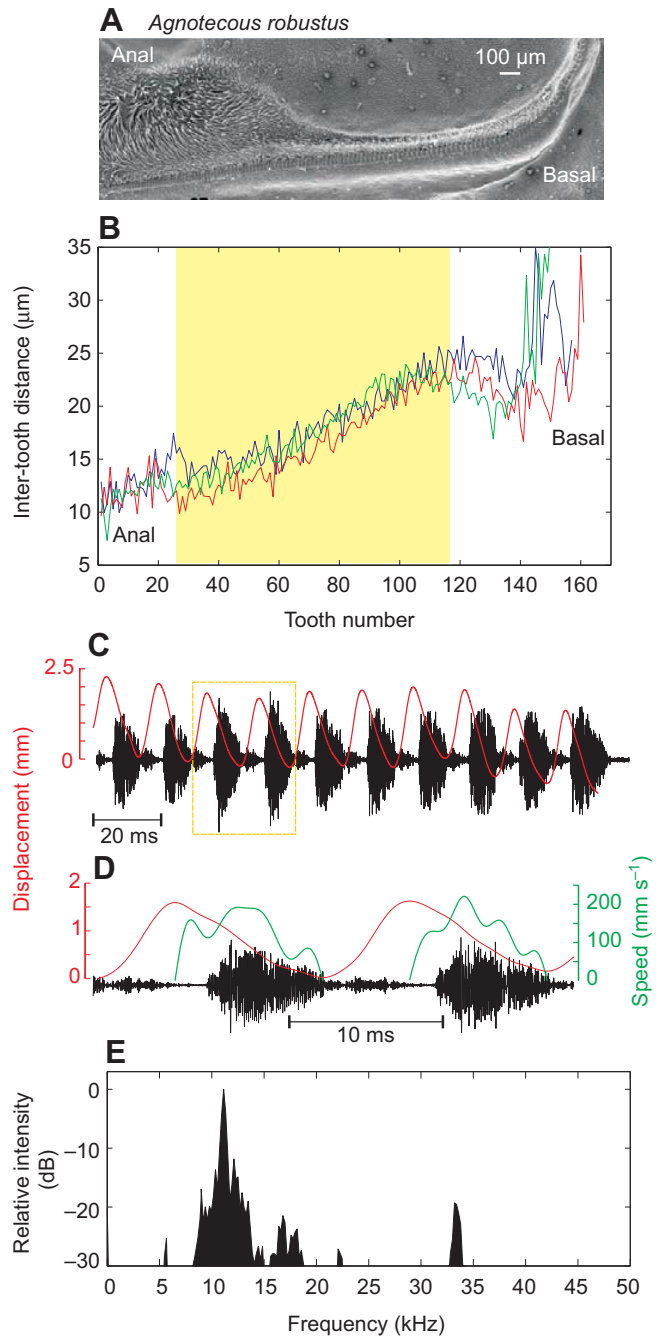


Fig. 5. Stridulation in *Agnotecous robustus*. (A) SEM of the stridulatory file. (B) File inter-tooth spacing measured in three specimens (shown in different colours). (C) Sequence of 10 calls (black) with associated wing movements (red). (D) Close-up view of the pulses enclosed in the dashed yellow box and associated wing motion (red) and derived wing velocity (green). (E) Power spectrum of the calls shown in D. The yellow area in B highlights the file region used effectively for sound production.

(Fig. 5E), and peaking at ca. 15 kHz. The broadband energy varies across wing cells, being higher in the anal region of both wings. High energy in the harps and mirrors tends to peak at similar values in the two wings (Figs 6, 7, Table 3).

DISCUSSION

Many cricket species communicate using low pure-tone f_c (2–8 kHz) (Otte, 1992; Michelsen, 1998). But high-frequency communication

Table 2. Analysis of acoustic variables

	Recording temperature (°C)	Dominant frequency (kHz)	Syllable duration (ms)		Syllable period (ms)		No. syllables per echeme			Echeme duration (s)			Echeme period (s)
			Start	Trill	Start	Trill	Start	Trill	Total	Start	Trill	Total	
<i>A. robustus</i> (N=4)	19–23.5	11.0±0.8	10±2.4		18±2.4		56±5			1.01			11.2±2.1
<i>Lebinthus</i> n. sp. (N=4)	27.5–29.5	16.7±1.3	28.1±9.8	13.3±2.2	219.9±127.7	21.6±3.7	11±5	24±3	38±5	2.6±1.6	0.6	3.3±1.2	30.1±16.5
<i>N. vittatus</i> (N=4)	30–32	7.3±0.1	6.9±1.4		16.1±6.9		3			29.9±5.5 ms			49±5.5 ms
<i>G. bimaculatus</i> (N=4)	23–25	4.8±0.6	20.3±1.2		42.7±3.8		4			0.156±0.004			0.39±0.007

has been documented in the subfamily Eneopterinae (Desutter-Grandcolas, 1997; Desutter-Grandcolas, 1998; Robillard and Desutter-Grandcolas, 2004b; Robillard and Desutter-Grandcolas, 2011a). In this paper, we investigated the morphological and mechanical specialisations allowing this group of crickets to broadcast high-frequency songs. We identified these specialisations as the key evolutionary novelties enabling the distinctive acoustic communication of eneopterine crickets, and compared our findings with what is known about other cricket species.

The evolutionary scenario emerging from the present acoustical and mechanical analyses suggests that high-frequency singing in Eneopterinae crickets probably started with the adoption of strategies that favour elevated TSR and the resulting acoustic radiation with higher carrier frequency. These crickets therefore exploit the initial multiplication step of stridulation by developing stridulatory files with a high spatial density of teeth.

The first strategy to increase TSR, and therefore f_c , is to use a regular wing closure velocity, either conventional or elevated. We found that some Eneopterinae crickets, *Agnotocus* spp. and *Lebinthus* spp., are able to produce higher TSR than other crickets (above 8000 teeth s⁻¹), driving the wing resonators at higher frequencies. These high frequency resonances were observed in the natural modes of vibration of the wings (Fig. 7). The increments in TSR frequency appear to have taken place in a stepwise manner,

rather than by a progressive increase of the ancestral dominant frequency. This way, higher harmonics may be used as the substrate to set up wing vibrations, and therefore sound radiation, at higher frequencies. In this scenario, evolution may favour the exploitation of high-frequency modes of vibration already existing in the natural wing f_0 of these crickets and perhaps in their ancestors. This pattern could stem from strong morphological and functional constraints due to the plesiomorphic vibratory properties of the FWs. TSR therefore, increases without losing the benefits of resonant sound radiation, i.e. the production of a pure tone. An analogous strategy of ‘harmonic hopping’ has been demonstrated at the level of populations in the large-eared horseshoe bat (*Rhinolophus philippinensis*), where morphs of the same or recently diverged species tend to switch between harmonics from the same fundamental frequency to echolocate (Kingston and Rossiter, 2004).

An alternative yet non-exclusive strategy for increasing TSR, and therefore f_c , is to alter the motor control of the wings during the closing stroke to produce a faster passage of the plectrum over the file and therefore generate an elevated TSR irrespective of whether tooth density is low or high. This strategy has been observed and measured in tettigoniids (Montealegre-Z et al., 2006). Montealegre-Z and colleagues found that the use of elevated wing closure velocity to increase TSR meets some physiological boundaries linked to f_c and file length (Montealegre-Z et al., 2006). For instance, a fast

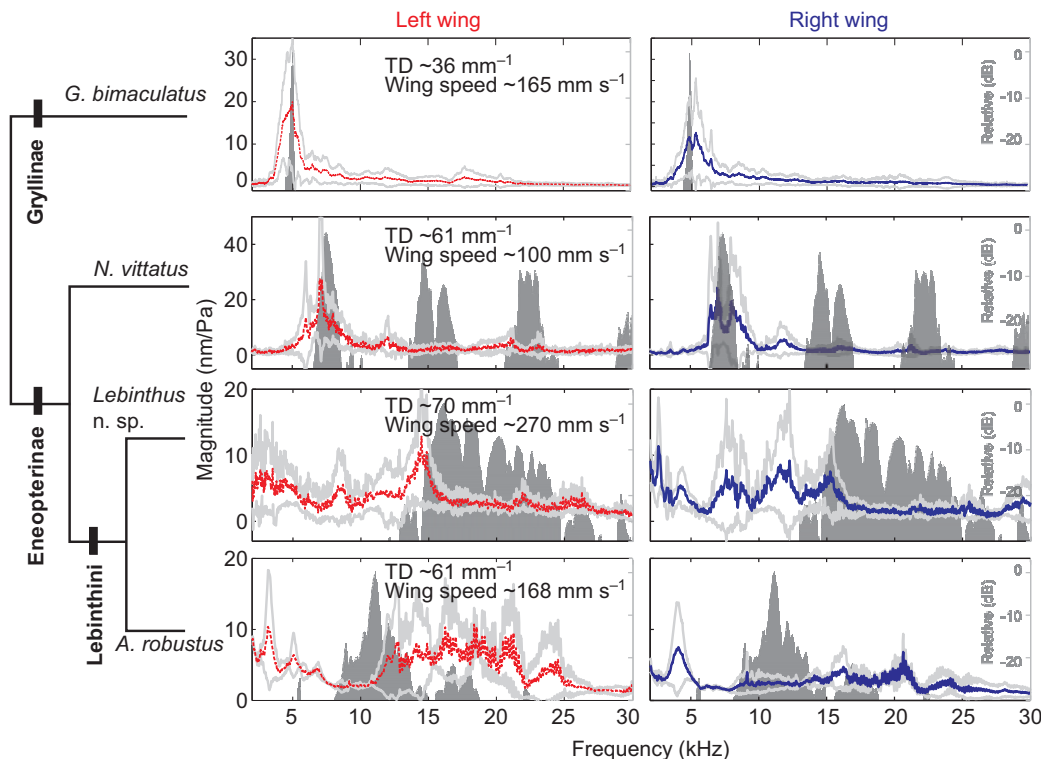


Fig. 6. Average resonance of entire wing vibration in response to acoustic stimulation for left (LW) and right (RW) wings. Red and blue lines show the LW and RW mean, respectively. Light grey spectra indicate standard deviation in both cases (*Gryllus bimaculatus*, N=44 individuals; *N. vittatus*, N=5; *Lebinthus* n. sp., N=4; and *A. robustus*, N=4). The typical power spectrum of the calling song of each species is illustrated in dark grey with relative dB scale given. File tooth density (TD) and average wing speed during closing are shown for each species. Phylogenetic relationships are represented by the cladogram on the left, which suggests that low-frequency pure-tones could correspond to the ancestral condition for Eneopterinae, and that the Eneopterinae have evolved motor and structural properties in their forewings to exploit high frequencies.

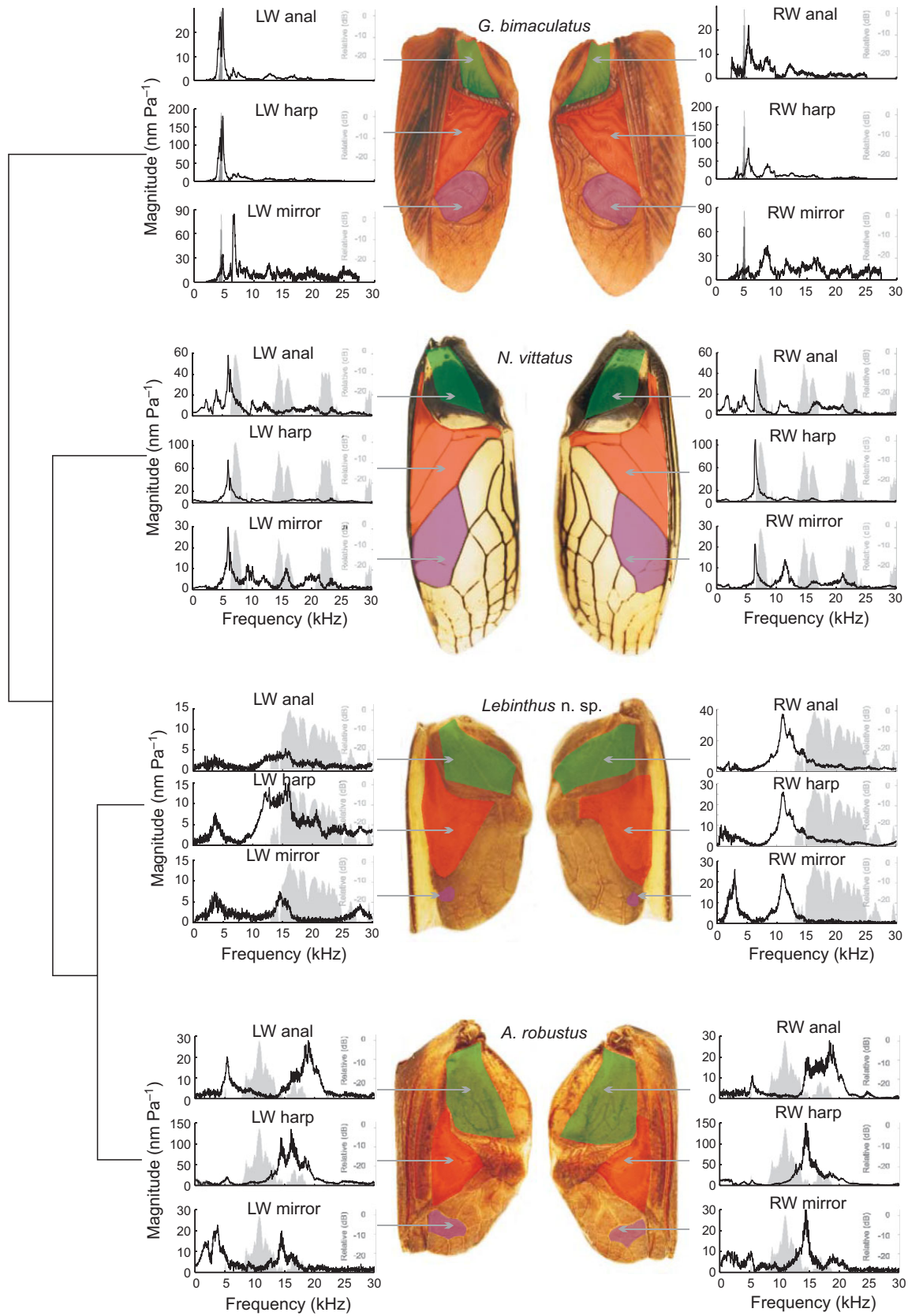


Fig. 7. Resonance of major wing cells in response to acoustic stimulation in a single individual of each group. Wing vibrations are all connected by a cladogram, suggesting that wing resonance has experienced a complex pattern during evolution. The observed change supports the hypothesis of the exploitation of harmonic relationships in some groups to enhance the production of tonal high frequencies. The diagram also highlights the fact that in the Eneopterinae, low-frequency narrow-band spectra may correspond to the ancestral condition for acoustic communication.

Table 3. Calculation of the quality factor Q from wing resonance and calling song

	f_c (kHz)	Q-call	LW f_o (kHz)	Q-LW	RW f_o (kHz)	Q-RW
<i>A. robustus</i> (N=4)	11.2	28.5±2.90	16.24±5.11	2.45±1.3	16.22±4.31	4.42±0.81
<i>Lebinthus</i> n. sp. (N=4)	16.7	3.6±1.10	14.47±3.50	3.60±1.1	12.25±3.52	7.6±1.12
<i>N. vittatus</i> (N=4)	7.3	19.8±2.22	6.91±0.68	19.80±2.2	7.0±2.40	16.8±4.20
<i>G. bimaculatus</i> (N=44)	4.8	17.1±3.54	21.20±8.11	17.11±3.5	18.4±8.50	17.1±3.50

Data are means ± 1 s.d.

f_o , wing resonant frequency; LW, left wing; RW, right wing.

closure ($>250 \text{ mm s}^{-1}$) produces a very short syllable, which might affect female perception. Therefore, tettigoniids commonly introduce silent intervals that promote the lengthening of the syllable. Silent intervals are caused by elastic deformation of the plectrum, producing faster TSR than can be achieved by the contractions of wing muscles alone (Morris, 1970; Morris and Pipher, 1972; Montealegre-Z et al., 2006). The trade-off incurred by this mechanism is the reduction of the tonal quality caused by the introduction of silent intervals and the loss of a coherent phase relationship between tooth strikes and wing vibrations. This strategy is used by *Lebinthus* n. sp., while in the other Eneopterinae crickets studied here, an increased tooth density combined with regular wing closure velocity seems to be a more common evolutionary path.

Analysed in a phylogenetic framework (Robillard et al., 2007), the main consequence of this pattern is that high frequencies evolved several times from a plesiomorphic low frequency condition where the first peak carried the dominant energy, either through convergence, i.e. independently in the two clades [*Cardiodactylus* versus (*Lebinthus*–*Agnotecous*)], or stepwise, with the dominant energy jumping from one harmonic peak to another. In effect, the present data do not support a progressive increase of the ancestral dominant frequency in the Lebinthini.

Mechanism of stridulation in the short-winged Lebinthini

Agnotecous robustus shows a reduction in file length and an increment in tooth density, but uses a similar number of teeth and a slightly higher wing closure velocity than *G. bimaculatus* (Table 1). The combination of these features clearly has an effect on pulse duration, forcing *A. robustus* to produce echemes of syllables lasting no longer than ca. 8 ms. In this species, f_c is 11.2 kHz, and wings are excited with a TSR of nearly 12,000 teeth s^{-1} by increasing both tooth density and wing velocity (Table 1). The LW also shows resonances at lower frequencies (4–5 kHz), which may explain the ghost frequency observed in the song at ca. 5 kHz. In this situation the wings do not exhibit a clear sharp f_o , as in *G. bimaculatus* and *N. vittatus*, but resonances are spread across a 15 kHz band (range 4–20 kHz). Prior to engagement, the resonant peak in the free wing resonance resides at 15–20 kHz, and shifts down to near 11 kHz during engagement. The mechanical cause, or causes, of such a shift remains uncertain.

Even though the file of *Lebinthus* n. sp. exhibits the typical organisation of teeth suitable to the production of a pure-tone signal, the spectrum tonal quality is decreased, in part due to the incorporation of silent intervals within the syllable and the variable natural frequencies from both wings involved. As evidenced in Fig. 4C,D, these silent intervals are produced by closing the wing in steps of short pauses: after each pause, a discrete pulse is produced by briefly accelerating the wings by plectrum deformation to reach the observed speed (Fig. 4D, green trace). The incorporation of silent intervals increases the duration of the syllable, as a continuous plectrum sweep occurring at nearly 270 mm s^{-1} would produce

extremely short syllables (estimated $<5 \text{ ms}$). Thus, taking into account the plesiomorphic condition observed in the wing mechanics that precludes tonal stridulation (sharp wing resonances, increasing inter-tooth spacing and sustained pulses), we conclude that the tonal quality of the spectrum in this species starts to deteriorate with the incorporation of the silent intervals produced by a discontinuous wing closing and the variable f_o in the wings. The fact that the file is adapted for a continuous plectrum sweep suggests that the inclusion of silent intervals is a recently adopted strategy. This notion is supported by the observation of continuous syllables as documented in other species of the genus (Robillard, 2009; Robillard et al., 2010).

Comparison between the short-winged Lebinthini and *Nisitrus*

The Lebinthini species studied here use the second peak of the spectrum as the dominant frequency, and present a reduced file length and an increased tooth density (compared with field crickets), a feature that could be shared by other eneopterines as suggested by the comparison with *N. vittatus*. In addition to this, Eneopterinae tend to maintain wing closing speed (*N. vittatus*, *A. robustus*) or use a combination of both higher wing speed and high tooth density (*Lebinthus* n. sp.). These strategies result in elevated TSRs, and in an increased song frequency. These species thus kept the ancestral grylline stridulation mechanism, irrespective of the f_c of their song, using one tooth per primary oscillation of the song and producing one syllable per FW closure. Contrary to the conclusion made by Robillard and colleagues (Robillard et al., 2007), the tooth impact rate is higher than the lower frequency component of the spectrum, but designed to match wing f_o and the song f_c . The increase of f_c in these genera thus mostly originates from modification of the stridulation step (which supports our second hypothesis; see Introduction). Unexpectedly, our results suggest that these changes may occur subsequently to more basal changes of both the stridulation and amplification steps, which would have led to higher tooth density and allowed ancestral Eneopterinae to exploit harmonic frequencies at the same time as the lower f_c . These plesiomorphic changes may have facilitated subsequent modifications of the stridulation step to exploit high frequencies as f_c in the Lebinthini, beyond the limits of 8 kHz usually observed in crickets using an escapement mechanism.

In terms of evolutionary patterns, these results imply a change of function of the harmonics in the Lebinthini and suggest a case of exaptation (Gould and Vrba, 1982). Although existing in most crickets with low levels of energy, the song's harmonics are usually not of prime importance in the calling song (Hung and Prestwich, 2004). In other Ensifera, harmonics are sometimes involved in the orientation of females during phonotaxis (Latimer and Lewis, 1986) or in competition between males (Hill, 1998). Whatever the role the harmonics play in field crickets, if any, they have gained the function of dominant frequency in Lebinthini. As such, harmonics acquired enhanced radiating power, and a new role,

distinct from the ancestral one, in the course of the evolution of acoustic communication in the Lebinthini tribe. These results provide an explanation of how such an evolutionary pattern could be achieved mechanically.

Bennet-Clark (Bennet-Clark, 2003) and Montealegre-Z and colleagues (Montealegre-Z et al., 2011) have shown that the f_0 between LW and RW is different in field crickets, and that such discrepancies in wing f_0 are alleviated when the wings engage during stridulation. In this situation, one wing seems to produce the main resonant components to produce the carrier frequency, while the other alters its resonance to produce a pure tone. We suggest that the short-winged Lebinthini may have increased their f_c by increasing drastically the wing closing velocity in addition to the plesiomorphic high tooth density. However, Fig. 7 shows that free wing resonance of the wings in *Lebinthus* and *Agnotecous* does not match the spectral peaks observed in the calling song. The variation of wing resonance produces the broad f_0 observed in the wings of *Lebinthus* and obscures a clear energy peak for a ghost frequency.

Comparison between Eneopterinae and cricket model species

Our data show that in the Eneopterinae crickets several wing cells are equally important in sound radiation, which supports previous studies on wing resonance in field crickets showing that the harp cell is not the only acoustic radiator (Bennet-Clark, 2003; Montealegre-Z et al., 2011). Modifications of the stridulatory apparatus endow the Lebinthini with high-frequency communication. In addition to alterations of wing venation and sound radiating cells (Robillard and Desutter-Grandcolas, 2004a), we show here that high-frequency communication in the brachypterous Lebinthini is associated with distinct changes in fine stridulatory structures, such as a reduction of file length and file inter-tooth spacing, an increment of tooth density, and changes in the wing f_0 . As explained above, these features in combination have a negative effect on syllable duration and can reduce it to less than 5 ms, while that of most field crickets lasts 15 ms or more (Walker and Moore, 2002).

Wing closure velocity during sound production changes very little in Lebinthini in comparison to Gryllinae (e.g. *G. bimaculatus*, *G. campestris* and *Teleogryllus oceanicus*). This strongly suggests that selection for increasing f_c , while maintaining sound purity, applies to the structure of the stridulatory file. Files with a higher tooth density enhance TSR, which in turn promotes the exploitation of high frequencies. However, the selected organisation of files with a high density of teeth should have some peculiarities (such as systematic tooth spacing) to produce the correct TSR that more or less matches the high resonant modes already occurring in the natural vibration of the wings. Such precision can be optimised by slightly adjusting wing velocity, which could explain the large variation observed in wing velocity across the studied taxa (Table 1).

File modifications might be also followed by the corresponding optimisation of wing resonance, whereby the f_0 of wing cells contributes to harmonic components differing from the plesiomorphic conditions. Evidence for this comes from *N. vittatus*, an eneopterine using low frequencies, unlike the Lebinthini. *Nisitrus vittatus* preserves most of the stridulation patterns observed in field crickets (e.g. wing anatomy and f_0), yet shows a remarkable reduction in file length, with an associated doubling of tooth density (Table 1). With this high density of teeth, a closing wing velocity of nearly 100 mm s⁻¹ results in a TSR of nearly 7000 teeth s⁻¹, which corresponds to the f_c and matches the wing f_0 (Fig. 7). Therefore, this strategy produces high TSR at the expense of slow closing speeds (Table 1). The f_c of this call occurs nearly 2 kHz above the normal range of field crickets (ca. 5 kHz), but lies within the range

of other crickets, which also show increments in tooth density, e.g. *Anaxipha bradephona* (~210 teeth mm⁻¹) and *Cycloptiloides canariensis* (~70 teeth mm⁻¹) (Dambach and Gras, 1995).

The evidence collected here suggests that the Lebinthini are exploiting high frequencies using variable wing f_0 , which does not require an escapement mechanism like that used by field crickets and perhaps other crickets using tonal calls between 2 and 8 kHz. The escapement mechanism used by field crickets serves the multiple functions of efficiently coupling vibrations of the two wings, of increasing the effective surface area for sound radiation and of maintaining a pure tone signal. This requires relatively sharp wing resonances, and also imposes some upper limit to the carrier frequency. It is suspected that such a system is best used for the single frequencies used by field crickets (Bennet-Clark, 1999). Above that limit, wing coupling becomes more difficult, requiring a high level of temporal synchrony to ensure a coherent phase relationship between the vibrations generated by the two wings.

CONCLUSION

Our results support the hypothesis that high-frequency songs may have evolved in a stepwise manner rather than only by a progressive increase of the ancestral dominant frequency. The biomechanics involved in sound production suggest that doubling the carrier frequency is more likely than a marginal increase of only 1–2 kHz. The basis for this resides in the pre-existing harmonic structure of songs generated by the resonant properties of wing radiators. As such, the wing structure and mechanical properties that often allow powered flight together constitute morphological constraints that may limit, yet also organise, the potential evolution of cricket acoustic communication.

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AUTHOR CONTRIBUTIONS

F.M.-Z and T.R. conceived and designed the experiments; F.M.-Z, T.R. and L.D.-G. performed the experiments/data acquisition; F.M.-Z, T.R., L.D.-G. and D.R. analysed the data; F.M.-Z, T.R., D.R., L.D.-G. and P.G. contributed reagents/materials/analysis tools; F.M.-Z, T.R., D.R., L.D.-G. and P.G. wrote the paper. T.R. and F.M.-Z. contributed equally to this work.

COMPETING INTERESTS

No competing interests declared.

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