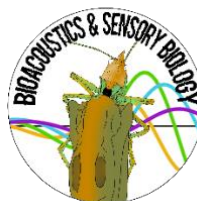




UNIVERSITY OF
LINCOLN



Invertebrate Sound and Vibration 2023 Lincoln, UK





ISV2023 Conference Programme

Thursday, 30th March 2023 (DoubleTree Hilton Hotel)

Arrival, registration and Cocktail Reception from 16:00 onwards in the Rooftop Electric Bar, DoubleTree Hilton. Brayford Wharf North, LN1 1YW

Friday, 31st March 2023 (Lincoln Medical School)

- 09:00: **Fernando Montealegre-Z and Ben Warren** Welcome address
- 09:10-10:10: **Plenary One - Nathan Bailey** Evolutionary mysteries of acoustic diversification in Insects: Wings that sing and genes that make them.

Session 1: Ecology and Evolution of Acoustic/vibratory Behaviour

Chair: **Fernando Montealegre-Z**

- 10:10: **Reinhard Lakes-Harlan** A mysterious signalling in *Stilpnochlora coulouana*
- 10:30: **Tatiana Tarasova** The processes in the hybrid zone between sibling grasshoppers
- 10:50: **Tony Robillard** Convergent evolution of harmonic hopping...
- 11:10: **Holger Braun** The ultrasound male-female communication of two short-winged bushcrickets
- 11:20-11:40, *Coffee break*
- 11:40: **UK Bioacoustics Network Pitch**
- 11:50: **60 Second Poster Pitch** (all delegates presenting a poster)

12:20-12:30 Conference photo

12:30-14:20 Buffet Lunch, Poster Session and Exhibitors' Display

Chair: **Darron Cullen**

- 14:20: **Leonidas-Romanos Davranoglou** Sexual selection and predation drive the repeated evolution of stridulation in Heteroptera and other arthropods
- 14:40: **Mario Vallejo-Marin** Buzz pollination: comparison of defence vibrations produced by bees...
- 15:00: **Monika Eberhard** Effects of vibratory performance during courtship of male spiders
- 15:20: **Jennifer Gleason** Running on empty: effect of starvation stress on courtship song of *Drosophila*
- 15:40: **Teddy Gaiddon**. Harmonic hopping in the songs of lebinthini crickets: using finite element ...
- 16:00: **Taina Conrad** The important role of parental acoustic signalling during brood care in the beetle...
- 16:20-16:40, *Coffee break*

Session 2: Biomechanics of sound and vibration production and reception

Chair: **Tony Robillard**

- 16:40: **Erin Brandt** Spider leg joint membrane as putative 'ears'
- 17:00: **Thorin Jonsson** Mechano-acoustic analysis of harmonic hopping in an eneopterine cricket
- 17:20: **Charlie Woodrow** Comparative analysis of the katydid 'ear canal' suggests acoustic niche expansion through isometry
- 17:40: **Ritesh Ghosh** Plant-sound vibration interaction: an ocean of possibilities



Saturday, 1st April 2023 (Lincoln Medical School)

- 09:00-10:00: **Plenary Two - Marta Andres** Auditory neuromodulation in mosquitoes: implications for mosquito behaviour and vector control

Session 3: Neurophysiology of acoustic signalling

Chair: **Ben Warren**

- 10:00: **Manuela Nowotny** Temporal dynamics of signal processing along the bushcricket's auditory pathway
- 10:20: **Mei-ling Joiner** Testing ginko2 in auditory neurons
- 10:40: **Daniel Eberl** Neurotransmitter effects on hearing in the mosquito, *Aedes aegypti*: mechanics and physiology
- 11:00-11:30, *Coffee break*
- 11:30: **Ali Cillov** Potential function of local and descending auditory neurons in a bush cricket
- 11:50: [Lincoln Lab Tours](#)

[12:30-14:00 Buffet Lunch, Poster Session and Exhibitors' Display](#)

Session 4: Emerging approaches to mechano-sensation

Chair: **Manuela Nowotny**

- 14:00: **Thomas Austin** Age resilience in an insect auditory system
- 14:20: **Ryan Palmer** Exploring electrical environments: the possibility and impact of electroreception
- 14:40: **Oscar Guadayol** Viscoelastic properties of the Auditory Vesicle of katydids
- 15:00: **Emine Celiker** Beyond the exponential horn: A comparison of bush-cricket acoustic trachea biomechanisms
- 15:20: **Christian Thomas** Single-cell sequencing approaches for invertebrate mechanosensors
- 15:40-16:20, *Coffee break*

Session 5: Prey and Predator Interactions

Chair: **Thorin Jonsson**

- 16:20: **Brandi Pessman** Vibratory noise may affect prey capture and web structure of the funnel-weaving
- 16:40: **Rohini Balakrishnan** Predation risk of signalling and searching in a multimodally duetting katydid

[17:00-18:00 Wine and Poster Session](#)

[18:45 Lincoln Cathedral Pre-dinner Tour and drinks, Miss Camille Marie Montealegre on Piano Forte](#)

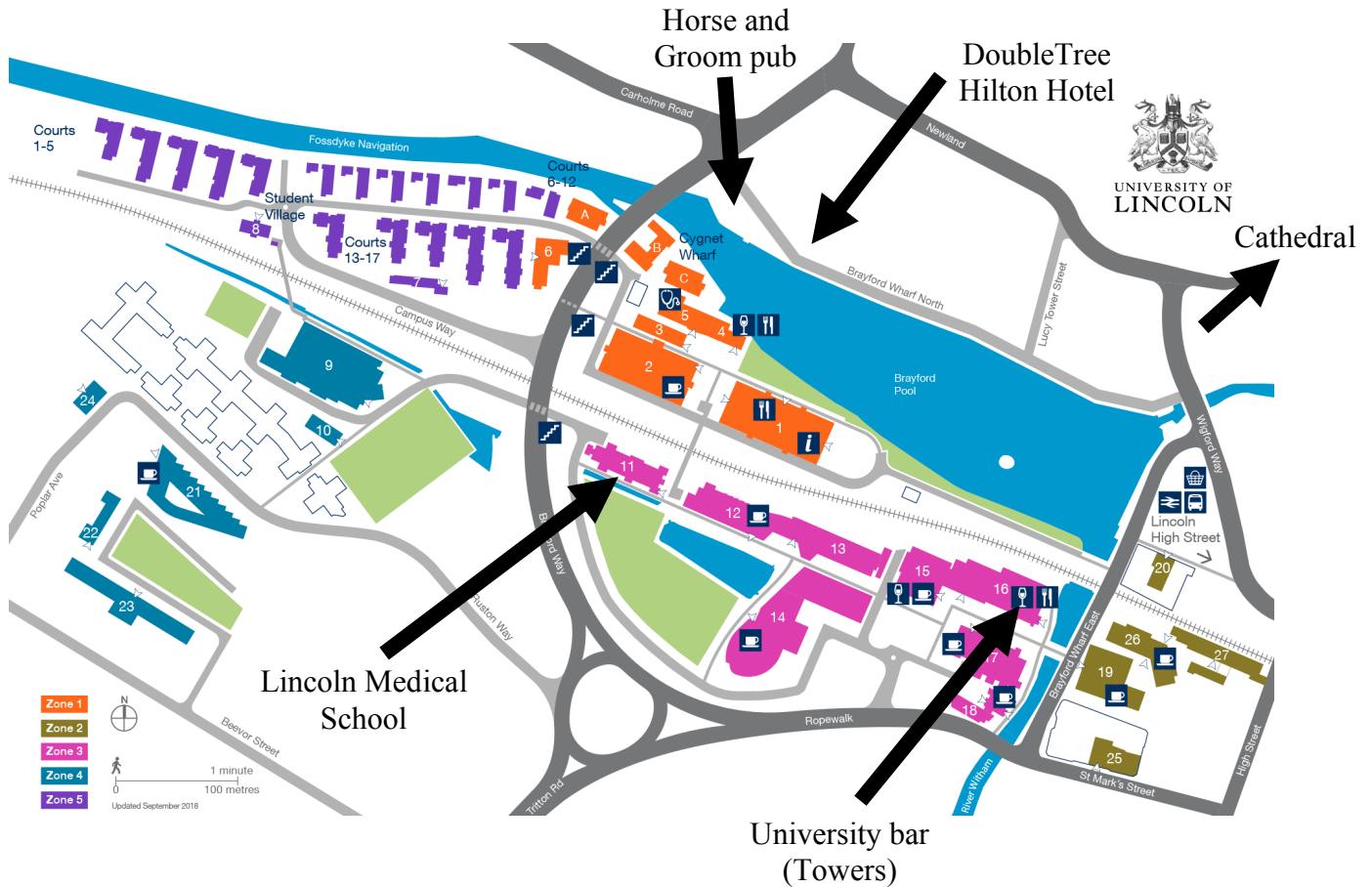
[20:00 Gala Dinner, Lincoln Cathedral](#)

Sunday, 2nd April 2023 (Lincoln Medical School)

Session 6: Final session

Chairs: **Ben Warren and Fernando Montealegre-Z**

- 09:00-10:00 **Plenary Three - Natasha Mhatre** Exploring morphological landscapes using biophysical models
- 10:00: **Marcelo Christian** Impact of conventional neonicotinoids and a novel alternative insecticide
- 10:20: **Atitheb Chaayasitdhi** Sound-evoked mechanics of auditory transduction machinery in desert locusts
- 10:40: *Farewell address and drinks*



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EVOLUTIONARY MYSTERIES OF ACOUSTIC DIVERSIFICATION IN INSECTS: WINGS THAT SING AND GENES THAT MAKE THEM



Nathan Bailey

School of Biology, University of St. Andrews, St. Andrews, KY16 9TH, UK

Singing insects must produce signals that are consistently recognisable to potential mating or competitive partners. However these signals have diversified extensively over evolutionary time. How have the genetic, morphological, and neurophysiological constraints maintaining signal consistency been repeatedly broken during this process? My talk will explore this question, focusing on *Ensiferan* tegminal sound production. I will discuss insights my research group has gained using Hawaiian populations of the field cricket *Teleogryllus oceanicus*, in which a remarkable array of male-silencing mutations disrupt forewing morphology but persist under selection from an eavesdropping parasitoid. I will explore the genetics and functional consequences of these tegminal variations and discuss the insights they provide about broader macroevolutionary patterns of signal diversification in *Ensifera*.

AUDITORY NEUROMODULATION IN MOSQUITOES: IMPLICATIONS FOR MOSQUITO BEHAVIOUR



Ellis D¹, Freeman E¹, Tytheridge S¹, Bagi J¹, Su M², Albert J¹, Andres M¹

¹ Ear Institute, University College London, 332 Gray's Inn Road, London, WC1X 8EE, UK

Mosquito ears are tiny acoustic sensors located in their antenna that detect the wingbeat frequencies of mating partners flying in close vicinity. The acoustic recognition of mating partners take place in aerial swarms that are composed of hundreds of male mosquitoes and few females flying together. The sensory ecology of the swarm, where faint female flight tones are recognized amidst the noise generated by hundreds of mosquitoes has acted as evolutionary driving force to shape highly unique ears. Apart from being highly sensitive and deeply sexually dimorphic, mosquito ears are innervated by an elaborate system of auditory efferent neurons- the only documented example across insects. The efferent fibres release the neurotransmitters octopamine, serotonin and GABA to modulate different aspects of mosquito auditory function and influence the detection of the mating partner. In the lab, we combine genetic, physiological and behavioural approaches to study the effects of this neuromodulation. Moreover, because mosquitoes act as vectors of diseases, we are also interested in exploiting its auditory system as target of control approaches to control disease-transmitting mosquito populations. In this talk, I will review our knowledge of this fascinating system, establishing links across mosquito species, and bridging the gap between mosquito auditory function and behaviour.

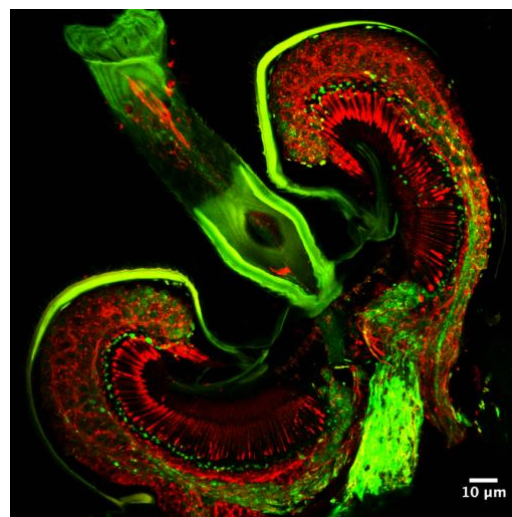


Figure 1. An elaborated network of efferent fibres innervates the mosquito auditory organ. Immunostaining with a presynaptic marker (in green nc46 antibody, anti-SAP47) labels the auditory neuron cilia and somata, and the auditory nerve suggesting efferent input. In red the neuronal marker anti-HRP shows the complexity of the mosquito auditory organ.

Key Words: hearing, mosquitoes, auditory efferent system, octopamine, serotonin, GABA

EXPLORING MORPHO-FUNCTIONAL LANDSCAPES USING BIOPHYSICAL MODELS



Natasha Mhatre

Department of Biology, Western University, London, Ontario, Canada

A key idea in biology is that structure determines function. Over the last few decades, we have seen a huge explosion in our ability to model and therefore predict the functional roles of biological objects, right from the sub-molecular to the organismal scale.

In studying sound and vibration communication, finite and boundary element modelling, is a well-established modelling system has been instrumental in developing new knowledge. What sets it apart from older techniques is that it allows us to simulate the mechanics and acoustics of the complicated morphology of real biological organisms in their environments.

An interesting direction that has grown out of our work, is that the targets of our explanation are no longer single 'platonic' animals. We are now using these models to think about the variation in animal's morphologies, whether over behavioural or evolutionary time scales. We now make ensembles of models, rather than targeting a single prototypical animal for explanation. This allows us to draw different representations describing how morphological structure and function covary. These representations capture the variations and possibilities within the structure, either as landscapes over the full state-space of the problem, or in terms of variation in the predicted behaviour.

Eventually, the hope is to develop more careful mechanistic arguments about the variation and evolution of structure and function. In my talk I will present some of our recent work where we use ensemble modelling. In particular, I will focus on using ensemble modelling to

understand the effect of morphology and environment on sound radiation efficiency as against just sound propagation in cricket and animal acoustic communication.

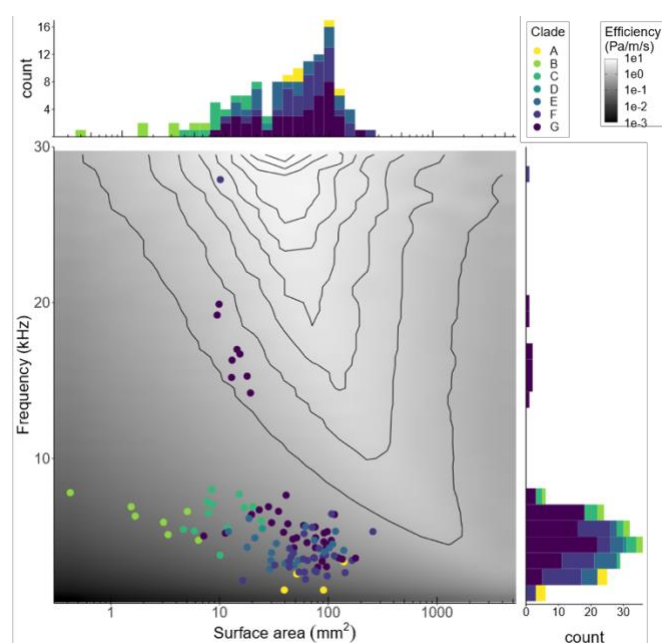


Figure 1. The sound radiation efficiency of 113 true crickets (Grylloidea) from the 7 clades defined in Chintauan-Marquier et al. 2016. This efficiency landscape shows the optimal size and frequency conditions for cricket-wing like radiators. All sampled crickets call in sub-optimal conditions and most do not use tools or acoustic aides such as baffles or resonant burrows. Using numerical modelling techniques, we investigated whether crickets and other animals have other means of increasing their sound radiation efficiency.

Key Words: biophysical modelling, crickets, tool use, alternative strategies, acoustic communication.

SOUND-EVOKED MECHANICS OF AUDITORY TRANSDUCTION MACHINERY IN DESERT LOCUSTS



Atitheb Chaiyasitdhi ¹, Benjamin Warren ²

¹ Laboratoire Physico-Chimie Curie, Institut Curie, Université PSL, Sorbonne Université, CNRS UMR168, Paris, France

² Department of Neuroscience, Psychology and Behaviour, University of Leicester, Leicester, UK

How insect convert sound-evoked vibrations into electrical signals that then insects eventually hear remains largely unknown. The sensory unit of insect auditory transduction is the scolopidium, an elongated multicellular unit that contains a 10- μm long single cilium. The cilium, a finger-like protrusion at the end of auditory nerve cells, is believed to be the site of mechano-electrical transduction. How the cilium move and gates the auditory transduction channel remain speculative.

Here, we describe our preliminary results on how the sound-evoked vibrations may gate the mechano-electrical transduction channels. We used a high-speed camera to detect motion of components within the scolopidium from ex-vivo preparations of desert locusts down to 10 nm at speeds over 10,000 times a second. The spatial resolution below the diffraction limit of light microscopy ($\sim 200 \text{ nm}$) was achieved by detecting differential activation of photosensors in the CMOS camera array (Figure 1).

Upon sound stimulation, we observed sound-evoked motion of the cap cell along the longitudinal axis of the scolopidium while the scolopidial walls and the cilium move orthogonally. In addition, we also observed rocking motion of the cap cell where the site of the cap cell that attaches to the cilium is the centre of rotation. These relative movements of the components within the scolopidium

suggest that sound-evoked vibration of the cap cell may stretch the cilium and thus activate the opening and closing of the mechanosensitive ion channels. These results are preliminary, yet they are the first direct recordings of sound-evoked mechanics of scolopidium.

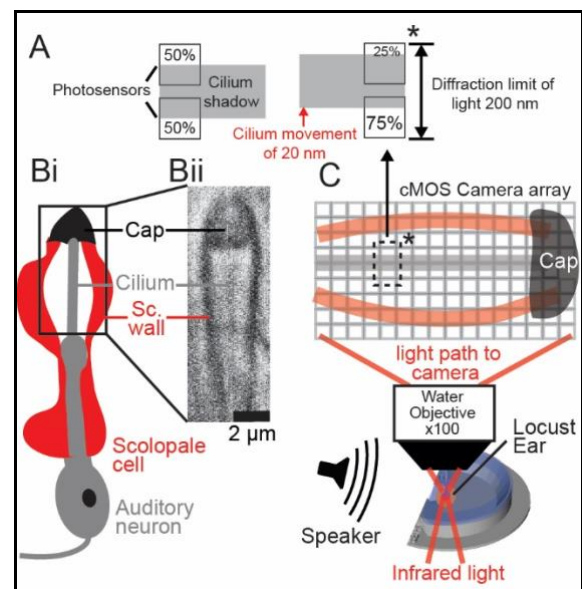


Figure 1. A. Nanometre detection using adjacent photosensors. Bi. Diagram of auditory neuron and distal scolopidium. Bii. ex-vivo image of scolopidium, 100 μs exposure time. C. Simplified microscopy setup and photosensor array.

Key Words: gating mechanism, transduction, mechanics, desert locust, hearing

VIBRATORY NOISE MAY AFFECT PREY CAPTURE AND WEB STRUCTURE OF THE FUNNEL-WEAVING SPIDER, *AGELENOPSIS PENNSYLVANICA*



Brandi Pessman¹ & Eileen Hebets¹

¹ University of Nebraska-Lincoln, Lincoln, Nebraska, United States

Mounting evidence suggests that air- and water-borne noise are pervasive and emergent threats to animal fitness. Yet we know little about the extent and consequences of substrate-borne (vibratory) noise, despite the ancient and ubiquitous use of substrate-borne information by animals. The funnel-weaving spider *Agelenopsis pennsylvanica* relies on web vibrations to detect prey and is prevalent across urban and rural habitats in North America (Figure 1). To determine if and how experience with different vibratory environments affects prey capture and (presumably) associated web-building behavior, we collected penultimate *A. pennsylvanica* from urban and rural habitats, demonstrated to differ in natural noise levels by 13 dB. Upon maturation, we exposed urban and rural females to ‘loud’ or ‘quiet’ vibratory playbacks (13 dB difference) using a fully crossed 2x2 design. We measured the latency to attack an artificial ‘prey’ stimulus twice a week for 6 weeks and web characteristics at the experiment’s end. Rural spiders attacked the artificial prey faster in loud rather than quiet conditions, while urban spiders did not differ between treatments. We also found that spiders may modify web structure to match current vibratory conditions, as spiders in loud conditions built denser webs than spiders in quiet conditions despite similar dry silk masses. Our results suggest that experience with different vibratory environments could affect prey capture and that *A. pennsylvanica* may adjust web structure to changing vibratory conditions.



Figure 1. *Agelenopsis pennsylvanica* builds funnel-shaped webs that transfer vibratory information about prey and mates. These spiders are prevalent in urban and rural habitats that vary in vibratory noise levels.

Key Words: anthropogenic noise, foraging, playback, urbanization

A COMPARATIVE ANALYSIS OF THE KATYDID 'EAR CANAL' SUGGESTS ACOUSTIC NICHE EXPANSION THROUGH ISOMETRY



Charlie Woodrow ¹ & Fernando Montealegre-Z¹

¹ School of Life and Environmental Sciences, Lincoln, Green Lane, Lincoln, LN6 7DL United Kingdom

Body size is a constraint on animal sound production and auditory tuning. Larger animals produce lower frequency sounds and have greater hearing sensitivity to low frequencies. This phenomenon, known as acoustic allometry, has historically been studied in vertebrates, where sounds vary substantially in their frequency, intensity, and temporal structure. Insects offer an ideal system to study acoustic allometry, as many insects can only produce a single signal. In katydids, males produce species-specific sounds using their forewings, and these sounds are received via an ear canal in the forelegs, which is believed to be biophysically tuned to the male song. It is known that larger katydids tend to produce lower frequency sounds, but the biophysical tuning of the ear canal, in a broad comparative context, is unknown. We used micro-CT imaging, phylogenetically controlled scaling analyses, and numerical modelling, to examine the morphological diversity and biophysical tuning of the katydid ear canal. It is revealed that ear canal geometry scales linearly with body size, and displays little morphological variation between species.

Numerical modelling and scaling of song frequencies with body size hints at a global acoustic isometry, whereby larger katydids have lower natural resonant frequencies (eigenfrequencies) of their ear canal to passively enhance the lower frequency acoustic signals of conspecifics. This suggests that katydids maintain essential frequency overlaps between signaller and receiver through simultaneous changes to the size of sound production and reception organs, permitting rapid and efficient acoustic niche expansion.

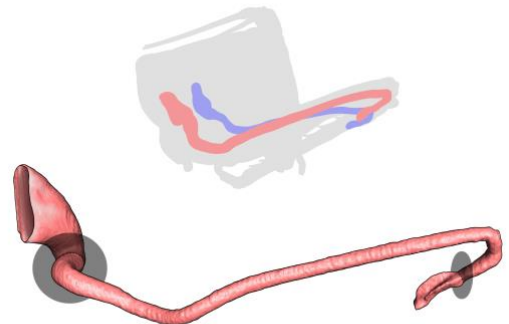


Figure 1. The katydid ear canal.

Key Words: hearing, bioinspiration, geometry, biophysics, modelling

SINGLE-CELL SEQUENCING APPROACHES FOR INVERTEBRATE MECHANOSENSORS



Christian Thomas¹, Tom Austin¹ & Ben Warren¹

¹ University of Leicester

As we age our auditory system's functionality decline. The pace at which this happens is accelerated if one is exposed to prolonged noise exposure. The Warren lab has previously shown the desert locust *Schistocerca gregaria* also exhibits noise induced auditory decline.

Whilst hearing loss in mammalian studies often point to a reduction in the number of hair cells, insect systems lack hair cells. Furthermore, recent studies in our lab reveal no difference in the number of auditory nerve or total cell count number in relation to age or noise exposure.

Single-cell sequencing is revolutionising the field of mammalian auditory organ function. Cell type as opposed to bulk RNA sequencing allows for the detection of much more subtle changes in gene expression. Recent single-cell sequencing experiments have helped elucidate the function of more cell types in the mammalian ear, including root and spindle cells.

Here, we hope to use single nuclei sequencing to help elucidate the cell types that exist in the Locust's Müller's organ. With this we hope to create a single nuclei atlas of the Locust's Müller's organ. We aim to determine the cellular function of each of the generated clusters based off previous mammalian single cell datasets and information from the *Drosophila* single cell atlas.

Once we have established a Locust auditory cell atlas, we hope to isolate specific cell types using FACS sorting, and elucidate how noise exposure, age and sex all affect auditory ability

in locust and potentially other invertebrate systems.

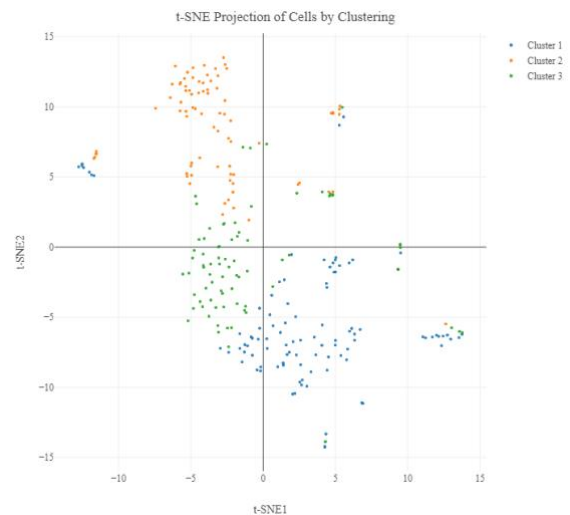


Figure 1. Preliminary data on 264 unsorted cells from the Müller's organs. Top genes from each cluster hint that cluster 1 may be neuronal cells, cluster 2 the epidermis, and cluster 3 a pumping type cell.

Key Words: Single Cell Sequencing, Transcriptomics, Locust

POTENTIAL FUNCTIONS OF LOCAL AND DESCENDING AUDITORY NEURONS IN A BUSH CRICKET



Ali Cillov ¹, Nataša Stritih Peljhan ² & Andreas Stumpner ¹

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² National Institute of Biology, Department of Organisms and Ecosystems Research, Večna pot 111, SI-1000 Ljubljana, Slovenia

Orthopterans have evolved acoustic communication >2-3 times independently. Their auditory systems share similar units, but exhibit divergent adaptations. A duetting bushcricket, *Ancistrura nigrovittata* (Phaneropteridae) has been studied in unprecedented detail. Males sing with a distinct temporal pattern at ca. 16 kHz and females respond with short delay and a click at ca. 28 kHz. The critical parameters for song recognition are known. Auditory neurons are local in the prothorax, ascend to the brain, descend posteriorly or do both. Ascending information is shaped by local processing in the prothorax. Two of the four known local (LN) and most descending neurons (DN) have not been studied in detail. Data from other bushcrickets are either missing or distributed over different taxa. We describe LN and DN in *A. nigrovittata* and ask what function they might have in song recognition.

Our data from intracellular recordings and stainings show morphologically similar but physiologically diverse DNs, including one that has a reduced descending axon, making it functionally an LN. Another LN is similar in its temporal responses but mainly differs in directionality. Both types might serve as reference neurons for sound. Most DN with central projections respond to acoustic stimuli only, while those with lateral projections respond also or nearly exclusively to vibration. We also report a neuron tuned to the male song frequency but also responding to vibration, possibly participating in the switch from long-distance acoustic to short-distance acousto-vibratory communication. Such short-distance acoustic and vibratory

behaviour is described for the first time in this species.

Key Words: Orthoptera, bushcricket, neuronal processing, descending neurons, local processing

EFFECTS OF VIBRATORY PERFORMANCE DURING COURTSHIP OF MALE SPIDERS (*PISAURA MIRABILIS*)



Monika J. B. Eberhard, Morgan M. Oberweiser, Stefan ter Haar, Paul-R. Franz & Gabriele Uhl

Zoological Institute and Museum, University of Greifswald, Loitzer-Str. 26, 17489 Greifswald, Germany

Male courtship can serve various purposes such as species recognition, mate localization, indication of individual quality or mating status. In the cursorial spider *Pisaura mirabilis*, where males present a nuptial gift to females, courtship includes visual, chemical, and vibrational stimuli. Courtship vibrations consist of repeated tremulation pulses which likely advertise the male's condition. Spiders are also known for sexual cannibalism; therefore, we can expect that male traits that reduce the risk of being cannibalized are selectively advantageous. Here we explore the functional role of vibratory communication within the framework of *P. mirabilis* reproduction.

We first assessed vibratory performance of 150 male spiders by collecting repeated recordings and analysing the pulse train associated with the courtship. These "screening" measurements were used to assess the variability within and between signalling males. Second, we investigated whether male vibratory courtship signals delay female predatory response. We tested this by playing back male vibratory courtship, white noise, or a silent control while simultaneously exposing females to prey (N=52).

Finally, we assessed the copulation probability of females with muted (pro- and opisthosoma glued together to restrict tremulation) vs. intact males to investigate whether females require courtship vibrations to accept a mate (N=15).

Our results suggest that male vibratory courtship exhibits enough between-individual variability to assess male quality. We found no delay of female predatory response to prey between treatments. Hence, vibrations might

not serve to reduce female aggression towards courting males. Our ongoing work will show if vibrational signals are needed for male acceptance by females.



Figure 1. Male *P. mirabilis* carrying a prey item wrapped in silk to present it to the female as a nuptial gift. Vibrogram of courtship pulses shown below. Photo by G. Uhl.

Key Words: Vibratory courtship, arachnids, biotremology, signal variability, signal repeatability, aggression reduction

NEUROTRANSMITTER EFFECTS ON HEARING IN THE MOSQUITO, *Aedes Aegypti*: MECHANICS AND PHYSIOLOGY



Daniel F. Eberl^{1,2}, YiFeng Y. J. Xu¹, YuMin M. Loh¹, Matthew P. Su^{1,3} & Azusa Kamikouchi¹

¹ Graduate School of Science, Nagoya University, Nagoya, Japan

² Department of Biology, University of Iowa, Iowa City, USA

³ Institute for Advanced Research, Nagoya University, Nagoya, Japan

Mosquito courtship and mating rely heavily on hearing function. Male mosquitoes demonstrate phonotactic attraction to the distinct sounds of flying females whilst in courtship swarms. Males can identify females despite swarms being vastly male-dominated partly due to the astonishing sensitivity and complexity of their hearing systems. These hearing systems, comprised of a flagellar sound sail coupled to a Johnston's organ (JO), the site of auditory mechanotransduction, are highly sexually dimorphic, with male JOs containing around twice as many neurons as female JOs.

Uniquely amongst insects, efferent pathways from the mosquito brain to the JO have been documented anatomically, with efferent fibers synapsing on JO neuron cell bodies (Andres et al, 2016). Furthermore, both octopamine (Georgiades, Alampounti et al, 2022) and serotonin (Xu, Loh et al, 2022) have been shown to alter the frequency tuning of the male antenna. Octopamine expression also influences afferent signals in the antennal nerve. However, the influence of other major neurotransmitter families on mosquito hearing function (and thus behavior) remains unclear.

Here, we report our progress in further investigation of neurotransmitter effects on hearing in the yellow fever mosquito *Aedes aegypti*. By combining functional analyses of flagellar mechanics by laser Doppler vibrometry, and JO physiology by extracellular recordings from the antennal nerve (Sound-Evoked Potentials (SEPs)), with anatomical and molecular assays, we can improve our understanding of the various roles

potentially played by neurotransmitters including dopamine and histamine.

Implications of our findings will be discussed in terms of improving our fundamental understanding of auditory mechanisms in this exquisitely sensitive ear.

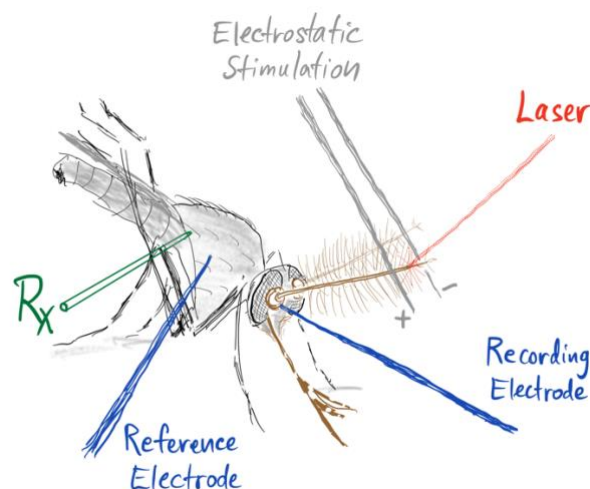


Figure 1. Preparation for recording electrophysiological activity from the *Aedes aegypti* antennal nerve in response to electrostatic stimulation with defined stimuli. Simultaneous monitoring by laser Doppler vibrometry allows measurement of precise movements of the flagellum. This preparation is used to measure the effects of neurotransmitter-related compounds.

Key Words: Monoamines, Johnston's organ, sound-evoked potentials, laser Doppler vibrometry

BEYOND THE EXPONENTIAL HORN: A COMPARISON OF BUSH-CRICKET ACOUSTIC TRACHEA BIOMECHANISMS



Emine Celiker ^{1,2}, Charlie Woodrow ², Aurora Y. Rocha Sanchez ³, Benedict D. Chivers ²,

Ludivina Barrientos-Lozano ³ & Fernando Montealegre-Z ²

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² University of Lincoln, School of Life and Environmental Sciences, Joseph Banks Laboratories, Green Lane, Lincoln, LN6 7DL, UK

³ Tecnologico Nacional de Mexico-I. T. de Ciudad Victorio, Blvd. Emilio Portes Gil No. 1301, Ciudad Victoria, C.P. 87010 Tamaulipas, Mexico

Bush-crickets (katydids) have ultrasonic ears located in the tibia of their forelegs, which work with a complex biomechanism analogous to the mammalian hearing system. Like mammals, bush-crickets also have tympanal ears with outer-, middle- and inner-ear components, although some morphological differences from the mammalian outer-ear lead to the bush-crickets having a dual input system. The sound will first reach the external side of the tympana directly, before reaching the internal side through the bush-cricket ear-canal (acoustic trachea, AT) with a time lapse and increased sound pressure (or volume) at certain frequencies. As a result, the bush-cricket hears the same sound twice. For many of the bush-cricket species, the increased sound pressure has been attributed to the AT being shaped and functioning as an exponential horn. However, the biomechanism behind other AT morphologies generally remains elusive. One such species is the duetting Phaneropterinae bush-cricket *Pterodichopetala cielo*, which also have a sexual dimorphism between their AT. In this study, we demonstrate that despite the sexual dimorphism, both male and female AT of *P. cielo* function as coupled resonators, producing sound pressure gains at the conspecific mating call frequency, and majorly dampening the sound at other frequencies. Through a comparison with an exponentially shaped AT, the main differences in their functioning mechanisms are also highlighted. The analysis has been carried out using an

interdisciplinary approach, where an experimental investigation was combined with finite-element analysis on the precise tracheal geometry to examine the mechanism behind the male and female *P. cielo* AT.

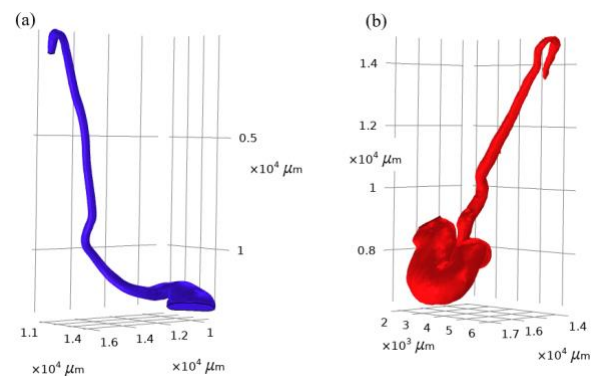


Figure 1. The acoustic trachea (AT) of two bush-cricket species. (a) The exponential horn shaped AT of a male *Copiphora gorgonensis*, and (b) the AT of a male *Pterodichopetala cielo* which functions as coupled resonators.

Key Words: Bush-cricket ear, acoustic trachea, finite-element analysis, laser Doppler vibrometry

SPIDER LEG JOINT MEMBRANES AS PUTATIVE 'EARS'



Erin E. Brandt¹ & Natasha Mhatre¹

¹Department of Biology, Western University, London, Ontario, Canada

Spiders are known for sensing the world through vibrations, which are detected by strain-sensitive lyriform organs embedded in the cuticle and concentrated near leg joints. Oddly, lyriform organs have the lowest neural thresholds at high frequencies (1-5 kHz), whereas the frequencies of most ecologically relevant vibrations, (courtship signals, prey movements), are much lower (~20-500 Hz). Recent work suggests that some spiders perceive high frequency airborne sound, potentially via web vibrations. However, it is unclear how this sound is perceived.

Spider legs are hydraulically actuated, and leg joints contain flexible membranes that expand to accommodate fluid pressure changes within the leg. We investigated whether this membrane could move in response to audio frequencies despite being backed by a high impedance fluid-filled cavity.

Here, we used laser Doppler vibrometry to measure joint membrane motion in two spider species: the western black widow (*Latrodectus hesperus*) and the European garden spider (*Araneus diadematus*). In both species, the femur-patella leg joint membrane (FPM) moved coherently in response to airborne sound within audio frequencies (Fig 1A, B). We also observed membrane motion in the FPM when the leg was bent at similar frequencies (Fig 1B). Motion in the FPM may therefore be induced in two ways: by airborne sound dis

placing the membrane directly, and indirectly through sound-driven web vibrations.

If the mechanical energy collected by the FPM is indeed transmitted to the lyriform organs at levels that cross the low neural thresholds in this frequency range, this would open up the possibility of direct 'air-to-fluid' ears.

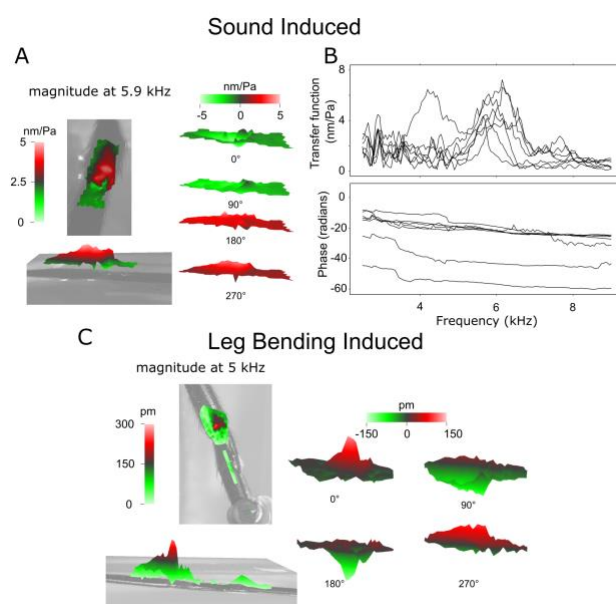


Figure 1. Leg joint membrane moves in response to sound and vibration of the leg in the western black widow. A. Magnitude of displacement across leg joint membrane in response to sound at 5.9 kHz, played at 80 dB. B. Frequency response of joint membrane. C. Response of leg joint membrane in response to leg bending at 5 kHz.

Key Words: spiders, sound perception, air to fluid sound transmission

RUNNING ON EMPTY: EFFECT OF STARVATION STRESS ON THE COURTSHIP SONG OF *DROSOPHILA STURTEVANTI*



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Stress can alter an organism's ability to court and produce high quality signals. In *Drosophila*, male courtship songs are produced through wing vibration and may consist of pulse and tonal trains, varying by species. In the *Drosophila saltans* species group most of the species sing with two types of pulse song. One member of the subgroup, *D. sturtevantii*, sings with pulse song and with a novel, tonal unit, a 'beep'. We hypothesized that the novel song was necessary for mating to occur and that it is altered with stress. We predicted that starved individuals are not able to perform song as well as fed individuals, and that mating success differs between stressed and unstressed flies. In addition, we predicted that songs differ between males that copulate and males that do not. As predicted, starved males mated less frequently than fed males, although they courted equally. In addition, song parameters differed such that the songs of starved males were less energetic. Males who mated were more likely to beep than males that did not.

Beeping was nearly necessary for mating but was not sufficient. Males that mated had a greater duty cycle than males that did not. We conclude that song enhances mating success and is altered by stress, but signals in other modalities may influence mating success.

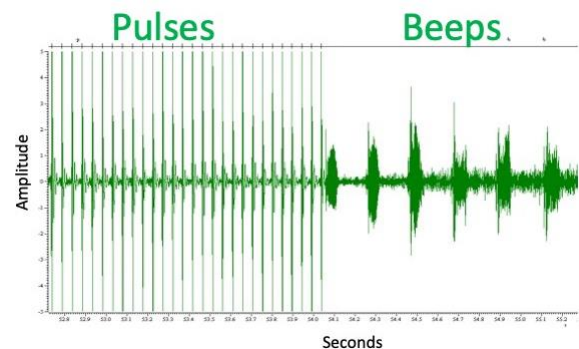


Figure 1. *Drosophila sturtevantii* courtship song with pulses and tonal beeps.

Key Words: *Drosophila*, courtship song, novel signal, stress

VISCOELASTIC PROPERTIES OF THE AUDITORY VESICLE OF KATYDIDS CHARACTERIZED BY *IN SITU* MICRORHEOLOGY



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The auditory system of katydids attains a levels of frequency detection and discrimination comparable to those of the mammalian ear, despite being much smaller. Part of this remarkable achievement is due to the existence of the *crista acustica* (CA), a triangular surface with an array of sensory cells tonotopically organized. The CA is covered by fluid believed to be part of an hemolymph canal. However, micro-CT scanning of the ear in several katydid species has shown that the CA is embedded in an enclosed independent cavity, described as the auditory vesicle (AV), which seems to enhance the formation and transmission of travelling waves (in the form of tsunami waves). Despite its importance in the hearing process, the very existence of the AV in katydids as an enclosed cavity is still a matter of debate, and the physical properties of the fluid covering the CA are unknown.

Here we seek to address both questions by using a novel microrheological application. We develop a method for *in vivo*, non-invasive multiple particle tracking microrheology (MPTM) that allows us to characterize both the dynamics and the viscoelastic properties of the fluid covering the CA in six neotropical katydid species with transparent ear cuticles. The use of MPTM proved unequivocally that the AV is a closed compartment with no direct connection to the circulatory system in the four species that have ear pinnae partially covering the tympanic membranes (*Conocephalinae*), whereas in the two species with naked tympani (*Phaneropteridae*), the CA was exposed to the general circulation.

Regarding the physical properties, no significant differences were found in any case in the rheology of the hemolymph and the AV fluid. We show that both fluids have a

Newtonian behaviour over a wide range of fluctuations, with no detectable elastic component and a dynamic viscosity close to that of water. The methodology we have developed can be of use to non-invasively study fluid mechanics in living organisms and provides accurate measurements of fluid properties that can be used in numerical modelling of the hearing system.

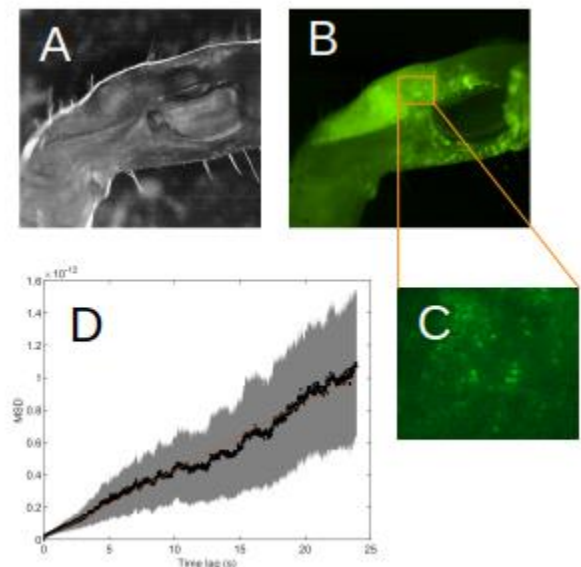


Figure 1. Microrheology on the AV of a male *Copiphora gorgonensis*. **A** shows the phase-contrast image of the leg after removal of the pinnae and micro-injection of fluorescent microspheres into the AV. **B** is the same image with epifluorescent illumination where the bright AV is clearly distinguishable. **C** shows a detail of the microspheres suspended in the AV fluid at high magnification. **D** is an example of the mean square displacement of the population of beads against time lag.

Key Words: katydids, fluid mechanics, *in vivo* microrheology, acoustic vesicle.

THE ULTRASOUND MALE-FEMALE COMMUNICATION OF TWO SHORT-WINGED BUSHCRICKETS (ORTHOPTERA, PHANEROPTERINAE) FROM ARGENTINA



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Two species of brachypterous phaneropterines found near the coast of the Río de la Plata (Argentina, Buenos Aires Province) produce interesting male calling songs and female responses. The first one was first recognized by its song to be different from a similar species, hence representing a new species of the genus *Anisophya*. The male produces long sequences of continuously repeated syllables with most energy between 30 and 45 kHz, whose impulse structure corresponds to the file morphology. The female responds right after some of these syllables with a brief signal, which is typical of the subfamily. The male of the second species, *Xenicola dohrni*, makes series of short ticks, with a carrier frequency range from 80 to 100 kHz. When the female responds, he shifts to more complex and much longer signals, depending on the proximity of the duetting female. In both species the female's signal comprises slightly lower frequencies compared to the male's song.

Key Words: *Anisophya*, bioacoustics, female response, *Xenicola*

TESTING GINKO2 IN AUDITORY NEURONS



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GINKO2 is a new genetically-encoded fluorescent biosensor, designed to detect and image potassium ions (K^+) in biological systems. Potassium plays a crucial role in auditory signal transduction in both insects and mammals, making the GINKO2 biosensor a promising tool for studying K^+ dynamics in various hearing model organisms. However, the pH sensitivity of GINKO2 complicates the interpretation of results, highlighting the need for caution when using the biosensor. Nevertheless, GINKO2 has potential for *in vivo* K^+ imaging. We present data on using GINKO2, tethered to the pH sensor pHuji, in the auditory sensory neurons of awake flies during sound stimulation.

Key Words: potassium, biosensor, auditory nerve, sound-evoked

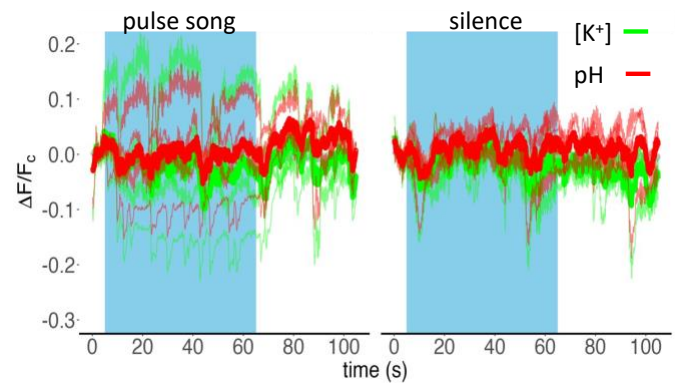


Figure 1. Auditory sensory nerve responses to the fly pulse song. Auditory sensory nerve responses recorded from female *Drosophila* expressing the reporters for both pH (pHuji) and K^+ (GINKO). Recordings are taken from the antennal mechanosensory and motor center in the brain while flies are awake with freely moving antenna and arista. Some individual recordings (thin lines) show changes to K^+ concentration and pH when the pulse song was played; these changes did not occur when recordings were done without sound stimulus. Blue areas indicate when sound was played (or silence).

SEXUAL SELECTION AND PREDATION DRIVE THE REPEATED EVOLUTION OF STRIDULATION IN HETEROPTERA AND OTHER ARTHROPODS



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Arthropods display a staggering diversity of vibroacoustic organs generating acoustic sound and/or substrate-borne vibrations. The primary mechanism that arthropods use to generate vibroacoustic signals is stridulation, which involves the rubbing together of opposing body parts. Although stridulation is common, its behavioural context and evolutionary drivers are often hard to pinpoint, owing to limited synthesis of empirical observations on stridulatory species. Here, we present the most comprehensive review to date on the systematic distribution and behavioural context of stridulation. We use the megadiverse heteropteran insects as a model, together with multiple arthropod outgroups (arachnids, myriapods, and selected pancrustaceans). We find that stridulatory vibroacoustic signalling has evolved independently at least 84 times and is present in roughly 20% of Heteroptera, representing a remarkable case of convergent evolution. By studying the behavioural context of stridulation, we find that predation pressure and sexual selection are the main behaviours associated with stridulation across arthropods, adding further evidence for their role as drivers of large-scale signalling and morphological innovation in animals. Remarkably, the absence of tympanal ears in most Heteroptera suggests that they typically cannot detect the acoustic component of their stridulatory signals. This demonstrates that the adoption of new signalling modalities is not always correlated with the ability to perceive those signals. By synthesising our understanding of stridulation and stridulatory organs across major arthropod groups, we create the necessary framework for

future studies to explore their systematic and behavioural significance, their potential role in sensory evolution and innovation, and the biomechanics of this mode of signalling.

Key Words: vibration, tremulation, tympana, bioacoustics, Heteroptera

IMPACT OF CONVENTIONAL NEONICOTINOIDS AND A NOVEL ALTERNATIVE INSECTICIDE ON AUDITORY PROCESSING IN THE DESERT LOCUST *SCHISTOCERCA GREGARIA*



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Since market introduction in the 1990's, neonicotinoids became the most widely used class of insecticides worldwide. Neonicotinoids act selectively as agonists at the nicotinic acetylcholine receptor of insects and show a low toxicity in vertebrates. However, the soaring agricultural use over the last decades is suspected to be one factor contributing to the decline of pollinators and other non-target insects, resulting in EU-bans on outdoor use in 2018.

Here we investigated the impact of the neonicotinoids Imidacloprid and Clothianidin as well as the emerging alternative Flupyradifuron on the auditory processing in locusts. We simultaneously recorded the neural responses of sensory afferents from the ear (auditory nerve recording) and ascending interneurons (neck connective recording) to different sound pulses (1, 2, 10, 20 and 30 kHz; at 35 - 80 dB SPL in steps of 5 dB). While recording, we replaced the haemolymph surrounding the prothoracic ganglion with Ringer's solution containing successively increasing concentrations from 10^{-13} to 10^{-3} M of the respective insecticide.

Our preliminary experimental data indicate that all three insecticides do not directly affect the signal transduction and spike generation of the sensory cells in the ear. However, the subsequent synaptic processing in the primary auditory neuropil of the thoracic ganglia is disturbed and finally disrupted in a dose-dependent manner starting at concentrations of 10^{-6} / 10^{-5} M.

Therefore, we conclude that in locusts, which

rely on auditory information for predator avoidance and/or intraspecific acoustic communication, sublethal intoxication by insecticides may have a significant negative impact on their biological fitness by reducing their chances for reproduction.

Key Words: insect, neurophysiology, hearing, neonicotinoids, flupyradifuron

TEMPORAL DYNAMICS OF SIGNAL PROCESSING ALONG THE AUDITORY PATHWAY OF A BUSHCRICKET



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Many insects use sound signals to find mating partners. Different strategies of acoustic communication have evolved in the family of bushcrickets (Tettigoniidae), and we study how adaptations of the ears and auditory processing in the central nervous system relate to the species-specific mate finding behaviour. We started our investigation with the bushcricket *Mecopoda elongata*. In this species, only 45 bipolar sensory neurons of the ear are involved in the process of signal transduction from sound stimuli to neuronal responses.

For acoustic stimulation we used pure tones of 20 ms duration from 2 to 62 kHz at 80 dB SPL and recorded neuronal activity in the ear and the nervous system. With a combination of different electrophysiological methods (intracellular single cell recordings and extracellular hook- and multielectrode recordings), we have now systematically characterized the auditory processing by the sensory cells, interneurons of the prothoracic ganglion and ascending neurons in the neck connective.

Our data show the temporal dynamics of local field potentials as well as the timing of neuronal spiking along the auditory pathway in *M. elongata* from the sensory neurons to the brain. Beside previously described inhibitory processes, we found by multielectrode-recordings in the prothoracic ganglion also a long-lasting increase in local field potential amplitudes, that point to amplification processes on the synaptic level.

Both processes may play a crucial role for temporal processing of the calling song chirps during acoustic communication between males and females in *Mecopoda elongata*.

Key Words: katydid, neuronal processing, spike timing, local field potentials, auditory pathway

PLANT-SOUND VIBRATION INTERACTION: AN OCEAN OF POSSIBILITIES



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Through millions of years of evolution, plants have gained a sophisticated molecular machinery to sense and respond to a plethora of environmental stimuli. Besides well-known biotic, abiotic and physical factors, sound vibration (SV) also causes remarkable developmental and physiological changes in plants, which include calcium spiking, cell cycle progression, improved seed germination, plant regeneration, and stress tolerance. However, there is a huge gap in our understanding of SV-mediated molecular alterations in plant cells.

Key Words: Plant-environment interactions, Plant acoustics, Plant stress tolerance, Plant molecular biology

Using transcriptomic and proteomic approaches, we have forwarded a hypothetical model of SV perception and signalling in plant cells for the first time. In addition to this, our study has also revealed several molecular players involved in SV-mediated stress tolerance in plants. Research on plant-SV interaction can open the door to future green technology for controlled environment agriculture.

Until now, plant-SV interaction studies were mostly conducted by playback of single-frequency synthetic sound, which does not answer why plants have evolved the ability to sense and respond to acoustic signals.

Future studies with ecologically meaningful sounds (e.g., caterpillar munching sounds, bees buzzing) are warranted to explore the evolutionary significance of plant-SV interaction.

A “MYSTERIOUS” SIGNALLING IN *STILPNOCHLORA COULONIANA*



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1

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Acousto-vibratory signalling is a basic feature in Orthoptera. These signals are often used in intraspecific communication, e.g. mate finding. Here, we describe signalling in the phaneropterine bush cricket *Stilpnochloa coulöniana*, with one type of signal which has a so-far unknown, “mysterious”, function.

Mature males produce nightly calls with single chirps of about 230ms duration and with 8.3 kHz peak carrier frequency. Females might acoustically respond to the calls, but mating might also take place without a bi-directional acoustic communication.

Interestingly, young males can produce a separate call of 400ms duration, when they are approached or disturbed during daylight. The conditions of this type of signalling have been analysed further. Typically, only one call is produced per stimulus and the behaviour habituates strongly. The sound production is triggered visually and with strong vibrations (handling of the resting places, but not the animal!). Thereby, the otherwise camouflaged animals draw attention to themselves, seemingly contradicting a survival function. By contrast to young males, mature males and females do not produce disturbance calls (figure 1). However, all adults and nymphs of later stages might produce single disturbance vibrations (tremulation).

We found no evidence that these vibrations (or sounds) address conspecifics as their behaviour is not changed. This signalling behaviour does not seem to have a clear defensive role or support survival. Therefore, the function of the disturbance signal remains unknown and will be discussed.

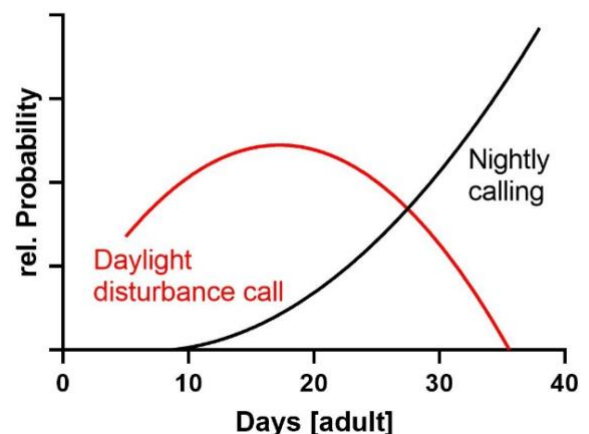


Figure 1. Relative probability of males producing acoustic signals in respect to age.

Key Words: behaviour, intraspecific communication, survival signal, katydid, airborne sound

PREDATION RISK OF SIGNALLING AND SEARCHING IN A MULTIMODALLY DUETTING KATYDID



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Mate-finding strategies such as signalling and searching have environmental and physiological costs for both sexes. In *Onomarchus uninotatus*, a canopy katydid, males produce acoustic signals and females reply with tremulations, producing vibratory signals, which are then used by males to locate females. Higher bat predation on females is proposed as the driving selection pressure for the evolution of such multimodal duetting.

We compared sex-specific predation risk of signalling and searching *O. uninotatus*, in enclosure experiments with their bat predator, *Megaderma spasma*. We hypothesized that (1) signalling is safer for females than males; (2) searching has a higher predation risk than signalling for females, explaining the evolution of duetting; and (3) signalling has a higher risk of predation than searching for males, hence they can minimize their time spent signalling by engaging in searching during duets. Since both males and females can make decisions on searching and signalling, this acoustic-vibratory duetting system allowed us to gain insights into the selection pressures that could have driven the evolution of such mate-finding behaviour.



Figure 1. Bat predator *Megaderma spasma* and female false leaf katydid *Onomarchus uninotatus*. Photo credits: Harish Prakash and Kasturi Saha.

Key Words: multimodal duetting, false-leaf katydid, predation risk, false vampire bat, signalling costs

MODELLING TO UNDERSTAND ELECTRORECEPTION VIA AERO-ACOUSTIC FILIFORM HAIR RECEPTORS



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It has been recently shown that bees, spiders and other arthropods can sense naturally occurring electrical fields. This discovery has potentially significant implications, expanding our view of how such organisms explore their environments using previously unknown sensory capabilities.

The putative electrosensitive receptors in terrestrial arthropods discovered to date are antennae (e.g., honeybees) and filiform hairs (e.g., bumblebees, hoverflies, and spiders). Both receptors are mechanoreceptive, responding neurologically to movements driven by changes in local the environment such as variations in electrical fields. The motion of these sensors occurs due to electrostatic forces that arise due to the electric properties of the receptors. That is, whether the sensor possesses an electric charge and/or can have one induced by local sources of charge.

These electroreceptive systems are also inherently multimodal (e.g., to tactile and/or aero-acoustic stimuli), such that electrostatic stimuli can be transduced through the same sensory and neurological 'hardware', likely reducing energetic cost compared to possessing unimodal sensors.

This talk consists of three topics: 1) the physical and biological feasibility of this electrical sense – with attention paid to the bimodality of filiform hairs, 2) how interactions between sensory hairs alter their spatio-temporal sensitivity to different stimuli, and 3) new sensory possibilities such as location detection, object identification and signal differentiation. I will remark on the

biological implications of this sense throughout.

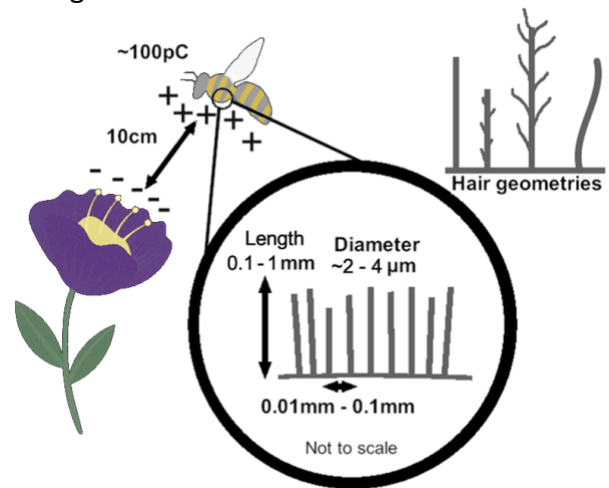


Figure 1. Schematic of electroreception and filiform hair scales.

Key Words: mathematical modelling, electroreception, mechanosensing, electrostatics.

IS THERE A CORRELATION BETWEEN BODY SIZE AND SOUND FREQUENCY IN CALLING FIELD CRICKETS?



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It was frequently assumed that female field crickets should prefer conspecific males that call with a lower sound frequency as a mechanism for sexual selection for larger mating partners, but several studies reported no correlation between male body size and the fundamental frequency of their calls.

Here we analysed how three different body size indicators (body length, pronotum width and fresh body mass) correlate with the fundamental sound frequency of the calling song within and across two populations of the Mediterranean field cricket *Gryllus bimaculatus*, that differ significantly in body size.

One test group of relatively small animals came from commercial cricket breeders and were purchased at a pet shop ('S'-males). The other group with rather large individuals came from the long-term inbreed colony of our lab ('L'-males).

The data show strong correlations between the different body size parameters within each of the two groups (S, L) and also across the animals of the two populations (S+L). Sound frequency of the calling song did not correlate with any of the body size parameters within either of the two populations (S, L), but when tested across all animals (S+L) significant correlations were found between body size parameters and sound frequency. Our study demonstrates that correlation between parameters at the species level do not necessarily mean that the same relation is also detectable in subpopulations, which highlights the potential limitations of correlation studies

due to insufficient sample range and sample size as well as differences between hidden subgroups within the data set.

Key Words: insect; acoustic communication; sound frequency; body size; sexual selection

THE IMPORTANT ROLE OF PARENTAL ACOUSTIC SIGNALLING DURING BROOD CARE IN THE BURYING BEETLE *NICROPHORUS VESPILLOIDES*



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Communication plays a vital part in any form of cooperative behaviour for numerous interactions. Apart from odor, acoustic signalling is common during various behaviours such as mating, defense, aggregation, and parental care - a prime example for cooperative behavior. One group that exhibits elaborate biparental care are burying beetles of the genus *Nicrophorus*, which makes them model organisms in behavioral ecology. In this study, we investigated the function of stridulatory signals during brood care in *N. vespilloides*, where parents cooperate in feeding and defending their young. Although their stridulations have been known since Darwin, and both parents stridulate, their function remains unclear to this day. We first recorded the signals produced by the beetles as well as their behaviour during the entire period of biparental care and examined differences between pre- and post-hatching care. Thus, we were able to characterize the signals and find a significant increase of stridulation activity with hatching. Using silenced parents, we were also able to show that a lack of stridulations has a significant impact on offspring fitness, leading to smaller larvae and smaller next generation adults. This clearly shows that stridulations play a vital role in biparental care. We believe that parents use them to communicate with their young as well as each other in order to improve brood care. This study provides evidence that stridulatory signals play an important role in the social communication of burying beetles, which is particularly important after hatching has occurred.



Figure 1. *Nicrophorus vespilloides* with clearly visible stridulatory organs (picture by Johannes Stökl)

Key Words: parental care, stridulations, burying beetles, offspring fitness

THE PROCESSES IN THE HYBRID ZONE BETWEEN SIBLING GRASSHOPPER SPECIES OF THE *STENOBOTHRUS EURASIUS* GROUP (ACRIDIDAE, GOMPHOCERINAE)



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Two sibling grasshopper species, *Stenobothrus eurasius* and *S. hyalosuperficies* have completely different acoustic behaviour (Tarasova et al., 2021). *S. eurasius* males produce their songs by common femoro-tegmina stridulation. A calling song consists of the prolonged echemes of compound syllables, generated by the complex leg-movement pattern. A courtship song of *S. eurasius* includes three elements (A, B and C) followed in a strict order. Element B has the same structure as the calling song. In contrast to *S. eurasius*, both sexes of *S. hyalosuperficies* produce sound not only by common stridulation, but also by wing clapping. The calling song consists of the short wing beats. In the courtship song, the wing clapping (element D) alternates with two elements generated by stridulation (A' and C').

Despite strong difference in the acoustic behaviour, the species hybridize in a contact zone in south-eastern part of European Russia (fig. 1).

In the contact zone, we found three populations, which we considered to be hybrid. In population №1 (fig. 1) hybrid males show a wide range of songs, which might be divided between *eurasius*-like and *hyalosuperficies*-like songs. The *eurasius*-like songs were dominant. They contained element B, being specific for *S. eurasius*, and element D typical for *S. hyalosuperficies*. In the *hyalosuperficies*-like songs changes in element C' were mainly found. In populations №2 – 3 almost all songs were the same as in *S. eurasius*, but some males produced element D. In general, intermediate song variants were extremely rare in the contact zone.

Behavioral playback experiments showed that *S. eurasius* females preferred conspecific songs, whereas *S. hyalosuperficies* females did not recognize between con- and heterospecific songs. Thus, incomplete behavioral isolation may suggest a higher prevalence of the hybrid song phenotypes than we found. This may indicate evolving post-zygotic isolation barriers as well.

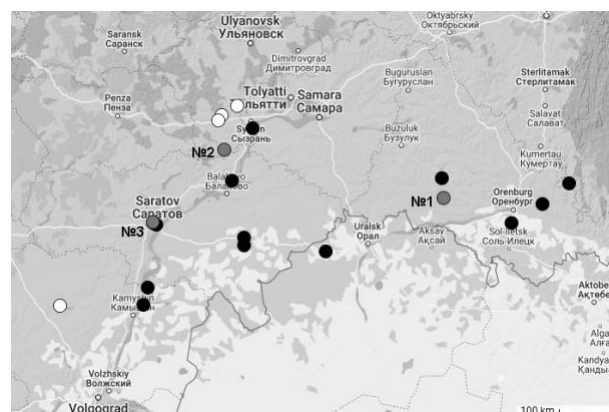


Figure 1. Map of contact zone of *S. eurasius* (white circles) and *S. hyalosuperficies* (black circles). Hybrid populations marked with grey circles and provided with numbers.

Key Words: grasshopper, mating behaviour, female response, hybridization.

HARMONIC HOPPING IN THE SONGS OF LEBINTHINI CRICKETS: USING FINITE ELEMENT MODELLING TO UNDERSTAND THE EVOLUTION OF ACOUSTIC COMMUNICATION



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Crickets are well known among insects for their ability to produce acoustic communication signals. These signals are produced by stridulation, the rubbing together of the forewings, and are used by males to call females for breeding purposes. This is an essential feature of these animals that has played, and continues to play, a key role in the evolution of this group. During my PhD I am interested in the tropical crickets of the tribe Lebinthini, subfamily Eneopterinae, which produce high frequency calls through a phenomenon of harmonic frequency amplification. This phenomenon, called harmonic hopping, has recently been shown to have played an important role in the rapid diversification of this tribe.

To elucidate the mechanisms underlying the harmonic hopping phenomenon, I use the finite element method to model the vibro-acoustic behaviour of the wings of these crickets. This mechanistic approach, which investigates the physical principles governing this phenomenon in extant species, may prove useful in understanding the mechanisms that led to the emergence of harmonic hopping during evolutionary history.

The first results of my work characterise the effect of variation in the physical properties of the wings on their vibro-acoustic behaviour, and suggest the possibility of a role played by greater stiffness of the wing veins. Ultimately, the identification of the key parameters governing

harmonic hopping in these models will allow us to identify the characteristics whose variation has led to the emergence of this phenomenon during evolutionary history.

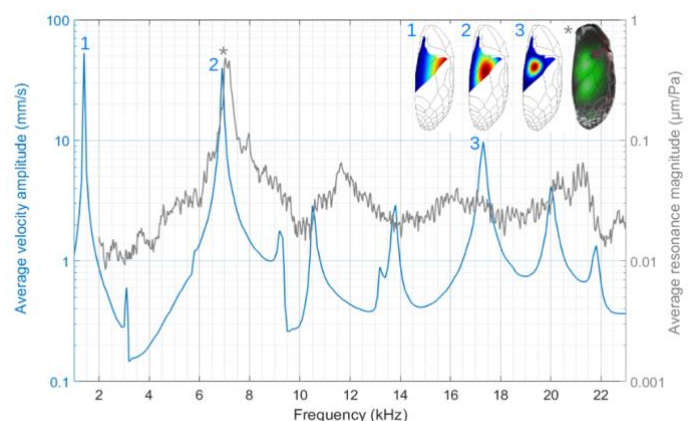


Figure 1. Mean velocity amplitude calculated on the finite element model of the harp of the *Nisitrus malaya* wing (blue line), and mean resonance magnitude of the left wing of *N. malaya* measured by Laser-Doppler Vibrometry (LDV) (grey line).

Key Words: harmonic hopping, Lebinthini crickets, finite-element modelling, acoustic communication, biophysics

MECHANO-ACOUSTIC ANALYSIS OF HARMONIC HOPPING IN AN ENEOPTERINE CRICKET



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Harmonic hopping has been described in mammals, birds and insects as a phenomenon by which acoustic character states change in discrete steps rather than in a gradual manner. In these cases, acoustic signals jump from low-frequency ancestral states to higher – harmonically linked – frequencies in closely related species, without apparent intermediate forms. Male crickets (Orthoptera, Gryllidae) usually produce low-frequency acoustic signals (2-8 kHz) to attract distant mating partners by rubbing their wings together. However, many species in the subfamily Eneopterinae have evolved calls with uncharacteristically high frequencies, some even reaching the ultrasonic range (>20 kHz). Here, we present a case of harmonic hopping towards high-frequency calls in the cricket genus *Pseudolebinthus* (Eneopterinae). Using laser Doppler vibrometry (LDV), microcomputed tomography (μ -CT) and inference from vibro-acoustic simulations using finite element (FE) analysis, we provide morphological and biomechanical support for a mechanism explaining the observed frequency jump from low to high song carrier frequencies. This proposed mechanism provides further support for the hypothesis that harmonic hopping events in eneopterine crickets occurred multiple times in various acoustical and morphological contexts independently, thereby constituting an example of convergent evolution of an acoustic trait (see talk by Robillard et al.).

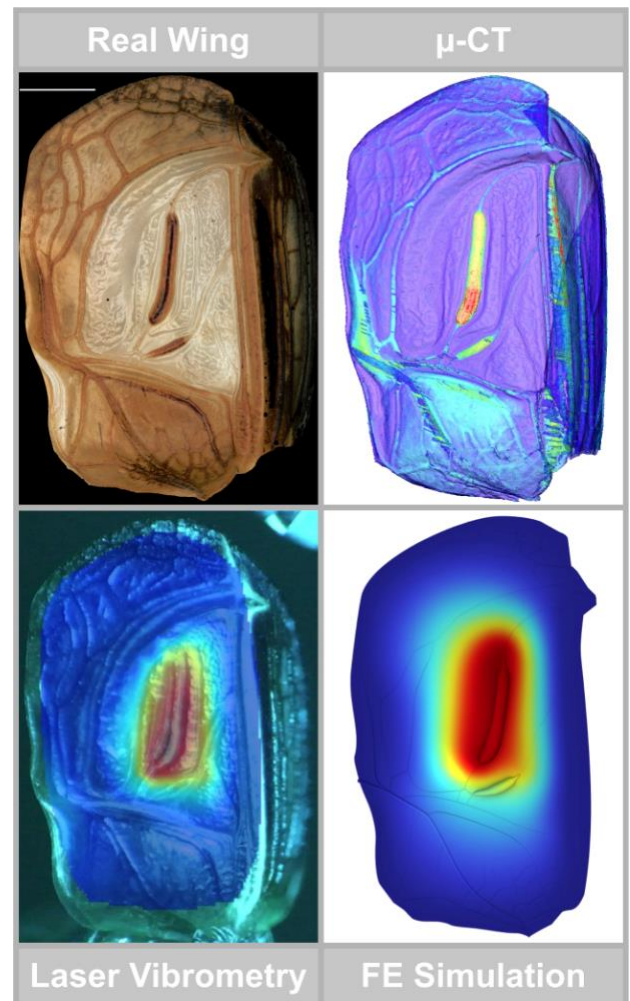


Figure 1. Left wing of a *Pseudolebinthus gorochovi* male. Top left: Image of a real wing (scale bar=1 mm). Top right: 3D model from μ -CT; thickness colour-coded. Bottom left: LDV recording showing vibration velocity at 4 kHz. Bottom right: Simulated vibration map at 4 kHz. Colour coding: Red=thick/high velocity; blue=thin/low velocity.

Key Words: Laser Doppler vibrometry, finite element modelling, micro-CT

DOES AN INSECT'S EAR BECOME LESS RESILIENT AS IT AGES?



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Age-related hearing loss is a poorly understood, multifactorial pathology that affects insects and mammals alike. A leading cause of age-related hearing loss is hypothesized to be a decrease in metabolism. However, there is a lack of research measuring metabolism in an auditory organ over a lifespan. Using Müller's organ of the Desert Locust (*Schistocerca gregaria*), we quantified metabolism as the ear ages. We also examine whether younger or older animals are more adept at dealing with high levels of noise exposure.

To better understand what might be causing age-related hearing loss, we manipulated the metabolism of Locusts through a variety of experiments. We also measured sound-evoked and baseline activity directly from the auditory nerve *in vivo* and from individual auditory neurons *ex vivo* as a function of age and noise exposure.

We also examine whether younger or older animals are more adept at dealing with high levels of noise exposure. Finally, we quantified expression of genes within the locust ear (RNA Seq.) as a function of age, as well as determining what biological processes are changing in response to both age and noise, and question what is causing this change in auditory resilience.

Key Words: ARHL, Metabolism, Transcriptome, Neurons, Resilience

CONVERGENT EVOLUTION OF HARMONIC HOPPING AND MULTIPLE ORIGINS OF HIGH-FREQUENCY CALLS IN ENEOPTERINAE CRICKETS



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Convergent evolution is the process whereby organisms evolve similar traits in independent evolutionary lineages, usually as a consequence of adaptation to similar ecological niches. Acoustic signals for intraspecific communication play a central role, as animals often depend on them for both survival and reproduction. Convergent signals can thus occur when analogous signals are generated by different combinations of structures and behaviours.

Acoustic communication signals produced by animals often depend on resonant structures that exhibit multiple, harmonically related, resonance frequencies. Over evolutionary time, the dominant frequency of an acoustic signal can shift from one harmonic to another, without intermediate frequencies. This phenomenon of 'harmonic hopping' has been reported in birds, mammals and insects; it allows signal's frequency to change in discrete steps in a form of punctuated evolution rather than in a gradual manner.

While most crickets produce acoustic signals at relatively low frequencies (2-8 kHz), some species of the subfamily Eneopterinae have evolved calls at much higher frequencies. High-frequency calls are used in several clades in the tribes Lebinthini and Eneopterini, and these high dominant frequencies correspond either to the first, second, third or fifth enhanced harmonic of the fundamental resonance (see talk by Gaiddon et al.).

In this study, we review these different types of harmonic hopping and document a new one in the African genus *Pseudolebinthus* (tribe Xenogrillini) caused by an original mechanism

(see talk by Jonsson et al.). We use a phylogenetic context to test the hypothesis of a convergent origin of harmonic hopping in these crickets.

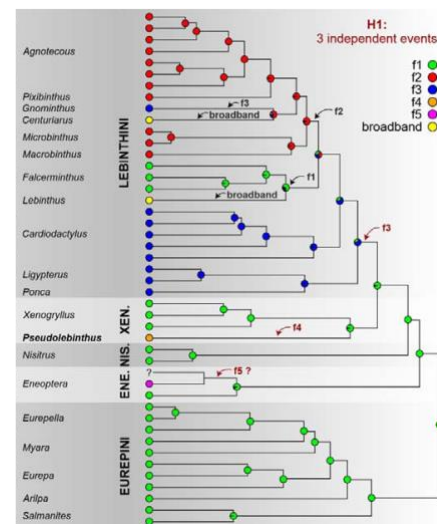


Figure 1. Bayesian ancestral state reconstruction of the call frequency of the Eneopterinae. Ultrametric tree corresponding to the consensus topology obtained from Vicente et al., 2017. Multistate coding of dominant frequency location among the harmonics of the spectrum: f1, green; f2, red; f3, blue; f4, orange; f5, pink; broadband, yellow. Dark red arrows mark the three independent changes in song frequency proposed by H1; ENE.=Eneopterini; NIS.=Nisitritini; XEN.=Xenogrillini.

Key Words: evolution, phylogeny, bioacoustics, Gryllidae, Eneopterinae

BUZZ POLLINATION: COMPARISON OF DEFENCE VIBRATIONS PRODUCED BY BEES IN MEXICO AND AUSTRALIA



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Buzz pollination occurs when vibration-producing pollinators, usually bees, apply high power vibrations to flowers to release pollen, incidentally pollinating flowers. The capacity to buzz-pollinate has evolved multiple independent times in bees and is known for 17% of bee genera, encompassing more than half of the 20,000 of bee species distributed around the world. Yet, why some bee species buzz-pollinate why others do not is still unknown. Here we will present the results of a recent expedition sponsored by National Geographic to record the mechanical properties of vibrations produced by bees in Mexico and Australia.

We used a portable set up to measure vibrations in the field from nearly 50 species in five bee families and compare the acceleration and force produced by bees with and without the capacity to buzz-pollinate. We used a phylogenetic framework to investigate how biomechanical properties of defence vibrations (which in buzz-pollinating bumblebees represent a lower estimate of the characteristics of floral vibrations) relate to morphological properties of different types of bees.

Our results suggest that both buzz-pollinating and non-buzz-pollinating bees can produce vibrations of similar mechanical characteristics, and that a limited capacity to produce vibrations of sufficient magnitude is unlikely to be a general explanation for why some bees do not buzz flowers. We discuss new hypotheses

that may help explaining the heterogeneous distribution of floral buzzing among bee species.



Figure 1. Australian *Xylocopa bombylans* (carpenter bee). Females of *Xylocopa* can produce some of the largest-amplitude defence vibrations in record and are excellent buzz-pollinators.

Key Words: Bees, biomechanics, buzz pollination, Mexico, Australia.

THE MUSIC IN MOSQUITO EARS: TWO TONES AT A HOPF BIFURCATION



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Hearing in vertebrates (and humans) emerges from an ‘active process’, which has previously been robustly explained by models using a so-called *Hopf bifurcation* (1). An equivalent model could explain hearing in fruit flies (2).

Mosquito ears also show the hallmarks of the *active process*. In male ears, large, self-sustained oscillations (SSO) are generated and appear to be key to their operation. Recent work also strongly suggests that mosquitoes use a complex, distortion-product based mode of hearing – meaning tones generated from a nonlinear mixing of the input tones (3). Yet, the mechanism by which those secondary tones occur is unclear.

Here, we examine the response function of the mosquito ear to test the *Hopf* model. Our stimuli are designed to address two important features of the model, namely compressive nonlinearity (where stimuli are amplified inversely proportional to their intensity) (2), and two-tone frequency suppression (4).

Preliminary work on *Anopheles* mosquitoes comparing flagellar motion and corresponding nerve responses shows qualitative differences in terms of distortion products observed between them. This points to a neurological response which does not map onto the input stimulus but exhibits a more complex nonlinear mechanism. Furthermore, these discrepancies show dependencies on the mechanical state of the flagellar ear (quiescent vs SSO).

This finding opens a promising path to modelling mosquito audition through a modified Hopf system and will be key to using mosquito hearing and acoustic mating for vector control.

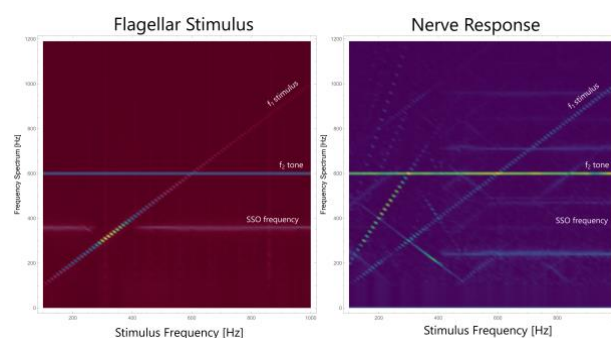


Figure 1. Mechanical (flagellar) and electrical (nerve) response to tonal stimulation recorded from *Anopheles* sound receivers while undergoing SSOs. Note the richer frequency landscape in the nerve response.

Key Words: nonlinearities, distortion-products (DPs), mosquito, audition

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BEHAVIOURAL REACTIONS OF SPIDERS TO VIBRATIONS MIMICKING PREY IN ORB AND FUNNEL WEBS

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It is widely accepted that while many spider species are diurnal, most Tarantula species are more active during the night. Spiders use vibrations to gather information from their surroundings. At night, when vision is limited, how would spiders use and react to vibrations in their webs that mimic a prey insect? We also wanted to see if spiders from both orb web and funnel web react the same to a vibrational stimulus or if different the different types display different reactions. This study investigated the difference in behavioural responses to vibrations in two different spider groups found on funnel and orb webs.

We tested the hypothesis that there would be no significant difference in the reactions exhibited by orb web spiders and funnel web spiders. Our experiment took place as part of an overseas field course in the Santa Lucia cloud forest in Ecuador which hosted a range of temperatures with a constant high level of humidity as we were in the clouds. We created a pure-tone signal (150Hz) and played it through a speaker which would cause a probe to vibrate and disturb the web. We tested 22 webs over 3 days and nights. The six categories of behavioural response were: hesitant attack, immediate attack, no reaction, retreat, stationary movement and walking up to vibration.

The results showed that the only significant difference in reaction between funnel and orb web spiders was when the spiders showed the behaviour “walking up to vibration”. For all other behaviours there

was no significant difference. Funnel spiders are known to capture their prey immediately, attacking them once they land in the web whereas orb spiders use their webs to capture the prey and are usually less immediate in attacking their kill. This difference in hunting strategy provides explanation as to the results we observed and presents the opportunity for further study into predator and prey interactions, as well as how vibrations are used by spiders.



Figure 1. Orb Web and Funnel Web examples.

Key Words: Araneae; behaviour; funnel; orb; vibrations; frequency; web; Theraphosidae.

COUPLED MEMBRANES: POSSIBLE ANALOGUE OF MAMMALIAN EAR BONES FOUND IN THE FIELD CRICKET EAR



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Most of the tonotopically arranged sensory neurons of the field cricket ear are sharply tuned to the male calling song carrier frequency (CF) of around 5 kHz. These neurons are separated from the primary sound input, the posterior tympanum (PT), by complex anatomy that includes two tracheal vesicles. For decades, the transmission pathway between the tympanum and the sensors has remained enigmatic. The associated biophysical frequency tuning has also been unclear.

For the first time, this in-between region has been imaged using micro-CT, which has informed the first finite element analysis (FEA) of its auditory mechanics. We have also assessed the tuning profile of the PT using further laser Doppler vibrometry (LDV).

Morphometrics suggest the cuticular wall separating the two vesicles, which we here refer to as the 'Dividing Membrane' (DivM), may previously have been overlooked. Thickness and surface area analyses of the micro-CT data indicate a lever configuration of three coupled membranes, including the tympanum, at an area ratio of approximately 30:10:1, that may be amplifying the energy transfer into the smaller of the two vesicles (Figure 1).

The numerical simulations correlate with the morphometrics, showing the DivM to maximally resonate at two biologically relevant frequencies including near to that of the calling song CF. Moreover, the FEA suggests the DivM may be oscillating more than the tympanum, and its frequency tuning to be sharper.

Together, morphometry and FEA demonstrate a hydraulic multiplication effect analogous to that of the mammalian ossicles.

The LDV results support the conclusion that the PT is too broad in its frequency response to match the sensory tuning. Three peaks are revealed, and phase recordings suggest the first to be due to a 'driving resonance' whereas the second to be the natural resonance.

It is expected these findings may contribute towards clarifying the pathway and even frequency filtering of this unique auditory system.

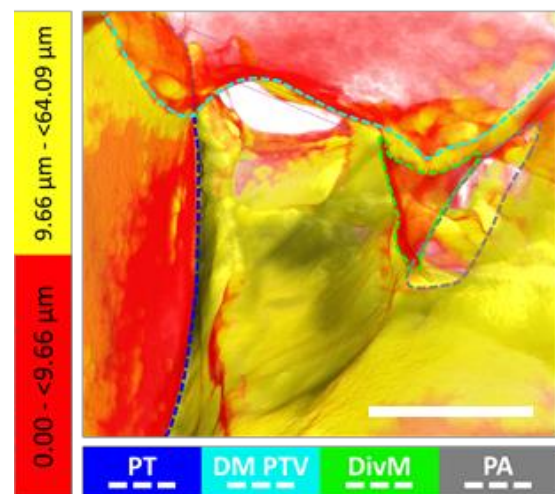


Figure 1. Colour coded micro-CT model highlighting thin (<9.66μm) features in red. Three coupled membranes are revealed: Posterior Tympanum (PT), Dorsal Membrane of Posterior Tracheal Vesicle (DM PTV), and the 'Dividing Membrane' (DivM). Proximal aperture (PA), communicating with the anterior vesicle, is also visible. Scale bar: 50 μm

Key Words: Cricket ear, micro-CT, finite element analysis, laser Doppler vibrometry

IMAGING AUDITORY PROCESSING IN CRICKETS USING EXTRACELLULAR LOADING OF Ca^{2+} TRACERS



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Polar calcium indicators like Oregon Green Bapta-1 can be introduced electrophoretically into the insect nervous system through the sheet of ganglia or nerves using surface electrodes. Here we explored this technique to label auditory sensitive neurons in the nervous system (brain and hearing organ) of crickets and bush-crickets in order to image sound evoked fluorescent responses generated by the Ca^{2+} indicator. Ca^{2+} -signals are picked up with a sensitive cooled CCD camera, attached to a compound microscope.

To study auditory processing in the brain, surface electrodes are used to deliver the dye into the anterior frontal auditory neuropil, which houses the axon terminals of the ascending auditory interneurons and of local interneurons involved in auditory pattern recognition. Dye delivery labels the population of auditory neurons and in some cases the corresponding cell bodies after sufficient diffusion time. Acoustic stimulation with pulse patterns derived from the cricket calling song pattern, activates neurons in the auditory neuropil and elicits sound evoked changes in the fluorescent signal. We aim to reveal details of the spatial organisation of auditory processing in the brain.

Auditory processing in the hearing organ is explored by iontophoretically labelling the auditory nerve and allowing the dye to diffuse into the *crista acustica*. Due to their black cuticle crickets require a challenging dissection of the hearing organ for imaging. However, some species of bush-crickets come with an almost transparent cuticle, which allows visualisation of the hearing organ in the intact

animal. We aim to successfully label the auditory organ with indicators and look forward to detect sound evoked Ca^{2+} -signals in the auditory afferents, which are tonotopically arranged in the *crista acustica*.

The experiments are ongoing and we report on the current state and outcome.

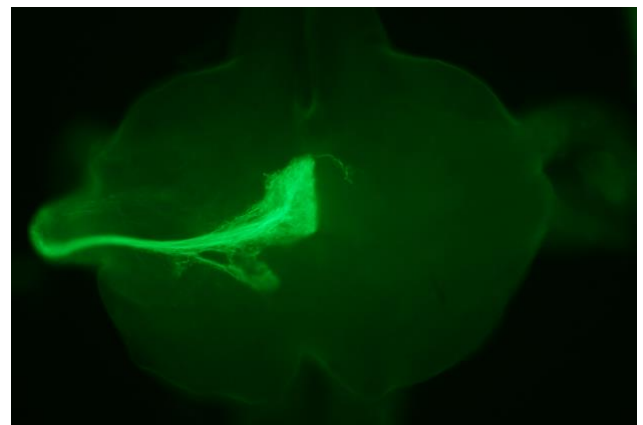


Figure 1. Projection of auditory afferents in the prothoracic ganglion of a bush-cricket (*Pholidoptera griseoaptera*) revealed with extracellular dye loading of Lucifer yellow into the auditory nerve.

Key Words: Auditory-Processing, Dye-Loading, Ca-Imaging, Good-Luck!

CHARACTERISING A NEW CLASS OF AERIAL ELECTRORECEPTOR IN BEES



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Recent research has demonstrated the capacity of bees to detect and respond to electric fields generated by electrostatically charged objects in their environment. This newly discovered sensory modality, termed aerial electroreception, has been shown to play a role in foraging decision making and social communication. In bumblebees (*Bombus terrestris*) and honeybees (*Apis mellifera*) respectively, charged mechanosensory hairs and antennae are the proposed electroreceptive structures, operating via electromechanical deflection of the putative electroreceptor. Herein, we propose that the antennal placode sensilla, which are considered to function exclusively as chemoreceptors, may be bimodal, also operating as an aerial electroreceptor in bees. Using electrostatic force microscopy, we show that the placode membranes hold a permanent electrostatic charge that differs from the surrounding antennal cuticle and is maintained long after death. Preliminary evidence from laser Doppler vibrometry experiments using a high-density scanning technique show that the placode membranes exhibit some electromechanical sensitivity to electric fields of biologically relevant frequencies and magnitudes. Specifically, the surface of bee antennae produce mottled patterns of electrostatic deflection that we interpret as the vibration of the placode membranes induced by electrical stimulation. Taken together, we suggest that the Coulomb interactions between the charged placode membrane and the external electric field result in a mechanical force over the placode membrane which can be used as a proxy for electrical sensitivity, offering an insight into

the function of these sensory units - a new class of electroreceptor.

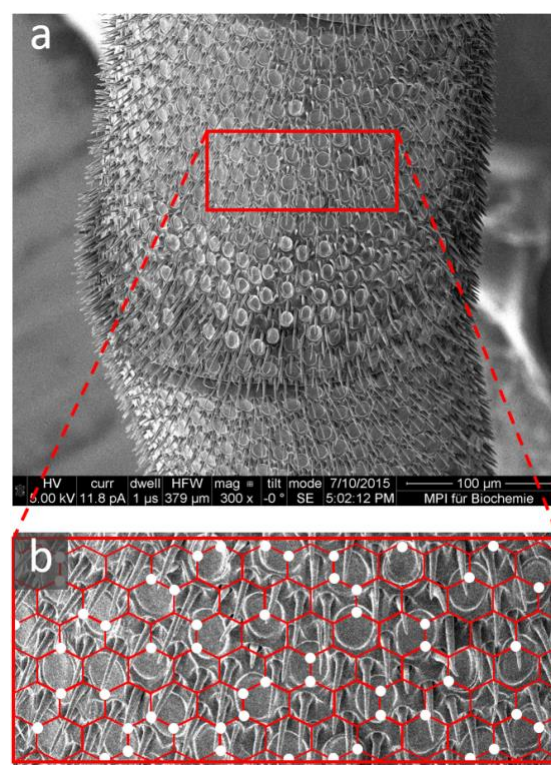


Figure 1. Visual representation of a high-density laser Doppler vibrometry scan (a, b). A region of the bee antenna is selected for the scan (a). The scan points are defined by a hexagonal grid. White circles represent scan points at which the laser focal position is located on a placode membrane. The vibrational velocity of each point within the grid is measured in response to electric field stimuli (b).

Key Words: Electoreceptor, charge, electromechanical, laser Doppler vibrometry, bees

KATYDID PINNAE ARE ALLOMETRICALLY SCALED RESONATORS FOR PASSIVE ULTRASOUND AMPLIFICATION



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Katydid have complex hearing organs. Located in their forelegs, the tympana can receive sound through two inputs: internally via the acoustic trachea (ear canal), or externally directly on the tympanum. Through the external path, many katydids possess cuticular pinnae surrounding the tympanum (Fig. 1).

Recent works demonstrated the role of these pinnae in high frequency reception, likely for enhanced bat detection. However, a comparative analysis is required to consolidate such findings in the wider context of acoustic communication and predator-prey dynamics.

Using micro-CT data to generate 3D printed models, we investigate the functional and morphological diversity of this external input to the tettigoniid ear, measuring morphology and resonance of 273 pinnae across 104 species of tettigoniid (~60% of subfamilies).

It is shown that pinnae provide broadband amplification of ultrasound, increasing sound pressure by up to 25 dB SPL; functioning as modified neckless Helmholtz resonators. In most species, this pressure gain is in the frequency range of insectivorous echolocating bats, further supporting the bat detection role of pinnae. Pinnae are negatively allometrically scaled to provide lower frequency resonances than would be expected under isometry, suggesting a selective pressure driving resonance at a global scale.

In addition, a comparison between anti-bat adaptations in katydids and moths reveals a potential global spectral overlap between acoustic predator cues and acoustic/auditory prey defences.

While the findings support a high frequency function of pinnae in many katydids, high frequency resonances in small and diurnal species are unlikely to share this anti-predator function.

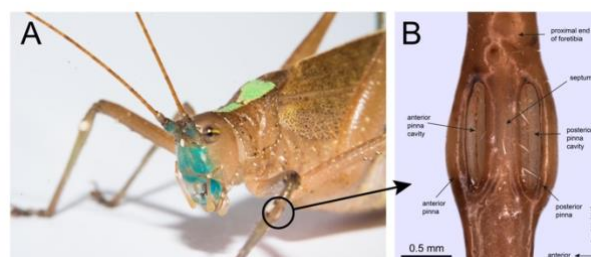


Figure 1. Auditory pinnae location and anatomy in *Docidocercus saggitatus*. A, *D. saggitatus*; B, pinnae anatomy.

Key Words: ultrasound, resonator, Ensifera, echolocation, arms-race

NON-INVASIVE CHARACTERIZATION OF NON-LINEAR DISTORTION PRODUCTS IN THE KATYDID 'COCHLEA' ORGAN



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Katydidids have complex ears in their forelegs. Two paired tympana transmit incoming vibrations in a tonotopic cochlea analogue, the *crista acustica* (CA). These vibrations reach the CA via a 'middle ear', the tympanic plate. These features make the katydid tympanal organ and CA an interesting model for cochlea research.

In the mammalian cochlea, non-linear and active auditory processes are important in the transduction process. Measurements of active and non-linear mechanics can be done in many ways, but one way is through measuring two-tone distortion products (DPs) and their emission from the cochlea using a microphone.

However accessing the site of origin in the cochlea of these DPs is challenging, as it requires invasive procedures which could damage the intact system.

Here, we investigated non-linear DPs in the katydid CA using non-invasive Laser-doppler vibrometry on katydidids with highly transparent cuticle. We recorded vibrations of the CA and tympana simultaneously to document DP amplitude with changing stimulus level and frequency ratio.

Experiments were repeated following CO₂ induced hypoxia, to test the physiological vulnerability of DPs.

We demonstrate that the DPs originate at their corresponding frequency-specific site in the CA, and visualise reverse travelling waves as hypothesised in the mammalian cochlea.

DP amplitude is greatly reduced following CO₂ hypoxia, but the continued presence of DPs during hypoxia and in recently dead specimens suggests the distortions are generated through mechanical intermodulation in the CA, and amplified by an active process in the mechanoreceptors at low stimulus levels.

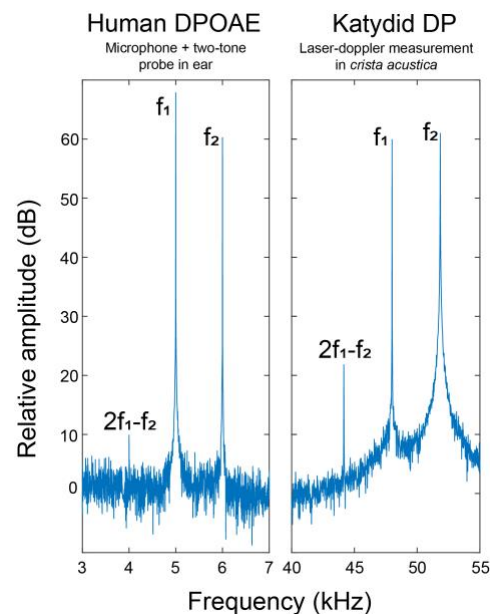


Figure 1. Example DPs in the human cochlea and the katydid *crista acustica*.

Key Words: Intermodulation, amplification, tonotopy, auditory.

CONFOCAL MICROSCOPY REVEALS CELLULAR ARRANGEMENT OF THE KATYDID EAR



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Katydids (Orthoptera: Tettigoniidae) have ears on their forelegs, with each consisting of two tympana on the tibia linked via an ear canal to a spiracular opening on the ipsilateral side of the thorax. These dual sound inputs at the tympana (external through the air, and internal via the ear canal), coupled with the interaural separation between the ears, lead to an exquisitely sensitive hearing system in which katydids can detect a broad range of sound frequencies with a highly accurate directionality, which is essential for both mate location and predator (bat) avoidance.

Neuronal mechanoreception in the katydid ear occurs at the crista acustica, a chordotonal organ consisting of 25–30 scolopidia arranged in a line. The cap cells of these mechanoreceptors are embedded within a 'tectorial membrane', which also covers the intermediate organ (which detects low-frequency vibrations) and attaches to the ear canal to form a tent-like structure. Despite its importance in maintaining the tonotopic arrangement of the crista acustica, little is known about the material composition of the tectorial membrane.

We used scanning laser confocal microscopy, combined with traditional fixation and histological staining, to visualise the tectorial membrane *in situ* and potentially identify its material composition. Despite a number of attempts, and approaches that include cuticular bleaching, Congo Red and Calcofluor White staining, and immunolabelling for tubulin, we were unable to obtain a clear

image of the tectorial membrane.

Nevertheless, the images we did obtain are among the clearest representations of the katydid chordotonal organs to date, showing F-actin within the scolopidia and the arrangement of resilin and chitin in the taenidial fibre of the ear canal. These results suggest that advances in non-destructive tissue fixation and clearing should reveal further details of the katydid hearing organ.

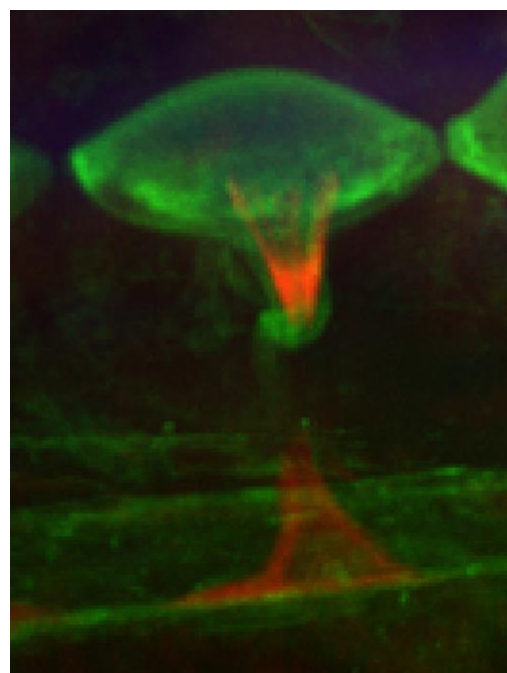


Figure 1. Confocal microscopy image of a scolopidium from the crista acustica of the katydid *Copiphora gorgonensis*, with F-actin labelled red and acetylated tubulin labelled green.

Key Words: Katydid, histology, scolopidia, mechanoreceptor, confocal microscopy

TWO VS ONE EAR: THE EVOLUTIONARY BENEFIT OF THE ABILITY TO LOCALISE SOUND FOR PRAYING MANTIS



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Insects have many forms of auditory detection. The ability to detect sound allows the animals to communicate with each other, avoid predation and detect prey. This is improved by the ability to localise where the sound is coming from. The order Mantodea is a diverse group of insects that were originally thought to be deaf but later found to have a single ear on the metathorax known as the cyclopean ear. They use this ear to detect echolocation from bats to avoid predation. While many species of mantids have one cyclopean ear, there are many other species that have been known to have two tympanal ears, known as the DK (deep groove and prominent knobs) ear and the meso ear. In this project, we examined how ultrasound being played at different angles can affect the praying mantis ability to localise sound frequencies depending on whether they have singular cyclopean or the two tympanal ears. Furthermore, we uncovered how their behaviour can link to antipredation defence and how that may link into localisation. Six mantids species from six genera (two with two tympanal ears and four with a singular cyclopean ear) were used during this experiment, and there were played sweep frequencies to the mantids at different angles along the horizontal plane. The reactions were video recorded to see how they behaved and how they orientated themselves to the sound if they were able to do so. We found that the frequency ranges of the mantids varied from species to species, and each species reacted differently to the frequencies played, with the most common reaction being non-directional cryptic behaviour. There was also little to no difference in the mantids' ability to localise the direction of

the sound between the two ear types. This experiment helps us understand the praying mantis ability to localise ultrasound, however it does not uncover why one ear may be beneficial for the praying mantis.



Figure 1. *Sphodromantis lineola*. This species has two tympanal. The smaller meso ear found between the mesothoracic coxae and metathoracic coxae. The Larger Dk (deep groove and prominent knobs) is found just below on ventral metathorax.

Key Words: Mantodea; Sound Localization, Predatory Insect, Tympanal Ears, Insect hearing.

TOWARDS A CHEAP, OPEN PLATFORM FOR HIGH-CONTENT PHONOTAXIS ASSAYS



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One defining feature of mosquito mating is the attraction of males towards the sound of a conspecific female's flight tone (also known as phonotaxis). The molecular control of this behaviour, and mosquito hearing more broadly, are highly complex. As such, high-sensitivity, high-throughput assays to interrogate phonotaxis would be greatly beneficial. We have developed a simple platform to monitor phonotactic responses across broad frequency and amplitude ranges, with temporal resolution throughout both light and dark. This platform is potentially scalable to different insects, allows freely-flying groups of animals to be assayed, and is unaffected by experimenter bias. In its basic form, the platform costs just over £350 to construct, with all software for control and analysis becoming open-source and freely available (in the near future). We are using this platform, as well as more rudimentary assays, to investigate the role of biogenic amine receptors and other neurotransmitter receptors in mosquito hearing and phonotaxis. In anopheles, we have used crispr to develop mutants of candidate receptors and are characterising their role in this complex, dusk-associated mating behaviour. Mosquitoes act as vectors for disease, and the benefits of understanding and assaying mosquito mating behaviour are manifold. Firstly, the ability to identify defects in mating could be important for the success of current and future control programmes. Furthermore, the disruption of mating behaviours may be a promising target for new interventions. We hope that this cost-effective platform improves accessibility to quantitative computational methods in behavioural science for disease vectors and beyond.

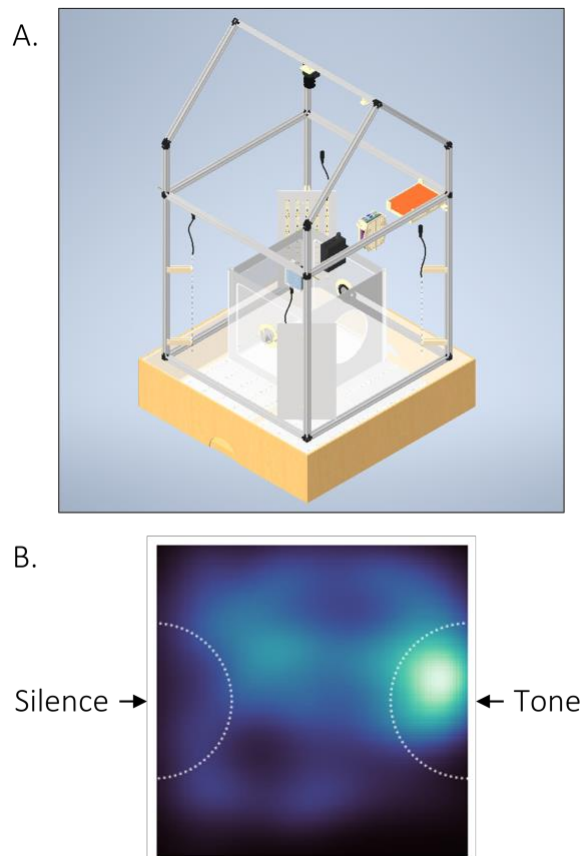


Figure 1. A) Platform setup. Setup consists of infrared light source below cage and infrared camera above. Speakers play tones, generated and randomised by Raspberry Pi, either side of the cage. Ambient lights controlled by Arduino. B) Example response (kernel density of flight paths) of male mosquitoes to a female flight tone.

Key Words: mosquito, behaviour, computation, neuroethology, efferent

DOES SPIDER LEG JOINT STIFFNESS VARY WITH LEG SIZE?



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Spiders are quite variable in body morphology, ranging from cellar spiders with long thin legs and small bodies, to giant tarantulas with thick short legs and large bodies. This size variation affects the mechanics of their body and will therefore ultimately influence the vibration frequencies that are sensed by the spider.

Here we focus on how leg segment size affects the stiffness of spider leg joints. Joint stiffness will influence what frequencies cause bending, and thus what vibrations are sensed. Joint stiffness should depend on the structures in the segment proximal to the joint. Anatomical structures within the segment are expected to stretch during joint movement and resist extension, with the greatest resistance offered by the largest structures, i.e. muscle. The same anatomical structures are also expected to scale with segment size, both in length and cross-sectional area. Therefore, we hypothesized that joint stiffness would vary with proximal segment area-to-length ratio.

To test this hypothesis, we used eleven species of spiders. We measured the stiffness of the femur-patella and tibia-metatarsus joints on legs one and two. We measured stiffness by applying a force to the distal leg segment and measured the change in joint angle. To quantify segment size, we measured the length of the segment proximal to the joint of interest and its diameter. We used mixed effects models to disentangle the relative effects of segment size, joint and species identity on joint stiffness. We found that the relationship between segment size and joint stiffness differed across species and joint type.

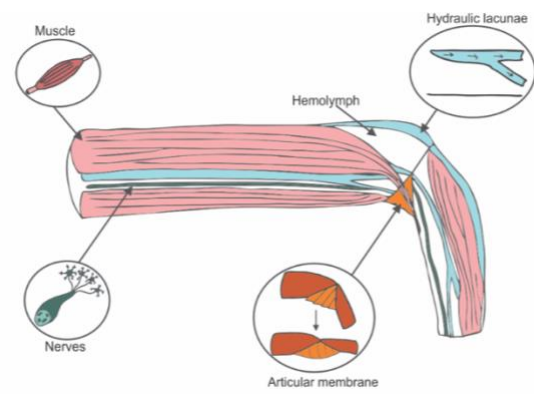


Figure 1. Stiffness is the resistance of a structure to deformation. For a spider joint, this is the joint's resistance to rotation. When the joint rotates, anatomical structures within the proximal segment are stretched. Muscle, typically the largest structure in the proximal segment, is expected to scale with segment size and likely contributes to joint stiffness the most. We expect the length (l) and cross-sectional area (A) of the muscle to be proportional to the segment. We expect the joint stiffness (k_r) to scale with segment size ($k_r \propto \frac{A}{l}$).

Key Words: spiders, stiffness, morphology, vibrations, biomechanics

A PHYSIOLOGICALLY INSPIRED MODEL FOR SPECIES-SPECIFIC SONG DETECTION IN GRASSHOPPERS



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Every grasshopper on a heated summer meadow faces the challenge of detecting the songs of its conspecifics from a cacophony of songs from numerous other *Caelifera* species. To better understand how a grasshopper's auditory system deals with this challenge, we develop and analyse a model that is based on the known neurophysiology of grasshopper auditory processing. A second goal of this project is to use this model for species recognition in field recordings of grasshopper songs in ecological surveys.

First, raw sound recordings are band-pass filtered to a frequency range that matches the songs of most grasshopper species and reflects the resonant properties of the tympanum. Then we summarize the processing steps of auditory receptor neurons and local interneurons in the metathoracic ganglion, namely transducer non-linearity, membrane time constant and spike-frequency adaptation, by envelope extraction, logarithmic transformation and high-pass filtering. The latter introduce intensity-invariance to the model. The pre-processed signal is then convolved with a set of different

types of Gabor kernels. Each convolved signal is thresholded and low-pass filtered with a time constant of about half a second. The sound is now represented by a set of slowly changing features. This way, the information contained in the original sound is dramatically compressed. This feature expansion reflects the neural representation at the level of ascending neurons. Finally, a perceptron decides whether the feature combination at each timepoint matches the pattern, i.e. the song of a specific species, that it has learned.

The proposed model needs only a few recordings of grasshopper songs for training and can then distinguish songs of a number of grasshopper species with impressive reliability. It deals well with songs of different intensities. However, the model is confused by mixed signals. More extensive investigations of model aspects such as time-warp invariance, robustness against background noise, and general applicability are currently on the way.

Key Words: grasshopper, auditory processing, classifier, signal detection, species recognition

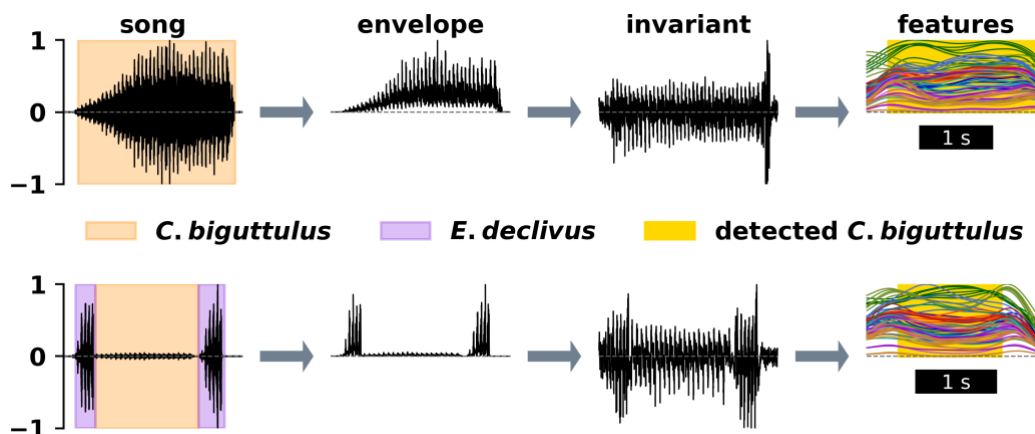


Figure 1. Schematic of the model's processing of songs of *C. biguttulus*, once in the foreground (top) and once in the background between songs of *E. declivus* (bottom).

AI-SUPPORTED MOTION TRACKING OF CRICKET WINGS DURING STRIDULATION



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For many Orthoptera species, singing is an important method of communication for mate finding, courtship or rivalry behaviour. In crickets and bush crickets (Ensifera), sound is created by stridulation (rubbing two body parts together) of the two forewings. A row of pegs (teeth) on the underside of the upper wing is rubbed against a rigid edge (scraper) of the bottom wing, producing vibrations with every tooth-scraper impact. The movements of the stridulation apparatus are a key part of sound production and can affect the amplitude and timing of the signal.

Key Words: motion recording, insect communication, DeepLabCut

In the seventies, a design for measurements of stridulation motion and the produced sound was first presented and further improved upon in the 2000s. Opto-electronic measurements were made using a position-sensing photodiode and reflective tape on hindlegs of grasshoppers or cricket wings.

Here, I present a new method based on high-speed video recordings and AI-supported tracking to analyse the wing movements of crickets during stridulation in detail. This markerless motion tracking allows for simple experimental setups where the animals can move and stridulate freely, without the need for precisely aligned equipment or manipulated wings. I show that AI-supported 2D tracking of wings is a possible alternative to currently used methods which also allows for the simultaneous tracking of multiple points on both stridulating wings. I will extent my experiments towards 3D tracking with a stereo camera setup to overcome the limitations in the depth plane of previous data acquisition methods.

A GENOMIC ANALYSIS OF ACOUSTIC SIGNALING BEHAVIORS IN HAWAIIAN CRICKETS



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The observation that sexual signals, and associated preferences, diverge rapidly among closely related species suggests that signal-preference co-evolution is a common occurrence in animal populations. In Hawaiian crickets, songs and acoustic preferences diverge coincident with speciation, as is true with most, if not all, animals that communicate acoustically during courtship. To characterize the genomic architecture of pulse rate and pulse rate preference variation between two closely related species of *Laupala* crickets, we combine quantitative trait locus (QTL) mapping

We find that male pulse rate QTL co-localize in the genome with female preference QTL in three fine-mapping analyses of separate linkage groups (chromosomes). We go on to identify candidate genes underlying acoustic behavior in these regions. Differential expression of some of these candidate genes is discussed.

Key Words: crickets, song, preference, genomics, candidate genes

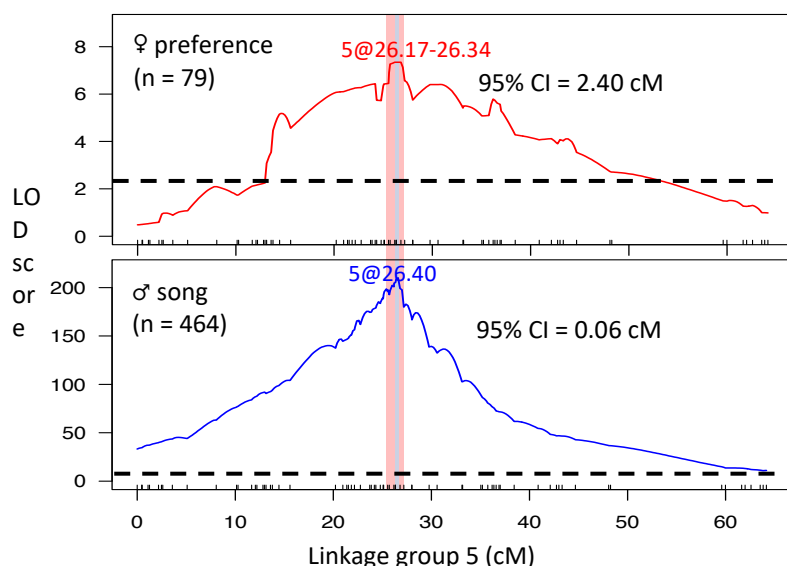


Figure 1. QTL mapping results showing co-localization of pulse rate and pulse rate preference loci on linkage group 5.

studies of F_2 hybrids with specific introgression lines along with transcriptomic results.

WING MECHANICS AND BIOACOUSTICS OF A NEW SPECIES OF *GNATHOCLITA* (TETTIGONIIDAE: PSEUDOPHYLLINAE) FROM THE CLOUD FOREST OF THE CENTRAL CORDILLERA IN COLOMBIA



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Male Katydid (Orthoptera: Tettigoniidae) rub together their specialised forewings to produce sound by stridulation. During wing closure, a sharp region on the anal margin of the right forewing (a scraper), engages with a row of teeth (file) on the left forewing. The movement of the scraper across the file produces vibrations which are amplified by a large wing cell adjacent to the scraper, the mirror. Katydid are known to stridulate with either sustained or interrupted sweeps of the file, generating resonant pure-tone (narrowband frequency) or non-resonant (broadband frequency) calls. However, some species can conserve some purity in their calls despite incorporating discrete pulses and silent intervals. This mechanism is exhibited by many Pseudophyllinae: Cocconotini. This study aims to measure and quantify the mechanics of wing vibration and sound production of a new species of the genus *Gnathoclita*, a Neotropical katydid from the montane forest of the central Colombian cordillera that can produce relatively narrowband calls at ~20 kHz. It was predicted that this species will use a stridulatory mechanism involving wing resonance to produce the observed call and spectral breadth. The calling behaviour and wing mechanics of eight males were studied using = micro-scanning laser Doppler vibrometry, microscopy, and ultrasound recording. Analysis of sound recordings and wing vibrations revealed a clear relationship between wing vibration and song carrier frequency. The stridulatory areas of the right tegmen exhibit a relatively narrow frequency response and the vibration outputs is mirrored in the calling song spectrum. As in most Pseudophyllinae, only one resonant area is activated during stridulation, the right mirror;

the left mirror shows an insignificant amplitude of vibration pattern. Under laboratory conditions the calling song duty cycle is relatively constant, with males spending most of the night singing from the burrows they guard and protect.

This new species is related to *G. sodalis*, but the distribution in different Andean branches, the differences in call frequency, and classical observations of anatomy support the proposition of a new species.

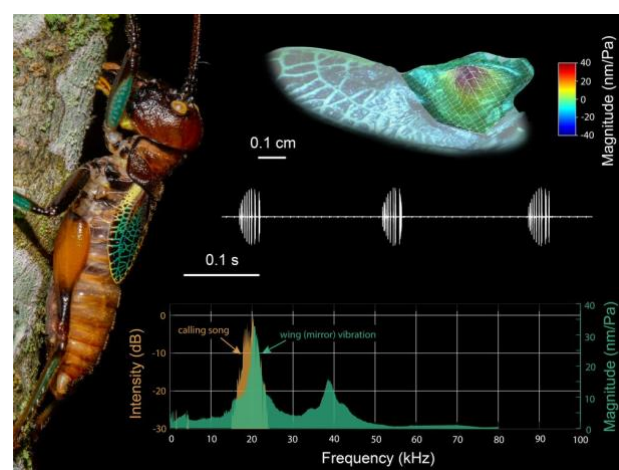


Figure 1. Habitus of a male *Gnathoclita* n.sp. with power spectrum, call oscillogram and wing vibrations.

Key Words: Laser Doppler Vibrometry. Cloud forest. Stridulation. Male aggression. Sexual dimorphism.

THUNDERSTRUCK: MECHANOSENSITIVITY OF ARTHROPOD FILIFORM HAIRS TO AC/DC ELECTRIC FIELDS



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Static electric fields (e-fields) pervade the natural environment from biological and physical sources. Such fields could be information-rich for electroreceptive organisms; encoding details of the local environment and weather, as well as signals and cues from con- and heterospecifics. Indeed, bumblebees and hoverflies can use electric cues during foraging, the well-known honeybee waggle dance appears to contain an electric component, and money spider dispersal is triggered by naturally occurring e-fields. The putative electroreceptors are mechanosensory filiform hairs or the antennal Johnston's organ, systems traditionally regarded as aero-acoustic sensors; yet, electrostatic physics renders them potentially bimodal. Due to an apparent charge on the hair or antenna Coulomb forces from external e-fields will attract or repel the structure causing movement likely triggering the same neurological 'hardware' used to detect aero-acoustic forces. How filiform hairs react to external e-fields remains largely unexplored, particularly with regards to ecologically relevant fields. Here, using laser Doppler vibrometry we explore the mechanical sensitivity of spider and cricket filiform hairs to such fields. Spider hair sensitivity is increased when time-varying e-fields are DC offset (analogous to a flying insect's electric signature), whereas, cricket hairs do not experience such an increase. This more general sensitivity of crickets as prey/hosts may reflect an increased cost to false negative responses compared to predatory spiders. These findings demonstrate that aerial electroreception, as with all sensory modalities, is likely variable among users, and hints that even analogous sensors such as filiform hairs may vary in the manner in which they transduce electrostatic

information.

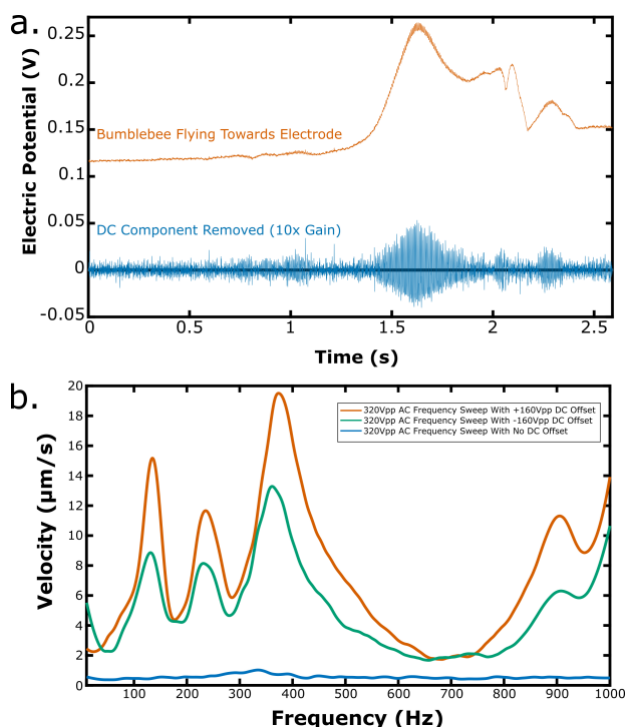


Figure 1. a. Recording of the aerial electric potential during a bee flight. The top trace demonstrates that the electric component of bee flight is always unipolar, despite the time-varying component of wingbeat, the trace never crosses zero, there is always a DC offset. The lower trace shows the same recording with the offset removed. **b.** Velocity spectra of a spider trichobothrium in response to electric fields with and without positive and negative DC offsets. Applying a positive or negative DC offset (akin to a flying insect) to frequency sweeps provided as external electric fields greatly increases the velocity of spider trichobothrium vibration.

Key Words: electroreception, spider, cricket, mechanosensory hairs, laser Doppler vibrometry

EVOLUTION OF COURTSHIP WITHIN SUBFAMILY GOMPHOCERINAE (ORTHOPTERA: ACRIDIDAE)



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Institute for Information Transmission Problems, Russian Academy of Sciences, Moscow, Russia

Gomphocerinae grasshoppers evolved complex mating behaviour and can produce several types of song. One of the song type, courtship song, can be very complex and be accompanied by visual elements. Here we analyse distribution of chosen courtship characters on the phylogenetic tree and reconstruct evolution of these characters.

First, we used four markers (COI, cytB, ITS1 and ITS2) for phylogenetic reconstruction. Our data set included 89 species of Palearctic Gomphocerinae. Second, we chose six universal characters of courtship: 1) difference between courtship and calling songs, 2) degree of divergence of the calling song element within courtship song, 3) the number of sound elements, 4) the number of different leg movement patterns, 5) the number of visual elements and 6) mechanisms of sound emission. We estimated phylogenetic signal and performed ancestral state reconstruction by means of using R software.

We found a relatively high phylogenetic signal ($0,5 > \text{Pagel's } \lambda > 0,7$) for difference between courtship and calling songs, the number of sound elements and the number of different leg movement patterns. These characters suggested to be relatively stable and so they could reflect evolutionary trends within considering taxon. Other characters were found to evolve rapidly and chaotically.

Our reconstructions suggest that common ancestor of the tribe Stenobothrini and *Chorthippus pullus* already evolved complex courtship song consisted of two sound elements. The song structure became to be more complicated independently within the genera *Omocestus*, *Myrmeleotettix* and *Stenobothrus*. The generation of visual elements was found to be an ancestral character state for *Stenobothrus* genus. The

presence of two sound elements in the calling or courtship songs was shown as an ancestral state for Gomphocerini. However, complex courtship was reliably detected as an ancestral state only for *Chorthippus albomarginatus* group. A controversial result of our work is the song simplification in *Chorthippus biguttulus* group, which is discussed and should be checked in future.

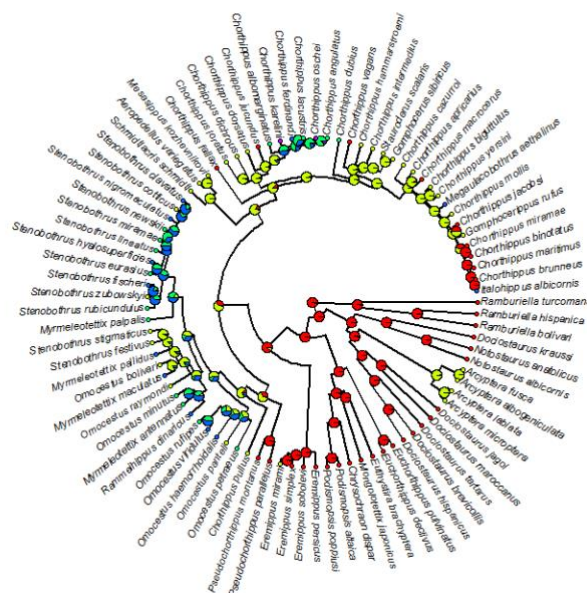


Figure 1. Ancestral state reconstruction of the number of sound elements in courtship: red – 1, yellow – 2, green – 3, blue – 4, purple – 5.

Key Words: Gomphocerinae, courtship, mating behaviour, evolution reconstruction

KINEMATICS SONG OF SONG IN THE RAPIDLY EVOLVING HAWAIIAN *LAUPALA* CRICKETS (ENSIFERA; TRIGONIDIIDAE)



Nicholai M. Hensley^{1,2}, Kerry L. Shaw¹, & Berthold Hedwig²

¹ Cornell University, Department of Neurobiology & Behavior

² University of Cambridge, Department of Zoology

During speciation, behavioural mechanisms are important in the origin and maintenance of reproductive boundaries between closely related, diverging populations. Here, we use the rapidly-radiating genus of Hawaiian *Laupala* crickets to understand how reproductive barriers evolve during speciation, focusing on mechanisms of stridulatory song. Like many crickets and their allies, males of the ~40 species of *Laupala* produce species-specific songs by rubbing their wings together to attract females, producing a rapid series of pulses over time. Analyzing high speed video and synchronized audio, we find that *Laupala* stridulation is similar to other crickets, as sound amplitude increases during wing closing. Further comparative work will analyze how species differ in the kinematics of sound production underlying song differences. Because behaviours may be a rate-limiting step in evolution of new species, understanding how they evolve provides key insight into the drivers and constraints that regulate this biodiversity.

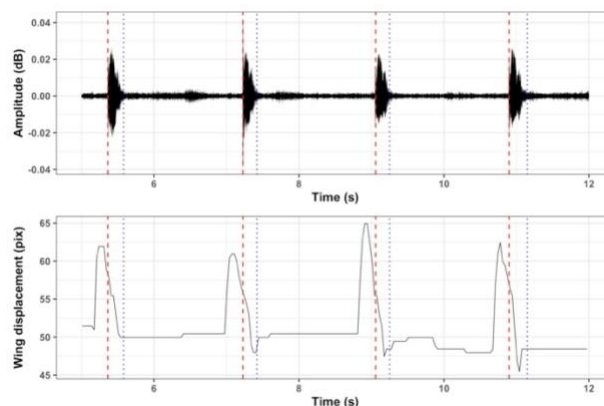


Figure 1. (Above) Sound amplitude over time during stridulation of a male *L. kohalensis*. Pulse consisting of relatively pure tones peak with characteristic periodicity. (Below) Tracking software identifies maximum (peaks) and minimum wing extension distances over time during stridulation. Sound pulses begin (vertical red-dashes) as wings close, and end during as wing position resets (vertical blue-dashes).

Key Words: behavioural evolution, *Laupala*, stridulation, kinematics, song

**PRELIMINARY OBSERVATION ON CALLING
SONGS AND REPRODUCTIVE BEHAVIOURS OF
THE TAIWANESE ENDEMIC CRICKET *LEBINTHUS*
LANYUENSIS OSHIRO, 1996**



Po-Wei Chen ¹

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The reproductive behaviour of crickets in the tribe Lebinthini is unique among other crickets in relatively higher frequency male calls, lack of female phonotaxis behaviour, and vibrotaxis behaviour in males (Tan *et al.*, 2021). In many Lebinthini species, calling songs and reproductive behaviours have never been documented. This study provides the first description and data on the song and behaviour of *Lebinthus lanyuensis*, a species endemic to Taiwan, in captive conditions. The duration of *L. lanyuensis* calling song is 2-7 seconds, consists of an initial part of multiple syllables and a second part of a short trill. Song frequency ranged between 12 and 35 kHz with a peak frequency of 19.4 kHz. The song characteristics and copulatory sequence of *L. lanyuensis* are mostly consistence with other known congeneric species, while no body tremulation and only few acoustic activities were observed. Antennae and femur movements were frequently observed. Males were occasionally observed producing substrate vibration by drumming the substrate with mandibles. In some instances, females were observed walking towards a calling male and subsequently copulate on the location.

Key Words: *Lebinthus*, song, reproductive behaviour, substrate vibration, Taiwan

EFFICIENCY OF SOUND PRODUCTION IN THE MONSTER HAGLID, *CYPHODERRIS MONSTROSA*



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Like many acoustic insects, male *Cyphoderris monstrosa* (Orthoptera: Prophalangopsidae) produce an advertisement call this is sustained over relatively long periods of time (several hours each evening during the active season). This acoustic call (song) is also known to mediate aggressive territorial interactions between males. Males also produced two types of nuptial gift that are consumed by females during or after mating, suggesting a significant energetic investment in mating by males.

Male song in *C. monstrosa* is an irregular trill, such that the temporal pattern (and quantity of sound output) is highly variable between males. Chirp Duty Cycle (the proportion of time a male spends producing sound) has been shown to predict the outcome of male-male aggressive contests, with more sustained singers being the contest winners.

There is also variation in the output level of male acoustic signals – some males produce louder signals than others – but this has not been systematically documented.

Our question was what information about male quality is available in *C. monstrosa* songs? We considered two hypotheses:

- 1) Acoustic signalling is an energetic contest. Males with greater energy reserves can sustain more continuous singing. Songs would therefore be a signal of a male's ability to acquire resources (foraging success).
- 2) Male songs are correlated with metabolic efficiency. Some males can generate acoustic energy at lower relative cost and songs therefore have information about inherent (physiological) quality.

We made calibrated measurements of sound production by male *C. monstrosa* to calculate the acoustic power output of individual males, while simultaneously measuring CO₂ production using open-flow respirometry. Our goal was to calculate the metabolic efficiency of sound production in *C. monstrosa*, controlling for variation in quantity of singing (chirp duty cycle) and output level among individual males. (It was great fun!)

Our data (so far) indicate that there is considerable variation in acoustic power. In addition to the previously documented temporal variation in sound production, individual males show considerable variation in output level and acoustic power. Output level appears to be repeatable within males (although there are limited data on this).

Variation in CO₂ production is correlated with acoustic output. Data suggest that some males are more efficient sound producers.

Key Words: signal function; Orthoptera; acoustic power.

3D-PRINTED SENSOR INSPIRED BY TRICHOID SENSILLA OF INSECTS, EARLY STUDIES OF THE MECHANICAL STRUCTURE



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Nature has, for the longest time, inspired mankind in the development of new technologies. In the past few decades, thanks to thorough biological studies on insects, we have come to better understand how their different sensory systems work. A fascinating sensing mechanism is the hair-like structure, often called trichoid sensilla or trichobothria, which are used to sense low frequency, near field, sound and air vibrations. Nevertheless, some iterations of this sensing mechanism are used as touch sensors, and it is believed that from this structure stem other sensilla that sense odour, temperature, and acceleration, as well as gyroscope-like mechanisms.

This project will use 3D printing techniques to create a sensor inspired by the trichoid sensilla of insects (mainly the hinged structure of the caterpillar *B. Brassicae*, and the cerci of crickets previously studied by the EU CILIA project). This would provide sensing of low frequency sounds at different frequencies based on small variations of the structure (e.g., different diameter or hair length). This can allow sensing of frequency specific sounds with great accuracy.

Early experiments have been based on the hair connected to a base containing a piezoelectric chip in order to record, with the aid of a vibrometer, the resonant frequency of the sensor structure due to vibration of the piezoelectric. The results showed the best response on a 4 mm long hair, where the hair had cantilever-like movements and the resonant frequency was a distinguishable single peak.

Further testing will involve experiments in the acoustic domain without the piezoelectric chip. This work was funded by the DGA-DSTL PhD program.

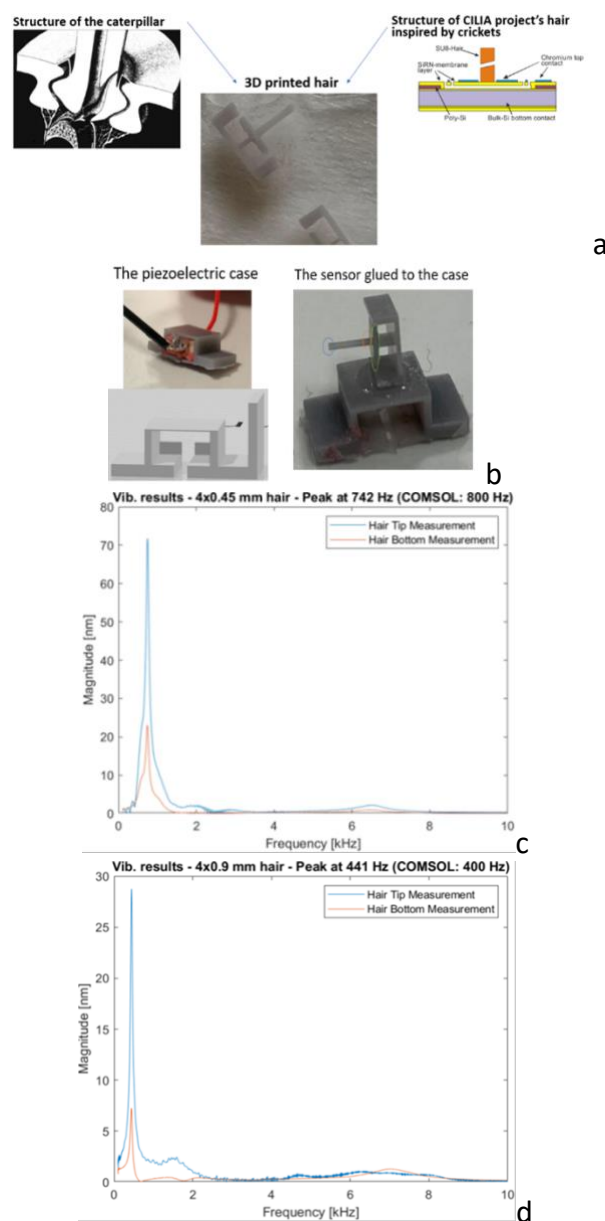


Figure 1. Structure of the 3D printed hair and its inspirations (a); piezoelectric case used for experiments (b); vibrometer results for the hair long 4 mm, with a 0.45 mm (c) and 0.90 mm (d) diameters.

Key Words: trichobothria, trichoid sensilla, 3D print, insects, sensors.

DO SIBLING GRASSHOPPER SPECIES OF THE *STENOBOTHRUS EURASIUS* GROUP USE DIFFERENT SONG RECOGNITION MECHANISMS?



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Two sibling species of Gomphocerinae, *Stenobothrus eurasius* and *S. hyalosuperficies*, hybridize in a contact zone in the south-east of European Russia. At the same time, the species not only sing the different songs but also use different sound-producing mechanisms. The males of *S. eurasius* sing by common stridulation that is typical for Gomphocerinae. Calling song in *S. hyalosuperficies* is produced by wing clapping, whereas courtship song is produced by both wing clapping and stridulation. The calling song of *S. eurasius* comprises two types of pulses repeated with different rate (100–120/s and 23–26/s) and duration, whereas only one pulse type (rate of 60–100/s) can be found in the calling song of *S. hyalosuperficies* (Fig. 1). Thus, the pulse rate of wing beats is close to the rate of one pulse type of *S. eurasius*.

To study the reasons and patterns of hybridization between the two species, we investigated female preferences for the natural and modified songs in playback experiments. The modified songs comprised either one ('high pulse rate') or another ('low pulse rate') pulse type of *S. eurasius* song (Fig. 1). Playback of the natural calling songs revealed clear preferences for conspecific songs in *S. eurasius* females. The females of *S. hyalosuperficies*, however, appeared to be less selective, since the response rate to con- and heterospecific songs did not differ significantly. At the same time, modified songs were significantly less attractive for females of both species.

Thus, behavioural experiments showed asymmetry in female preferences in these two species, which allows us to suppose the direction of hybridization in the contact zone. We also can propose different recognition mechanisms in these sibling species. A

relatively high selectivity in *S. eurasius* females suggests a necessity of two elements with different pulse rate for the calling song recognition. The results on *S. hyalosuperficies* females are harder to interpret. Almost equal attractiveness of con- and heterospecific songs for *S. hyalosuperficies* females could be explained by that both songs contain pulses of similar 'high pulse rate'. However, modified song with pulses repeated with high rate was rather unattractive for them. We propose that the overall duration of calling song rather than the pulse rate could be important for the process of recognition in *S. hyalosuperficies*.

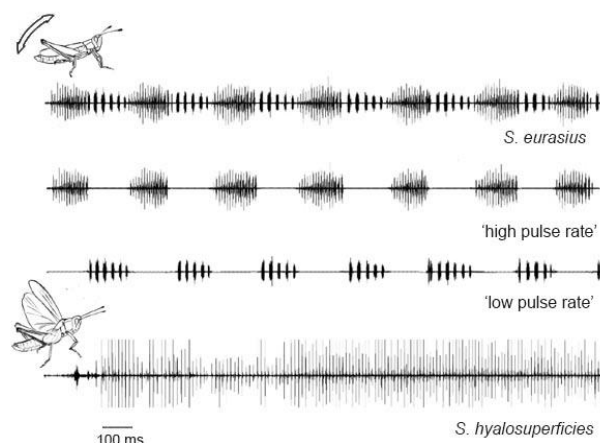


Figure 1. Oscillograms of the calling songs of *Stenobothrus eurasius* and *S. hyalosuperficies* and two modified songs used in playback experiments.

Key Words: grasshopper, calling song, stridulation, wing clapping, playback

QUANTIFYING NANOSCALE MECHANICS OF TAENIDIA FIBRES USING ATOMIC FORCE MICROSCOPY



Eleftherios Siamantouras¹, Charlie Woodrow¹, Emine Celiker¹, Darron A. Cullen¹, Claire E Hills¹, Paul E Squires¹, Fernando Montealegre-Z¹

¹ School of Life & Environmental Sciences

Derived from the respiratory tracheae, bush-crickets' acoustic tracheae are hollow tubes evolved to transmit sounds from the external environment to the interior ear. Due to the location of the ears in the forelegs, acoustic tracheae function as a structural element that can withstand large stresses during locomotion. Tracheae gain their mechanical strength from tightly coiled taenidia, which are organized as spring-shaped fibers in the inner wall of the trachea tube. Here, we report a Atomic Force Microscopy Force Spectroscopy (AFM-FS) approach to quantify the mechanics of single taenidia fibers in *Mecopoda elongata* and *Copiphora gorgonensis*. Microsamples of taenidia stripes were extracted from dissected acoustic and respiratory trachea tubes and immobilized on the substrate of a petri dish. Analysis of force-displacement curves at low strains using the Hertzian contact model showed an Elastic modulus distribution between 13.9 MPa to 26.5 GPa for *Mecopoda elongata* and 1.8 MPa to 96.1 MPa for *Copiphora gorgonensis* for acoustic tracheae. Microsamples obtained

from the respiratory trachea were in range between 3.2 and 52 MPa. Differences in elasticity between acoustic and respiratory tracheae indicate sophisticated evolution with elasticity playing a key role in optimization of function. In conclusion, apart from the significance in ear canal modelling, nanomechanical characterization of taenidia can inform the development of innovative bioinspired materials.

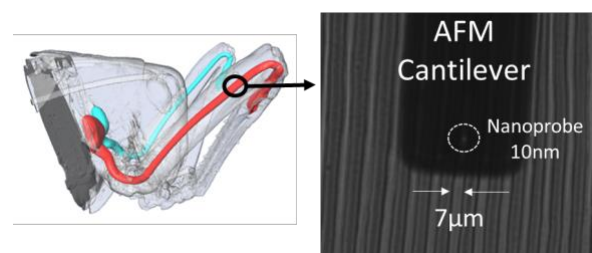


Figure 1. AFM-based nanomechanical characterisation of single taenidia fibres.

Key Words: AFM, nanoindentation, trachea, taenidia, nanomechanics

POSTERS

Poster 1: The music in mosquito ears: two tones at a hopf bifurcation.

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Poster 2: Behavioural reactions of spiders to vibrations mimicking prey in orb and funnel webs.

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Poster 3: Coupled membranes: possible analogue of mammalian ear bones found in the field cricket ear.

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Poster 4: Imaging auditory processing in crickets using extracellular loading of Ca²⁺ tracers.

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Poster 5: Characterising a new class of aerial electroreceptor in bees.

Beth H. Harris & Daniel Robert.

Poster 6: Katydid pinnae are allometrically scaled resonators for passive ultrasound amplification.

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Poster 7: Non-invasive characterization of non-linear distortion products in the katydid 'cochlea' organ.

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Poster 8: Confocal microscopy reveals cellular arrangement of the katydid ear.

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Poster 9: Two vs one ear: the evolutionary benefit of the ability to localise sound for praying mantis.

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Poster 10: Towards a cheap, open platform for high-content phonotaxis assays.

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Poster 11: Does spider leg joint stiffness vary with leg size?

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Poster 12: A physiologically inspired model for species-specific song detection in Grasshoppers.

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Poster 13: AI-supported motion tracking of cricket wings during stridulation.

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