Tenors not sopranos: Bio-mechanical constraints on calling song frequencies in the Mediterranean field-cricket

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Abstract

Male crickets and their close relatives bush-crickets (Gryllidae and Tettigoniidae, respectively; Orthoptera, Ensifera) attract distant females by producing loud calling songs. In both families, sound is produced by stridulation, the rubbing together of their forewings, whereby the plectrum of one wing is rapidly passed over a serrated file on the opposite wing. The resulting oscillations are amplified by resonating wing regions. A striking difference between Gryllids and Tettigonids lies in wing morphology and composition of song frequency: Crickets produce mostly low-frequency (2-8 kHz), pure tone signals with highly bilaterally symmetric wings, while bush-crickets use asymmetric wings for high-frequency (10-150 kHz) calls. The evolutionary reasons for this acoustic divergence are unknown. Here, we study the wings of actively stridulating male field-crickets (*Gryllus bimaculatus*) and present vibro-acoustic data suggesting a biophysical restriction to low-frequency song. Using laser Doppler vibrometry and brain-injections of the neuroactivator eserine to elicit singing, we recorded the topography of wing vibrations during active sound production. In freely vibrating wings, each wing region resonated differently. When wings coupled during stridulation, these differences vanished and all wing regions resonated at an identical frequency, that of the narrow-band song (~5 kHz). However, imperfections in wing-coupling caused phase shifts between both resonators, introducing destructive interference with increasing phase differences. The effect of destructive interference (amplitude reduction) was observed to be minimal at the typical low frequency calls of crickets, and by maintaining the vibration phase difference below 80°. We show that, with the imperfect coupling observed, cricket song production with two symmetric resonators becomes acoustically inefficient above ~8 kHz. This evidence reveals a bio-mechanical constraint on the production of high-frequency song whilst using two coupled resonators and provides an explanation as to why crickets, unlike bush-crickets, have not evolved to exploit ultrasonic calling songs.

1 Introduction

Male crickets (Ensifera, Gryllidae) produce loud musical songs to attract conspecific females by rubbing their raised forewings together, a process known as stridulation. During stridulation, the plectrum – a sharp sclerotized region at the anal edge of the left wing (LW) – engages with the file, a row of teeth on a modified, serrated vein on the underside of the right wing (RW) in a clockwork-like manner (Elliott and Koch, 1985; Prestwich et al., 2000). In Gryllidae, the RW usually sits on top of the LW, and during stridulation, both wings open and close in a rhythmic cycle, with sound being generated during the closing phase only (Koch et al., 1988; Bennet-Clark, 1999). The dorsal field of each bilaterally symmetric wing displays a number of clearly delineated wing cells involved in sound radiation. These are the harp, mirror, chord, and the hardened, non-membranous anal surface (Montealegre-Z et al., 2011) (Fig. 1).

The male is under strong sexual selection to sing at a high amplitude in order to effectively attract and provide phonotactic information for distant females (Forrest and Green, 1991; Römer, 1998). In most cricket species, acoustic energy is concentrated within a narrow-band, pure-tone signal centred on a single low-frequency carrier (~5 kHz in the case of the field-cricket *Gryllus bimaculatus* De Geer) which is amplified and radiated by wing regions functioning as natural resonators (Bennet-Clark, 1999, 2003). A loud, pure-tone calling song extends the signal range, aiding the females in determining the direction of the sound source through the enhancement of binaural hearing (Kostarakos et al., 2008; Michelsen and Larsen, 2008) and makes it possible to obtain a large signal-to-noise ratio for transmission across the environment (Michelsen, 1998; Warren et al., 2006; Wiley, 2006)**.** For optimal power transfer from sound source to the surrounding medium, a resonator like the cricket wing should have a radius of at least 1/6 of the sound wavelength λ(λ = ~7 cm at 5 kHz; assuming a monopole radiator; the radius increases to 1/4-1/3λ for dipoles) (Fletcher, 1992; Bennet-Clark, 1998). Small, sound-producing insects like crickets with wings about 0.5-1 cm in size are therefore under strong selection to optimize power output in order to maximize signal range. Crickets approach this optimization problem by using both symmetric forewings together as sound radiators during stridulation to increase the sound radiating surface for low-frequency songs (Bennet-Clark, 1999, 2003; Montealegre-Z et al., 2011). In contrast, their close relatives bush-crickets (Tettigoniidae) have evolved high-frequency singing using asymmetric wings as a derived trait where the overlying LW bears the file and is usually mechanically dampened, while the plectrum-bearing RW is highly adapted for efficient sound radiation (e.g. Montealegre-Z and Postles, 2010; Sarria-S et al., 2016; Song et al., 2020). The drivers for the evolution of this asymmetry are unknown but it has been hypothesised to be linked to ultrasonic sound production and signal purity (Montealegre-Z, 2005; Gu et al., 2012)

Signal transmission is facilitated by resonance– an inexpensive way of enhancing sound output while conserving metabolic energy – whereby the call's carrier frequency (f_c) is determined by the resonance frequency f_0 of the wings, which implies that both wings in a symmetric system should resonate at similar f_0. Reliance on two coupled resonant structures requires that crickets have to achieve and maintain a high degree of phase locking between the two wings in order to add vibrations constructively (Prestwich et al., 2000). Only when the two resonators are vibrating at similar f_0 with minimal phase differences (φ) is constructive wave superposition providing the desired effect of increasing the amplitude of radiated sound energy. When optimal (φ=0), this constructive interference results in a doubling of the amplitude of the combined output (Rossing, 1990). How can this behaviour, defined here as in-phase, take place?

The in-phase resonance between wings is facilitated by an escapement mechanism that allows both wings to vibrate together and radiate sound efficiently (Koch et al., 1988). However, prior mechanical

analyses of cricket stridulation showed that the mechanism of sound production is asymmetrical (Bennet-Clark, 2003; Montealegre-Z, 2005; Montealegre-Z et al., 2011): While the RW receives its energy input along the file's ca. 200 teeth distributed over a distance of some 4 mm, the underlying LW receives energy only through the small region of the plectrum (0.1 mm^2, Fig. 1B). Figure 1B shows that as the plectrum is dragged on the file from left to right, it generates mechanical impacts at different locations along the file. The input of mechanical energy therefore varies in time and location, potentially resulting in a complicated dispersion of substrate-borne waves across the surface area of the RW (Fig. 1B left). On the other hand, the LW has only one input, the plectrum, and vibrations will travel constantly to the various LW regions from that input (Fig. 1B right). Therefore, the LW should vibrate with constant phase, independently of the plectrum's position on the RW. In contrast, the RW should be more vulnerable to phase changes as the moving plectrum delivers energy impulses along the file. If these assumptions hold true, the constant phase generator (LW) and the variable phase generator (RW) are expected to interact and generate beats in their summed acoustic output, in particular at locations where LW and RW vibrations cancel each other out (Sismondo, 1993). Yet, the natural song of the male does not exhibit such beats; instead, song pulses have sustained and regular amplitude and phase profiles.

In addition, it is also implied that the wings' resonances are perfectly in tune with the input stimulus, each wings' f_0 is equal to the song carrier frequency f_c. However, previous studies revealed that the left and right wings exhibit different f_0, above and below the output f_c (<5 kHz>) (Nocke, 1971; Bennet-Clark, 2003; Montealegre-Z et al., 2011). Non-contact laser Doppler vibrometry (LDV) measurements showed that the left and right wings of field-crickets are mechanically different, with resonant frequencies differing by as much as 2 kHz ($f_{0\,left} < f_{0\,right}$; Montealegre-Z et al., 2011). It remains unclear how the seemingly imperfect and differently tuned resonators can generate the high quality pure-tones observed in crickets.

Using LDV, focal microinjection of the neuropharmacological neuroactivator eserine, and specialized acoustic equipment, we measured wing vibrations in actively stridulating Mediterranean field-crickets (*Gryllus bimaculatus*). From physical acoustics, we hypothesise that efficient, high gain, pure-tone radiation results from the in-phase oscillation of both wings when coupled during the stridulation process. We furthermore formulate and test a second hypothesis: different wing regions vibrate in phase, despite differential tuning and inputs, and thereby generate the coherent acoustic radiation typical of field-cricket songs.

As a consequence, any imperfections in the coupling of the wings that lead to temporal and phase shifts between the resonators should result in sub-optimal amplitude of the output signal and ultimately impose constraints on signal frequency.

2 Material & Methods

2.1 Animals

Adult male crickets (*G. bimaculatus*) obtained from a breeding colony maintained at the University of Bristol were used. Animals were kept at room temperature (20-22 °C) under a 12h:12h light:dark cycle and were fed with oats, dry dog food and water *ad libitum*. Adult males were randomly taken from the colony, their wings inspected for damage and kept individually in cages prior to the experiments. After isolation, 18 males that sang for prolonged periods of time were chosen for the experiments, as these

119 animals usually responded better to pharmacological stimulation. All males recorded were singing with
120 the usual wing overlap (RW over LW).

121 2.2 Neuropharmacological stimulation

122 To elicit persistent stridulation in tethered crickets, we followed methods established and described in
123 detail in earlier studies (Hedwig and Becher, 1998; Wenzel et al., 1998; Wenzel and Hedwig, 1999;
124 Montealegre-Z et al., 2011). In short, we used borosilicate glass microcapillaries (1B120F-3; ID=0.68
125 mm; World Precision Instruments, Inc., Sarasota, FL, USA) pulled with a Sutter microelectrode
126 puller (Sutter Instrument Company, Novato, California, USA) to produce ca. 10 µm wide tips. These
127 microcapillaries were then filled with eserine/ringer solution (10^{-2} mol l^{-1}; Sigma-Aldrich Company
128 Ltd., Dorset, UK) and connected to a picospritzer (Picospritzer II, Parker Hannifin, Pneutronics
129 Division (formerly General Valve), NJ, USA). Small quantities of eserine (an acetylcholinesterase
130 inhibitor) were injected into a brain neuropil, located in between the pedunculus and the α-lobe of the
131 mushroom bodies. Successful procedures elicited sustained stridulation in the typical calling song
132 pattern (see Supplementary Video 1). Crickets were removed from the study if we recorded no
133 singing activity within 1 hour after the first injection.

134 Crickets exhibit frequency modulation (FM) in their calls, and the envelope of this modulation has
135 been shown to be a fingerprint of each individual (Montealegre-Z et al., 2011). The quality of the
136 pharmacologically elicited calls was examined by correlating their FM pattern with that of the natural
137 calls obtained by zero-crossing analysis. Calls were judged of sufficient quality when the correlation
138 was higher than 0.85 (see Montealegre-Z et al., 2011, for more experimental details).

139 2.3 Recordings of wing vibrations in stridulating animals (wings engaged)

140 Vibrations from the tegminal surface were successfully quantified from 11 of the 14 stridulating
141 animals using two coupled laser Doppler vibrometers (Polytec PSV-300-F, and a PSV-400; Polytec
142 GmBH, Waldbronn, Germany) and corresponding scanning heads (OFV-056) fitted with close-up
143 attachments. The velocity output of the PSV-300-F served as an input channel for the PSV-400
144 vibrometer, thus allowing for synchronization of the recordings. Sound signals were recorded using a
145 1/8" condenser microphone Brüel & Kjær Type 4138, connected to a Brüel & Kjær 2633
146 preamplifier (Brüel & Kjær, Nærum, Denmark), which was in turn connected to the PSV-400
147 acquisition system. Measurements were performed in single-shot mode (one recording per chosen
148 spot on the wing, no averaging) mode in the temporal domain (1024 samples at 512 kHz sampling
149 rate, leading to recordings with 2 ms duration and a temporal resolution of ~1.95 µs). Acoustic and
150 vibrational measurements were recorded with Polytec Scanning Vibrometer software (PSVSoft,
151 Version 8, Polytec GmbH, Waldbronn, Germany). The microphone was positioned posterior to the
152 specimen, 3-4 cm away from the wings as to not interfere with the laser beams. Simultaneously, wing
153 vibrations were recorded with the laser beams focused on the anal regions, harps, chords and mirrors
154 (Fig. 1 and see Supplementary Video 1 showing a singing male after pharmacological stimulation).
155 Through the video feed of the two LDVs, we were able to visually place the laser points with some
156 acuity within the regions in question, ensuring that the recordings from left and right wing came from
157 equivalent locations. Results for the chord regions are shown in the supplementary material section
158 but are not included in the main results as we were able to obtain chord recordings in only 7 out of
159 the 11 animals used (the left chord regions are usually covered by the RW during stridulation and
160 thus not easily accessible). The laser spot position and signal strength (the amount of laser light
161 reflected from the target) was monitored and controlled via the live video feeds to the controlling
162 computers of both laser systems. Using earlier LDV systems, signal strength often had to be
163 increased by applying minute reflecting beads or powder to the wing surfaces. This was not the case
here as the focussed laser light (λ ~ 630 nm) waswell

reflected by the wing cuticle, which allowed us to perform contactless vibration measurements without further manipulation of the wings.

The microphone signal was used as a measurement trigger, so only wing vibrations involved in sound production were recorded. Data acquisition was programmed to last for 2 ms during the maximum amplitude event of a song pulse. This duration was chosen to minimise the movement of the wings during recording (~ 8-10 teeth) while still gathering sufficient data for analysis (see also Montealegre-Z et al., 2011).

2.4 Individual resonances of unengaged fixed wings (free vibration)

After the previous experiment, each of the wings of each live specimen (n=14) were extended and separated from each other by fixing the axillary sclerites with a bee's wax (Fisher Scientific UK, Limited, Leicestershire; product code W/0200/50), and Colophony (Sigma-Aldrich Co. St. Louis, MO, USA; Product No. 60895-250G) mixture (1:1). The wings were extended to not be in contact with the pronotal lateral and posterior edges. A loudspeaker (ESS AMT-1; ESS Laboratory Inc., Sacramento, CA, USA) was used to broadcast periodic chirps in the range 1-20 kHz, with a flat (55 dB SPL ± 1.5 dB) spectrum. The microphone was placed dorsally in the middle of both extended wings (Fig. 4). The laser system was set to record in the scan mode. A complete scan of the extended wings in response to the periodic chirps was performed with the PSV-400 LDV, using 250-300 scanning points per wing with 10 measurements averaged per point. FFT with a rectangular window and a sampling rate of 512 kHz, 128 ms sampling time, and a frequency resolution of 7.81 Hz were generated for each point.

2.5 Data Analysis

Experimental data was either analysed directly with the PSV software or with custom written scripts in Matlab (R2019a; The MathWorks Inc., Natick, MA, USA). Instantaneous phase in the time domain was obtained with Hilbert transform using custom Matlab code (Hartmann, 1997). We tested whether the frequency differed between left and right wings, and between areas (mirror, harp, chord, anal 1, anal 2) using linear mixed effects models run in R 4.0.0 (R Core Team, 2020). Models were run separately for free and engaged wings, with male ID included as a random effect. Models were run using lme4 (Bates et al., 2015) and lmerTest (Kuznetsova et al., 2017), with *post hoc* testing carried out using emmeans (Lenth, 2020). We also test the difference in the normalised amplitude of the mechanical response (μm/Pa), between left and right wings using a paired t test.

3 Results

Using focal microinjection of the neuroactivator eserine into the cricket's brain (Wenzel et al., 1998; Hedwig, 2000), long-lasting and stable stridulation was elicited in 14 restrained males (Supplementary Video S1). Using two synchronized micro-scanning LDVs, we successfully measured the spatially resolved vibration of both wings simultaneously during the 'engaged' phase of the stridulation process in 11 of the 14 actively singing males, following a previously established protocol (Montealegre-Z et al., 2011). After the cessation of singing, the wings of each specimen were extended and fixed basally and stimulated with sweeps of broadband sound to reveal their natural resonances f_0 and relative magnitudes of vibration. The surface area of these 'unengaged' wings was scanned in its entirety, providing a detailed map of vibrational patterns (Figs. 2 & 3 and Supplementary Video 2, showing wing vibrations of one male at resonance of 4.6 kHz).

3.1 Natural frequencies of wing vibrations

Full wing scan recordings of unengaged (extended and fixed) wings show that the RW f_0 is significantly higher than the LW (RW=5.168 ± 0.434 kHz, SE 0.116; LW=4.827 ± 0.396 kHz, SE 0.106; LMM: $F_{1,152.60}$= 15.93, p<0.001). However, when comparing vibration amplitudes at the average f_0 of both wings, no difference between left and right wings was found. This was true for both average vibration amplitudes per wing and maximum vibration amplitudes of the harp areas alone (RW_{harp}=0.32 ± 0.24 µm/Pa; LW_{harp}=0.40 ± 0.35 µm/Pa; t=0.988, df=13, p=0.34). When each wing is stimulated at its average f_0, one always exhibits a higher vibration amplitude (on average by a factor of ~1.7; Fig. 2b, c), but this dominant wing can be either LW or RW (cf. Supplementary Video 2, where the animal's left wing vibrates with higher amplitude). In a previous study, we reported a trend of LW dominance which we could not identify here, which is most likely due to our low sample size (n=44 in Montealegre-Z et al., 2011).

Examining wing vibrations in more detail, LDV measurements reveal that each wing region exhibits its own resonance spectra with varying peak frequencies (Fig. 3A); there were significant differences in the f_0 between areas (LMM: $F_{4,152.56}$= 72.55, p<0.001). Post hoc testing revealed that the mirror of each wing consistently showed higher f_0 than the average wing f_0 (LW_{mirror}= 6.858 ± 0.540 kHz, SE 0.127 kHz; LW average f_0 of other areas = 4.827 ± 0.396 kHz, SE 0.106; RW_{mirror}= 7.007 ± 0.865 kHz, SE 0.204 kHz; RW average f_0 of other areas = 5.168 ± 0.434 kHz, SE 0.116; n=18) (Fig. 3A), with other areas of the wing not significantly different from each other.

3.2 Wing vibrations in stridulating animals

Wing vibrations were recorded during active stridulation using two LDVs in single shot mode, enabling vibration measurement at defined locations and times (see Supplementary Video 2). Remarkably, vibrations of engaged wings during stridulation (Fig. 3B) differ from sound-evoked vibrations in unengaged wings (Fig. 3A). When the wings are engaged, all regions exhibit near identical, narrow vibrational frequency spectra with maximum power concentrated at the carrier frequency f_c of the calling song (here 5.125 kHz; LMM: $F_{3,66.29}$= 1.56, p=0.208; Fig. 3B). There is also no difference between the left or right wing (LMM: $F_{1,65.20}$= 0.77, p=0.383). The convergence of all resonators towards one very narrow frequency band of oscillation is reminiscent of entrainment, a process similar to synchronization between Huygens' clocks (Peña Ramirez et al., 2016).

Apart from identical oscillation frequency, an additional key feature of synchronized resonators is their phase relationship. Time-resolved LDV data were obtained by recording vibrations from different regions of both wings at synchronised points during stridulation (see methods). Results across 11 specimens show that the wings are not perfectly in phase during sound production, but that phase lags φ exist over a wide range between left and right wings (Fig. 4). In some individuals, φ is small and relatively constant between wings (both over time and between regions, Fig. 4A), while others show larger differences in phase (Fig. 4B and Fig. S1). Within an individual, average phase lags across wing regions seem to be relatively consistent, although considerable variation exists (see Fig. S1).

Time domain recordings of single point measurements at the harps, anal regions and mirrors also show that LW vibration amplitudes are mostly higher than RW amplitudes (red and blue lines in Fig. 4A, respectively) but there is also considerable variation in amplitudes across animals and wing regions (Fig. 4B). The high variation in vibration amplitude can be explained by the limitations of the experimental set up. As two lasers had to be manually aligned on the stridulating animals, space restrictions and changes in the way the animals held their wings during stridulation often prevented a perfect orthogonal alignment of the laser beams to the vibrating surfaces, resulting in absolute

displacement amplitudes that are hard to compare between wings. Relative phase relationships between the wings, however, can be measured with high accuracy, as they are not affected by laser beam–target orthogonality. In theory, mathematical superposition of LW and RW vibrations allows estimating the resulting combined output vibration. For example, the net vibratory response at a given place and time caused by the two harps is the sum of the responses which would have been caused by each harp individually (Fig. 5AB). These calculations show that the greater the phase lag φ (and thus time lag Δt for a given frequency; cf. Figs. S1 & S2) between LW and RW, the lower the amplitude of the resulting vibration and therefore the gain as compared to using only one wing (Fig. 5AB and C). Without exact amplitude information for engaged wings, we can nevertheless show the effect of phase shifts between wings on the overall output amplitude assuming that vibration amplitudes are equal for both wings (as shown in Fig. 5A). Thus, Fig. 5C shows normalised RMS (root mean square) gain as a function of phase lag φ of three different wing regions using normalised vibration velocity amplitudes. In ideal conditions, where both wings exhibit equal vibration amplitudes at equal frequencies, perfect phase locking (φ=0°) produces a gain of 2, while a phase lag of 120° (Δt=67 µs at 5 kHz) would produce a gain of 1 or no amplification of the resulting output as compared to using only one resonating wing. For example, the phase lags recorded from left and right harps (median values ranging from 6º to 79º across all specimens; this equates to Δt values between 3-43 µs; see Fig. S1, S2) produce relative amplitude gains ranging from 1.97 to 1.34 (Fig. 5C, blue stars). Other wing regions (mirror and anal regions, red circles and yellow squares, respectively), exhibit similar values.

Fig. 5D illustrates the effect imperfect coupling of the wings has on the overall combined output amplitudes at different song carrier frequencies (assuming both wings vibrate with the same frequency and amplitude). While animals producing pure-tones at 5 kHz can afford to have relatively uncoupled wings with time lags up to ~67 µs before destructive interference occurs (Fig. 5D, intersection of blue and grey dashed lines), Δt at which destructive interference starts is reduced to ~48 µs and 34 µs when singing at 7 or 10 kHz, respectively (red and yellow lines). The inset in Fig. 5D showing the average time differences and standard deviations between wings for the 11 specimens recorded shows that the span of Δt values (like φ) is generally small enough to ensure amplitude gains well over 1.5 when singing with a 5 kHz carrier frequency.

4 Discussion

Here, we have revealed the presence of an elegant additional mechanism at work in crickets that contributes to generating high amplitude, pure tone signals using distinct yet coupled sound generators: the two forewings and their cellular structures. Although the wings appear to be mirror images of each other (Fig. 1), they are asymmetrical in their mechanical properties and structure (Fig. 2A), as previously reported (Simmons and Ritchie, 1996; Bennet-Clark, 2003; Montealegre-Z et al., 2011). For *G. bimaculatus*, it is known that the RW on top is slightly larger in surface area and exhibits a higher f_0 than the LW (Montealegre-Z et al., 2011).

In addition, differences in resonant properties between both wings and among single wing regions are characterised in some detail. The biomechanical data demonstrate that, within a single wing, different regions have variable resonance peaks close to that of the harp f_0 value and overall resonance curves also differ in their spectral composition (Fig. 3). Interestingly, the observed differences between both the individual wing regions and between the wings themselves (Fig. 3A), vanish when the wings engage in active stridulation (Fig. 3B). These results confirm for the first time that all regions of both wings actively radiate sound at the carrier frequency during stridulation and that the resonance

properties of the LW dominate the frequency output. This suggests that, during stridulation, the LW harp vibrations, generated through plectrum-teeth impacts, drive the vibrations of all other wing regions, including those of the RW, so that the engaged wings vibrate together at the LW f_0.

In order to produce the best possible signal output from both coupled resonators, we hypothesised that both wings and the wing regions therein should not only oscillate at one common frequency, but also, ideally, in-phase (φ=0°), thereby creating maximal constructive interference (and thus a two-fold amplitude gain as compared to using only one wing). Whilst the whole system is indeed driven and oscillating at one specific frequency, we find considerable incoherence in the phase relationships between LW and RW and their respective regions. Figures 4 and 5 clearly show that individual wing regions are not phase-locked to each other but exhibit average phase differences φ ranging from ca. 6° to 79°, equating to temporal differences Δt between the wings of 3-43 µs at the carrier frequency (f_c=5.125 kHz). Figs. 4 and S1 also show that individuals exhibit roughly similar phase differences within their wing regions but phase shifts between individuals are quite variable. This leads us to suggest that the ability to tightly control the wing movements and the coupling of the resonators is an individual trait depending on either wing morphology or neuro-muscular control of the stridulation process or a combination thereof. As a consequence, the phase differences φ and corresponding time lags Δt seen across the recorded individuals would approach the distribution of this trait over the population.

Fig. 5AB depicts the consequences of these phase shifts in two male crickets on the opposite sides of the range of observed φ. While the lower φ of Male 1 (=φ5.3Δ ,°t=2.9 µs, Fig. 5A) results in a considerable output gain in comparison to the individual harp amplitudes (ca. 1.85 times the highest LW amplitude), the higher phase differences of Male 2 (φ=58°, Δt=28 µs, Fig. 5B) result in only a moderate gain (ca. 1.3). For this animal, a further increase in φ and consequently Δt would result in destructive interference, whereby the combined output of both wings would be less than the output of one wing alone, negating the advantage of using coupled resonators. This is shown in more detail for three major wing regions over all animals in Fig. 5C. It is noteworthy that no instance of destructive interference was observed in the specimens studied.

Fig. 5D shows the effects frequency has on the overall gain of this imperfect coupling in the temporal domain. While a cricket singing at 5 kHz will experience an increase in combined output amplitude (gain>1, above dashed grey line, Fig. 5D) for temporal differences between the wings of up to 67 µs (corresponding to a 120° phase shift and assuming equal vibration amplitudes), crickets singing at higher frequencies will encounter this threshold much earlier (at 48 µs and 33 µs for 7 kHz and 10 kHz, respectively). Consequently, the animals' observed inability to tightly synchronise the wing movements in time will act as an acoustic constraint for crickets to exploit higher song frequencies using two (imperfectly) coupled resonators. In addition, Fig. 5CD demonstrate that the observed imperfections in wing coupling in *G. bimaculatus* are still sufficiently low to ensure theoretical amplitude gains well above 1.5 times in comparison to the output of one wing alone. It is unknown, however, if φ and Δt are, for example, dependent on temperature. Due to the clockwork escapement mechanism involved in stridulation (and different from wing motion dynamics; Prestwich and Walker, 1981) tooth strike rates and f_c are largely independent of temperature in many Gryllidae, as are the resonant properties of the wings (Elliott and Koch, 1985; Bennet-Clark and Bailey, 2002). However, some species can show slight changes in f_c with temperature. Furthermore, the temporal song patterns, including syllable duration, are often affected by changes in ambient temperature (Pires and Hoy, 1992; Walker and Cade, 2003). It would therefore be conceivable that φ is also temperature dependent, potentially increasing with temperature and changes in singing behaviour. Further experiments

including other cricket species and varying recording temperaturesare planned to address inter-species variability and temperature dependence of the animals' wing coupling abilities.

If the higher values of Δt we observe in *G. bimaculatus* (Fig. 5D for averages and std; see Fig. S2 for a depiction of the range of observed values across all animals) are an indicator for the minimal amount of temporal control crickets in general are able to exert during stridulation, then one can attempt to calculate a cut-off frequency above which the sound production with two symmetrical and coupled wings becomes inefficient. The highest median value for φ we measured for the three wing regions were between 72° and 80°, equating to Δt values between 38-43 µs at f_c=5.125 kHz. Using simple trigonometric relationships between phase, amplitude, Δt and frequency of waves and under the simplified assumption that both waves have the same frequency and amplitude, one can calculate the frequency f_{max} at which the gain of the combined output of the superimposed waves becomes 1:

$$f_{max} = \arccos\left(\frac{Gain}{2}\right) / (\pi * \Delta t) \quad (1)$$

Using (1) and the range of Δt stated above, theoretical f_{max} values range from 7.8 kHz to 8.8 kHz (for 43 µs and 38 µs, respectively), denoting frequencies above which stridulation using the mechanism described above becomes inefficient for some animals in the population. Taking the mean and standard deviation values for Δt shown in Fig. 5D as rough population measure (harp: 19.3±14.1 µs; mirror: 23.9±10.8 µs; anal region: 18.7±12.5 µs; see also Fig. S2 & S3), one could state that ~16% of males would not be able to produce song above ~10 kHz with an amplitude gain above 1 when using both wings as active resonators.

These cut-off frequencies correspond very well with maximal carrier frequencies observed in the majority of Gryllidae, which lie between 2-8 kHz (Bennet-Clark, 1989; Robillard et al., 2015). A notable exception are members of the subfamily Eneopterinae, which produce calling songs with frequencies of up to 26 kHz (Robillard et al., 2013). Interestingly, in this subfamily, there is a clear gap between species singing at low frequencies and species singing at high frequencies. This gap is located between 7.9 and 12.2 kHz and members of the high-singing species form a distinct clade within the Eneopterinae (the Lebinthini) (Desutter-Grandcolas and Robillard, 2004). Additionally, Robillard *et al.* found that these species exhibit resonance patterns and stridulation mechanisms quite different to the ones employed by other Gryllids and other Eneopterinae (Robillard et al., 2013). Here, the resonances in the LW and RW are clearly asymmetrical, only partly (or not at all) overlapping the carrier frequencies and they generally show lower vibration magnitudes when compared to e.g. the wings of *G. bimaculatus*. Furthermore, instead of employing constant tooth strike rates (like *G. bimaculatus* and most other gryllids), some Lebinthini employ a stridulation mechanism (resembling those commonly observed in bush-crickets) whereby the wing stops during the closing phase to build up elastic energy which is then quickly released to produce highly increased tooth strike rates and therefore higher frequency calls (Robillard et al., 2013). These adaptations for high-frequency song production are similar to those encountered in bush-crickets. In bush-crickets, the wings are generally highly asymmetric as well, both morphologically and acoustically: The LW (lying on top of the RW and bearing the active stridulatory file) is often thicker, usually shows no clear stridulatory fields and is highly damped, therefore playing only a minor role in sound radiation (Montealegre-Z and Postles, 2010; Baker et al., 2017). The RW on the other side (which receives its mechanical input vie the plectrum) often exhibits extremely thin to translucent stridulatory fields with clear resonance properties, thus constituting the acoustically active wing (e.g. Sarria-S et al., 2014; Baker et al., 2017). Thus, the sound production system in Tettigoniidae only contains one resonator, reducing the surface for sound radiation, whilst eliminating the problems inherent to two imperfectly coupled resonators as described here for crickets. This allows for a shift to higher song frequencies (and shorter wavelengths)

without destructive interference from a second resonator, and simultaneouslyensures that the size of the remaining resonator is still (closer to) optimal for pure tone sound radiation.

In conclusion, the results presented here suggest a mechano-acoustical constraint on the bilateral near-symmetrical, dual resonator sound production mechanism common to most Gryllidae which prevents the exploitation of higher song frequencies above ~8-9 kHz whilst still being able to produce loud and pure-tone calling songs to effectively attract mates. This could have been an important constraint for the majority of Gryllidae (restricting them to the role of tenors) which the Tettigoniidae (the sopranos within the Ensifera) seem to have overcome by evolving a highly asymmetric singing mechanism (Montealegre-Z et al., 2017; Song et al., 2020) which allows them to produce high-frequency songs without the drawback of undesirable destructive interference reducing song amplitude.

5 Author contributions

F.M.-Z., T.J., and D.R. designed research; F.M.-Z., and T.J. performed experiments; CS conducted statistics; F.M.-Z., T.J. and CS analysed data; and F.M.-Z., T.J. and D.R. wrote the paper. F.M.-Z. and T. J. contributed equally to this work.

6 Conflict of interest

The authors declare no conflict of interest.

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10 Figure captions

Fig. 1: Extended tegmina of *Gryllus bimaculatus*. (*A*) The main regions involved in sound production are highlighted. Nomenclature of wing regions follows Montealegre-Z et al. (2011). (*B*) The problem of phase interference during tegmino-tegminal stridulation. For the left, plectrum-bearing wing (PBW), energy from tooth impacts will travel a constant distance (D) from the plectrum region to a specific region of the same wing (e.g., the red dot; arbitrarily chosen). Conversely, for the file-bearing right wing (FBW), the point of energy input will change as the scraper moves over the file. Energy will travel different distances (D1, D2, D3), reaching the red dot at variable times t, resulting in varying phases of vibration as the scraper moves.

Fig. 2: Amplitude response of extended wings to sympathetic acoustic stimulation. (*A*) Orientation image relating tegmen topography to the position of the scanning lattice. (*B*) Scanned area and deflection shapes of the tegmen dorsal surface (harp and mirror). Dashed lines illustrate the sections through which the deflection envelopes in C were built. (*C*) Envelope of mechanical deflections along transects shown in B for a series of phases (in steps of 10º) in the full oscillation cycle. For this specimen: RW f_0=4.71 kHz, LW f_0=4.62 kHz).

Fig. 3: Wing region resonances of unengaged and engaged wings of a male *G. bimaculatus*. (*A*) Natural resonances of wing regions measured with LDV in unengaged wings. (*B*) Wing resonances measured in the same individual during stridulation (engaged). Vibration amplitudes have been normalized to a relative dB scale.

Fig. 4: Vibration displacements and phase relation in three major wing regions during stridulation in two *G. bimaculatus* males. Wing vibration measurements were obtained simultaneously from two homologous wing regions using two LDVs. (*A*) An individual with nearly perfect phasing of the wings (median φ between 6º and 15º). (*B*) An individual with more prominent phase differences and variation between the wings (median φ between 60º and 68º). Each panel represents an independent recording showing RW in blue, LW in red and phase lag φ in grey. φ is measured as the difference in phase between LW and RW at the LW local maxima and minima. Boxplots show the median (red line), 25th, 75th percentiles (box) and 1 IQR whiskers for all φ per wing region. Outliers are marked as red +.

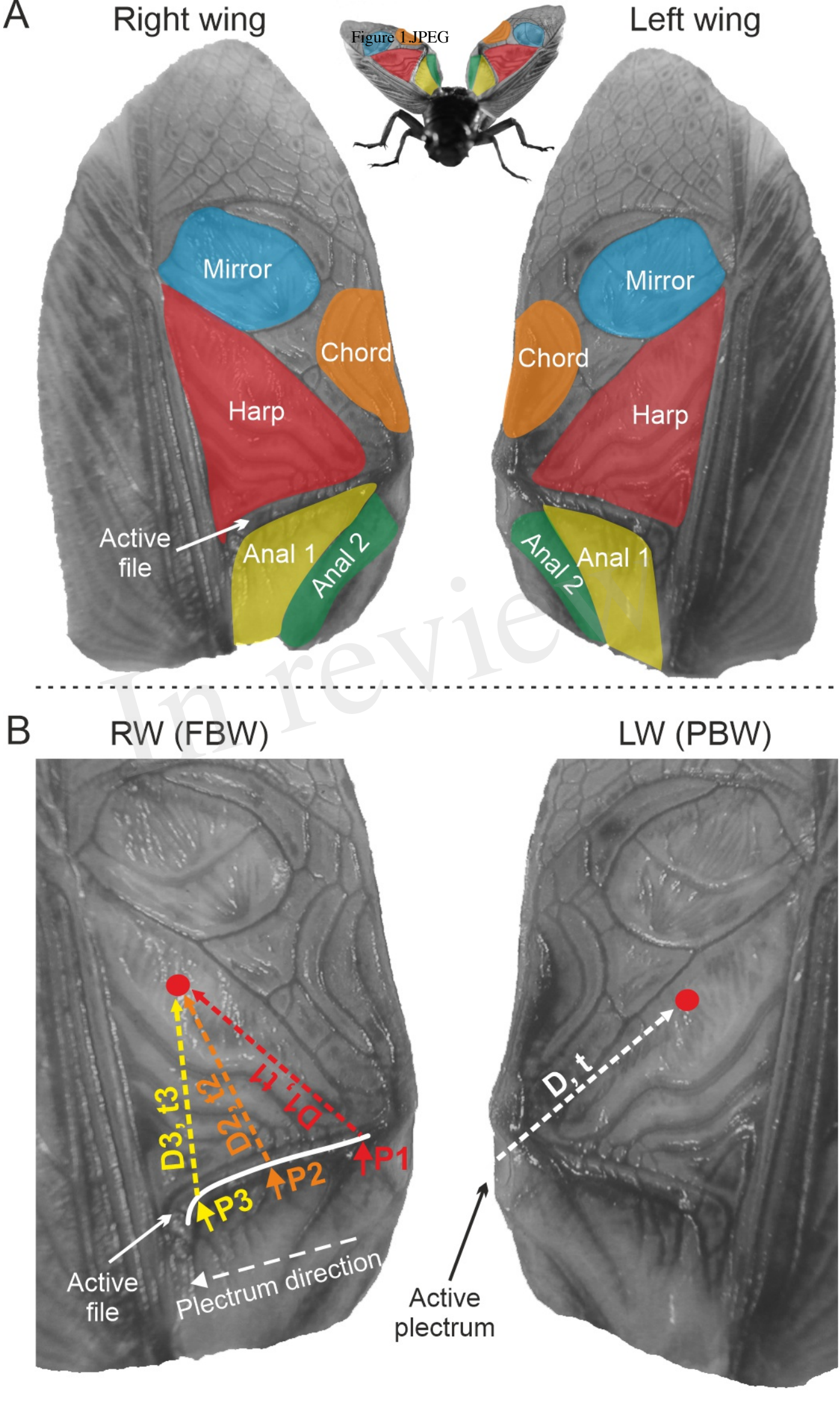
Fig. 5: Sound wave superposition to illustrate amplitude gains. (*A*) Theoretical harp output calculated from a *G. bimaculatus* showing small phase differences between both harps (φ~5º; Δt~3 µs; f_c=5.07 kHz). (*B*) Harp output from an individual with large phase differences (φ~58º; Δt~28 µs; f_c=5.7 kHz). Note that in spite of large phase differences, the output (black outline) shows a gain, which is larger in A. In both cases, tracks have been normalised to the highest amplitude. (*C*) Comparison of median absolute phase lag per specimen and RMS gain of three major wing regions. Vibrations were obtained simultaneously from the paired respective regions (harps, mirrors and anal) of LW and RW. RMS gains were calculated from the superposition of normalised LW and RW displacement responses measured with each laser. Each data point per region represents one individual; n=11. The solid line shows theoretical gains with increasing φ assuming equal vibration amplitudes and frequencies. (*D*) Mean absolute time lags Δt (black circles) and standard deviation between LW and RW for three major wing regions and 11 animals. Coloured solid lines show the theoretical amplitude gains (right y-axis in grey; equal amplitudes and frequencies) as function of Δt for three different carrier frequencies (blue, red and yellow for 5, 7 and 10 kHz, respectively). Values below 1 (dashed grey line) signify lower combined output amplitudes compared to using only one resonator.

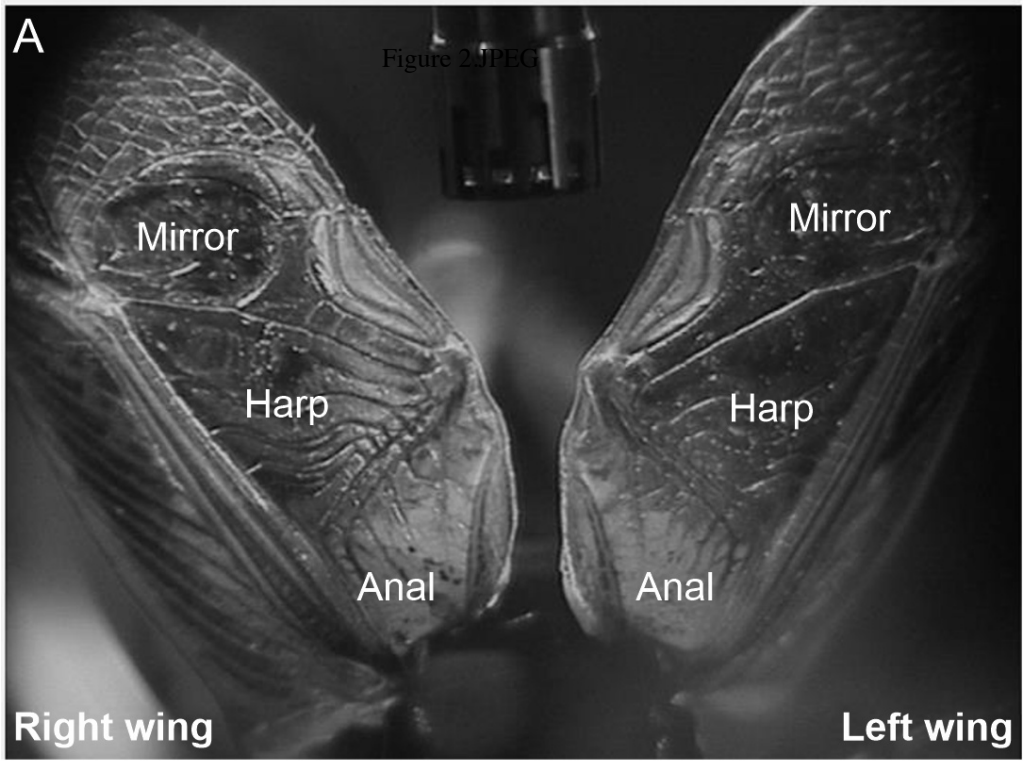
11 Video captions

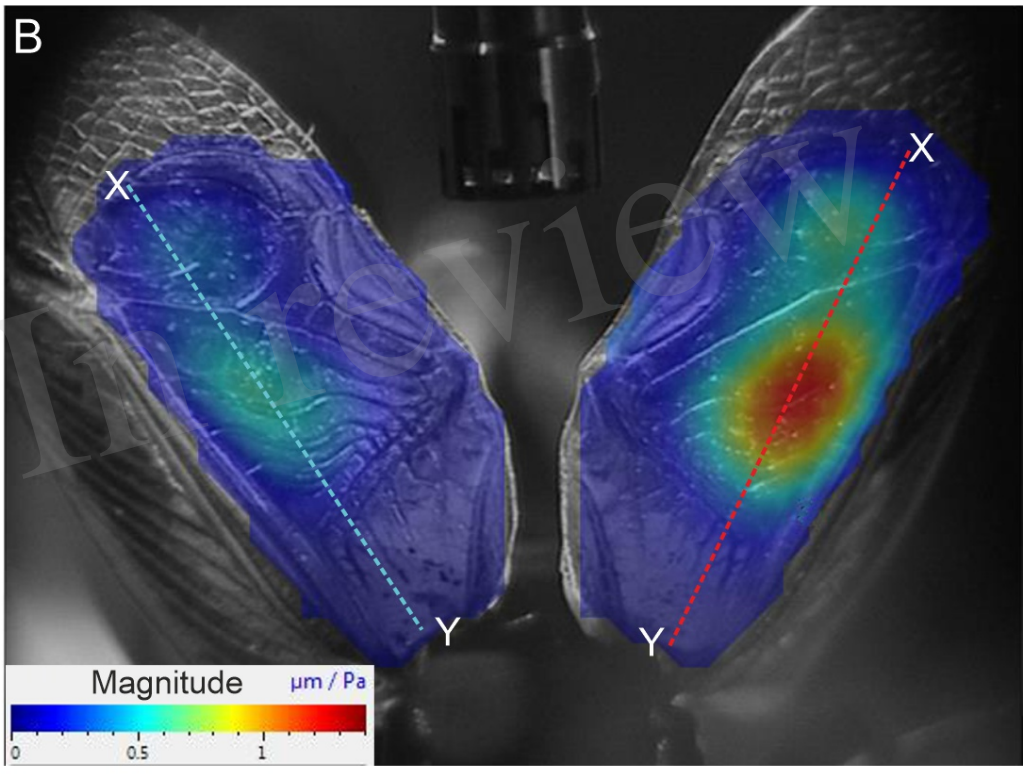
Video 1: A male *Gryllus bimaculatus* producing calling song in the experimental setup after pharmacological injection of Eserine (10^{-2} mol/l) into the brain. The cricket is mounted and fixed on a holder in front of the LDV. The LDV's laser dot is visible on the harp area of the right wing.

Video 2: Animation of the vibration map of unengaged left and right wing of a male *Gryllus bimaculatus* as derived from LDV recordings. The wings are elevated upwards from the animal's body at a similar angle to the natural singing position, spaced apart and imaged from the front; the reference microphone is visible between and slightly behind the wings. The overlaid vibration map shows the colour-coded relative displacement (µm/Pa; red=max. positive displacement; blue=max. negative displacement) of the wing surface as a response to acoustic stimulation at the wings' overall resonance frequency (4.62 kHz). Here, the LW displacement amplitude is higher than the RW's.

Figure 1.JPEG







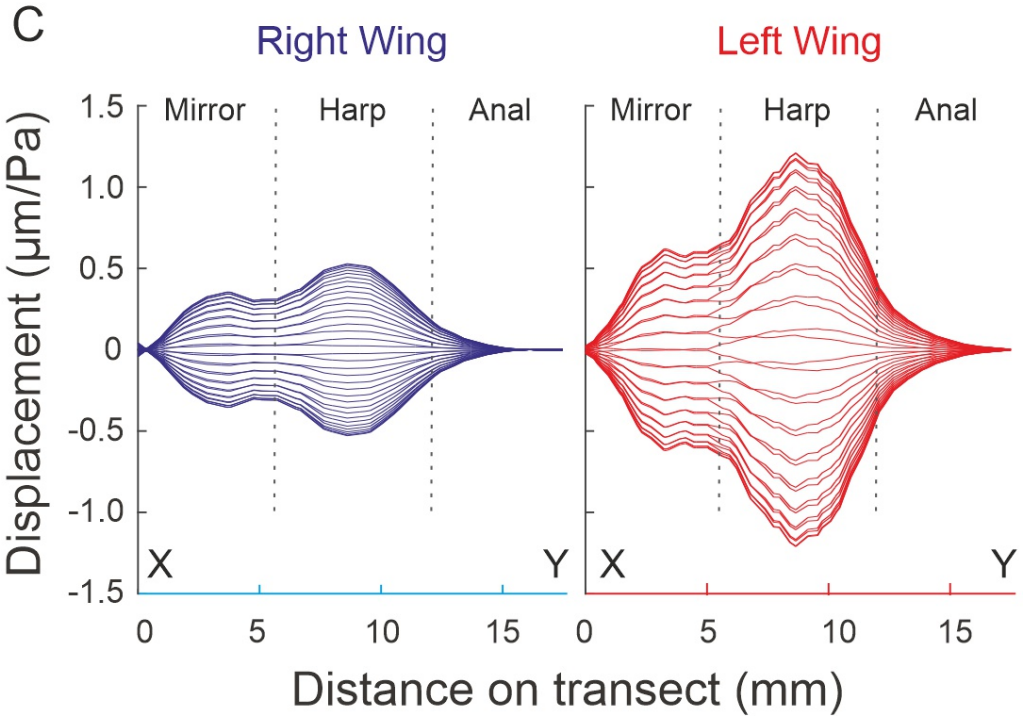


Figure 3.JPEG

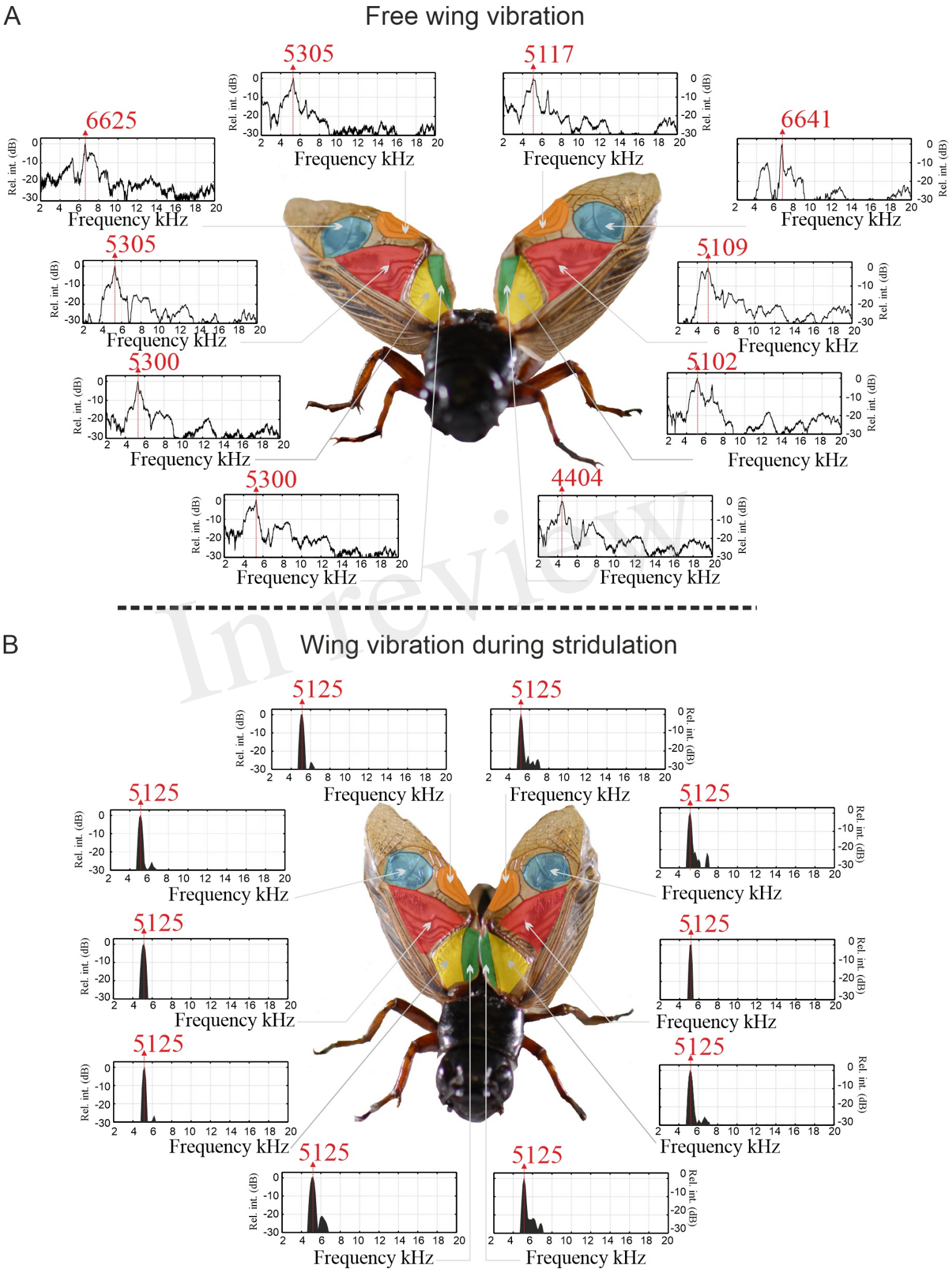


Figure 4.JPEG