Reverse stridulatory wing motion produces highly resonant calls in a neotropical katydid (Orthoptera: Tettigoniidae: Pseudophyllinae)

Fernando Montealegre-Z *

School of Biological Sciences, University of Bristol, Woodland Road, Bristol, BS8 1UG, UK

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ABSTRACT

This paper describes the biomechanics of an unusual form of wing stridulation in katydids, termed here 'reverse stridulation'. Male crickets and katydids produced sound to attract females by rubbing their forewings together. One of the wings bears a vein ventrally modified with teeth (a file), while the other harbours a scraper on its anal edge. The wings open and close in rhythmic cycles, but sound is usually produced during the closing phase as the scraper moves along the file. Scraper-tooth strikes create vibrations that are subsequently amplified by wing cells specialised in sound radiation. The sound produced is either resonant (pure tone) or non-resonant (broadband); these two forms vary across species, but resonant requires complex wing mechanics. Using a sensitive optical diode and high-speed video to examine wing motion, and Laser Doppler Vibrometry (LDV) to study wing resonances, I describe the mechanics of stridulation used by males of the neotropical katydid *Ischnomela gracilis* (Pseudophyllinae). Males sing with a pure tone at ca.15 kHz and, in contrast to most Ensifera using wing stridulation, produce sound during the opening phase of the wings. The stridulatory file exhibits evident adaptations for such reverse scraper motion. LDV recordings show that the wing cells resonate sharply at ca. 15 kHz. Recordings of wing motion suggest that during the opening phase, the scraper strikes nearly 15,000 teeth/s. Therefore, the song of this species is produced by resonance. The implications of such adaptations (reverse motion, file morphology, and wing resonance) are discussed.

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1. Introduction

Stridulation, sound production by rubbing body parts, is regularly used by insects, and the Orthoptera (e.g., Ensifera and Caelifera) are probably one of the most well-known examples. In these groups sounds are usually generated by males, mainly for the purpose of mate attraction (Gwynne, 1977, 1982). In Ensifera (e.g., Gryllidae, Gryllotalpidae, Tettigoniidae and Haglidae), with some exceptions, sounds are produced on a tegmino-tegminal basis, rubbing together specialised regions of the forewings (tegmina) (Morris, 1999). Typically, one wing bears a row of teeth, the stridulatory file, and the other harbours a scraper. The scraper is swept along the file to produce a series of stimuli (strikes) against the file teeth. These strikes create vibrations that are subsequently amplified by wing cells specialised in sound radiation.

During sound production the forewings show two phases of motion, opening and closing, repeated in cycles, but the main amplitude and temporal components of the call are usually produced during the closing phase (Heller, 1988; Koch, 1980; Kutsch, 1969; Morris and Pipher, 1972; Pasquinelly and Busnel, 1954; Pierce, 1948; Suga, 1966; Walker, 1975). A complete cycle of wing movement is termed phonatome (Leroy, 1966; Walker and Dew, 1972). I will refer to this form of stridulation (sound production during closing phase of the wings) as conventional wing stridulation.

The wing generator is therefore a frequency multiplier. The scraper on the posterior edge of the contralateral wing engages with the teeth on the file, and multiplies the low wing-closing frequency (usually of only a few hertz) to the song frequency produced by tooth impacts (Michelsen, 1998; Michelsen and Nocke, 1974).

Sustained pure-tone calls are common in Ensifera using tegminal stridulation, from the low audio to nearly 40 kHz. In these species, the number of teeth struck by the scraper corresponds to the number of sustained oscillations in the sound pulse (Bennet-Clark, 1999a; Koch et al., 1988; Montealegre-Z and Mason, 2005; Montealegre-Z and Postles, 2010). This one-to-one relationship is attained by a resonant mechanism, where the number of teeth struck per unit time matches the wing resonant frequency (f_o). A particular characteristic of these species is that tooth spacing gradually increases from the

anal to the basal file region, i.e., in the direction of the wing closure (Bennet-Clark, 2003; Koch et al., 1988; Montealegre-Z, 2005; Montealegre-Z and Mason, 2005; Prestwich et al., 2000).

Although males of most species produce the main amplitude component of the song during wing closure, in some species low amplitude sounds are also generated during the opening phase of the wings (Morris and Walker, 1976). To distinguish these from sounds made during the closing phase of the wings, I will call them minor emission (sounds produced during the opening phase) and major emission (sounds produced during the closing phase). These minor emission sounds preserve a broadband nature when compared with major emission sounds (reviewed in Montealegre-Z, 2005). In other species minor emission sounds have been adopted as part of the acoustic repertoire, so that the complete call is composed of a rapid sequence of sounds produced in both phases of the wing cycle (opening and closing). The combination of both might help to prolong phonatome duration used for species recognition (Deily and Schul, 2004).

Here, using a highly sensitive opto-motion detector, high-speed video, and a Laser Doppler Vibrometer (LDV), I document the mechanism of reverse stridulation in *Ischnomela gracilis*, a large, slender katydid of the subfamily Pseudophyllinae. Males of this species produce short pure-tone calls, with two main frequencies peaking at 15 and 80 kHz, to attract their mates. This paper investigates if the carrier frequency of the call results from resonant stridulation, where the tooth impacts match the wing's f_o, as in most species using conventional wing stridulation. Finally, I show that file and scraper in *I. gracilis* are morphologically adapted for sound output during a reverse sweeping motion.

2. Methods

2.1. Acoustic recordings in the lab

Specimens were collected in the Parque Nacional Natural Gorgona, Colombia, in December 2003, and November 2009, and were transported to the University of Toronto (2003), and to the University of Bristol (2009), UK, where their songs were recorded with wide bandwidth equipment (1–100 kHz). Calling songs were simultaneously recorded with associated wing movements, as described below.

2.1.1. Recordings of stridulatory wing movement

Stridulatory wing movements and associated sound production were recorded from seven males. Sound production was monitored with a Brüel & Kjær (B&K) 1/4″ microphone type 4939. Wing movements were recorded using a highly sensitive opto-electronic device (Hedwig, 2000). The motion detector used, gives enough resolution to capture and extract the low amplitude vibrations caused by the tooth strikes; these are seen as small oscillations within the line tracing the low-frequency wing motion. A small piece of reflective tape (Scotchlite 7610 and 8850 retro-reflective tape (manufactured by 3 M and distributed by Motion Lab Systems Inc.) was placed on the left forewing and its position was monitored with the motion detector, as described by 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 on each channel, via a data acquisition board (USB-6251, National Instruments, Austin, TX, USA) using Labview Software (National Instruments; Austin, TX, USA). Digitised signals were low-pass filtered at 100 kHz to avoid aliasing. Power spectra and spectrograms were calculated in Matlab software (The MathWorks, Inc., Natrick, MA, USA). The temperature in the room was 23.9 ± 0.85 °C. Tooth strikes captured with the motion detector were then isolated from the wing motion by band-pass filtering

(1–30 kHz) the wing movement trace. Isolated tooth strikes were then used to study the phase relation between tooth impacts and sound vibration using Lissajous diagrams (see below).

Wing movements were also recorded using high-speed video (Redlake Motionscope PCI1000s, San Diego, CA, USA) from two of the specimens transported to Canada in 2003. The high-speed video system was synchronised with a computer data acquisition board (National Instruments PCI6023e, 16 bit, 200 kHz sampling rate) using Midas software (2000 Xcitex Inc. Cambridge, MA, USA) for simultaneous recording of sound production. Recordings were acquired at 1000 frames/s for high-speed video, and a sound sampling rate of 200 kHz. Specimens were put on an artificial perch and the camera was aligned and focused directly on the stridulatory field. A 1/4″ microphone (B&K 4939), connected to a B&K Nexus Amplifier (Type 2690), was directed to the specimen in dorsal view. Data were analysed frame-by-frame using MIDAS software. These recordings were intended to study the wing velocity during sound production and to identify the functional parts of the file.

2.1.2. Zero-crossing analysis

In order to make detailed comparisons of sound generation, anatomy of the stridulatory file and wing movements (see below), I analysed songs with the zero-crossing (ZC) v.7 programme provided by K.N. Prestwich. Songs were low-pass filtered with a 25 kHz cut-off to isolate the dominant frequency. Processed signals were saved as wave files and imported from ZCv.7. ZC analysis computes the signal frequency cycle-by-cycle by detecting the timing of zero crossings to compute the reciprocal of the period of individual cycles of sound production, and is therefore suitable for pure-tone signals. Some signals were low-pass filtered en vivo at 20 kHz to remove higher frequency components using a digital filter installed in the recording system. These filtered signals were used to study the cycle-by-cycle variation in carrier frequency (f_c) to investigate any association of the stridulatory file morphology and the instantaneous frequency of each pulse.

2.2. Forewing resonance

Seven specimens collected in November 2009 were transported to Bristol, UK, for laser vibrometry experiments. Wing resonance was measured in four males using a microscanning 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's controlling computer.

For the experiments, the entire stridulatory field in both tegmina was measured using 250–300 measurement points, as described by Montealegre-Z and Postles (2010). Tegminal vibrations were examined in the frequency domain in response to broadband acoustic stimulation (periodic chirp) in the range 5–100 kHz. The spectrum of the stimulus at 60 dB (re 20 µPa SPL) was corrected and flattened with an error of ±0.6 dB. The acoustic signals were generated by the PSV 300 internal data acquisition board (National Instruments PCI-4451; Austin, TX, USA), amplified using a Sony amplifier (TAFE570, Tokyo, Japan) and passed to a loudspeaker (SS-TW100ED; Sony, Japan) positioned 15 cm from the specimen. For recordings, an intact specimen was mounted on a silicone holder using metallic clamps to fix legs. The wings were laterally extended by fixing the axillary sclerites with bee's wax (see (Montealegre-Z et al., 2011), for details of the preparation).

The quality factor Q, is defined to be the ratio of the frequency of the peak response divided by the full-width of the frequency spectrum at the two points where its amplitude is $1\sqrt{2}$ times the peak value (Fletcher, 1992). Q can also be calculated from the time domain as π divided by the natural logarithm of the free decay of the oscillation, π/Ln decrement (Bennet-Clark, 1999b). Wing resonant

frequency (f_o) vs. call f_c; and Q measured from call spectrum and from wing vibration, were compared using standard 2-tailed *t*-test.

2.3. Morphology of the stridulatory apparatus

2.3.1. The stridulatory file

Before describing the stridulatory file of katydids, it is useful to mention some novel terminology (Fig. 1). The following glossary is adapted from that used to described the structure of wood saws (www.disstonianinstitute.com/glossary.html).

Tooth angle: The angle between the support face of a file tooth and the baseline. File on the left tegmen seen from a caudal aspect, and angle rotating clockwise (Fig. 1). This angle varies among katydid species but in most it would be acute, as shown in Fig. 1.

Baseline of teeth: An imaginary line connecting the bottom of each file tooth, parallel to the front of the file to the long-axis of the file.

Point line: An imaginary line connecting the points at the cusp of each file tooth, parallel to the base line of the file or to the long-axis of the file.

Height: The physical distance between the baseline and the point line.

Gullet: The space between a saw's teeth. The term can be used to refer to the space between teeth of the insect file.

Face of attack: the aspect of the tooth first encountered by the scraper.

Support face: the aspect of the tooth that is not struck by the scraper.

2.3.2. The stridulatory file

The stridulatory file was studied by Scanning Electronic Microscope (SEM) using a Hitachi electronic microscope at the Dept. of Zoology of the University of Toronto. Analysis of the file morphology was performed on digitised SEM photographs using the dimension tool of a drawing programme (Corel Draw X4, Corel Inc.). Inter-tooth spacing was measured from the edge of the cusp of one tooth to the cusp of the next tooth along the point line (Fig. 1).

The morphology of the scraper was also studied under electron microscopy. Parts of the tegmina containing scraper and mirror were dissected and then embedded in Spur's solution; transverse sections across the scraper were made with a microtome following Di Sant' Agnese and De Mesy Jensen (1984). See Montealegre-Z and Mason (2005) for details of the procedure. For comparative purposes, scraper morphology of other katydid species producing song by conventional wing stridulation was obtained from the literature and included in the analysis.

3. Results

3.1. Call description

The phonatome consists of a short pulse (8.31 ± 0.14 ms, *n* = 7, Fig. 2A and C); phonatomes are usually given in bouts of two with a duty cycle of 1.3–2.3% (Fig. 2A), between bouts the duty cycle is
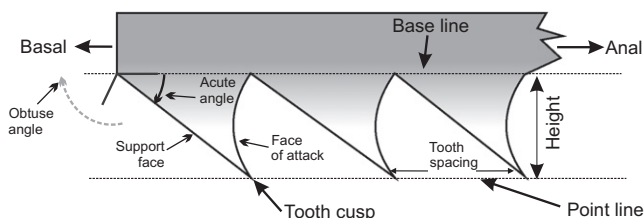

Fig. 1. Proposed nomenclature for the stridulatory file teeth structure and arrangement. File seen from a caudal aspect.
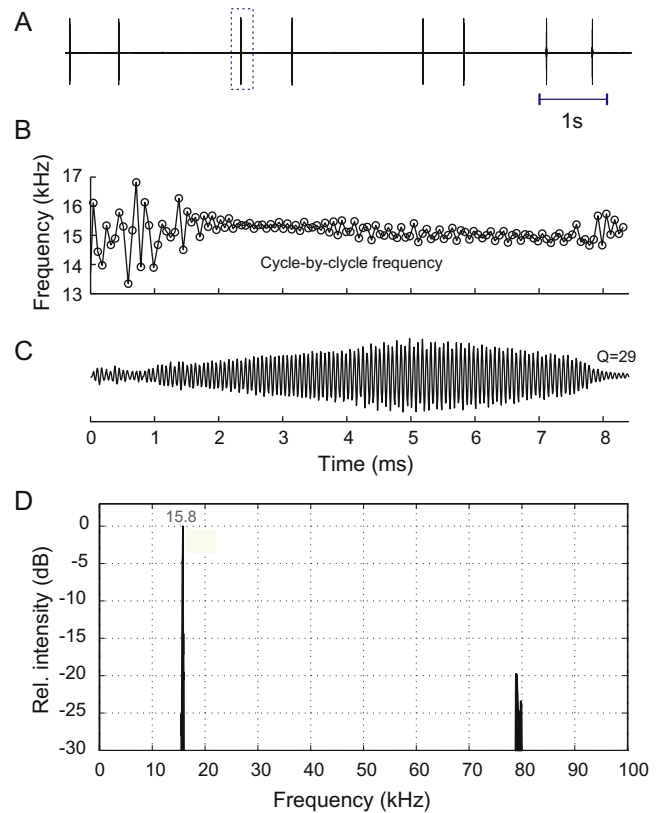

Fig. 2. Call analysis of *Ischnomela gracilis*. (A) A complete sequence of eight phonatomes. (B) Zero-crossing analysis of the pulse shown in C. (C) A song pulse extracted from A (dashed box). Q measured from the free decaying oscillations of the sound pulse. (D) Power spectrum of the pulse in B.

lower 0.2–0.25% (*n* = 7). At 10 cm dorsal from the stridulatory organ, the sound pressure level (re 24 μPa) was 91.86 ± 2.61 (*n* = 7). ZC analysis shows that the instantaneous frequency decreases for about 1 kHz, from 15.8 to 14.7 kHz, along the pulse (Fig. 2B). These short calls are audible tones at ca. 15 kHz (15.78 ± 0.16 kHz); a narrow amplitude peak in the high ultrasonic emerges near 80 kHz (Fig. 2D). The higher peak in the spectrum appears to be a 5th harmonic of the fundamental frequency. Similar frequency values of the call were reported by ter Hofstede et al. (2010), in males of this species collected in Panama. The quality factor Q, measured from the free decay of the pulse was 28.14 ± 1.68, SE 0.63, *n* = 7.

In the field males perch and sing from understory vegetation between 1 and 1.5 m. I did not observed these animals singing from spiny bromeliads as documented for its Panamanian congeners *Ischnomela pulchripennis* (Lang et al., 2006). Instead, all singing insects can be 'localised with some effort by a human listener. The seven males studied here were observed interacting acoustically in the field.

3.2. Stridulatory wing movements

Stridulatory movements and associated sound were simultaneously monitored and recorded using a highly sensitive motion detector and a condenser microphone. Different from most neotropical katydids recorded, males of this species clearly generate the calling song during the opening phase of the wings (Fig. 3A). The wings slowly open while the scraper traverses the file from the basal end to the anal end. At the anal end, the wings suddenly stop producing a silent phase without motion; at this stage the
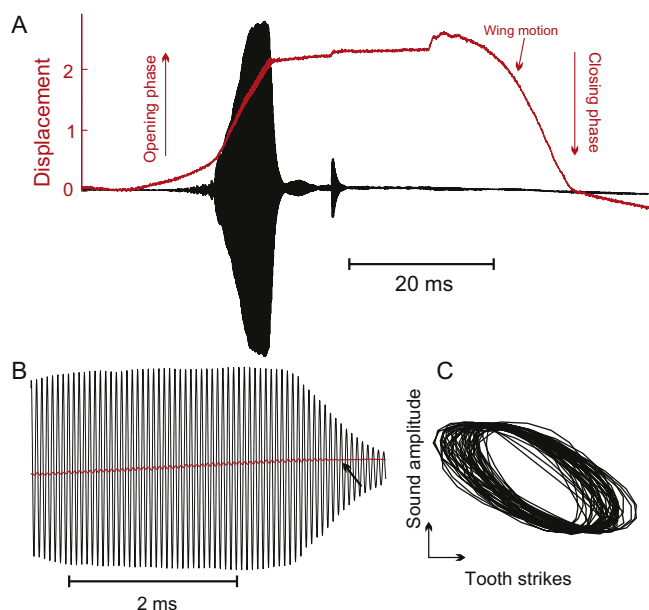
Fig. 3. Wing motion during sound production. (A) A single pulse (black trace) and associated wing motion (red trace). Notice that sound is produced during the opening phase. (B) High resolution of the same pulse and wing trace in A, correlating sound oscillations with tooth strikes. The wing trace contains the vibrations produced by tooth strikes and these are highly associated with sound oscillations. The arrow at the end of the pulse shows when scraper and file disengage. (C) Lissajous plot of tooth strikes and sound vibrations, when both signals have been band-pass filtered. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

scraper seems to have found a block (Fig. 3A, see Supplementary movie 1). The wings then are forced to break apart from this obstacle. In some specimens the process of separation occasionally produces incidental short clicks detected by the microphone, as observed in the recording shown in Fig. 3A; however the appearance of these click sounds was rare and variable, even within same individual.

The wing motion trace should contain the low frequency displacement of the wings in the complete phonatome but also the high frequency impacts produced by the interaction of scraper and file teeth. In fact, the impacts caused by this interaction are observed in the wing motion trace as low amplitude ripples. Oscillations in the sound pulse are highly associated with these ripples produced by tooth impacts (Fig. 3B). By band-pass filtering wing motion trace (to remove the low frequency component of the wing motion, as well as frequencies above 25 kHz), vibrations produced by tooth strikes were extracted and plotted against the sound vibrations obtained from the same recording. The result is the elliptical Lissajous figure shown in Fig. 3C. Lissajous figures tells us about the phase relation between the two signals (an input and output) and the ratio of their frequencies. Elliptical Lissajous figures only occur when the two signals being compared have the same frequency and a stable phase relationship (Koch et al., 1988; Gray, 1997). In other words, tooth strikes occur at a constant phase in relation to the wing vibration. In Fig. 3C both input frequencies are identical, but the punctual phase variance between them creates the shape of an ellipse.

Since the wing motion is measured as displacement per voltage, the speed of the wing cycle involved in sound production (opening phase) was calculated as the derivative of displacement over time (Fig. 4). Results show that the scraper is dragged in reverse with an average speed of 260 (±17, $n = 5$) mm/s. The speed of the opening phase is not constant but slightly decreases along the pulse by some 150 mm/s (Fig. 4).
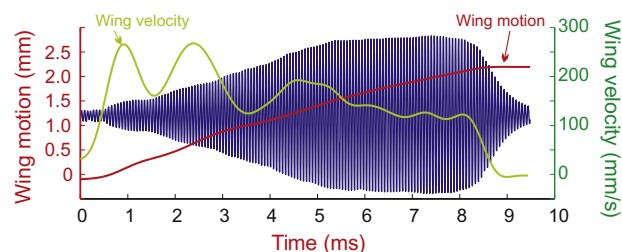

Fig. 4. Calculation of wing velocity during sound production (opening phase). The wing velocity (green outline) was estimated from wing displacement trace (red outline), as the change in motion in time, at 300 k-samples/s. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

3.3. Wing resonance

Five males were tethered in a holder with their wings extended, and the dorsal sides of both stridulatory fields facing the LDV scanning head. A speaker was positioned on the back of the specimen broadcasting broadband sounds between 5–100 kHz, and wing vibrations were monitored with the LDV system. These recordings indicate that the mirror and surrounding areas resonate at ca. 15 kHz (14.8 ± 1.2 kHz, $n = 5$), a second peak of lower amplitude is observed at 28 ± 1.8 kHz, and a third resonant peak, of smaller amplitude that the first peak as well, emerges at 77 ± 3.2 kHz (Fig. 5). Q measured from the spectrum of wing vibration was 26.4 ± 3.36, SE = 1.50, $n = 5$. This value was not significantly different from that calculated from the free decay of the song pulse (2-tailed t-test, $p = 0.26$). The left stridulatory area does not show a particularly sharp resonance, however vibrations of very low amplitude were observed around 20 kHz, 6× lower than the right wing f_o peak (Fig. 5).

There were significant differences between wing f_o and f_c ($f_o = 14.88 ± 0.31$, SE 0.14; $f_c = 15.8 ± 0.2$, SE 0.09; tailed t-test, $p = 0.00054$, $n = 5$), the wing f_o was always lower than the average value measured for f_c. The dominant peak at nearly 15 kHz corresponds to the f_c; the second resonant peak is observed in the calling song of a few specimens but usually 25 dB lower than the dominant peak. The third peak at nearly 80 kHz is a feature of all
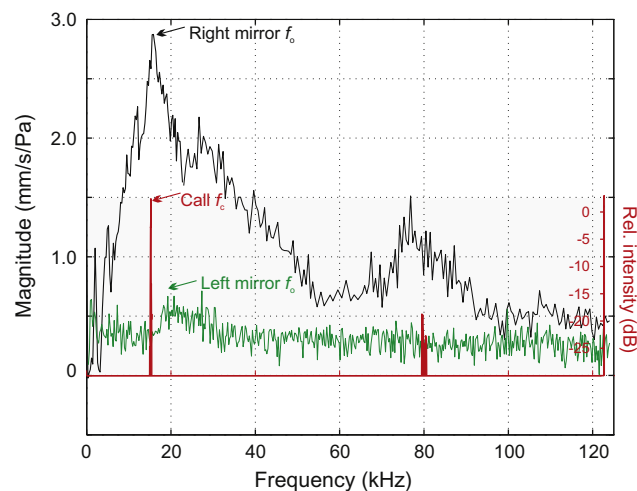

Fig. 5. Vibration of the stridulatory field of both wings in response to acoustic stimulation. The graphic shows the transfer function between surface velocity and sound pressure of the mirror and adjacent cells. Ghost area depicts the spectrum of the calling song (red outline) in the same specimen. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)
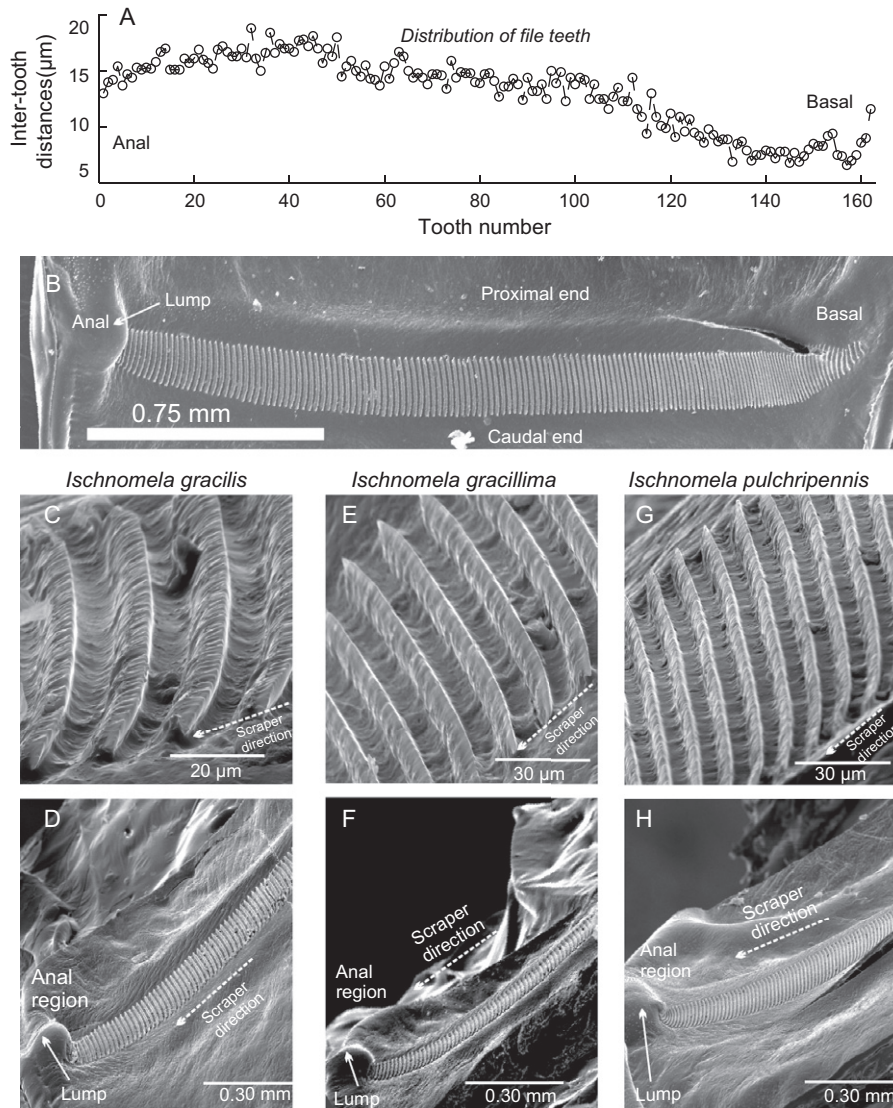
Fig. 6. Morphology of the stridulatory file of *Ischnomela gracilis* and congeners. (A) Inter-tooth spacing in the file of a male of *I. gracilis* shown in B. (B) SEM of the entire stridulatory file of *I. gracilis* (basal close to the wing hinge). (C, E, G) Close view of the file (mid region) of three species of *Ischnomela* showing tooth orientation; note that a basal orientation of teeth cusps. (D, F, H) Close view of the anal area of the stridulatory file in the same species. C, D: *I. gracilis*; E, F: *I. gracillima*; G, H: *I. pulchripennis*.

specimens studied, and relates to the highest-frequency peak observed in the call of the specimens studied.

3.4. Morphology of the stridulatory apparatus

3.4.1. The stridulatory file

The stridulatory file of *I. gracilis* is relatively short for the size of the specimens. Measured from the anal to the basal end, the file is 2.6 ± 0.21 mm ($n = 7$) and holds 160–162 teeth (Fig. 6A and B), of which some 140 are used in sound production. Tooth angle obtuse, producing a basal orientation of the tooth cusps (Fig. 6C); which contrasts with the pattern shown by most katydid species studied so far (Heller, 1988; Montealegre-Z, 2005; Montealegre-Z and Morris, 1999). Tooth spacing increases gradually from the basal end to the anal end (Fig. 6A), and this feature also differs from what has been observed in most katydid species producing pure-tone calls. On the anal end the file possesses a lump, which seems to work as a stopper or break to control scraper motion (Fig. 6D).

Since most of the file is used for sound production, the lump prevents complete separation of both wings during the opening phase (see Section 4, and Supplementary Movie 1). These morphological adaptations of the stridulatory file are also observed in other species of the genus *Ischnomela* (Fig. 6E–H).

3.4.2. Morphology of the scraper

Fig. 7 shows cross sections of the scrapers of *I. gracilis* and two more katydids producing pure tones by conventional stridulation (i.e., during the closing phase of the wing motion cycle): *Panacanthus pallicornis* and *Artiotonus captivus*. The scraper anatomy of *P. pallicornis* and *A. captivus* was taken from the literature (Montealegre-Z et al., 2006), and is included here only for comparative purposes. The scraper of *I. gracilis,* as in most bushcrickets, is a strongly sclerotized edge of the anal margin; an extension of the scraper margin folds ventrally (folded part in Fig. 7, see also Montealegre-Z, 2005). In all three species an enlargement of the vein A3 backs the scraper region; in *I. gracilis* this vein is massive, while in *P. pallicornis* and *A.*
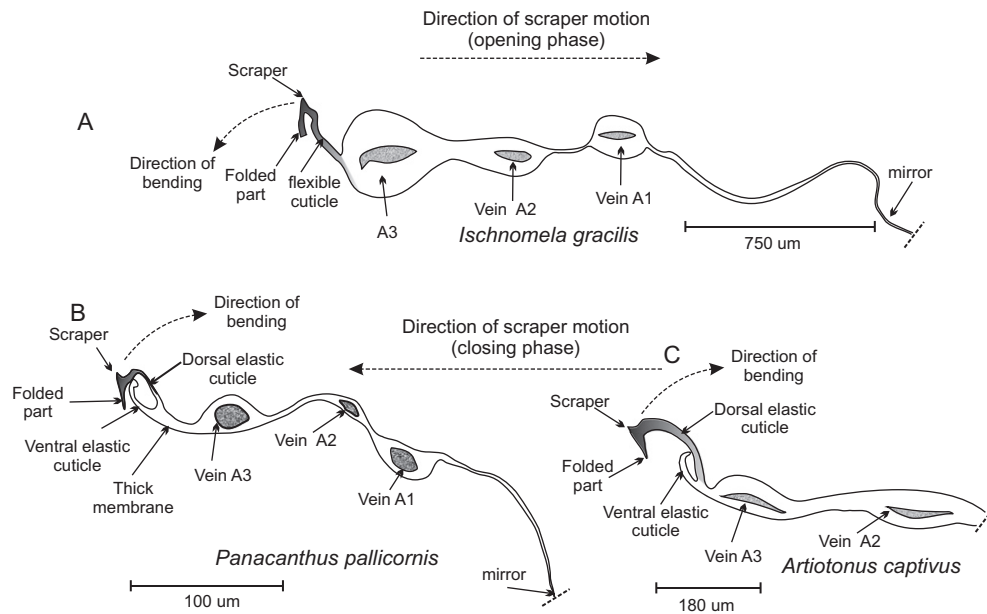
Fig. 7. The scraper morphology of *Ischnomela gracilis*, and that of other species producing calls by conventional stridulation. The line drawings show a cross section of the scraper region, as indicated in methods, seen from the anterior part of the wing. B, C, redrawn from Montealegre-Z (2005).

captivus A3 is of smaller diameter (Fig. 7B and C). In *P. pallicornis* and *A. captivus* a subsclerotized (sub-membranous) area is in between scraper and A3, this region is very reduced or almost absent in *I. gracilis*. There is also a small flexible region between A2 and the thin mirror frame A1 (Fig. 7A), which may allow bending during the interaction of the scraper-file. Two layers of cuticle, dorsal and ventral, converging in the anal margin of the tegmina, form the scraper region and are clearly seen in *P. pallicornis* and *A. captivus* (termed here dorsal and ventral elastic cuticles, Fig. 7B and C). These two layers are not clearly seen in the scraper of *I. gracilis*, they seem to have been fused into one cuticle layer.

4. Discussion

With a few exceptions, ensiferan species using tegmino-tegminal stridulation studied to date generate songs during the closing phase of the wings. This emphasis on closing loudly still allows males to produce either broadband or pure-tone calls, depending on the species. *I. gracilis* illustrated here, and its congeners, have evolved to produce calls on the opening of the wings, while maintaining high Q.

In species producing the main amplitude components of the call during the closing phase of the wing cycle, the muscles used for sound production are bifunctional, i.e. they are also used in flight and walking (Josephson and Halverson, 1971; Kutsch, 1969). The muscles that power the closing of the wings during stridulation are a group of dorsal longitudinals on each side, all of which insert onto the phragma; they therefore act indirectly (Josephson and Halverson, 1971). The main muscles responsible for the opening of the wing during stridulation, the basalar and subalar muscles, insert onto the wing and so act directly (Josephson and Halverson, 1971; Pfau and Koch, 1994). The closing phase of the stridulatory movement is produced by lowering the tergum (indirect muscles), whereas the opening phase depends on pulling against the subalar and basilar sclerites in the wing hinges while rotating the tergopleural arm on each side (Kutsch and Huber, 1989). So one would expect major sound output to be produced by contraction of the muscles causing the downstroke (direct muscles) of the wing during flight, as those generate more output power than the upstroke

muscles – most of the aerodynamic forces are produced during the down stroke (Alexander, 2002). But paradoxically during stridulation, these direct muscles control not the closing, but the opening phase. One then wonders about the relative mass of the antagonistic muscle sets of species using conventional and reverse stridulation: what is the relative development of the muscles that make the effective file-stroke in comparison to those for the ineffective return? Are indirect muscles usually larger than the direct in most katydids, is this situation reversed in *Ischnomela*?

It is not clear why stridulation evolved to employ the muscles used during the upstroke in flight (those with less power). The twitch of these opening muscles might permit a smoother control of the driving force to maintain the correct frictional interaction of the scraper and file than would the muscle twitch involved in the opening phase (those involved in the flight down stroke). The muscle twitch producing the closing phase might provide for a better control of the start and end of the scraper sweep on the file (Bennet-Clark, 2003; Kutsch, 1969; Pfau and Koch, 1994). For example, for sounds produced during wing closure the wings tend to accelerate, reaching maximum velocity towards the middle or last quarter of the file, and then they deccelerate, continuing by inertia until the closing is complete [e.g. *Gryllus campestris* (Koch et al., 1988); *P. pallicornis* (Montealegre-Z and Mason, 2005); *Gryllus bimaculatus* (Montealegre-Z et al., 2011)]. Therefore, during the closing phase the wings never experience erroneous overlapping as the scraper traverses the file and might gradually stop reaching its basal end. During the recovering phase (the opening) sensory feedback might control the limit of the separation to avoid unwanted wings overlapping in the next closings. Appropriate wing overlapping is critical in katydids as forewings are very asymmetrical and only one wing overlapping works for effective sound production. For instance, male crickets have special campaniform sensilla, which are located and oriented on the wing in such a way that they are able to measure thrust forces along the file (Schaffner and Koch, 1987a,b). Without information from the campaniform sensilla male crickets produce faulty calling songs (e.g., loss speed control during opening and closing). Although never searched for across species, campaniform sensilla have been observed in some katydids (Montealegre-Z and Morris, 2003).

A possible hypothesis as to why most Ensifera evolved to produce effective sound emissions primarily during the closing phase of wing movement is that a scraper moving basad on the file offers an even control of stridulation, and provides an obligatory stop at the body. The silent phase (the opening) on the other hand offers no such obligatory halt; wing halt on opening must be controlled by sensorial feedback.

The success of such adaptation (sound production during the closing phase) is easily seen in species producing calls with extremely high duty cycles (Heller, 1988; Josephson and Halverson, 1971; Koch et al., 1988; Walker, 1975; Walker et al., 1970; Walker and Dew, 1972). Males of these species produce the main amplitude components of the sound during the closing phase of the wings, and in spite of their extreme rates of wing movement, never swap wing overlap. At present I know of no katydid species that produces the major amplitude components of its call during the opening phase, and that at the same time, exhibits high duty cycles. The species shown to stridulate in reverse, all exhibit relative low duty cycles, including *I. gracilis*. The effective phase of wing motion for these species is the opening, during which the wings undergo a combination muscular forces and inertia, no doubt different to those involved in the silent opening phase seen in most ensiferan species.

An advantage to producing major song components during the opening phase of the wings is yet to be answered. At least three aspects of possible adaptiveness deserve consideration: (1) Changing the combination of muscular forces implicated in engaging both wings. During conventional sound production (sound on wing closing), one wing is lifted while the other is depressed so that the wings (the scraper on the file) advance against each other. In species using conventional sound production this combination of forces needs be maintained during a continuous closing phase controlled by closing direct muscles. For sound production during the opening phase the forces implicated automatically change in magnitude and direction. (2) Conspicuous change in the orientation and organisation of file teeth. Compared with most extant katydid species, the morphology of the stridulatory file has drastically changed in *Ischnomela* spp. to provide a better reversing engagement of scraper and file teeth during wing motion in the opposite direction (basal to anal), and in uniformity to maintain a constant rate of strikes during an accelerated effective opening phase. (3) High wing resonances. The above suggests that the generator structures in Tettigoniidae can evolve quickly in response to biomechanical selective pressures. *A phylogenetic* scenario of the genus *Ischnomela* and allies is needed to address this topic.

4.1. Reverse stridulation in Ischnomela and wing resonance

In most species employing resonant generation, tooth impact rate matches or closely approaches wing resonance [field crickets: (Bennet-Clark, 2003; Montealegre-Z et al., 2011; Montealegre-Z et al., 2009; Nocke, 1971); bushcrickets: (Bailey, 1970; Montealegre-Z and Mason, 2005; Montealegre-Z and Postles, 2010)]. *I. gracilis* produces a tone at ca. 15 kHz and a high frequency component at 80 kHz. Using a LDV, I showed here that these frequencies are discernible from the natural vibration of the wing, although wing f_o falls nearly 1 kHz below song f_c. Similar differences between wing f_o and song f_c have been observed in other katydids, e.g., *Copiphora gorgonensis* (Montealegre-Z and Postles, 2010). When the wings engage in stridulation, f_o of the wing radiator seems to increase to achieve the frequency value obtained in the song recordings.

I have also demonstrated that tooth strikes and sound vibrations at the fundamental frequency (15 kHz) conserve a similar phase relation during the entire pulse, and that the speed of the opening phase of the wings matches a tooth impact rate of nearly 15,000/s. Consequently, the sound generator is excited at its f_o as

the scraper is pushed by the wings at the speed necessary to hit ca. 15,000 teeth/s. This is confirmed by the Lissajous plot, which shows that the tooth impact rate and sound vibrations are phase-locked (Fig. 3C). The other high frequency resonances (30 and 80 kHz) observed in the wing vibration and in the calling song are likely to be excited by the silent 'tick-sound' produced during the catch and release of the scraper. Although speculative, such 'tick-sounds' are expected to take place at suitable phase points upon the fundamental vibration of the wing; therefore higher wing vibration modes occurring at these phases can be excited (Bennet-Clark and Bailey, 2002). The ear in this species is indeed more sensitive to ca 15 kHz, but a secondary range of low thresholds for high frequency sounds at 70–85 kHz is also evident (ter Hofstede et al., 2010). Therefore the high-amplitude peak observed in the calling song at ca. 80 kHz could also have a function in intra-specific communication.

4.2. The scraper

The importance of scraper morphology for sound production has been discussed by various authors (Bennet-Clark, 2003; Montealegre-Z and Mason, 2005; Montealegre-Z et al., 2006; Prestwich and O'Sullivan, 2005). From the particular cases offered by these authors and data collected from different species, it is evident that variation in scraper design is associated with file morphology and perhaps with certain characteristics of the sounds generated (Montealegre-Z, 2005). For instance, scrapers with large elastic cuticles are usually associated with ultrasonic frequencies and with pulse trains (Montealegre-Z and Mason, 2005; Montealegre-Z et al., 2006).

In Fig. 7 the scraper of species producing song during the closing phase presents a ventral reinforcement (the ventral cuticle layer), which might account for short bending during each tooth impact and so ensure that the maximum scraper distortion does not cause over-long scraper-file engagements or abrupt leaps (Montealegre-Z and Mason, 2005). The scraper of *I. gracilis* exhibits a different adaptation, possibly associated with its reverse stridulation: the ventral cuticle seems to be absent and the bending area is limited to the dorsal elastic cuticle (or a fusion of the two, Fig. 7A).

4.3. The stridulatory file

The stridulatory file of *I. gracilis* shows remarkable adaptations for stridulation produced during the opening phase of the wings, which are not observed in the majority of other katydids. For example, basal orientation of tooth cusps, anal increments of tooth spacing, and a lump or wing stopper on the anal end of the file. The genus *Ischnomela* incorporates three species: *I. pulchripennis* (Panama), *I. gracilis* (Ecuador and Colombia) and *I. gracillima* (Colombia); they all exhibit these features (Fig. 6C–H). This suggests that all three species use the same technique of stridulation.

In almost all species of katydids and crickets, examined by the author and others, the tooth angle is acute (Fig. 1) and tooth cusps always lean toward the anal region of the file (i.e., opposite to the direction (anal–basal) of scraper motion). At release, the scraper always hits the face of attack of subsequent teeth (Fig. 1). This morphology likely optimises engagement and augments the impacts caused by successive catches and releases of the scraper (Bennet-Clark, 2003). In contrast to this typical katydid morphological design the tooth angle of *Ischnomela* spp. is obtuse, and all tooth cusps lean toward the basal end of the file, i.e., face opposite to the direction of scraper motion (Fig. 6C, E, and G). This special tooth orientation favours engagements of a scraper that moves in the direction basal–anal. Therefore, in *Ischnomela* the support tooth face (Fig. 1) has switched to become the face of attack, and vice versa (Fig. 6C, E, and G).

The distribution of file teeth deserves discussion as well. In most species producing pure-tone calls during the closing phase of the wing cycle tooth spacing increases in the same direction as scraper motion, i.e. widening anal to basal. Conversely in *Ischnomela*, tooth spacing gradually increases towards the anal margin of the wing (basal to anal, Fig. 6A). This reversed morphology is unquestionably an adaptation for reversed scraper motion (in the direction basal to anal). As the speed of scraper motion gradually decreases during the formation of a song pulse (Fig. 4), it is likely that tooth-strike rate also decreases, causing the observed frequency modulation in the pulse (Fig. 2B). In conclusion, independent of the direction of scraper motion, for conventional and reverse stridulation, the intertooth spacing is essential to generating a pure tone, and the tooth angle fundamental for optimising scraper-file tooth engagement.

As the scraper moves in reverse, a lump on the anal region of the file (Fig. 6D, F, and H) arrests its motion, and prevents the wings from undue separation. Recordings obtained with the motion detector and HSV, show that the wings clearly pause at the lump's location, and occasionally a sound tick of small amplitude is produced (Fig. 3A, Supplementary Movie 1). One might hypothesise that the lump is needed to generate the sound tick occasionally observed. For instance, in some Phaneropterinae species the female responds acoustically to the male's call, and the male must find her phonotactically (Heller et al., 1997). The male produces two song elements, a complex one for recognition and a simple one (a short tick) for triggering the fast female response (Dobler et al., 1994). Because females of *I. gracilis* do not sing, and males are sedentary, the possibility that these final clicks occasionally observed in the male song function as a trigger for female response is ruled out. Therefore, I preferred to accept a mechanical explanation for the lump.

The lump or stop is necessary to avoid complete separation of the wings and subsequent erroneous wing overlapping during the ongoing cycle. An effective opening phase brings frictional forces and inertia, not involved in a silent opening phase, hence, the border line of wing separation in *Ischnomela* relies on mechanical control (the lump) and not on sensory feedback.

'Reverse' stridulation has been reported in a few European species of the genera *Phaneroptera* (Phaneropterinae) and *Uromenus* (Bradyporinae) (Heller, 1988). While very little is known about the morphological and mechanical adaptations of the sound generator in these species, it is known that they all produce broadband calls. Some of the morphological adaptations of the stridulatory file described here can be observed in species of the genus *Phaneroptera*. *Phaneroptera nana* for example exhibits a complex stridulatory file: a continuous large file segment extends between the basal and anal sides of the wing, but abruptly narrows and curves in the anal region, where another set of teeth are formed (Heller, 1988). This produces a file partition on the anal end, which in turn causes a discontinuous scraper motion. Such discontinuity might work as a scraper stopper. Although these details were not discussed by Heller (1988), from the SEM shown one can observe that the tooth spacing increases from the basal end to anal end, and that the tooth angle is also obtuse, just as in *Ischnomela*.

Acknowledgements

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.jinsphys.2011.10.006.

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