

The mechanics of sound production in *Panacanthus pallicornis* (Orthoptera: Tettigoniidae: Conocephalinae): the stridulatory motor patterns

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Accepted 27 January 2005

Summary

To examine whether sound production in katydids relies on an escapement mechanism similar to that of crickets we investigated the functional anatomy and mechanical properties of the stridulatory apparatus in the katydid *Panacanthus pallicornis*. Males of this species produce sustained pulses with a sharp low frequency peak of ~5 kHz and a broad band spectrum between 15 and 25 kHz. Simultaneous recordings of movement and sound indicate that the entire stridulatory file is used for sound production and there is nearly a 1:1 correspondence between the number of cycles in a song and the number of teeth on the file. There is an overall tendency for both the spacing of teeth to increase along the file and the velocity of wing closure to increase as the scraper traverses the file. There is considerable variation, however, in the evenness of tooth spacing and in the instantaneous velocity of wing closure during sound production. The production of sustained pulses appears to depend on resonance in the right tegmen, with the left tegmen acting primarily as a damping element. This resonance is not strongly coupled to the scraper and, unlike crickets, the timing of file-scraper interactions, and therefore the phasing of energy input to wing oscillations, is variable. Similarly, the quality of the sound spectrum

varies over the course of a single stridulatory wing-stroke. Based on measurements of tooth spacing on the stridulatory file and cycle-by-cycle frequency of sound output, we predicted the velocity of wing movement that would provide consistent phasing of file-scraper interactions with respect to sound-radiating wing oscillations and compared this with measurements of wing velocity. Acceleration of wing velocity during stridulation results in a closer match to the velocity required for optimal phasing during a portion of the call, and this corresponds with higher amplitudes of radiated sound and the excitation of higher order modes of vibration (evident as distinct harmonic peaks in spectrograms). Our results suggest that in katydid stridulation, the movement of the scraper along the file is not regulated by an escapement mechanism as it is in crickets. Instead, katydids that produce pure-tone songs sweep their wings over a range of velocities, within which some portion matches file tooth spacing to give optimal phasing of energy input to excite a resonance in the right tegmen.

Key words: sound production, katydid, *Panacanthus pallicornis*, resonance, stridulatory mechanisms, biomechanics, bush cricket, wing movements, stridulation.

Introduction

Males of several families of Ensifera (especially crickets, Gryllidae, and katydids, Tettigoniidae) produce calling songs by stridulation, i.e. rubbing together specialized regions of the forewings (tegmina). Typically, one wing bears a row of teeth, the file, and the other bears a scraper that is pushed along the file to produce a series of impacts with the file teeth. The mechanism of ensiferan stridulation is best understood through studies of a few species of cricket (Koch, 1980; Elliot and Koch, 1985; Bennet-Clark, 1987, 1989, 1999a, 2003). All cricket species studied to date produce pure-tone calling songs in the low audio range using resonant vibration of the tegmina (Bennet-Clark, 1987, 1989, 2003). The production of pure

tones in crickets depends on an escapement mechanism that couples the scraping of the stridulatory apparatus with the sound-radiating oscillation of wing membranes. This mechanism allows a controlled movement of the file and scraper, and a precise phasing of energy input to result in resonant vibration, i.e. the tooth contact rate matches the resonant frequency (f_0) of the tegmina (Nocke, 1971; Koch et al., 1988; Bennet-Clark, 1999a, 2003; Prestwich et al., 2000). In crickets the two tegmina are closely similar. The tonality and output power of cricket sound production depend on symmetrical vibration of the two wings. The mechanism of stridulation, however, is inherently asymmetrical, as one wing

is stimulated through the file (located on the underside) and the other through the scraper (located on the anal edge). Recent evidence indicates that this asymmetry in the roles of the two wings during stridulation is compensated by a subtle asymmetry in their mechanical characteristics to result in identical in-phase vibration of the tegmina (Bennet-Clark, 2003).

The mechanism of stridulation in katydids has received less attention (Bailey, 1970; Morris and Pipher, 1972). In contrast to crickets, katydids include both pure-tone and broadband signals (although most species produce broadband signals), and the fundamental frequency, f_D , of pure-tone katydid calls can range from the low audio up to extreme ultrasonic (>100 kHz; Mason et al., 1991). Moreover, katydids produce acoustic signals without the collateral vibration of the two tegmina, which in katydids are highly asymmetrical. It is unknown whether the existing model for stridulatory sound production (i.e. the clockwork cricket; Elliot and Koch, 1985) applies to katydids, or whether the same mechanism can also be used to produce broadband signals. Bailey (1970) concluded that the mirror frame (wing veins surrounding sound radiating membranes) was essential for pure-tone production in katydids, stating that it behaves as a tuning fork. Morris and Pipher (1967) suggested that the mirror frame vibrates as a cantilever, independent to the rest of the tegmen. More recently, Bennet-Clark (2003) speculated that pure-tone sound production in katydids was similar to that used by crickets, i.e. an escapement mechanism.

The genus *Panacanthus* is a monophyletic group of katydids with well-resolved phylogenetic relationships and in which a wide range of song types is represented (Montealegre-Z and Morris, 2004). In this paper, we investigate the mechanics of sound production in the katydid *Panacanthus pallicornis* Walker. In the phylogeny of *Panacanthus* this species succeeds *P. cuspidatus*, the most basal species in the cladogram. *P. cuspidatus* is the only species of the genus producing a pure-tone song (at 10 kHz), while *P. pallicornis* produces sustained sound pulses with a low sharply tuned frequency peak of ~5 kHz and also a broad band frequency range between 15 and 25 kHz (see sound description for more details). None of the other members of this genus produces sustained sound pulses or narrow spectral peaks. It thus appears that the evolution of signal characteristics in this genus has proceeded from a pure-tone ancestral condition to more broadband sound along the phylogeny (Montealegre-Z and Morris, 2004). The song of *P. pallicornis* appears to be intermediate between pure-tone and noisy song types. It has a dominant spectral peak in the audio range below 10 kHz, but also contains strong broadband components. In this paper we will focus on the characteristics of the sound produced, the anatomy and some physical properties of the stridulatory apparatus and the stridulatory motor patterns. We repeat some of the measurements made on crickets by Bennet-Clark (2003) to facilitate comparisons with that group, and address the question of whether similar stridulatory mechanisms can account for sound production in both katydids and crickets.

Materials and methods

Fieldwork

Panacanthus pallicornis Walker inhabits mountain forests in Western and Central Colombia (Montealegre-Z and Morris, 2004). Males have a high duty cycle and their calling song is one of the dominant sounds heard at night in their habitat. Specimens were collected in Colombia at the Reserva Natural de Yotoco (West of the Departamento del Valle del Cauca) between May 17 and June 10, 2001. Yotoco forest is a preserve of subtropical wet forest (3°52'N, 76°23'W; elevation 1400–1600 m) in the Departamento Valle, 50 km north of Cali, near Buga between the municipalities of Yotoco and Restrepo. It is also on the eastern slope of the western cordillera; the annual rainfall here is 1000–2000 mm. We used acoustic data recorded from specimens collected between April 1996 and June 2000 in Bitaco, which is a settlement close to Yotoco; climatic conditions are similar in both habitats.

Acoustic recordings and analysis

Acoustic recordings in the field

Males of *Panacanthus* spp. were located by their loud calls. Some specimens were recorded in the field with audio-limited equipment (1–20 kHz) using a Sony Walkman WM D6C Professional cassette tape recorder and ECM 909 Sony microphone. Ambient air temperatures were taken using either an Omega HH23 digital thermometer (Stamford, CT, USA) or an alcohol thermometer.

Acoustic recordings in the laboratory

Specimens were transported to the University of Toronto, Canada, where their songs were recorded with wide bandwidth equipment (1–100 kHz). These recordings were performed using Brüel & Kjær (B&K, Nærum, Denmark) equipment: a 1/4" (Type 4135) condenser microphone was connected to a sound level meter (Type 2204). Each insect sang from an individual small cylindrical cage constructed of aluminium screen (mesh size 6/cm), pinned to a base of sound-absorbent material. The output from the sound level meter went to a Racal instrumentation tape recorder running at 30" s⁻¹ or was digitised (Tucker Davis, System II, Alachua, FL, USA) at a sampling rate of 100 or 170 kilosamples per second and stored to the hard disk of a computer. Digitised signals were low-pass filtered at 100 kHz to avoid aliasing. Sound levels (re 20 µPa) were measured with the 2204 sound level meter (Fast or Impulse/Hold as indicated), usually at a distance of 10 cm from the dorsum of the insect singer. Power spectra and spectrograms were calculated using DADISP 4.1 (DSP Development Corp., Newton, MA, USA) or Matlab software (The Mathworks, Natick, MA, USA). All statistical analyses were carried out using R software (www.r-project.org).

Zero-crossing analysis

In order to make detailed comparisons of sound generation, anatomy of the stridulatory file and wing movements (see below), we analysed songs with the Zero-crossing module for Canary software (Cornell University, Laboratory of

Ornithology). Zero-crossing v.3 was provided by K. N. Prestwich. Songs were low-pass filtered with a 7.5 kHz cutoff to isolate the f_D . Zero-crossing 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. By low-pass filtering at 7.5 kHz to remove higher frequency components (Krohn-Hite Model 3382 filter 8 pole LP/HP Butterworth/Bessel; Brockton, MA, USA) *in vivo*, we could examine cycle-by-cycle variation in f_D for comparison with the spacing of teeth on the stridulatory file and the velocity of wing movement during sound production (see below).

Morphology of the stridulatory apparatus

Mirror morphology and the main areas of activity

We examined the morphology of the mirror and scraper using electron microscopy. We measured the thickness of various regions of the mirror and determined the detailed anatomy of the scraper. Parts of the tegmina were dissected and then embedded in Spur's solution; transverse sections were made with a microtome, according to the process of (Di Sant' Agnese and De Mesy Jensen, 1984).

The vibration modes of a membrane have nodal lines or curves that divide the membrane into areas vibrating with opposite phase (Fletcher, 1992). In preliminary experiments,

the mirror surface was studied to find its main areas of activity. The method used was similar to that described by Sismondo (1979). Specimens were lightly anaesthetised in CO₂ and a thin layer of talcum powder spread over the mirror surface. The large size of the specimens allows excitation of the right tegmen by manually engaging the scraper and pushing it over the left stridulatory file.

Observations were made under a dissecting microscope: manual excitation of the system generated sound and produced a displacement of powder particles in areas of maximum vibration. Three areas of major activity were detected inside the mirror (areas 1, 3 and 4, Fig. 1). Other regions were chosen to represent parts of the tegmina of different thickness and position relative to the mirror so that we could study in more detail how all parts of the tegmina contribute to sound radiation. These data will be presented in a subsequent paper.

The stridulatory file

The stridulatory file was studied by scanning electronic microscope (SEM) using a Hitachi (Tokyo, Japan) electron microscope at the Department of Zoology, University of Toronto. Analysis of the file morphology was performed on digitised SEM photographs using the dimension tool of a drawing program (Corel Draw 10, Corel Inc.). Inter-tooth distances were measured from the edge of the cusp of one tooth

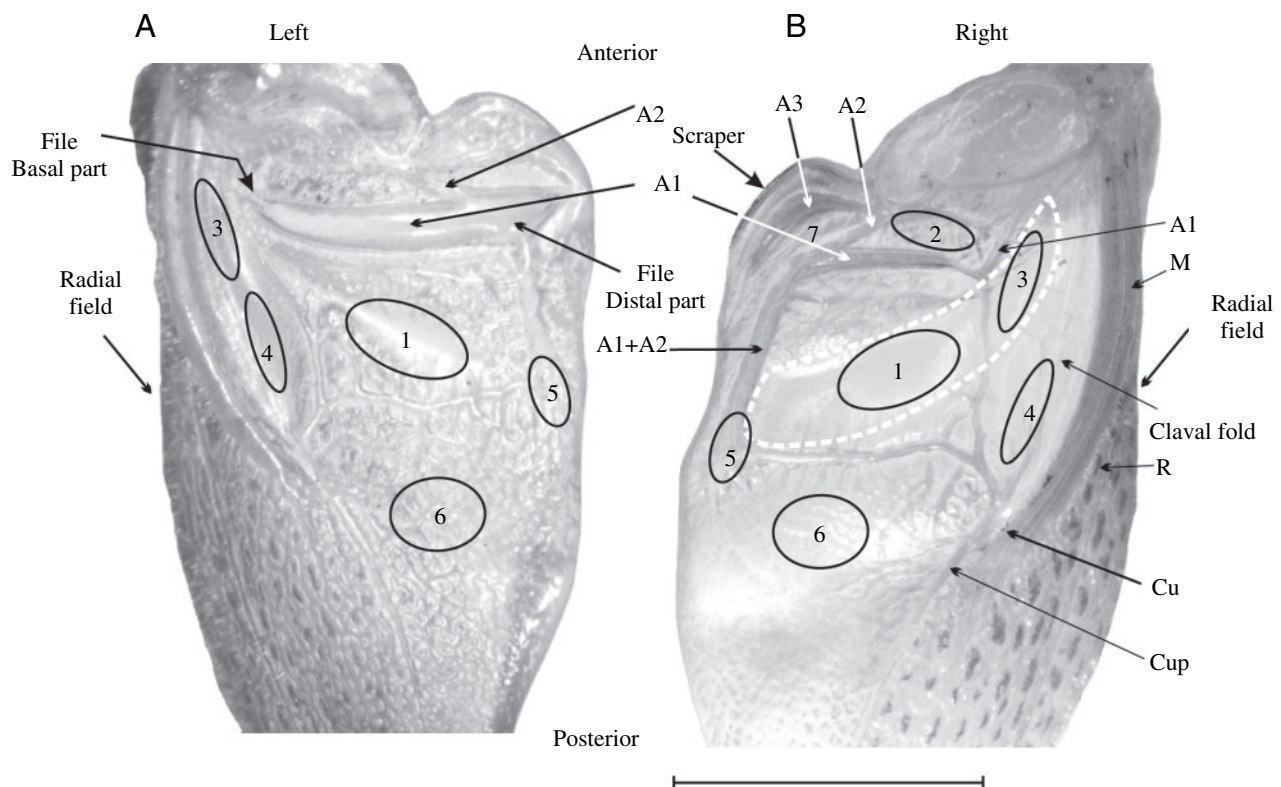


Fig. 1. Tegminal structure. Left (A) and right (B) tegmina. Marked regions indicate areas of maximal vibration in particle displacement studies of the right tegmen, and homologous regions on the left tegmen. Scale bar, 6 mm. Regions 1, 3 and 4 were experimentally found only for the right tegmen, but homologised for the left. The rest were chosen according to the thickness of the wing and used in complementary studies of wing vibration (data not published here). Venation nomenclature and vein identification were adopted from Desutter-Grandcolas (2003), and when explicit names or locations of some veins were not provided, we inferred them.

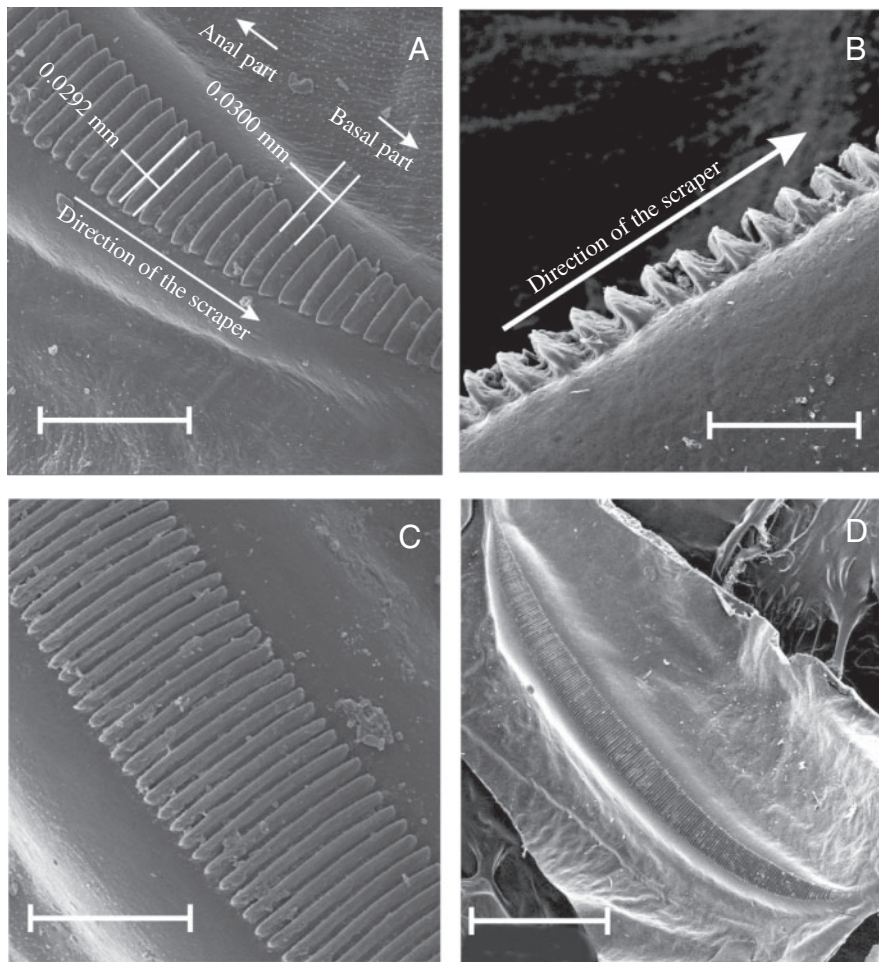


Fig. 2. Scanning electron micrographs of the stridulatory file. (A) Proximal end of the file showing variation in inter-tooth distances towards the costal margin; scale bar, 231 μm . (B) Detail of teeth from the mid portion of the file in lateral view showing teeth asymmetry and orientation angle; scale bar, 100 μm . (C) Mid portion of the file as seen in dorsal view; scale bar, 231 μm . (D) Entire file showing its curvature and variation in width; scale bar, 1.36 mm.

to the cusp of the next one (Fig. 2). For comparison, we also made similar measurements of the stridulatory file for other species in this genus (*P. cuspidatus*, *P. gibbosus*, *P. varius*, *P. intensus*). Only single specimens of some of these species were available.

Stridulatory wing movement recordings

We recorded stridulatory wing movements and associated sound production from eight males. Sound production was monitored with either a $\frac{1}{2}$ " microphone (Larson Davis Laboratories model 2540, Provo, UT, USA) or a B&K $\frac{1}{4}$ " microphone type 4939. Wing movements were recorded using an opto-electronic device (von Helversen and Elsner, 1977; Hedwig, 2000). For recording wing movements, a small piece of reflective tape (Scotchlite 7610 and 8850 retro-reflective tape manufactured by 3M and distributed by Motion Lab Systems Inc., Baton Rouge, LA, USA) was placed on the forewing and its position was monitored with a photodiode.

Movements of the forewing evoke changes in the current of the diode, which were recorded simultaneously with sound output. Sound and wing-movement signals were recorded on separate channels of a computer data acquisition board and analysed using Dadisp or Matlab software (in initial recordings sampling rates were 250 kHz for sound and 31.25 kHz for wing movements, and 100 kHz for both in most recordings). The temperature in the room was $23.9 \pm 0.85^\circ\text{C}$. The reflective tape (1×3 mm) was attached to the left tegmen, in a manner that allowed movements to be recorded in either dorsal (perpendicular to the wing surface) or posterior view (in the same plane as body axis). Most recordings were obtained from the posterior view.

After the specimens had been recorded several times and the wing movements characterised, several teeth of the file were removed using a dentist's turbine drill, whose cutting edges were reduced to 0.1 mm in diameter. Teeth were removed carefully in two or three regions, mainly from the middle portion of the file. A space of several teeth (10–30) was left between gaps. This allowed us to associate sound oscillations with tooth-scraper contacts or with jumps of the scraper over several teeth, and also to evaluate the functional parts of the file.

We also recorded wing movements using high-speed video (Redlake Motionscope PCI1000s, San Diego, CA, USA). The high-speed video system was synchronised with a computer data acquisition board (National Instruments PCI6023e, Austin, TX, USA; 16 bit, 200 kHz sampling rate) using Midas software (2000 Xcitex Inc., Cambridge, MA, USA) for simultaneous recording of sound production. In some cases the position detector was connected as a third channel, so that wing movements were simultaneously recorded in two modalities: high-speed video and photo-response of the diode. Recordings were acquired at 500 or 1000 frames s^{-1} for high-speed video, and a sound sampling rate of 100 or 200 kHz. Specimens were put on an artificial perch and the camera was aligned and focused directly on the stridulatory field, either dorsal, lateral from the right side or focusing the file and scraper from the posterior. A $\frac{1}{4}$ " 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.

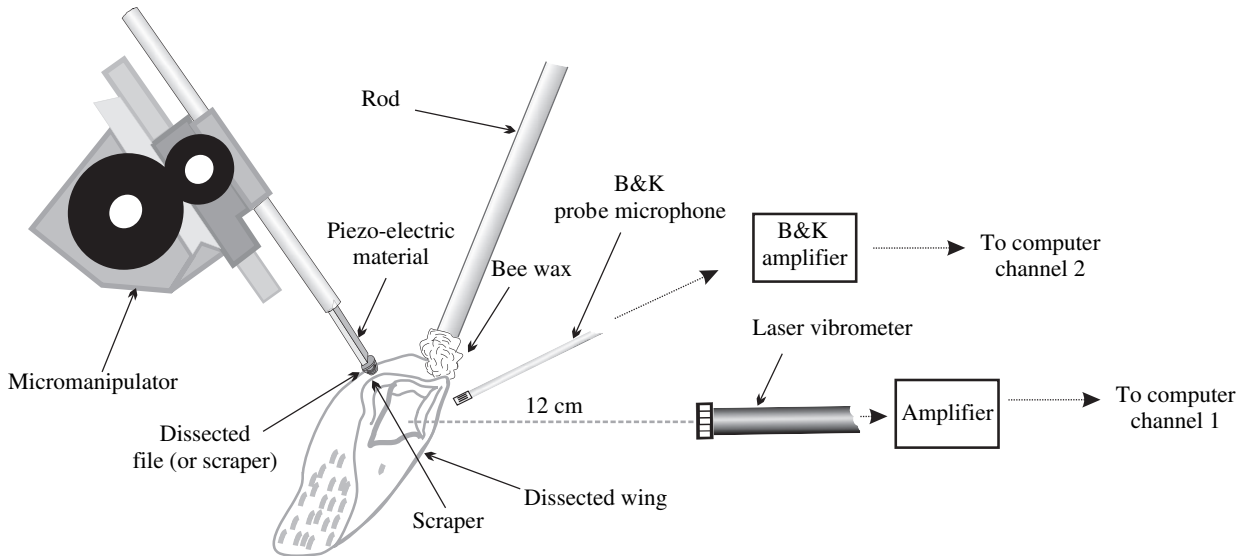


Fig. 3. Diagram of the setup for measuring the natural frequency of vibration f_0 of isolated wings. A dissected segment of file (or scraper) was mounted on a probe held in a micromanipulator. This was used to simulated single file-tooth impacts and generate free vibration in the wing. Wing vibration was measured directly using a laser vibrometer and radiated sound was recorded using a probe microphone.

Experimental manipulation of wing membranes

As a preliminary investigation of the contribution to sound production by specific regions of the mirror, we recorded songs from five specimens before and after removal of one or more mirror membranes. Wing membranes were removed with a fine soldering iron. Specimens were initially recorded in a soundproof room with intact wings, as described previously. Subsequently area 1 was removed in three specimens and their songs re-recorded after 48 h. Then the membrane of area 3 was removed and the specimens were recorded again after another 48 h. Finally, the membrane of area 4 was removed and the process repeated. The remaining two specimens were treated in similar way, but instead of removing parts of the mirror in different steps, areas 1, 3 and 4 were removed at the same time.

In a separate set of experiments ($N=4$), we modified single tegmina by painting them with liquid latex. This material dried as a layer of latex that adhered to the wing surface and could be removed by simply peeling it away. This allowed us to measure the effect of loading each wing individually in a single specimen. Specimens were recorded before application, with the latex applied to one wing, and again following removal of the latex.

Simulation of tooth-scraper contacts

After the calls and wing movements of intact specimens were recorded, some specimens were anaesthetised in CO_2 and the right or left tegmen removed. The wing hinge was sealed with bees' wax in order to halt bleeding. The tegmen was attached to a metallic rod by bees' wax at the position of the wing hinge (Fig. 3).

Wing vibrations produced by artificial tooth-scraper contacts (clicks) were recorded for areas 1, 3 and 4. In these experiments, clicks were generated with a dissected file or

scraper. Thus the right tegmen was driven *via* its scraper using an excised file and the left tegmen was driven with a dissected scraper *via* its file. In both cases fine movements of either the dissected file or scraper were performed with a micromanipulator, in order to control for individual tooth impacts. We took into account the angle of the scraper and file during stridulation as observed in our video recordings of wing movements. The preparation was mounted in a similar way for every experiment.

The reaction of the membrane was transduced by the laser vibrometer. A probe microphone (B&K Type 4138) was placed within 5 mm of the specimen. Alignment of the microphone and laser beam is shown in Fig. 3. The microphone and laser vibrometer signals were digitized and stored in a computer (TDT System II, 100 kHz sampling rate). All experiments were performed on an anti-vibration table. The laser allows for accurate measurements of vibration in specific regions, while the microphone was used to monitor the output song of the particular area studied and all surrounding regions. The three main areas found by particle displacement of talcum powder were studied with laser vibrometry (Fig. 1). The same regions were studied in the left tegmen and three plucks were recorded in each area.

The quality factor Q is a measure of the of the response peak of a system. Q is given by the ratio between the frequency at peak output and the bandwidth at half power (Fletcher, 1992; Arya, 1998; Bennet-Clark, 1999b). Systems with higher values of Q have greater response at the peak frequency, therefore large amplitudes at resonance and small bandwidth. Systems with low Q values have lower peak response and broad bandwidth. Resonant biological systems tend to have small Q values in the range from 1 to about 30 (Fletcher, 1992). We calculated the Q -factor for both forewings based on the

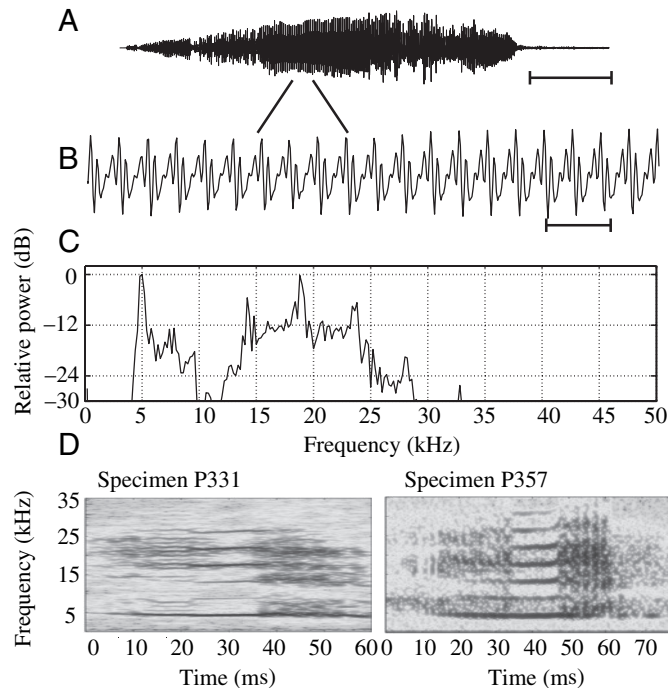


Fig. 4. Calling song analysis. (A) Oscillogram of a single *P. pallicornis* song; scale bar, 10 ms. (B) Detail of waveform shown in A; scale bar, 0.5 ms. (C) Power spectrum of the song in shown in A. (D) Spectrograms of two different specimens (note inter-individual variation of call). While the overall spectrum (C) is noisy, spectrograms (D) reveal a segment in the middle of the call with clearer tonality and harmonic structure.

recordings mentioned above, using the method proposed by Bennet-Clark (1999b).

Results

Call description

The calling song of 52 males is known from recordings collected during the last 5 years. A call is composed of a single phonatome (sound produced during one cycle of wing movement) with a gradual rise in amplitude (Fig. 4A,B). The spectrum is complex with a fundamental frequency (f_D) near 5 kHz and several higher harmonic components (i.e. multiples of f_D ; Fig. 4C,D). Each song is composed of 225.8 ± 24.4 cycles of f_D (mean \pm s.d.; range=182–256 cycles, $N=12$) and is 54 ± 7 ms in duration ($N=52$). Specimens recorded in the field at 18°C had a mean principal frequency peak of 5.0 ± 0.4 kHz (mean \pm s.d., range 4.8–6.0, $N=12$). In laboratory conditions (22 – 25°C) males have $f_D = 4.7 \pm 0.2$ kHz (range 4.3–5.1, $N=21$). The bandwidth at 12 dB below the main spectral peak is 293.7 ± 61.8 Hz (range 197–459, $N=20$). Higher harmonic components are noisier (i.e. comprise a broader spectrum of frequencies). There is a band of energy from ~ 12 – 28 kHz with a peak near 14 kHz (mean $= 14.7 \pm 1.1$, 13.1–16.3, $N=12$) and 9.4 ± 3.5 dB (range 4.0–12.5, $N=12$) below f_D . Another peak near 20.0 ± 1.2 kHz (range 17.5–21.4, $N=12$) is 6.1 ± 3.6 dB

(range 0.9–12.1, $N=12$) below f_D . Finally, a third peak of high energy occurs near 25.0 ± 1.3 kHz (range 23.5–27.0, $N=12$) and $\sim 8.61 \pm 3.5$ dB below f_D . No significant energy occurs above 28 kHz (Fig. 4C). Measured with the microphone at 10 cm dorsal to the animal, the average intensity of the song was 97.6 ± 2.6 dB ($N=17$).

Spectrograms were performed for 20 different individuals. From these studies it is clear that each individual produces a complex but consistent spectrum and different individuals produce somewhat different spectra (Fig. 4D). In all individuals there is a portion of the call in which the relative amplitude of higher harmonic peaks in spectrum increases. We refer to this as the harmonic portion of the song. This tends to occur in the middle one quarter to one third of the song; however, there is considerable individual variation in the timing of this harmonic portion (Fig. 4D).

Morphology of the stridulatory apparatus

Anatomy of the right tegmina

As in most tettigoniids, in *Panacanthus* the left forewing overlaps the right one. The right tegmen has most of the specialised structures for sound radiation (i.e. the mirror cells, veins and surrounding areas) and these are located in the dorsal field. Homologous regions can be identified in both wings, but in the left wing they are thicker. Sound is generated when a specialized region of the right tegmen (the scraper, Fig. 5) is pushed across a specialized vein (the file, Fig. 2) on the under surface of the left tegmen. The teeth of the file deflect the scraper, causing the right wing to bend until the scraper is released. This catch-and-release mechanism induces oscillations in the mirror membranes resulting in the radiation of sound from the tegmina (Pierce, 1948).

The mirror of the right tegmina consists of complex cells (Fig. 1B). There are actually two major cells. One is a sub-quadrilateral region enclosed by the thick veins A1 and A1+A2. The mirror, measured from the vein A1 to the distal part of the frame, is 4.11 ± 0.20 mm in length ($N=20$). The other region is an adjacent sub-triangular region bounded by the Cubital veins and A1. This cell is longitudinally divided by a fine vein and the claval fold (Desutter-Grandcolas, 2003). The surface of the sub-quadrilateral is diagonally shallowly depressed; the depression extends from the left inferior corner to the right superior (Area 1, Fig. 1B, broken white line). On the left side, this cell is isolated from the rest of the wing by a thicker vein, formed by the fusion of A2 and A1. The surface of the membrane of the triangular region is even more complex, presenting a small but deeper concavity on the region adjacent to A1 (Area 3) and another, larger but shallower, parallel to the claval fold (Area 4, Fig. 1B). However, the concave surfaces of Area 1 and Area 3 form a continuous curvilinear depression that is interrupted just by a fine vein probably derived from A1 (Fig. 1B, broken line); these zones differ from the rest of the membrane in their transparency. Another elongated cell is formed by veins A2 and A3 in the basal portion of the anal margin; we recognized this cell as

Area 7 (Figs 1, 5). Different regions of the tegmina differ in thickness (Fig. 5B).

The scraper is a very complex structure (Fig. 5A,B). It is framed by an extension of vein A4 (Fig. 5A); its active area of contact is a sharp edge 2.58 ± 0.06 mm in length (range 2.5–2.7 mm, $N=12$) and 35 ± 4.5 μm thick in its widest part (range 31–40, $N=3$, Fig. 5B). The sharpest part of this edge is narrower than the widest part described above; hence the scraper is triangular in cross-section (Fig. 5B,C). As the file teeth are triangular in profile (see below), the triangular shape of the scraper allows it to fit between adjacent teeth on the stridulatory file despite variation in inter-tooth spacing. The scraper active area is easily distinguishable because it is strongly sclerotized and darkened. There is a space of 1.56 mm between the scraper edge and the robust vein A1+A2 that surrounds the mirror. Dividing this space there is a thick vein formed by a branch or an extension of A3. As a result of this division, this complex region comprises two cells: one close and almost perpendicular to the mirror plane ($\sim 100^\circ$), formed by a thick mass of soft tissue and elastic cuticle (thick membrane in Fig. 5A,B); the other adjacent to vein A1+A2, is the subsclerotized Area 7. Microtome sections reveal that the scraper is dorsally connected to the rest of the tegmen by a layer of elastic cuticle. Another layer, which seems not to be directly connected to the scraper but partially isolated, is positioned ventrally. There is a gap between this area and the scraper, because the cuticle beneath is not continuous; this gap is covered with soft tissue (Fig. 5C). Both dorsal and ventral layers of cuticle are connected basally, i.e. they are part of same region (the thick membrane) that bifurcates to form the dorsal and ventral layers. The scraper morphology differs from that of crickets, and this model seems widespread in Tettigoniidae (Anstee, 1971; Montealegre-Z and Morris, 2003; F.M.-Z, unpublished), suggesting that it might operate differently (see Bennet-Clark, 2003).

The stridulatory file in Tettigoniidae has traditionally thought to be a modification of the vein Cu2 or cubital posterior (Ragge, 1955; Desutter-Grandcolas, 1995). However, more recent studies suggest that the stridulatory file originated from the vein A1 (Desutter-Grandcolas, 2003); our description is based on the latter wing venation nomenclature. In the ventral part of the right tegmen, the vein A1 does conserve some features of a stridulatory file, although it is dramatically shorter and narrower than its counterpart in the left wing (the functional stridulatory file).

Anatomy of the left tegmina

Like most tettigoniids, *P. pallicornis* is unable to switch

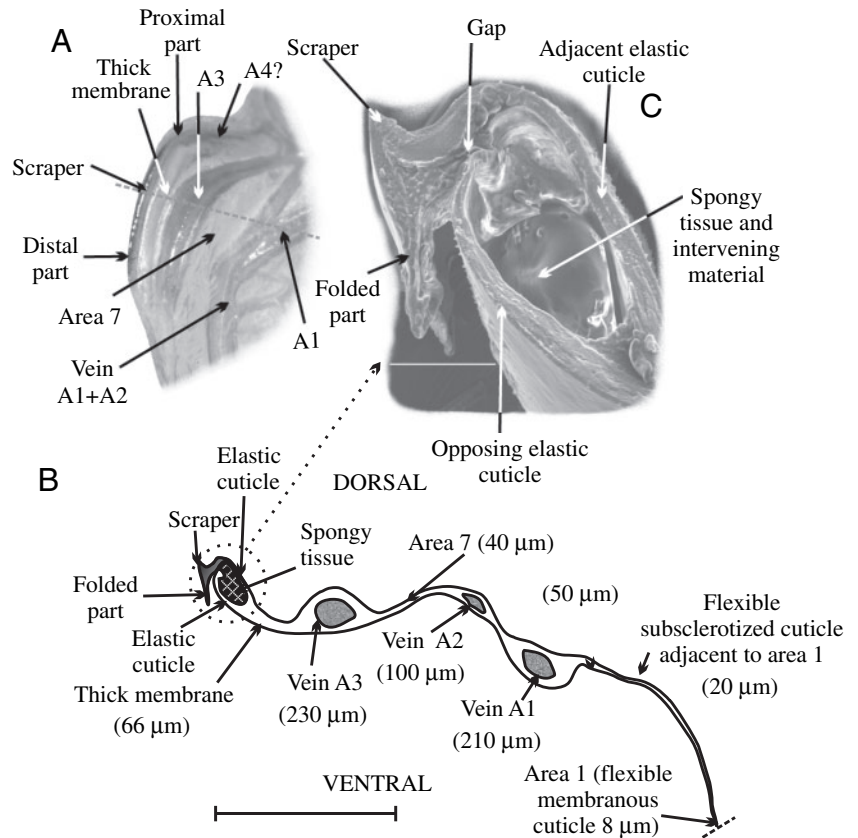


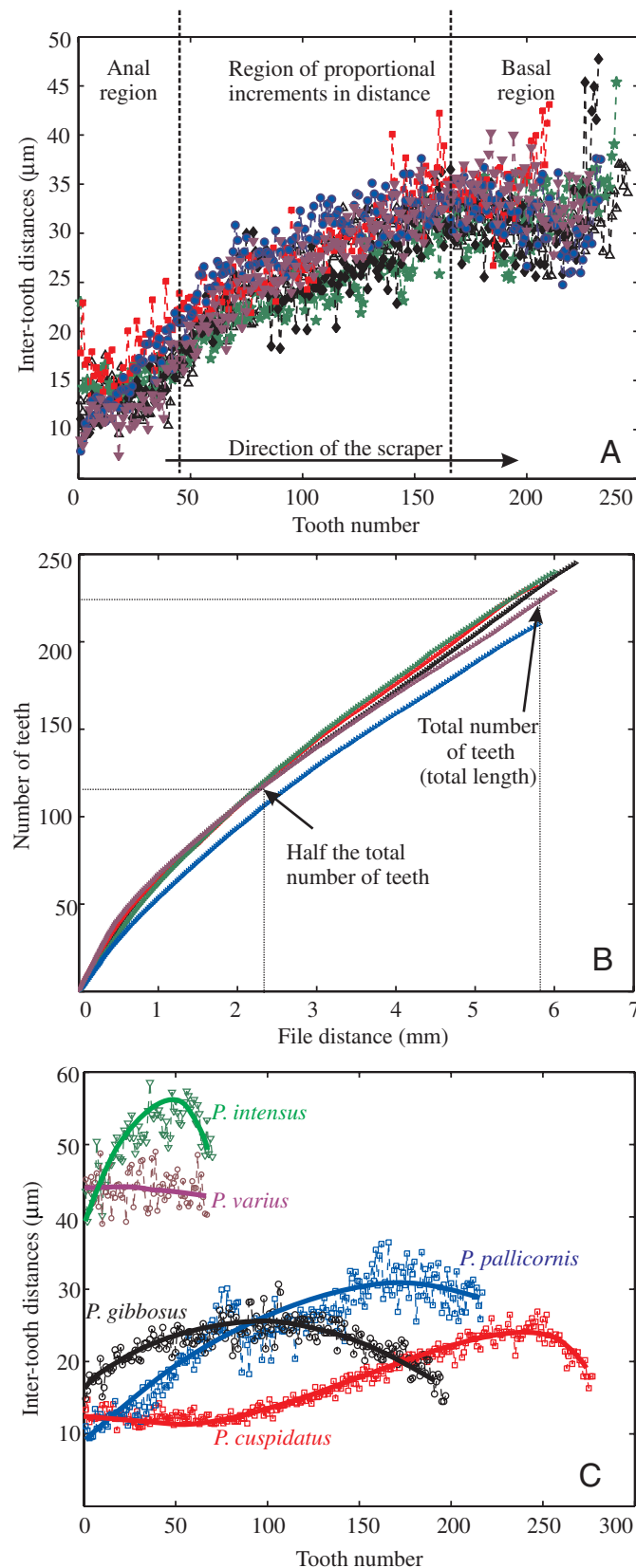
Fig. 5. Scraper morphology. (A) Scraper region of the right tegmen, viewed dorsally, with adjacent veins and cells indicated. (B) Cross section of the scraper-bearing region of the right tegmen through the area indicated in B (blue broken line); scale bar, 1 mm. Dotted circle indicates the region shown in C. (C) A scanning electron micrograph of the edge of the right tegmen at the location of the scraper. Labels refer to structures described in the text; scale bar, 100 μm .

wing overlap as the two tegmina are highly asymmetrical (Fig. 1A). The most important component of the left wing for sound production is the stridulatory file, through which the wings are excited during stridulation.

The stridulatory file of *P. pallicornis* is 6.47 ± 0.09 mm in length ($N=16$, mean \pm S.D.). It is laterally curved (Fig. 2D), and dorsoventrally arcuate. The number of teeth varies from 210–253 ($N=16$). In profile, teeth are laterally compressed, but the engagement side (distal part) is vertical with respect to the horizontal base; its contra-lateral side is more obtuse (Fig. 2). When seen in lateral view, this asymmetry gives a triangular aspect to every tooth, so that teeth present a steep face to the scraper during the engagement. The file is massive, therefore it seems mechanically unlikely that tooth-scraper interactions and vibrations of the rudimentary left mirror (F. Montealegre-Z and A. C. Mason, manuscript in preparation) generate bending vibrations of the file at the frequencies radiated by the tegmina with sufficient amplitude to release the scraper during each oscillation, as suggested for the escapement mechanism of crickets (Bennet-Clark, 1999a, 2003).

There is an overall trend for teeth to be more widely spaced towards the end of the file (Figs 2A, 6A), but spacing is highly

variable. Average inter-tooth distance increases over the anal approximately one fifth of the file length, then stabilises and decreases slightly in the last basal one fifth or one quarter. Half



of the total number of teeth are concentrated in the anal third of the file, i.e. where the teeth are engaged at the beginning of sound production (Fig. 6B). In this portion of the file, teeth occur at an average density of ~ 46 teeth mm^{-1} , while in the basal two thirds of the file, teeth occur at an average density of 29 teeth mm^{-1} . Thus, the file presents regional differences in inter-tooth spacing at the anal and basal ends (Fig. 6A). The tooth spacing seems to be less consistent than that of gryllids studied as models of pure-tone generators (Bennet-Clark, 2003). Comparison of the file-tooth spacing among several *Panacanthus* species (Fig. 6C) shows a range of patterns and variability in tooth spacing. *Panacanthus cuspidatus* and *P. pallicornis* have the most regular distribution of file teeth, and a pattern of increased tooth spacing along the length of the file. In *P. gibbosus*, *P. intensus* and *P. varius*, tooth spacing is less regular and inter-tooth distances are greatest in the middle portion of the file. Details of file structure and sound analysis of these species can be found in Montealegre-Z and Morris (2004).

Both tegmina have the common feature of having the costal and apical fields made of a very flexible soft tissue. This ductile region extends towards the distal portion of both wings. Another remarkable feature of both forewings is that the radial field is very swollen, giving the tegmina a globular contour in this region. The radial field is completely isolated from the costal field by the veins R and M. When the insect is singing, the costal fields of both tegmina remain attached to the abdominal pleura and these swollen portions and stridulatory field form a chamber.

Identification of main areas of vibration

We used observations of particle vibration over the mirror to identify maximal tegminal areas, but for the purposes of this paper we did not attempt to quantify the relative vibration of different wing regions. Quantitative vibration measurements of both tegmina were made using a laser vibrometer, but these results will be presented in a subsequent paper. Areas 1, 3 and 4 presented the major displacement of talcum powder particles. Area 1 vibrated with greater amplitude than 3 and 4, where

Fig. 6. Distribution of teeth on the stridulatory file. (A) Plot of the inter-tooth distances over the length of the file for six specimens of *P. pallicornis*, each indicated with a different colour and symbol. Inter-tooth distances increase towards the basal end of the file (i.e. where the scraper contacts the last teeth during the closing stroke), but this increase is not smooth. (B) Plot of the cumulative number of teeth vs total distance along the file of five specimens (each shown in different colour). This indicates that the inter-tooth distance gradually increases basally, and that half of the total numbers of teeth are located in the anal one third of the file. (C) Inter-tooth distances of five *Panacanthus* spp. Trend lines are Lowess curve fits. Second degree polynomial regressions of inter-tooth distance against tooth number showed lower variability of tooth spacing in species using sustained pulses [*P. cuspidatus*, $r^2=0.95$ (red outline) and *P. pallicornis* $r^2=0.87$ (blue)]. Tooth spacing was more variable in species using transient pulses with broad band spectrum [*P. gibbosus*, $r^2=0.75$ (black), *P. intensus*, $r^2=0.74$ (green), *P. varius*, $r^2=0.02$ (purple)].

particle movement was reduced. Particles move from these areas and tend to accumulate outside of the mirror, on a sharp depression between Area 6 and the posterior part of the mirror frame (Fig. 1B). Areas 3 and 4 are concave regions that are more transparent than the rest of the thicker surrounding membrane in which they are embedded. These regions show strong phase independence during particle displacement. Phase independence was first observed under a stereomicroscope and confirmed with laser vibrometry (data not included in this paper).

Wing movements

Sound emission coincides with the entire course of tegminal closure (Figs 7, 8). Both wings are raised above the body to an angle of about 45° (viewed laterally and taking the wing's resting position as 0°). If viewed dorsally, when the scraper begins to contact the first teeth of the file, each wing is opened at a maximum of 27° (from a resting position). Viewed from the posterior, in the same action each wing is lifted about 49° from the horizontal plane of the body. A small change in amplitude of the wing-movement signal occurs during the closing

movement as the scraper first contacts the file of teeth (Fig. 7B). The pattern of stridulation involves a single opening and closing stroke without pause, and a single type of phonatome. The total opening and closing of the forewings (phonatome period) lasts ~ 120 ms, and the effective time for sound production varies from approximately 40–60 ms ($N=10$).

High-speed video and opto-electronic recordings show that during sound generation (closing stroke), the entire length of the stridulatory file is used and the velocity of the scraper gradually increases (Figs 7, 8). The velocity of the closing stroke was calculated by measuring the displacement of a specific point on the wing every five frames (i.e. over time intervals of 5 ms for 1000 frames s^{-1} or 10 for 500 frames s^{-1}).

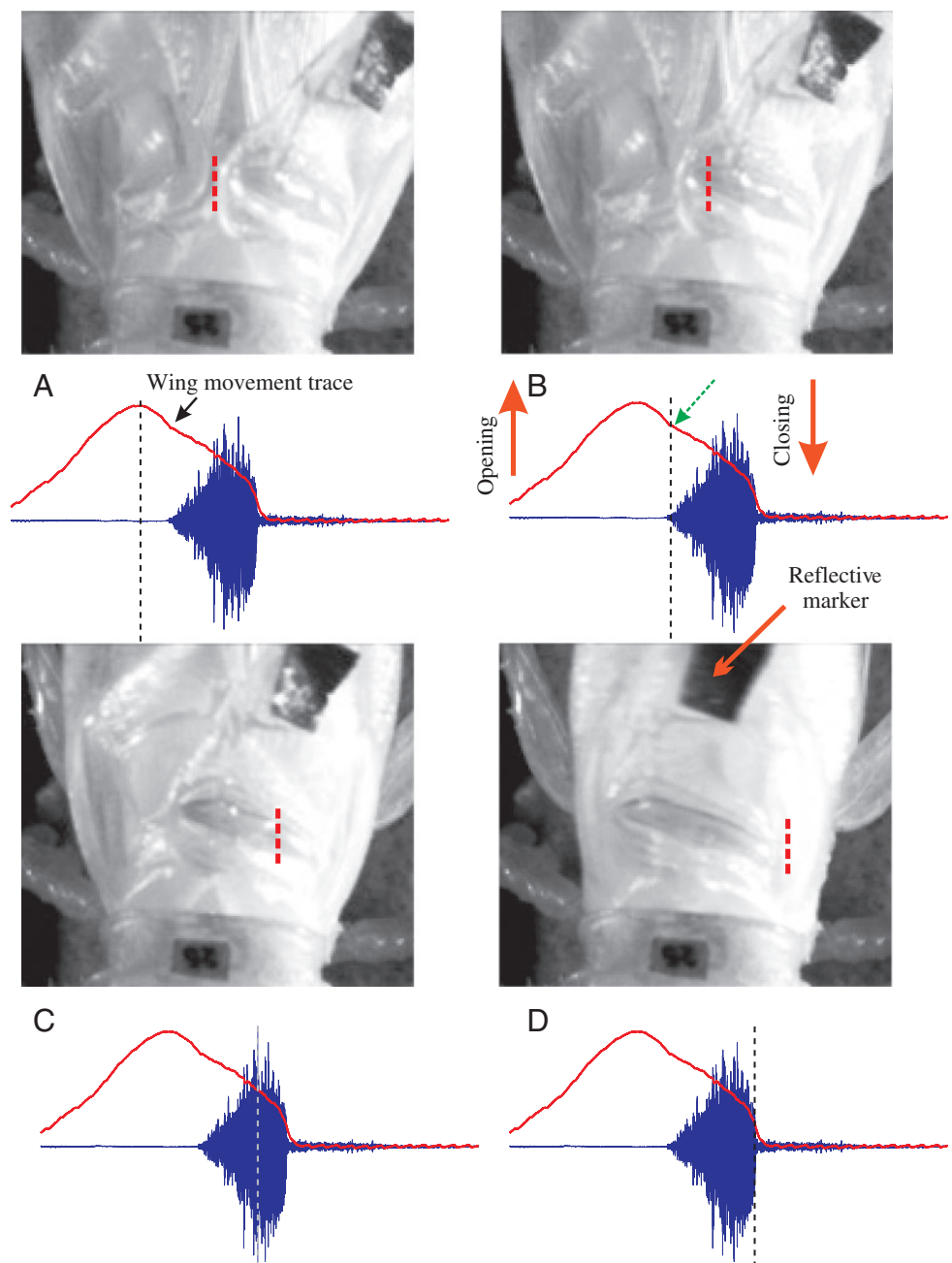


Fig. 7. Wing movements during sound production recorded simultaneously with two different methods: high-speed video (top) and optical position detector (bottom). Four frames from one complete wing stroke are shown. Each frame is accompanied by an oscillogram of the sound (blue) and simultaneous trace of wing position vs time (red). Vertical broken lines (red) indicate the position of the scraper in the video frame. In the simultaneous sound and wing position traces, the vertical broken line indicates the time corresponding to the video frame. (A) Maximal opening of wings, just prior to engagement of file and scraper and beginning of sound production. (B) The scraper contacts the first teeth of the file and the first sound waves are generated. Note decrease in the slope of wing position (red trace) when the scraper contacts the first teeth (green broken arrow). (C) The scraper crossing the middle portion of the file, maximum amplitude of the song pulse, increasing slope of wing position trace. (D) The scraper contacts the last few teeth of the file and comes to rest with the end of the sound pulse.

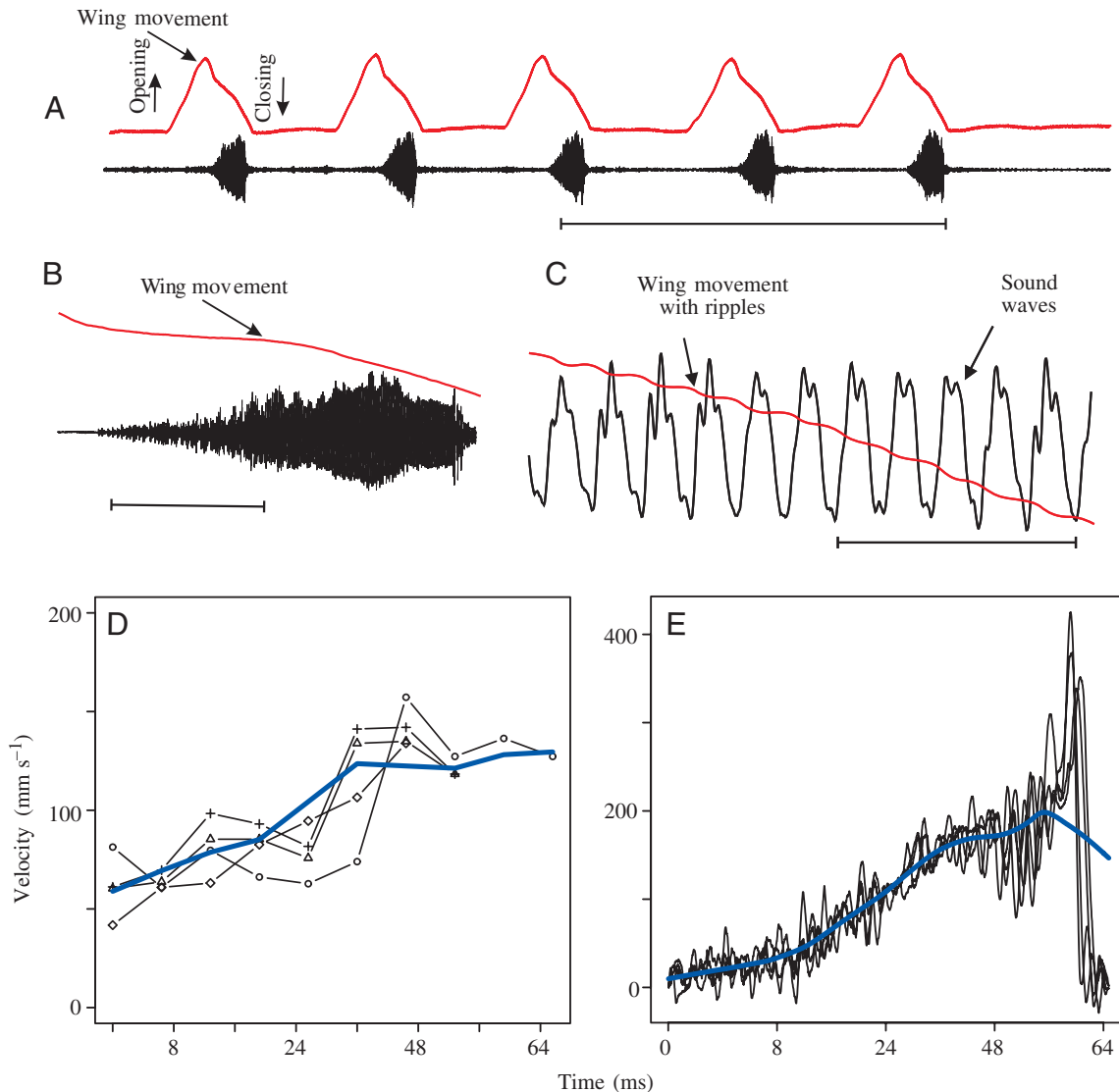


Fig. 8. Sound oscillograms and wing movements as recorded with the optical position detector. (A) Five consecutive songs (black) with their corresponding wing position trace (red); scale bar, 0.5 s. (B) Enlargement of one pulse of the sequence shown in A; scale bar, 20 ms. (C) Further enlargement of a segment of the pulse shown in B. Ripples in the wing position trace match sound waves; scale bar, 1 ms. (D) Velocity of wing closing, calculated from frame-by-frame analysis of high-speed video. Results for one song from each of four individuals are shown (symbols) along with a Lowess curve fit to the data (blue). (E) Velocity of wing closing, calculated from the wing movement trace of the position detector. Wing velocity traces for four songs from the same specimen are plotted (black) and line fitted to the average using the Lowess curve fit (blue).

This measurement was repeated for about eight consecutive stages during a single wing-stroke ($N=3$ specimens). The average velocity of the scraper movement along the file is $\sim 120 \text{ mm s}^{-1}$. Wing velocity data calculated by taking the derivative of the position sensor signal give higher resolution (Fig. 8) and indicate variation in velocity over the course of a single wing cycle, but still show an overall increase over the course of a wing-stroke. The wing velocity abruptly, but briefly, increases when the pulse is complete and the scraper disengages from the file before the forewings recover their resting position.

The engagement of the scraper can be studied precisely from these recordings. The active contact area of the scraper changes

during one closing stroke, as described for the cricket *Teleogryllus oceanicus* (Bennet-Clark, 2003). The first teeth of the file are contacted by the distal part of the scraper; then the contact area is gradually shifting to the anterior mesal portion during the closing stroke. That is, there is a moderate but detectable relative movement of file and scraper perpendicular to the direction of wing closing (data not shown).

Recordings using the opto-electric position sensor show that high frequency 'ripples' in the position signal are detectable when sound and wing movements are shown at full resolution, corresponding to the sound-radiating oscillations of wing membranes likely generated by tooth impacts (Fig. 8C). This is consistent with previous observations

indicating that tooth contacts occur at a frequency similar to the calling song frequency (Suga, 1966; Koch et al., 1988; Hedwig, 2000).

Manipulation of wing membranes

Removal of mirror membranes of the right wing

After removal of Area 1, there was no change either in the song envelope or in the spectrum. A minor reduction in intensity was noticed in all cases, especially at frequencies between 15 and 25 kHz, similar to the observations of Morris and Pipher (1967) and Keuper et al. (1988). Removal of regions 3 and 4 resulted in a reduction of the relative power of f_D and a significant increase in the frequencies of spectral peaks (Fig. 9, Table 1). Increments in frequency, after removal of mirror regions, have also been reported in other katydids (Morris and Pipher, 1967; Keuper et al., 1988) and are consistent with a decrease in the mass component of a resonant system (Arya, 1998). In some specimens (previously recorded intact) we excised all regions at once and results were similar. Fundamental and harmonic components increased in frequency and the relative power of the fundamental decreased after ablation of Areas 3 and 4.

Loading of wings with latex

When the left tegmen was loaded, the overall characteristics of the song were not changed, but there was a decrease in frequencies in the harmonic segment of the pulse (paired Wilcoxon test $P < 0.029$, Fig. 10A,B, Table 2), as well as a decrease in the amplitude of the call. These results are consistent with increased damping in a resonant system (Arya, 1998). The original spectral properties of the song were restored following removal of the latex (Fig. 10C). When the right tegmen was loaded, the harmonic segment of the pulse no longer occurred (Fig. 10D).

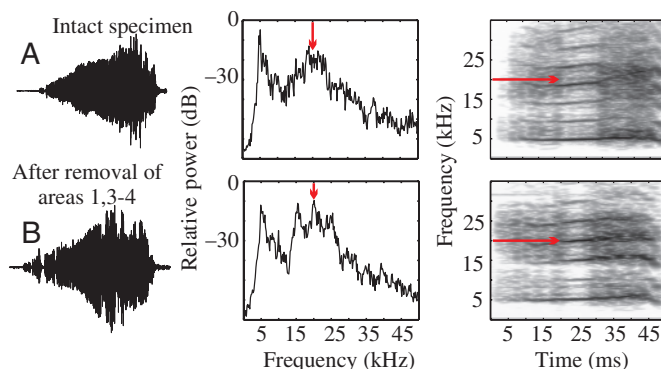


Fig. 9. Removal of mirror regions. Analysis of calls from one individual before (A) and after (B) removal of the membranes of Areas 1, 3 and 4 of the right tegmen. Oscillograms are shown in the left, power spectra in the middle and spectrograms on the right. Red arrows indicate the same frequency in the spectral displays (middle and right). After removal of Areas 1, 3 and 4, there was a decrease in the relative intensity of the fundamental and an increase in harmonic frequency components.

Table 1. Descriptive statistics for dominant frequency before and after some mirror membranes were removed

Specimen	Intact specimen	Removal of mirror membranes	Paired <i>t</i> -test	<i>P</i> -value
P464*	4592.9±36.1	5155.5±203.7		
P466†	4972.2±38.5	5411.1±25.5		
P467‡	5072.7±54.6	5478.8±123.8	-4.8	0.040
P476‡	5049.5±130.0	5469.1±28.0	-5.3	0.035

*Only Area 1 removed.

†Only Areas 3 and 4 removed.

‡Removal of Areas 1, 3 and 4, with 1 removed first.

Temperature 24.5±0.5°C.

Values are means ± S.D. three songs (pseudoreplicates) recorded from intact and treated specimens and compared in a paired *t*-test.

Removal of file teeth

Recordings of specimens in which two or three groups of teeth were removed (separated by a segment of intact teeth) corroborated our results showing a one-to-one correspondence between file teeth and cycles of the fundamental song frequency. Gaps in the song corresponded to the segments of missing teeth, and the number of cycles generated in the intervening segment matched the number intact teeth between the regions of ablation (Fig. 11A,C). Removal of file teeth had little effect on the overall spectrum of the song. But a comparison of the spectrograms and wing movements from the same individual before and after ablation of file teeth showed differences in the spectral quality of the sound produced, as well as the obvious changes in the time-domain (Fig. 11). The harmonic segment of the song was not produced after removal of file teeth, and the velocity of wing movement was erratic and did not show a clear acceleration towards the end of the pulse as in the intact song (Fig. 11B,D).

Zero-crossing analysis, wing velocity and sound quality

Zero-crossing analysis indicates that the f_D is quite constant over the course of a single pulse, particularly during the harmonic portion of the call. There is no 'glissando' as observed in crickets (Simmons and Ritchie, 1996; Bennet-Clark and Bailey, 2002; Bennet-Clark, 2003) and in some katydids (Bailey and Broughton, 1970). The frequency is more or less constant as the pulse progresses, regardless of increments in inter-tooth distances, except on the anal and basal ends of the file, where tooth distribution is different (Figs 6, 12). For one specimen we were able to obtain complete measurements of the inter-tooth distances for the stridulatory file, as well as sound and simultaneous optical recordings of wing movement. Using cycle-by-cycle frequency measurements and file tooth spacing, we calculated the instantaneous velocity of wing movement over the course of an entire wing stroke that would be predicted if each cycle of the f_D of sound output corresponded with a single impact of file and scraper, as in crickets (Elliot and Koch, 1985). In other words, using the actual sound output and file morphology, we

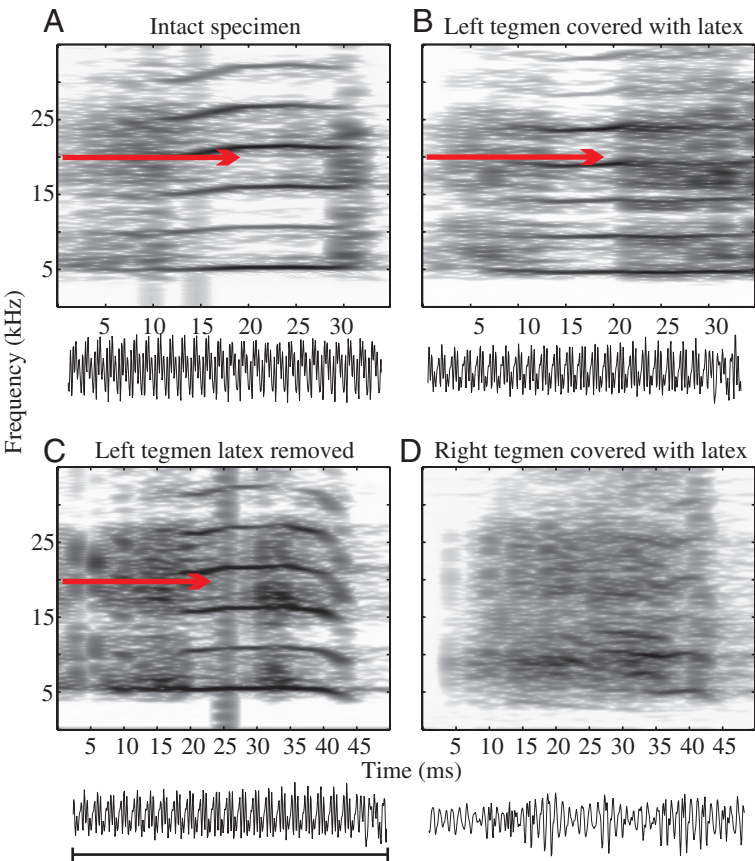


Fig. 10. Effects of loading the left tegmen. Spectrogram of songs of the same specimen intact (A), after loading the the acoustic regions of the left tegmen (B), following removal of the latex from the left tegmen (C) and after loading the mirror of the right tegmen (D). Red arrows in A–C indicate the same frequency. Harmonic components decreased in frequency when the left tegmen was loaded and recovered following removal of the latex. Loading of the right tegmen eliminated the harmonic structure of the call. A segment of the oscillogram at high resolution in every case is presented below each panel; scale bar, 60 ms.

calculated the wing closing velocity that would be required to maintain regular phasing of tooth impact with respect to sound-radiating oscillations of the wings. We compared this ‘ideal’ wing-velocity trace with the measured wing velocity data derived from optical recordings of wing movement (Fig. 13). The wing velocity required to match cycle-by-cycle frequency with the timing of tooth impacts is also quite stable, whereas the actual wing velocity accelerates over the course of sound production (Figs 7, 13B). There is a discrepancy between the expected and calculated velocities; initially, the actual wing velocity (Fig. 13, blue line) is lower but approaches the predicted velocity (green line) near the strongly harmonic region of the pulse and tends to become more erratic towards the end of the pulse, sometimes oscillating around an average velocity similar to the predicted velocity (Fig. 13C). In general, both (measured) wing velocity and cycle-by-cycle sound frequency were more variable during non-harmonic portions of the call.

Simulation of tooth-scraper contacts

The excitation produced by simulations of tooth-scraper contacts was analysed on the three areas of vibration in the right tegmen and on the homologous areas of the left. The purpose of these recordings was to measure directly the natural frequency of wing vibrations, f_0 . Clicks generated by single tooth-scraper interactions show a single abrupt build up and a gradual free decay in each case (Fig. 14B,C). In the original recordings, there was a strong component at very low frequencies (306.7 ± 69.7 Hz, $N=7$). We suspected that this represented oscillation of the entire tegmen around its attachment point, i.e. as a cantilever. We calculated the frequency of vibration for a wing attached to one end moving as a cantilever (Morris and Pipher, 1967) using published values for Young’s modulus (Fletcher, 1992). A wing with the physical characteristic of that of *P. pallicornis* would move with frequencies between 227 and 300 Hz when glued from its hinge to a rigid rod. We concluded that these low frequencies are the result of the movement of the tegmina from the tip of the rod to the tegminal apex, and not of the normal vibration of the mirror and adjacent zones. In the data presented here, this ‘whole-wing oscillation’ component has been removed by high-pass filtering (600 Hz cutoff).

Simulation of tooth scraper contacts was simultaneously measured with a probe microphone and with a laser vibrometer. Both measures gave similar values for f_0 of the wings (Fig. 14A). In all cases, the f_0 was significantly lower for the left tegmen (mean \pm S.D., 4311 ± 235 Hz, $N=4$) than for the right tegmen (mean \pm S.D., 5101 ± 472 Hz, $N=4$), which was close the f_D of the song (mean \pm S.D., 5050 ± 173 Hz, $N=4$). The free vibration of the right tegmen was moderately lower or higher than the frequency of the calling song, while that of the left was always lower.

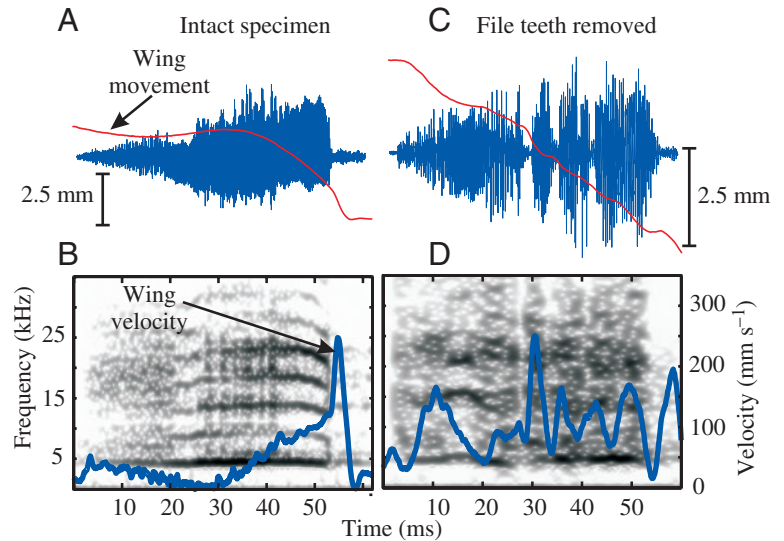
Lower free vibration of the left tegmina, in relation to the species calling song, might have occurred because of changes in the resonant properties of isolated wings. The

Table 2. Descriptive statistics for carrier frequency of four specimens, before and after the left tegmen was loaded with liquid latex on the dorsal and lateral parts

Specimen	Mean carrier frequency (Hz)		<i>t</i>	<i>P</i> -value
	Intact specimen	Left wing with latex		
p22	5390.7 \pm 37.0	4728.3 \pm 104.0	–29.9	0.001
p25	7453.0 \pm 450.0	6047.0 \pm 190.0	–5.0	0.038
p26	6196.0 \pm 156.0	4820.0 \pm 20.0	–15.2	0.004
p34	5049.0 \pm 111.0	4575.3 \pm 53.2	–6.6	0.022

Values are means \pm S.D. of three songs recorded from intact and treated specimens and means compared with paired *t*-test. Temperature 24.5–25.0°C.

Fig. 11. Effect of removing teeth from the stridulatory file. (A) Sound oscillogram (blue) and wing position (red) of an intact specimen. (B) Sonogram of the sound presented in A, along with the instantaneous velocity of wing movement (blue). Note the gradual and constant increase in velocity during the region with strong harmonics. (C) Sound and wing movement of the same specimen after ablation of teeth in three different parts of the file. First gap corresponds to the removal of 11 teeth between teeth 125 to 135 (gap= ~ 0.29 mm); the second gap results from absence of 7 teeth between tooth 142 and 148 (gap= ~ 0.17 mm) and the last gap from removal of 7 teeth between 184 and 190 (gap= ~ 0.21 mm). Note the abrupt changes in wing movement when the scraper encounters these gaps. The number of sound produced in segments between the gaps in the oscillogram matches the number of teeth in the intact regions of the file. (D) Sonogram of the sound shown in C, including the wing velocity (blue). Wing velocity is more variable than when the file is intact (B) with abrupt changes in velocity corresponding to gaps in the file. Scale bars in A and C refer to wing position traces.



interaction between file and scraper might add mass and stiffness to the system, as suggested by Bennet-Clark (2003). Conversely, the mean values of Q did not differ significantly for both tegmina, i.e. 17.2 ± 5.2 for the left wing and 16.2 ± 8.9 for the right ($F=1.01$, $P=0.32$, $N=5$). Other aspects of the physical properties of both wings will be presented in a subsequent paper.

Discussion

Our results indicate that some aspects of stridulation in *P. pallicornis* are similar to those of crickets while others are not. Similarities with most crickets are that large portions of the file are used in the production of sustained sound pulses (some species, such as the mole cricket *Scapteriscus vicinus*, use only small fractions of its file), there is approximately a one-to-one correspondence between the number of teeth on the file and the number of cycles of the fundamental sound frequency produced; sound radiation appears to result from resonant vibration of the tegmina, and forewings are excited at their natural frequency of vibration. Other details, however, are not consistent with a cricket-like mechanism. The irregularity of tooth spacing on the file, the corresponding fluctuations in the velocity of wing movement, and the variability in the spectral quality of sound produced over the course of a wing stroke are not consistent with sound production by a narrowly tuned resonant system in which precise phasing of input energy is maintained by an escapement mechanism. Below we discuss the comparison between *P. pallicornis* and cricket stridulatory mechanics in more detail and present a hypothesis for the mechanism sound production in katydids that accounts for these differences.

Cycle-by-cycle analysis showed that the f_D is relatively constant and does not show the glissando effect observed in crickets (Leroy, 1966; Simmons and Ritchie, 1996; Bennet-

Clark, 2003). There is, nevertheless, variation in spectral quality within a pulse. Spectrograms of individual calls show that a segment of each pulse has a more prominent harmonic structure while other portions of the same pulse are noisier. The portion of the call having a more sustained and pure-tone structure varies between individuals, but is consistent within individuals and corresponds to periods of stable or accelerating velocity of wing movement during the closing stroke, and in which the wing velocity matches the inter-tooth distances

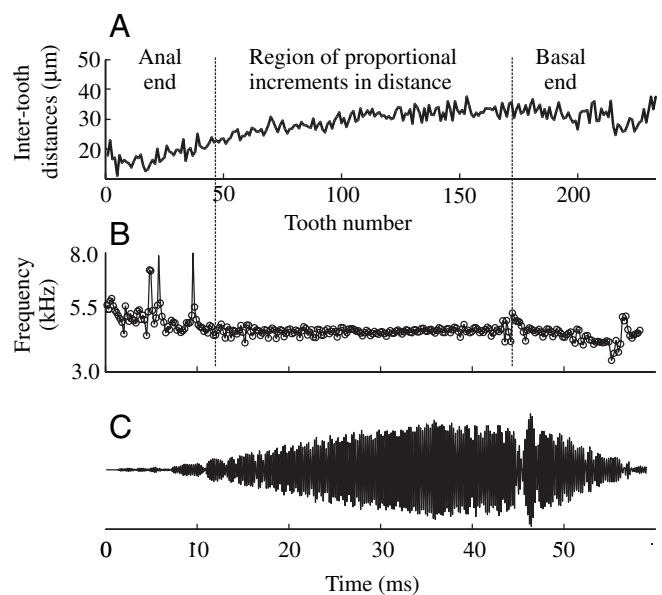


Fig. 12. Morphological measurements of the stridulatory file compared with sound characteristics of *P. pallicornis* (specimen P473). (A) File tooth distribution (B) Graph of the cycle-by-cycle frequency vs time of the song pulse shown in C. (C) Oscillogram the song pulse after low-pass filtering (7.5 kHz, see Materials and methods) high-frequency components.

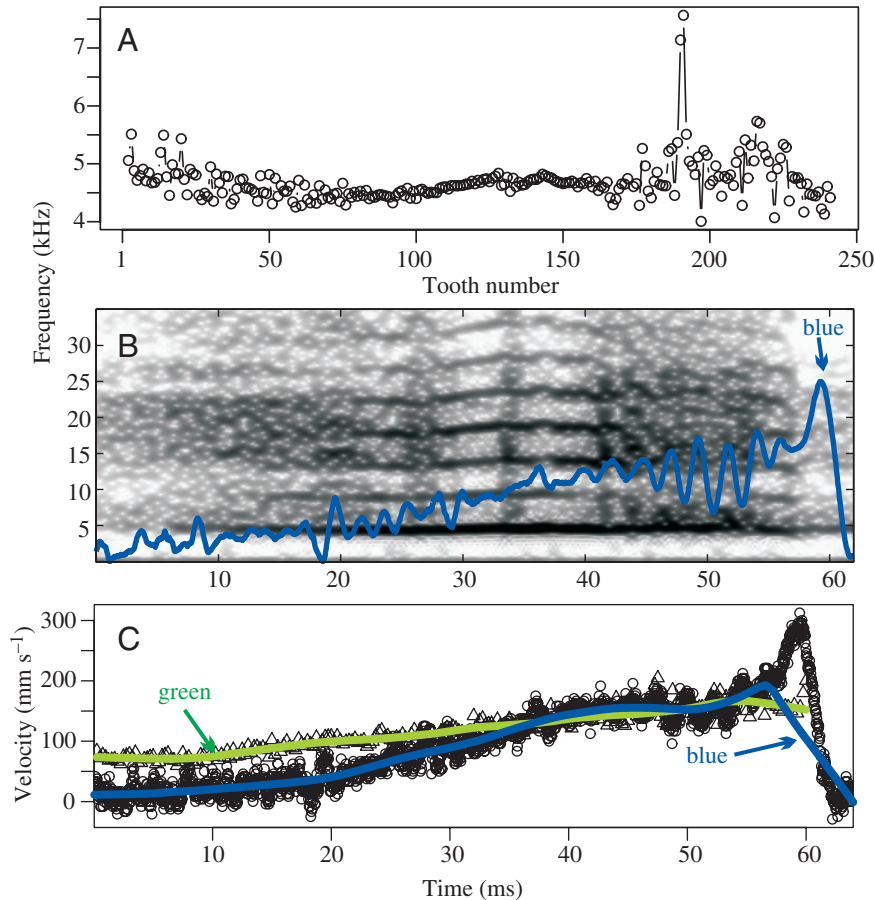


Fig. 13. (A) Cycle-by-cycle frequency analysis of a calling song. The fundamental frequency is more constant in the middle portion of the song, corresponding to the strong harmonics evident in the spectrogram of the same song (B). The blue trace represents the instantaneous velocity of the wing, calculated from the wing movement recordings. (C) Plot of predicted wing velocity (triangles, green line) for the song shown in A and B, and measured wing velocity (circles, blue line) for this song and three others from the same individual. Predicted wing velocity is calculated from the cycle-by-cycle frequency data and the measured distances between adjacent file teeth in this specimen. For each cycle of sound output, we obtained the period and calculated the wing velocity required for the scraper to traverse the corresponding inter-tooth gap in that time. This indicates the instantaneous wing velocity required for tooth-scraper impacts to occur at the same phase in every cycle of sound output. The blue and green lines are Lowess curve fits to the data. The harmonic portion of the song occurs when the actual wing velocity most closely approaches the predicted velocity for consistent phasing of tooth impacts.

allowing regular phasing of tooth-scraper impacts with respect to the sound waveform (Figs 6, 12, 13). This suggests that resonance is the main factor setting the f_D , but that the arrangement of file teeth has a strong effect on the quality of tegminal oscillation, and that tooth impact rate is not strictly regulated during the course of a stridulatory wing stroke, as it is in crickets. In crickets the scraper is released by vibrations of the file and other regions of both tegmina at the f_D (Koch et al., 1988; Bennet-Clark and Bailey, 2002). This model requires a very flexible file that controls movement of the scraper from tooth to tooth. In crickets the stridulatory file is believed to act as a spring, releasing the file as it bends upward with each oscillation of the wing (Bennet-Clark, 2003). *P. pallicornis* and its congeners, as well as several other katydid species, possess a very rigid and massive file. The amplitude of vibration of the file itself tends to decrease continuously over the course of a stridulatory wing stroke (F. Montealegre-Z and A. C. Mason, in preparation). In other words, the stridulatory file in crickets appears to bend with the tegminal oscillations, consistent with its being located on a wing that forms part of the sound radiator, whereas the file in *P. pallicornis* shows little evidence of bending and is located on the thicker left tegmen, which does have a major role in sound radiation (F. Montealegre-Z and A. C. Mason, in preparation). Oscillation of the file in this species, its congeners and most katydids, should therefore not be sufficient to control the scraper movement during the

closing stroke. The massiveness and rigid nature of the file and complex scraper anatomy suggest that sound production is governed by the mechanics of the scraper as it moves along a passive file. It should be noted, however, that the escapement mechanism of crickets does not strictly require the degree of flexibility in the file that is usually assumed. Oscillations of the scraper-bearing wing alone could dislodge the scraper from file teeth by a mechanism analogous to what we propose here (see also Bennet-Clark, 1989, 1999a).

The mechanics of the scraper

Fig. 15 outlines the mechanism we propose for the regulation of movements of the scraper during stridulation in *P. pallicornis*. When the scraper is released from a tooth, it accelerates forward until it strikes a subsequent tooth. This impact is associated with the emission of high frequency sound (red asterisk, Fig. 15B, see Bennet-Clark and Bailey, 2002). Likely the complete scraper region from the vein A3 to A1 (or A1+A2) bends upward (Fig. 15A,B). During this action, the thick membrane reduces its extent (i.e. extends downward) and will force the adjacent elastic cuticle to strongly bend towards the right side (if seen from the posterior), acquiring a maximum distortion just as it is released (Fig. 15C). At this point, the gap between the opposing and adjacent cuticles is also maximal, and all the intervening material of this region is stretched (Fig. 15C). The resulting deflection of the tegmen constitutes

a half-cycle of sound-radiating oscillation (~ 0.11 ms; see red wave in Fig. 15B,C). While this partial cycle occurs, the wings continue to move past each other and oblige the scraper to dislodge (Fig. 15C), creating a second high-frequency click sound upon its release (blue asterisk in Fig. 15D). Elasticity of the system, due to the bifurcation of the cuticle in this region,

will cause the scraper to return to equilibrium during the next 0.11 ms, while it travels to and contacts the next tooth, completing the cycle of sound-radiating oscillation. The cycle repeats when the scraper contacts the next tooth (Fig. 15E). Some of the energy released by each impact will be absorbed when the adjacent cuticle flexes right. The rest of the energy should be dispersed during the distortion of the scraper region in the form of sound-radiating vibration to the rest of the tegmina, primarily radiated by the mirror of the right tegmen and to a lesser extent by the mirror of the left (F. Montealegre-Z and A. C. Mason, manuscript in preparation). Put simply, the time required by the scraper to hit a tooth and travel between two adjacent teeth corresponds to the period of the sound cycle (~ 0.22 ms). To produce a pure frequency, a consistent interval of 0.22 ms is required between tooth contacts, and the scraper must increase its velocity as the wings' closing stroke progresses because file teeth are more separated at the basal end of the file (see Fig. 5A). Our data are consistent with this. Wing velocity also gradually increases during the production of a sound pulse (see Figs 11B, 13B,C), which predicts that tooth impacts will be stronger as the closing stroke progresses. This is consistent with observations that the sound pulse increases in amplitude towards the end. Sound amplitude decreases again in the last one quarter of the pulse, corresponding to decreasing inter-tooth distances in the last one fifth or one quarter of the file length (Fig. 5A). These observations were confirmed with our high-speed video recordings; the complete file is used during stridulation and wing velocity increases over the course of a wing-stroke, but decreases at the very end (Fig. 8D).

File morphology

File morphology may strongly affect the sound generation (Morris and Pipher, 1972; Walker and Carlyle, 1975; Montealegre-Z and Morris, 1999; Morris and Montealegre-Z, 2001). Bailey and Broughton (1970) showed that when the tooth-scraper contact rate and the natural frequency of the tegmina match, sound energy is greatest and the wave form most pure. We have evidence that average tooth-contact rate in *P. pallicornis* matches f_D of the radiated sound. We also show, however, that there is a significant overall increment from the anal to the basal of the file in inter-tooth distance, and that inter-tooth distances do not increase continuously but in an erratic fashion. These discontinuous changes in distances among teeth along the file will result in irregular phasing of tooth-contact with tegminal oscillation, inducing non- f_D

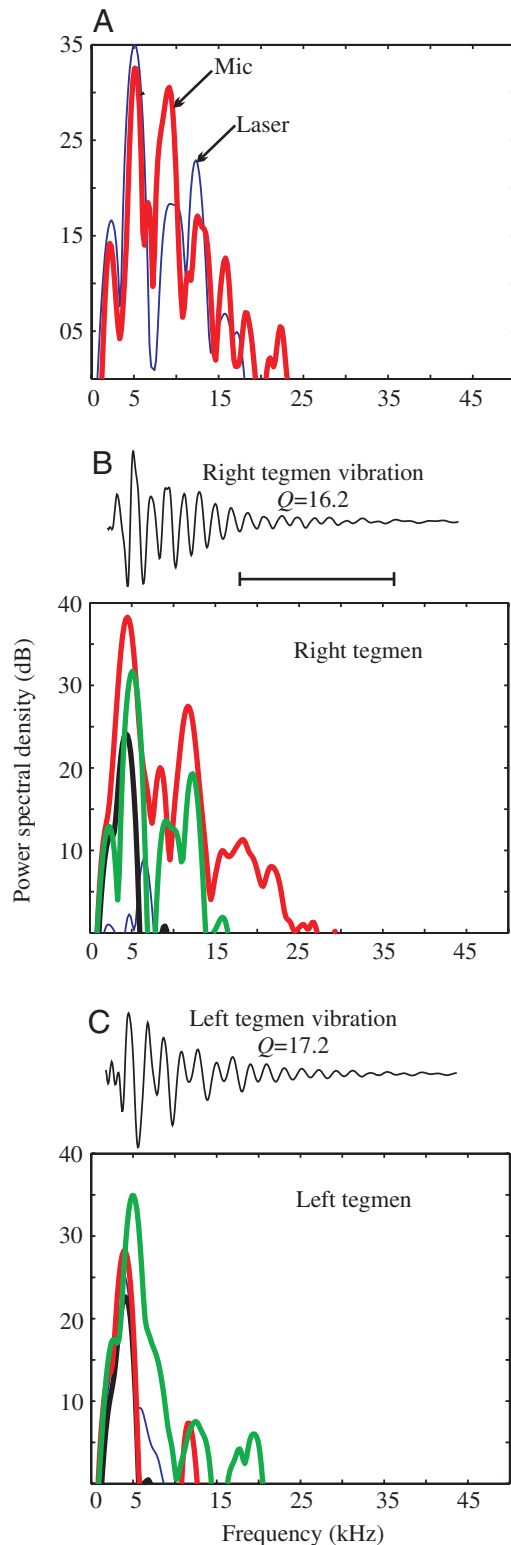
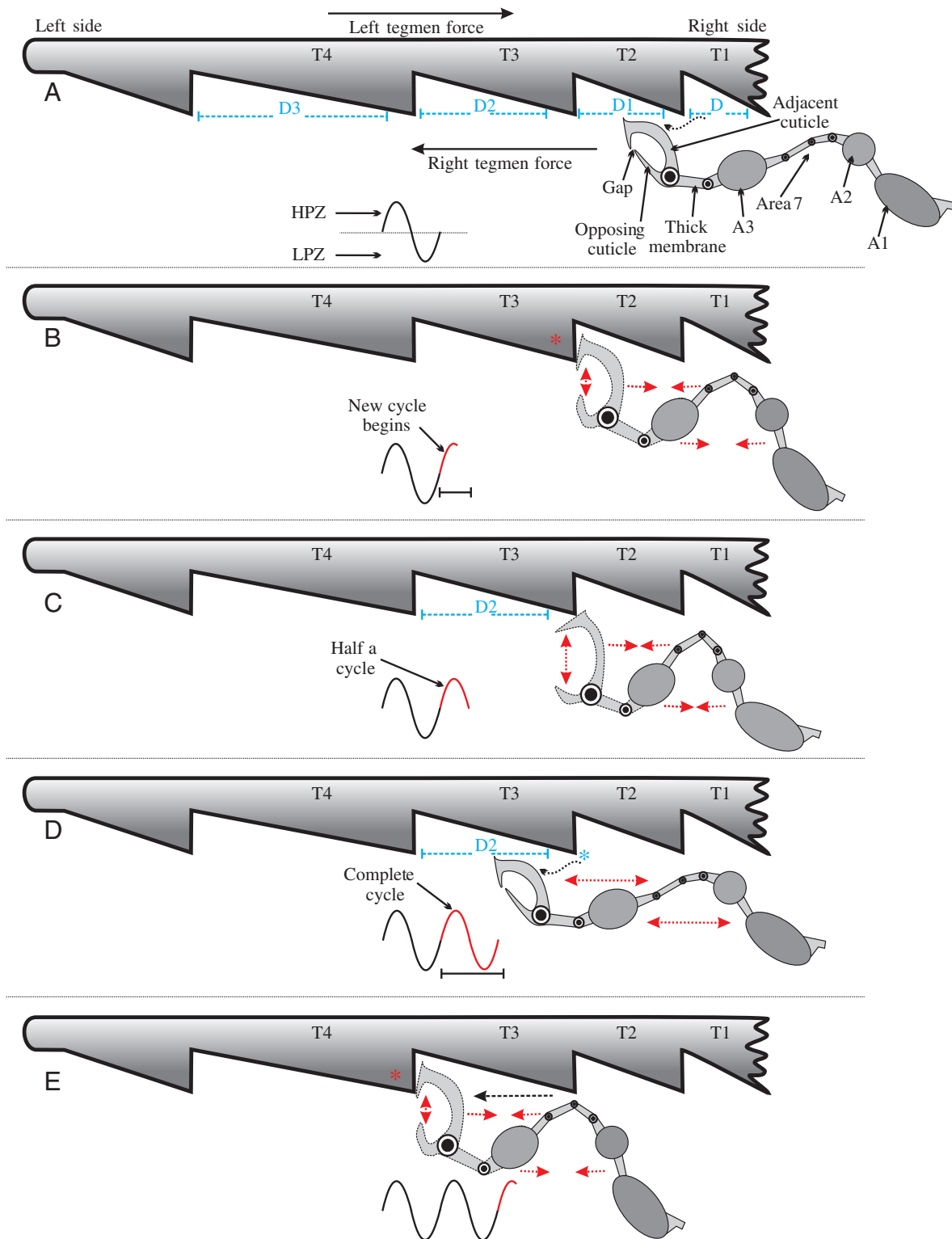


Fig. 14. Resonances of the forewings. (A) Free vibration of the right tegmen as recorded from region 4 with a probe microphone (red outline) and with a laser vibrometer (blue outline). Free vibration of the right (B) and left (C) wing of *P. pallicornis* recorded by laser vibrometer over region 1 of both wings; scale bar, 2 ms. Vibrations were induced by simulating single file-tooth/scraper impacts in dissected wings. The Q values presented above each oscillogram are means of four specimens. Power spectral analysis is shown below each oscillogram, with specimens shown in different colour outlines.

components, causing changes in the amplitude of the f_D (compared to what it would have been), and in the sharpness of high frequencies (harmonics) of the spectrum. In stridulatory files of pure-tone singers (e.g. those of most crickets and *P. cuspidatus*), the inter tooth distances increase systematically from the anal to the basal portion of the file

(Fig. 6; see also Bennet-Clark, 2003), an arrangement that favours a constant tooth strike (and in turn a constant frequency) as the closing velocity increases (Fig. 15). However, for *P. pallicornis*, inter-tooth distances tend to increase more erratically than systematically, and this may account for some of the variability in the call spectra. Bennet-



Clark (1970) observed that the file-tooth arrangement resembled the instantaneous amplitude of sound pulses in the mole crickets he studied. Increments in inter-tooth distances corresponded with increments in amplitude in the pulse. We found a similar pattern in *P. pallicornis*. Increments in inter-tooth space may generate stronger high frequency transients during engagement and release of the scraper (Bennet Clark and Bailey, 2002) due to corresponding increments in velocity.

Nevertheless, there is a net increase in the inter-tooth space towards the basal end of the file. Consequently, the tooth-contact rate is maintained close to resonant frequency of the tegmina (~5 kHz; Figs 12, 15). Notably, this pattern of increasing tooth spacing was similar in *P. cuspidatus* and *P. pallicornis*, the only species in the genus that produce sustained sound pulses, with *P. cuspidatus* having the most pure-tone song (Montealegre-Z and Morris, 2004). The other species we examined, which produce transient pulse trains, show most variable tooth spacing with largest inter-tooth distances in the middle of the file (Fig. 6C).

Resonance of the tegmina

In this section, we discuss the roles of the right and left tegmina in the sound radiation, leaving aside those structures responsible for the initial generation of tegminal vibrations (i.e. the file and scraper). Bennet Clark (1999a) discusses the general

principles of resonant vibration as they relate to sound production by insects. Our results indicate that sound radiation in *P. pallicornis* is based on resonant vibration of the tegmina. Similarly to previous work (Broughton, 1964; Morris and Pipher, 1967; Bailey, 1970; Keuper et al., 1988), our findings show that sound radiation is primarily a function of the right tegmen. Furthermore, the function of membranes of the mirror appears to be amplification rather than tuning. Removal of mirror membranes caused only a small increase in the output frequency, consistent with a decrease in the mass element of a resonator (Arya, 1998), as well as a decrease in output amplitude. It has been proposed that the mirror frame acts as a cantilever whose frequency is directly proportional to the f_D of the calling song (Morris and Pipher, 1967; Bailey, 1970; Sales and Pye, 1974). Based on this argument, we estimated the frequency of vibration of the mirror of *P. pallicornis*, which has a span of about 4.1 mm. Morris and Pipher's fig. 12 (Morris and Pipher, 1967) and Bailey's fig. 8 (Bailey, 1970) indicate a frequency of vibration of the mirror frame of *P. pallicornis* of nearly 5 kHz, which corresponds to the f_D of the calling song. This supports previous findings that the tuning of tettigoniid tegminal resonators resides, in part, in the structure of the veins surrounding the mirror, rather than the mirror itself (Broughton, 1964; Morris and Pipher, 1967; Bailey and Broughton, 1970).

The natural vibration frequency f_0 differed for the two tegmina, being higher for the right than for the left tegmen. The f_0 of the right tegmen matched the f_D of the calling song close. Differences in tuning of the two forewings is not rare in Orthoptera; similar results were presented by Bennet-Clark (2003) for the cricket *T. oceanicus*. Bennet-Clark concluded that the f_0 of the file-bearing tegmen, which is always lower when compared with the contra-lateral wing, might increase during the interaction of both forewings, as stiffness is added to the system. During the interaction both wings compensate for optimal values of f_0 , generating the f_D of the calling song.

The primary role of the left tegmen appears to be to provide damping. Loading of the left tegmen resulted in decreased amplitude and frequency of output, as predicted for an increase in the damping coefficient in a forced resonant system (Arya, 1998). In addition to reduced amplitude and a shift of the resonance peak to lower frequencies, increased damping in a forced resonator also results in a broadening of the relationship between output amplitude and driving frequency; this effect is described by the quality factor, or Q value. For high- Q systems, damping is light and there is a dramatic increase in vibration amplitude when the driving frequency matches the resonant frequency. But this resonance peak is narrowly tuned and the driving frequency must match the resonant frequency very closely to maintain this output. In lower- Q , more heavily damped systems, there is a broader relationship between output amplitude and driving frequency such that variation in the driving frequency causes more gradual changes in vibration amplitude. In other words, a more strongly damped system can be driven over a broader range of frequencies than a more lightly-damped, higher Q system. In many mechanical systems, where consistent operation is required, damping

Fig. 15. Model of scraper–file interactions and their relation to sound. (A) Hypothetical file segment and scraper region. Note the increments in inter-tooth distances (in the direction of scraper movement) indicated with blue dotted lines and letters; the structures of the scraper involved are also labelled. The sound cycle, shown below, was generated when the scraper was released from T2 (tooth 2) and crossed the region with distance D1. Note that the scraper has been released and is moving forwards, travelling over D1 to find the following tooth (T3). HPZ and LPZ, higher and lower pressure zones of the sound wave, respectively. (B) The scraper hits T3, generating a reaction of the system at its natural frequency (~ 5 kHz for *P. pallicornis*). The oscillatory reaction is shown in the diagram with a red outline (half a cycle), which is added before the previous cycle, indicated in A, decays. The impact will also generate a vibration of high frequency (see Bennet-Clark and Bailey, 2002), represented with a red asterisk but not indicated in the oscillation of the diagram. The scraper is temporarily trapped by T3 but, as the forewings continue in motion, the scraper regions bend upwards. In this model, the initial deflection of the oscillator is upward, to generate the higher pressure zone (maximal amplitude of the sound cycle, see A); scale bar, 0.11 ms. (C) The motion of the wings causes the scraper to dislodge while the first half of the oscillation is completed. (D) The distorted adjacent membranes temporarily recover their original shape, passing through equilibrium. The adjacent membranes and the rest of the oscillator of the right tegmen continue their vibration downward to generate lower pressure zone, completing the second half of the cycle (red outline). The scraper completely dislodges from T3, generating a click sound also of high frequency (blue asterisk), and travels over D2. Scale bar, 0.22 ms. (E) The scraper travels D2 and strikes T4 before the previous oscillation decays; this action repeats over and over. Note that the time spent by the scraper to go from one tooth to the next is equal to the period of 5 kHz (0.22 ms \cong 1/5 kHz).

serves to eliminate undesirable peaks in vibration due to varying frequencies of input energy. In insect sound production, resonance is usually discussed as a strategy for maximizing output power at the expense of bandwidth (Michelsen and Nocke, 1974), or regulating output frequency (Bennet-Clark, 1999a). Our results suggest that katydids producing broadband acoustic signals, such as *P. pallicornis*, may also make use of resonant vibration of sound radiating structures to improve output efficiency by opting for a more or less low-*Q* resonator with a wider bandwidth (Fig. 14).

The quality of sound output in *P. pallicornis* depends on matching the timing of tooth-scraper impacts to the frequency of sound produced. Males accelerate their wings during stridulation, generating a frequency sweep of tooth-scraper impact rates. A broader resonance in tegminal vibration should allow a greater portion of the wing-stroke (i.e. a greater range of tooth-scraper impact rates) to result in efficient sound generation than would be possible with a narrower resonance, given the irregularity of the driving force. Therefore, *P. pallicornis* song may reflect a trade-off between output amplitude and the duration of the song over which a clear frequency structure can be maintained in the absence of a mechanism to directly regulate the tooth-strike rate (such as the escapement mechanism of crickets).

Conclusion

Our results show that the escapement mechanism of the 'clockwork cricket' (Elliot and Koch, 1985; Bennet-Clark and Bailey, 2002) is probably not at work in katydids, even those that produce pure-tone songs. Sound production in *P. pallicornis* does rely on the tuned vibration of tegminal resonators. The excitation of tegminal vibration, however, relies on a more variable mechanism than the precisely regulated escapement of crickets. A rigid file and a scraper mounted on a flexible joint allow stridulatory wing movements to occur at a range of different speeds. Engagement with file teeth will displace the scraper by deflecting it around its flexible joint. The subsequent release will allow the scraper to spring back to its natural position and, combined with continued wing movement, will result in an impact between the scraper and the next file tooth. This impact will induce vibrations in the tegmina. When the wing velocity is such that successive tooth impacts occur at an interval that matches the natural vibration frequency of the tegmina, resonant vibration will build up in the wing. By sweeping their wings over a range of velocities during stridulation, *P. pallicornis* males, in the absence of a mechanism that specifically matches the rate of tooth impacts with tegminal vibration cycles, are able to produce a complex song spectrum that includes a resonant portion when wing velocity and tooth spacing coincide with the natural mode of tegminal vibration.

This study was supported by Natural Sciences and Engineering Research Council of Canada, operating grant (238882) to Andrew Mason and (4946) to Glenn K. Morris. Thanks to Dr Glenn K. Morris for his useful criticisms and

impetus, and for his constant support. We immensely appreciate the help of Dr Bruce Schneider for the guidance and valuable remarks during this research. We also thank Dr Kenneth Prestwich and Dr Henry C. Bennet-Clark for his feedback and opinions. Dr Uwe W. Koch and Dr Berthold Hedwig offered suggestion during the construction of one of the position detectors (PD) used in this work. The circuit system of our PD was designed by Dr Koch and assembled by Mr Andrew Veglio at the University of Toronto at Mississauga. We thank Dr Otto von Helversen and people in his lab for their direction and advice during the recordings of wing movements of the specimens transported to Germany. Christine Belanger made microtome sections and staining solution. Special thanks to Dr James Fullard for allowing us to use his equipment. Eric Lin (University of Toronto) helped with the use of the electron microscope. The Colombian Ministry of the Environment granted permits to collect and export the specimens used in this study under decree No. 077, of April 23, 2002. Special thanks to government officials Sandra Navarrete and Francisco de Paula Gutierrez. We are extremely grateful to the Corporación Autónoma Regional del Valle del Cauca (CVC) for providing permits and accommodation during our field work at the natural reserve of Yotoco, particularly to Efrén Salcedo, Joaquín Romero, Wilmar Bolivar and to our field assistants Manuel and Fernando Vargas-S. Liliana Castano-R. helped to take care of insects in captivity. This manuscript was improved thanks to the constructive comments of our reviewers.

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