

# 2<sup>nd</sup> International Symposium on Biotremology



## Abstract Book

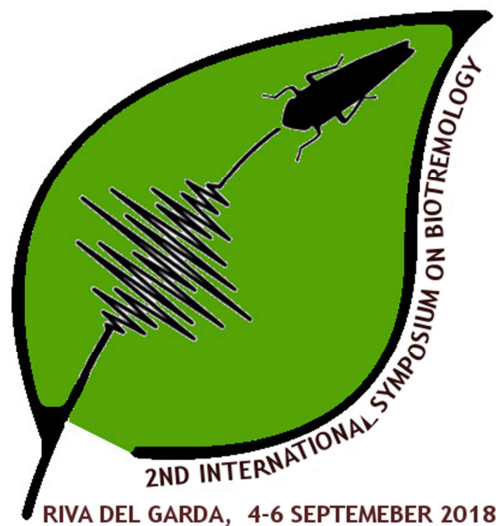
Centro Congressi

Riva del Garda, Trento, Italy

September 4-6 2018



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*Conference Chair: Valerio Mazzoni*



## Welcome to the 2<sup>nd</sup> **International Symposium on Biotremology!**

*The Organizing and Scientific Committees welcome each of you to **Biotremology 2018**, at Riva del Garda. How we have grown and expanded in two short years!*

*After our first standalone conference of biotremologists in 2016, we have returned even stronger, and certainly still filled with enthusiasm and excitement for the future of this movement. We represent 16 countries from all continents except Africa and Antarctica...and likely more if we consider country of origin, rather than where we now reside. The registrations for the meeting have increased by 30%, even though a number of researchers from the US are already in the classroom for their fall term, but wishing they were here! Some of us first met in person in 2016 and have returned. Others are colleagues or collaborators of those who attended in 2016 and are taking their turn in finding their way to Italy while the colleagues continue to work at home. Others have found us for the first time because they have no close colleagues at home, and want to share their ideas with new colleagues here.*

*This is no passive, casual aggregation of people on holiday or vacation, though we do expect to have a great time in this beautiful venue. We are reporting on taxa from spiders to elephants, some of which we are just now starting to study, while for others we have continued to study in different and multiple contexts, using tools from molecular biology to landscape ecology, examining relationships from mating and parental care to the vibroscape. We use all tools we have at hand, and build others that not so long ago lived only in our imagination. We are not a group of passive individuals. We are members of an emerging discipline, studying behaviors so old we cannot grasp the origin, so widespread we are in awe of the scope and range. We are researchers representing all stages of career paths from graduate students to emeritus professors, who can learn from each other in the days we have together, and form relationships that will continue long after the meeting. We are biotremologists!*

On behalf of the organizing committee,

**Peggy Hill**

**Valerio Mazzoni**

**Meta Virant-Doberlet**

## **ORGANIZING COMMITTEE**

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# Conference Maps



- (1) Conference Venue: Centro Congressi di Riva del Garda, Parco Lido 1
- (2) Public Event Venue: MAG Museo Alto Garda, Piazza Cesare Battisti 3/A
- (3) Social Dinner Venue: Centrale Idroelettrica, Via Giacomo Cis 13
- (4) Pier (Boat Excursion): Piazza Catena 25



# PROGRAM

TUESDAY 4<sup>TH</sup> SEPTEMBER

08:00 - 09:00: **Registration**

09:00 - 09:20: **Welcome** (**Andrea Segré**, President of Fondazione Edmund Mach, **Ilaria Pertot**, Director of the C3A - Center Agriculture Food Environment, University of Trento)

09:20 - 10:00: **Opening Session (Ch: P Hill)**

- Biotremology: past, present, future (**Meta Virant-Doberlet**, Slovenia)
- “Insect Drummer Awards” Ceremony (**Peggy Hill**, USA and **Rafael L Rodriguez**, USA)

10:00 - 10:30: Coffee Break

10:30 - 11:30: **Keynote Lecture 1: Philip Brownell** (introduced by P Hill)

- **Vibrational sensory ecology of sand scorpions**

11:30 - 13:05: **Talk Session 1: Morpho-Functionality & Physiology (Ch: B Mortimer)**

- Cicadas using two mechanisms and communication channels for acoustic and vibrational communication (**Matija Gogala**, Slovenia)
- Complex arrays of chordotonal receptors in the first two abdominal segments of froghoppers, leafhoppers, and planthoppers (**Peter Bräunig**, Germany)
- Vibrational signal production in *Aphrodes makarovi* (**Anka Kuhelj**, Slovenia)
- Transitions of vibration-dependent behavioral states in *C. elegans* population through a ‘fight-or-flight’ response circuit (**Takuma Sugi**, Japan)
- Can postural change in insects modify vibratory sensitivity? New insights from the study of leg vibration in cave crickets (**Nataša Stritih Peljhan**, Slovenia)
- QUESTION TIME

13:05 - 15:00: Lunch Break

15:00 - 16:00: **Keynote Lecture 2: Reginald B Cocroft** (introduced by RL Rodriguez)

- **The vibrational ecology of insect-plant interactions**

16:00 – 17:50: **Talk Session 2: Mating Behavior (Ch: RL Rodriguez)**

- Social ontogeny in the mating communication system of an insect (**Desjonquères**, USA)
- Risky ripples: multiple selection pressures operating on a multisensory sexual display (**Halfwerk**, Netherlands)
- Noisy neighborhoods are a challenging environment for courtship (**Polajnar**, Slovenia)
- Correlation between mating behavior and ovarioles development in the meadow spittlebug *Philaenus spumarius* (**Avosani**, Italy)
- Species delimitation and recognition of allied species of psylloids (Hemiptera: Psylloidea) based on vibrational communication (**Liao**, Taiwan)
- Mating behavior of *Orientus ishidae* (Hemiptera: Cicadellidae) (**López Díez**, Slovenia)

- QUESTION TIME

17:50 – 19:00: **Steering Committee**

19:30 – 22:30: Public Event at MAG: **Caitlin O’Connell-Rodwell** – Stanford University organized under the **project E-STAR** - Bando "I comunicatori star della scienza" - Provincia Autonoma di Trento

- Soft Dinner
- The Secret Life of Elephants (Caitlin O’Connell-Rodwell, USA)

## WEDNESDAY 5<sup>TH</sup> SEPTEMBER

08:30 - 09:30: **Keynote Lecture 3: Daniel Robert** (introduced by M Gogala)

- **Bee electroreception and the potential role of Coulomb force in terrestrial arthropods**

09:30 – 11:00: **Flash Talk Session: (Ch: R Mühlenthaler & M Gogala)**

11:00 - 11:30: Coffee Break

11:30 – 13:20: **Talk Session 3: Social Insects (Ch: A Wessel)**

- The differences in the vibrational signals between male mason bees in Europe (Conrad, Germany)
- Adult-larvae vibrational communication in paper wasps: the role of abdominal wagging in *Polistes dominula* (Nieri, USA)
- Excitation signal extraction of ant walking pattern under the influence of noise using a biomechanical bipedal mathematical model (Oberst, Australia)
- Evolution of vibroacoustic signals in Myrmicinae ants (Casacci, Poland)
- Ants respond to tree vibrations caused by browser chewing (Krausa, Germany)
- Tiny ants, whistling acacias and devastating elephants (Hager, Germany)
- QUESTION TIME

13:20 – 14:45: Lunch Break

14:45 – 18:30: Excursion: Garda Lake boat trip

19:30 – 22:30: Social Dinner at Centrale Idroelettrica of Riva del Garda

## THURSDAY 6<sup>TH</sup> SEPTEMBER

08:30 - 09:30: **Keynote Lecture 4: Caitlin O'Connell-Rodwell** (introduced by P Hill)

- **The vibration sense in elephants: lessons for human hearing**

09:30 – 10:50: **Talk Session 4: Applied Biotremology (Ch: M Virant-Doberlet)**

- Trapping of male and female Asian citrus psyllids exposed to a 6-h Series of synthetic vibrational communication calls (Mankin, USA)
- Acoustic communication and mating behaviour in the tomato potato psyllid, *Bactericera cockerelli* (Sulc) (Sullivan, New Zealand)
- Vibration sensitivity in a cerambycid pest and its potential for pest management (Takanashi, Japan)
- Vibrational signals as semiophysicals: a comparison between pheromonal and vibrational mating disruption (Mazzoni, Italy)
- QUESTION TIME

10:50 – 11:15: Coffee Break

11:15 – 11:30: **Springer Young Researcher Awards**

11:30 – 13:05 **Talk Session 5: Environmental and Behavioral Ecology (1) (Ch: RB Cocroft)**

- Insect phonology: combinatorial signal processing in *Enchenopa treehoppers* (Hemiptera: Membracidae) (Rodriguez, USA)
- Biotremology in heelwalkers: population differentiation, information content and female choice (Eberhard, Germany)
- Tropical vibes: vibration use and detection by the hermit crab *Coenobita compressus* (Roberts, USA)
- The use of bimodal acoustic signals by red-eyed treefrogs, *Agalychnis callidryas* (Caldwell, USA)
- Mating behavior in spotted wing species suggests a modular model of vibratory communication in *Drosophila* (Rota Stabelli, Italy)
- QUESTION TIME

13:05 – 15:00 Lunch Break

15:00 – 16:30: **Talk Session 6: Environmental and Behavioral Ecology (2) (Ch: N Stritih Peljhan)**

- Bioacoustics and ecoacoustics for the analysis of the soundscape (Pavan, Italy)
- Vibration landscapes: the role of materials in information transfer (Mortimer, UK)
- “Bad vibes”: anthropogenic vibrations affect anuran calling activity (Zaffaroni-Caorsi, Brazil)
- Effect of wind on tremulatory signaling of a Neotropical katydid (*Copiphora brevirostris*) (Velilla, Netherlands)
- Is it time for ecotremology? (Sturm, Slovenia)

16:30 – 17:00 Coffee Break

17:00 – 18:30 **Workshops**

- Building a solid networking in Biotremology (Eberhard, Mazzoni)
- Recording techniques and sound analysis” (Mühlethaler, Wessel, Conrad)

18:30 – 18:40 End of Works (Mazzoni, Hill, Virant-Doberlet)

## LIST OF POSTERS / FLASH TALKS

1. Neuronal regression of chordotonal vibroreceptor organs in the cave cricket *Dolichopoda araneiformis* (Orthoptera: Rhaphidophoridae) (Stritih Peljhan, Slovenia)
2. Investigation of the vibratory interneurons in the ventral nerve cord of *Halyomorpha halys*: morphology and physiology (Stritih Peljhan, Italy)
3. Vibrational behavior of psylloids (Hemiptera: Psylloidea): functional morphology and mechanisms (Lin, Taiwan)
4. Leafhoppers got ears! The abdominal chordotonal organ of leafhoppers - a new type of vibration receiver in the Hemiptera (Hemiptera: Tymbalia: Cicadomorpha: Cicadellidae) (Elhers, Germany)
5. From wing vibration to loudspeaker design, a Netlogo modelling approach (Hartbauer, Austria)
6. Calling, courtship, and post-mating tremulations in a Neotropical katydid (Kernan, USA)
7. Interference of female rivalry signal of stink bugs (Hemiptera: Pentatomidae) in the courtship and mating behavior of their conspecifics (Moreira Dias, Brazil)
8. The role of variability of the different elements of courtship songs in mating recognition and selection of conspecific partner in *Carpocoris fuscispinus* (Shestakov, Russia)
9. Age-related changes in female responsiveness in *Aphrodes makarovi* (Fras, Slovenia)
10. Telltale Vibrations – are male signals in the spider *Pisaura mirabilis* dependent on condition? (Machnis, Germany)
11. I've got that silky touch! Impact of female status on male vibratory courtship in *Pisaura mirabilis* spiders (Möller, Germany)
12. Do tap-dancing birds use vibrational signals? Mutual multimodal courtship display in a socially monogamous songbird (Ota, Japan)
13. Rustling ants: percussive communication performed by two *Camponotus species* in Borneo (Bota, Germany)
14. When time is tight – Drumming in swarming alates (Hager, Germany)
15. Vibrational signals alert the foraging force (Krausa, Germany)
16. Interspecific differences of vibro-acoustic signals in four species of bark beetles of the genus *Polygraphus* Er. (Coleoptera: Curculionidae, Scolytinae) inhabiting the island of Sakhalin (Kerchev, Russia)
17. Insights into some temporal features of the female signals of Brown Marmorated Stink Bug (*Halyomorpha halys*) to improve attraction towards males (Torriani, Italy)
18. Preliminary results on the pair formation process and pest management strategy of two newly discovered treehopper vectors (Nieri, USA)
19. Evaluation of social behavior based on vibrational signals of *Philaenus spumarius* in semi-field conditions (Akassou, Italy)
20. Vibroscape variability within hay meadow habitat (Rexhepi, Slovenia)

# KEYNOTE LECTURES

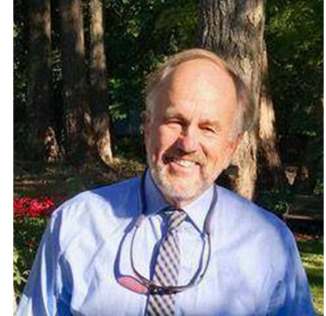




## VIBRATIONAL SENSORY ECOLOGY OF SAND SCORPIONS

**Philip BROWNELL**

Department of Integrative Biology, Oregon State University, Corvallis, OR, USA



Although among the largest and most ancient of terrestrial invertebrates, the world's >1600 identified species of scorpion are poorly known to physiological and behavioral science. For behavioral studies, those living on sand dunes and fluorescent under black-light at night are particularly accessible for field and laboratory study. My research has focused on neurobiological studies of sensory systems used by these highly adapted animals to find prey, cannibalistic mates and the home burrow refuge.

Primary among these are the vibrational sensory organs at the tips of their 8 legs, the basitarsal slit sensilla. The individual slits within each organ are variably sensitive to compressional and surface waves that conduct through loose sand at unexpectedly slow velocity (~50-60 m/s) and broad bandwidth (300-7000Hz). The 7 to 9 slits within each slit organ are exquisitely sensitive to accelerations of ~1 to >1000 nm amplitude at signal-appropriate peak frequencies of 800-5000 Hz. Importantly, a robust plexus of axo-axonic synaptic connections between these sensory neurons mediates lateral inhibitory interactions that 'protect' the more sensitive slits from saturating responses to the noisy and highly amplitude-variant signals generated by slippage of sand grains when disturbed. The effect of these interactions within and across the slit organ sensory field should be adequate to encode both amplitude and temporal cues known to assess surface location of the target disturbance.

Beyond vibrational sensing, scorpions employ a fascinating array of adaptations for life in hot dry deserts. These include the pectinal chemosensory system for detection of pheromonal signals deposited on sand, sensitivity to polarized light and a resting metabolic rate that rivals that of a tick. Fascinating as they are, further integrative studies of sand scorpions and their dune co-inhabitants should improve our understanding of this fragile and too often abused environment.

## THE VIBRATIONAL ECOLOGY OF INSECT-PLANT INTERACTIONS

**Reginald B COCROFT**

Division of Biological Sciences, University of Missouri, MO, USA



Plant-borne vibrations are the currency of myriad social and ecological interactions in animals: between potential mates and competitors; between parents, offspring and other social partners; among mutualists; and between predators and prey. Because the 'substrates' for these propagating mechanical waves are themselves living organisms, well equipped with mechanosensors, plants are a potential audience for the mechanical vibrations produced by their herbivores and mutualists. Many sources of plant-borne vibration contain information of potential importance to plants, and most have been present throughout the long history of plant-insect interactions: arthropod social behavior, herbivore feeding and locomotion, pollinator activity, airborne sound, and abiotic factors including wind and rain.

I will discuss some of the vibrational interactions studied in my laboratory, including insect communication and herbivory, and I will present results of recent studies of plant responses to insect-generated vibrations. I will also outline some of the efforts in my laboratory to make the methods for studying plant-borne vibrations more accessible and widely available.



## **BEE ELECTRORECEPTION AND THE POTENTIAL ROLE OF COULOMB FORCE IN TERRESTRIAL ARTHROPODS**

**Daniel ROBERT**

School of Biological Sciences, University of Bristol, UK



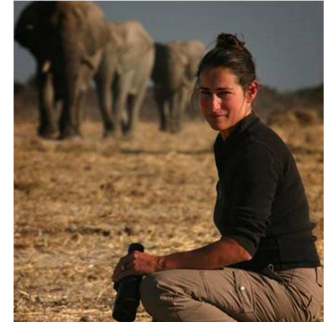
Working together to exchange nutrition for pollination services, bees and flowers interact relying on vision, olfaction, touch, and humidity sensing. Recently, we have discovered that bees can also detect and learn about the weak electric field that arises as they approach a flower. This electric field is generated because flying bees are usually electrically positively charged whilst flowers tend to be negatively charged. A third component contributes to this electric interaction, the atmospheric potential gradient (APG) that is a consequence of the ionization of the atmosphere and the global electric circuit.

I will present our current understanding of this triadic interaction (Bee-flower-APG), but also specifically discuss the role that triboelectrification may play in the sensory ecology of terrestrial arthropods and plants. It will be shown that physical contact and friction between insects and their environment generates charge separation and a Coulomb force. It will be proposed that this triboelectric charging may play a role in the biology, and sensory ecology, of plants and insects. The enticing possibility that many arthropod species, beyond bees, are capable of electroreception will be proposed and discussed.

## THE VIBRATION SENSE IN ELEPHANTS: LESSONS FOR HUMAN HEARING

**Caitlin O'CONNELL-RODWELL**

Department of Otolaryngology, Head & Neck Surgery, Stanford University School of Medicine, CA, USA



The vibration sense is employed by a wide range of taxa as a mechanism of prey detection, predator warning and avoidance, habitat sensing, as well as communication. Elephants generate vibrations used in communication through percussion, using their feet or trunk, or through coupling of their vocalizations to the ground. Elephants may use vibrational cues in a number of contexts to interpret their social and physical environment. Responses to playback studies demonstrate the sophistication of the elephant's ability to detect and respond appropriately to vibrational cues.

Elephants have two possible pathways for the detection and interpretation of vibrations, either through bone conduction to the cochlea, with processing in the auditory cortex, and/or through vibration sensitive mechano-receptors in their feet and trunk that are transmitted to the somatosensory cortex. In addition to a review of our previous published studies in this area, I will present new data on the sensitivity of elephants to bone conduction relative to humans and discuss the implications of these findings for human medicine.

# ORAL PRESENTATIONS





## **BIOTREMOLOGY: PAST, PRESENT AND FUTURE**

**Meta VIRANT-DOBERLET**

Department of Organisms and Ecosystems Research, National Institute of Biology, Ljubljana, Slovenia

Surface-borne mechanical waves provide organisms with information about their environment that is crucial for their survival and reproduction. This information is not limited to intraspecific vibrational communication, but also includes prey detection and predator avoidance, and potentially also about habitat quality. However, due to our own perceptual bias in favour of air-borne sound communication, the fact that the majority of organisms lives in a vibratory world has long been overlooked.

The increased awareness that substrate vibrations are an ancient and widespread form of animal communication, as well as that vibration receptors are ubiquitous in organisms, lead to the establishment of biotremology as a new discipline of study of mechanical communication. More than any other communication modality, vibrational communication is an evolutionary result of an interaction between physical properties of the environment and the animal's anatomy, physiology, as well as behaviour. Taking into account the enormous diversity of organisms relying on vibrational signalling, research in this emerging field offers numerous opportunities to unravel mechanisms that are central to our understanding behaviour in general.

While in the 21<sup>st</sup> century sophisticated recording equipment and computer analysis programmes enabled fast progress in some areas, there are still many basic questions that remain largely neglected, like details of signal production mechanisms and vibration reception in many taxa. Moreover, even for researchers working in the field of biotremology, it is hard to imagine what it is like to live in a vibratory world. We should keep in mind that vibrational communication did not evolve in a sound proof-room, but in a rich and complex natural vibroscape.

## CICADAS USING TWO MECHANISMS AND COMMUNICATION CHANNELS FOR ACOUSTIC AND VIBRATIONAL COMMUNICATION

**Matija GOGALA<sup>1</sup>, Tomi TRILLAR<sup>2</sup>**

<sup>1</sup>Slovenian Academy of Science and Arts, Ljubljana, Slovenia

<sup>2</sup>Slovenian Museum of Natural History, Ljubljana, Slovenia

Cicadas *Cicadatra persica* Kirkaldy, 1909 and a closely related species *Cicadatra* sp. from the Greek island of Milos use for intraspecific communication continuous song produced by tymbals typical for this genus, and phrases comprising short echemes produced by tymbals and following a series of wing clicks, produced most probably by banging tegmina against the abdomen. Other species of this genus like *C. atra* or *C. platyptera* produce single clicks between short echemes during courtship using the same mechanism. There are also cicadas from other genera and families worldwide, producing similar clicks as a part of their songs or as female signals to their mates. Our question was why they use two different mechanisms in acoustic communication.

These clicks, recorded as airborne sound are faint broad band signals. Therefore we wanted to know how these clicks are transmitted as biotremulation signals through the substrate. We used a Bruel & Kjaer 4338 accelerometer with the vibration pick-up preamplifier 2625 for recording vibrational signals in the field. Cicadas *Cicadatra* cf. *persica* were kept in a cage with the branch of *Pistacia terebinthus*. The accelerometer was fixed to the lower end of the branch. We recorded continuous songs and phrases with short echemes and wing clicks as acceleration and/or velocity parameters.

As expected we found that biotremulation signals of wing clicks were very intense low frequency vibrations and tymbal echemes have completely different spectral characteristics with the main energy in higher parts of the spectrum up to 20 kHz.

The explanation of our results is that cicadas of the genus *Cicadatra* use tymbal sounds to communicate through air and wing clicks for biotremulation through substrate, branches of bushes and trees on which they usually feed and sing.

## COMPLEX ARRAYS OF CHORDOTONAL RECEPTORS IN THE FIRST TWO ABDOMINAL SEGMENTS OF FROGHOPPERS, LEAFHOPPERS, AND PLANTHOPPERS

**Peter BRÄUNIG**

Department of Zoology, RWTH Aachen University, d-52074 Aachen, Germany

Staining the peripheral nerves of the thoraco-abdominal synganglion revealed complex arrays of scolopidial organs in the first two abdominal segments of froghoppers (*Philaneus spumarius*), leafhoppers (*Graphocephala fennahi*, *Cicadella viridis*) and planthoppers (*Issus coleoptratus*).

Two scolopidial organs occur on each side of the first abdominal segment, up to four on each side in the second segment. Some of them are large and may contain some 80 sensory neurons. No such organs were found in the other abdominal segments.

This arrangement of sense organs deviates very much from that found in other insects. Most likely the arrays participate in the special communication of these insects where they may function as proprioceptors during the production of vibratory signals, or might serve as sense organs for the reception of conspecific signals.

**VIBRATIONAL SIGNAL PRODUCTION IN *APHRODES MAKAROVI***

**Anka KUHELJ<sup>1</sup>, Aleš ŠKORJANC<sup>2</sup>, Miloš VITTORI<sup>2</sup>, Nada ŽNIDARŠIČ<sup>2</sup>, Hannelore HOCH<sup>3</sup>,  
Andreas WESSEL<sup>3</sup>, Meta VIRANT-DOBERLET<sup>1</sup>**

<sup>1</sup>Department of Organisms and Ecosystems Research, National Institute of Biology, Ljubljana, Slovenia

<sup>2</sup>Biotechnical Faculty, Department of Biology, University of Ljubljana, Ljubljana, Slovenia

<sup>3</sup>Museum für Naturkunde, Leibniz-Institut für Evolutions und Biodiversitätsforschung, Berlin, Germany

Communication between partners is an essential part of reproductive behaviour and, as such, it is subjected to sexual selection. Considerable indirect costs result from high energy expenditure during signalling. We already know quite a lot about leafhoppers' behaviour and their vibrational signals, yet the production of their signals is not clear. We investigated the mechanism of signal production in the leafhopper *Aphrodes makarovi* with focus on: (a) structure of the tymbal, (b) tymbal muscles and (c) mechanism of vibrational signal production.

We combined scanning electron microscopy (SEM) with micro tomography ( $\mu$ CT) to investigate the structure and position of the tymbal and associated muscles. By histological sections and transmission electron microscope (TEM) we looked at the muscle structures. We simultaneously recorded vibrational signals with laser vibrometer and movements of the tymbal membrane during signalling with a high speed camera. To see the mechanics of the tymbal muscle, we recorded electromyograms (EMG) from the main tymbal muscle dvm1.

We identified the tymbal region and main muscles. The tymbal membrane in females is clearly a paired structure with 7 ribs. Pulses in female vibrational signals appear to be associated with bending of both tymbal membranes and they also matched with the EMGs. In males, the tymbal area is more complex and during production of advertisement calls the whole abdomen is vibrating. The timing of muscle action potentials was synchronous for both dvm1. The main tymbal muscle dvm1 is larger in males than in females.



## TRANSITIONS OF VIBRATION-DEPENDENT BEHAVIORAL STATES IN *CAENORHABDITIS ELEGANS* POPULATION THROUGH A 'FIGHT-OR-FLIGHT' RESPONSE CIRCUIT

**Takuma SUGI**

Molecular Neuroscience Research Center, Shiga University of Medical Science, Otsu, Shiga, Japan

Animals transition between opposing behavioral states such as fight and flight, sleep and wakefulness, or aggression and tranquility. However, how much stimulus difference induces the entry, maintenance and exit of behavioral states, and its underlying neural mechanisms, remains unknown.

Here, we show that dominant vibration-dependent behaviors in a *Caenorhabditis elegans* population transition through a bandpass filter circuit. We first found that the *C. elegans* population exhibits bimodal behavioral states, forward acceleration and backward movements, in response to a vibration with frequency less than 210 Hz; whereas, they dominantly show a backward response to vibration with frequency more than 210 Hz, suggesting that a 210 Hz frequency is a bifurcation point of behavioral states. Habituation memory can be formed by a vibration with frequency only more than, but not less, than 210 Hz.

We constructed a mathematical model based on calcium imaging of two parallel sub-circuits, each driving forward and backward responses, indicating that the band-pass filtering in the circuit allows the behavioral state transitions.

Thus, our study proposes a neural circuit model describing experience-based behavioral transitions between 'fight and flight' responses.

## CAN POSTURAL CHANGE IN INSECTS MODIFY VIBRATORY SENSITIVITY? NEW INSIGHTS FROM THE STUDY OF LEG VIBRATION IN CAVE CRICKETS

**Nataša STRITIH PELJHAN<sup>1</sup>, Johannes STRAUSS<sup>2</sup>**

<sup>1</sup>Department of Organisms and Ecosystems Research, National Institute of Biology, Ljubljana, Slovenia

<sup>2</sup>AG Integrative Sensory Physiology, Institute for Animal Physiology, Justus-Liebig-Universität Gießen, Germany

The influence of leg and body mechanics on vibration detection has been studied previously on large arthropods, such as spiders and crabs, and on small insects such as bees and treehoppers. We studied the mechanical leg response to vibration stimuli in the cave cricket *Troglophilus neglectus*, a representative of Orthoptera with relatively long and slender legs that produces vibratory signals by tremulation in mating. We aimed to determine the gain of the vibration input available for the leg vibrosensory organs, which we studied under different conditions of the stimulation set-up.

While varying leg attachment, leg flexion and body posture in the restrained or free-standing animals, we recorded the response to vibration at different points along the leg, using laser-Doppler vibrometry.

The response was the strongest on the tibia, where specialized vibrosensory organs occur. Its peak frequency varied between 200 and 2000 Hz in strong dependence on the level of leg flexion. The muscle tension and/or the load force on the leg seemed to have an important influence, too. As a consequence, the largest difference in the response amplitude to the same stimulus ranged between 20 and 40 dB on the same leg, presumably strongly influencing sensitivity of the receptors to detect these stimuli. The response peak amplitude increased with increasing frequency, suggesting the cave cricket legs as the most suitable for detecting high-frequency vibration. In free-standing animals with the abdomen touching the vibrating substrate, the legs showed a secondary response peak below 150 Hz, induced by body vibration, expected to significantly increase sensitivity of low-frequency vibratory leg receptors.

In light of comparative data, a differential mechanical influence of the legs and the body on the detection of vibratory signals may be considered for different insect and arthropod species, while their ability to influence vibration detection via postural change appears to be general.

## **SOCIAL ONTOGENY IN THE MATING COMMUNICATION SYSTEM OF AN INSECT**

**Camille DESJONQUÈRES, Rafael L RODRÍGUEZ**

Department of Biological sciences, University of Wisconsin-Milwaukee, WI, USA

Elaborate components of language, such as social ontogeny, are being uncovered in a diversity of vertebrate species. Although acoustic communication in insects is usually considered fully innate, more and more species of insects are found to communicate with sound both as adults and nymph. Those lifelong interactions are the basis of social ontogeny of signals in other species. Here we report on a novel form of social ontogeny in the communication system of an insect that uses plant-borne vibrational signals.

Weekly monitoring during the development of treehoppers reared singly revealed that signal repertoires differ between nymphs and adults. Moreover the ontogeny of signal features and signaling activity is sexually dimorphic even in nymphs. We also found evidence that variation in ontogenetic trajectories project onto the adult stage. The signals and mate preferences varied between adults reared in isolation or in groups; however individuals raised in isolation were able to produce species typical signals.

Thus this social ontogeny of signals and mate preferences is novel as it is non-obligate and involves social interactions of individuals of the same stage.

## **RISKY RIPPLES: MULTIPLE SELECTION PRESSURES OPERATING ON A MULTISENSORY SEXUAL DISPLAY**

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Elaborate sexual displays are favoured by sexual selection, but their evolution is often limited by environmental factors that can influence both signallers and receivers. The environmental impact is in particular complex for multimodal communication signals which are received through a variety of sensory modalities. Incorporating secondary signal components into a sexual display may therefore not only increase a signaller's attractiveness to females, but also influence its detection by unwanted receivers, such as rivals and eavesdropping predators. I will demonstrate how the production of additional signal components, namely the call-induced water ripples of male túngara frogs (*Physalaemus pustulosus*) provides cues to rival males as well as predatory bats.

Water ripples are a by-product of calling from a water surface, but function as important secondary component of the frog's sexual display. When added to a male's airborne-acoustic call component, ripples enhance the response of rivals by two-folds. Furthermore, bats are also able to detect a male's call-induced ripples and may use them to locate their prey. Interestingly, when the environment is manipulated, either by mimicking climatic conditions such as wind or rain noise, or biotic conditions such as aquatic vegetation, both signalling behaviour as well as detection by receivers is impacted.

Our data demonstrate that a trait that evolved as a production constraint of a primary signal component can be under strong sexual and natural selection as a secondary component in a multimodal display. The co-option of multiple components in sexual displays can be beneficial to signallers and intended receivers but costs, such as increased predation risk, have to be taken into account when reconstructing the evolution of multimodal communication.

## NOISY NEIGHBOURHOODS ARE A CHALLENGING ENVIRONMENT FOR COURTSHIP

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We studied the effect of two fundamental discoveries regarding vibrational communication of the leafhoppers in the genus *Aphrodes*: that producing vibrational signals is costly and that animals may face significant noise in their communication channel. *A. makarovi* males were put through weekly orientation trials throughout their lives, with the task to find the source of female signals when exposed to vibrational noise on a host plant. We measured their effort and efficiency in different scenarios, *i.e.* control, female reply only, female reply overlapped by male rival signals, continuous duetting, biotic noise from a congeneric species (*A. bicinta* “dragonja”), and anthropogenic noise.

As predicted, males exposed to a continuous playback of male-female duets were the most efficient, switching to a satellite tactic, which enabled them to find the source of female signals without calling themselves. On the other hand, biotic noise severely hampered communication by overlapping parts of the duet, while constant anthropogenic noise represented by a lawnmower recording both caused many males to stop calling and reduced efficiency of active males. Behavioural analysis was supplemented by measuring the resting respiratory rate and reactive oxygen species (ROS) in brain and tymbal muscle tissues, as well as examining the structure of tymbal muscles by transmission electron microscope. By comparing treated and untreated individuals, oxidative damage due to intense activity could be correlated with lifetime calling and searching activity, thus providing additional proof for the causative link between the cost of vibrational signaling and shorter lifespan of very active individuals as demonstrated in earlier studies.

We will present preliminary analysis of physiological parameters showing tentative support for this hypothesis.

## CORRELATION BETWEEN MATING BEHAVIOR AND OVARIOLES DEVELOPMENT IN THE MEADOW SPITTLEBUG *PHILAENUS SPUMARIUS*

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The recent development of innovative applications of biotremology to manipulate the mating behavior of some species of Hemiptera has opened new possibilities for pest control. *Philaenus spumarius* (Hemiptera: Cicadomorpha) is the main vector of *Xylella fastidiosa*, the causal agent of the Olive Quick Decline Syndrome in Southern Italy. To assess whether a behavioral manipulation technique by means of playback of vibrations is feasible for *P. spumarius*, it is necessary to know when females reach their peak of mating receptivity. Females and males of *P. spumarius* exchange vibrational signals during the pair formation process, and the mating duet can induce female acceptance to copulation. On the other hand, female receptivity depends also on its physiological state, thus the development of ovarioles could be a cue to evaluate the availability to mate of females. Such a knowledge would provide significant information on the best time to employ a behavioral manipulation technique.

To evaluate when female acceptance starts to occur, we investigated a possible correlation between female mating behavior and female physiology. Our hypothesis is that the responsiveness of females to the male courtship song (MCRS) may occur only at a certain stage of ovarioles development. We individually tested 20 virgin females every 15 days from the day of eclosion as adults (mid-May) to the end of summer. Vibrational signals were recorded via two laser vibrometers. A female was placed on a plant and, after 15 minutes during which we monitored her calling activity, a playback of a pre-recorded MCRS was played onto the plant (through a mini-shaker) to assess whether they were available to establish a normal duet. The female was removed 1 minute after the end of the playback. Half of the females were dissected and their ovaries were examined under a light microscope. We also tested 15 couples every 15 days to investigate whether the physical presence of the male affected the female behavior.

## **SPECIES DELIMITATION AND RECOGNITION OF ALLIED SPECIES OF PSYLLOIDS (HEMIPTERA: PSYLLOIDEA) BASED ON VIBRATIONAL COMMUNICATION**

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Vibrational communication plays an essential role in mating behavior in Psylloidea. These vibrational signals convey species-specific information and contribute to reproductive isolation among different species. However, the study of signal variation and species recognition among closely related species of psylloids are scarce. The purposes of this study were 1) to examine the species delimitation of allied psylloid species using vibrational behavior; and 2) to understand the role of species recognition in pre-reproductive isolation of psylloids.

Four groups of closely related psylloid species, including three species of *Cacopsylla*, two species of *Trioza*, two species of *Macrohomonotoma*, and five species of *Stenopsylla* were studied for comparison of vibrational signals. Within these, two species of *Cacopsylla* and two species of *Trioza* were used in species recognition via cross experiments.

In the results, species within each group were distinguished based on vibrational characteristics including temporal and spectral characteristics. It indicates that vibrational characteristics are invaluable to distinguish closely related species of psylloids. Consequently, the results of species recognition suggest that psylloids recognize conspecific signals to facilitate mating and prevent unnecessary hybridization via refusing of heterospecific signals.

This study provides evidences that vibrational signals of psylloids work as the systematic characteristics in delimitation of closely related species and the significant role in reproductive isolation of psylloids.

## MATING BEHAVIOR OF *ORIENTUS ISHIDAE* (HEMIPTERA: CICADELLIDAE)

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We present the first analysis of the mating behavior of the Mosaic Leafhopper *Orientus ishidae* (Matsumura) (Hemiptera: Cicadellidae), an invasive Asian species shown to be associated with 16SrV phytoplasmas, related to the grapevine disease “Flavescence dorée” (FD). *O. ishidae* vector capacity for FD was confirmed in different studies and even though grapevines are not its main host, its presence has been confirmed inside vineyards. Although the life cycle of this insect is known, no information about its mating behavior was available. As common for the family Cicadellidae, we expected substrate-borne vibrational signals to play an important role in pair formation, location of the females and copulation.

Calls by single males and females, male-female communication, and behavior of rival males exposed to an ongoing courtship were recorded using a laser vibrometer.

We found that males exhibit “call and fly” behavior, producing a loud search signal that, if answered by the female, develops into a “pair recognition sequence” where short male and female calls alternate until the female stops answering, the moment in which the “courtship phrase” starts. During the courtship, males emit an elaborate phrase composed of different ratios of harmonic calls, “taps”, abdomen hits, “clicks” and “scrapes”. When the female starts answering courtship calls, ratios of the elements within the courtship phrase change and new elements are added. Towards the end of the courtship female signals overlap with the male’s, followed by the end of the courtship with the male approaching the female with fast wings movements alternated with two short calls, and, finally, copulation. When additional males were added to the pair, the rivals produced a loud signal that masked the female one and confused the courting male as he tried to mate with the rival male.

While a basic study, we hope our results may pave the way for development of innovative techniques to control this potential pest.



## THE DIFFERENCES IN THE VIBRATIONAL SIGNALS BETWEEN MALE MASON BEES IN EUROPE

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Solitary red mason bees, *Osmia bicornis*, are known to use vibrations in female choice, where the females not only evaluate a male's fitness through their vibrational signal but also use them to distinguish between males of different origins. This was shown conclusively via bioassays, in which females from Germany rejected English males unless they were imbued with an artificial German signal and vice versa. However, an investigation into which parameters of the signal might differ between species and populations has been lacking so far.

We therefore recorded vibrational signals of males of *O. bicornis* from Germany, England and Denmark as well as the closely related species *O. cornuta* using a laser vibrometer.

Our results clearly showed significant differences in the signal between *O. bicornis* and *O. cornuta* males regarding frequency, modulation range and average pulse duration, meaning that there definitely is a species difference. Moreover, *O. bicornis* populations from Germany and Denmark surprisingly also differed significantly in frequency and modulation range, with England lying in between the two. From the bioassays in our previous study we would have expected Germany and England to differ the most, and Denmark lying in between. We conclude that the females probably use another signal parameter that we have not evaluated yet in order to choose their mates.

From our knowledge about the system to date we believe that we are looking at the first steps of speciation through female choice and female preference in this system, which could help us with new insights into evolutionary processes in the future.

## ADULT-LARVAE VIBRATIONAL COMMUNICATION IN PAPER WASPS: THE ROLE OF ABDOMINAL WAGGING IN *POLISTES DOMINULA*

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Communication through substrate-borne vibrations is widespread among social insects and regulates fundamental aspects of social life. Females of paper wasp, *Polistes dominula*, by performing an abdominal oscillatory behavior known as “abdominal wagging” (AW), are able to produce vibrations which propagate through the nest. Because it is widely recognized that AW is strictly associated with the presence of larvae in the comb, it has been suggested that AW might represent an adult-brood vibrational signal. Indeed, substrate-borne vibrations would have short-term effects related to food and trophallactic exchanges between adult and larvae. According to this, two opposite hypotheses have been proposed: a) vibrations could prepare larvae to receive food by decreasing the amount of salivary secretion, or b) they could be used by adult to stimulate the release of nutrient larval saliva.

Here, we used an electro-magnetic shaker to play back the *P. dominula* vibrations on nests containing larvae. We assessed, for the first time, the short-term effect of abdominal wagging on larval behavior by recording larval response and by measuring the amount of saliva released immediately after abdominal wagging playback.

Our results show that larvae a) are able to perceive the substrateborne vibrations and b) react to abdominal wagging by increasing the movement of their body, likely to attract the attention of adult females during feeding inspection. Nevertheless, vibrations neither increase nor decrease the release of larval saliva.

Although our results support the alleged role of vibrations in adult–larvae communications, they do not support the hypothesis about salivary release modulation.

## EXCITATION SIGNAL EXTRACTION OF ANT WALKING PATTERN UNDER THE INFLUENCE OF NOISE USING A BIOMECHANICAL BIPEDAL MATHEMATICAL MODEL

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It has been shown in recent years that vibration and acoustics play an important role in insect communication and cue detection. It is well known that ants use acoustic signals detected by sensory organs within their legs. Termites communicate mainly using vibrations and our recent research shows they are able to detect their predators solely on vibrational cues of their footsteps. As the vibration response of a substrate to walking ants depends strongly on its mechanical properties and a biological source might adapt and change its behaviour for different substrates and preys, the excitation signal will be of more interest than merely the substrate response.

Here we test the extraction of the excitation signal from the substrate response by employing a bipedal model of walking or running ants within a Tikhonov regularisation procedure. The vibration response is contaminated with noise and the effects of spectral, wavelet and geometric filters on the extraction procedure are tested.

Our results indicate that the geometric filter algorithms provides the best results while spectral filters, even though the most robust, do not preserve the dynamics and are also the least accurate which leads to wrong excitation signal estimations.

## EVOLUTION OF VIBROACOUSTIC SIGNALS IN MYRMICINAE ANTS

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In social insects effective communication is fundamental to live in complex hierarchical societies. In ants, the primary method of communication involves chemical and, to a lesser extent, tactile cues but also vibroacoustic signals play an important role within ant societies. Although acoustic cues have generally been regarded as ‘weakly developed’ in ants, they are also the least studied communicational signals. Stridulation is the most widespread way of vibroacoustic emission in ants, involving the rubbing of a ‘plectrum’ against a ‘file’ (pars stridens) located on opposite segments of the anterior abdomen, which has evolved independently in multiple ant lineages within the subfamilies Nothomyrmecinae, Ponerinae, Ectatomminae, Pseudomyrmecinae, and Myrmicinae.

We investigated the extent of the variation of vibroacoustic signals of 25 European ant species belonging to the subfamily Myrmicinae and the factors promoting the evolution of these signals. Recordings of vibroacoustic emissions were collected from individuals of each caste from three colonies per species and temporal and spectral features of the signals were then measured. The vibroacoustic patterns obtained from the studied ant species were then compared to composite evolutionary trees based on species “DNA barcodes” to verify the hypothesis that signals have evolved across species and genera following phylogenetic trajectories. In addition, we verified whether nest substrates or other environmental factors may have driven vibroacoustic signal evolution in ants.

The preliminary results of the study will be presented and discussed in a wide evolutionary context. This work has been carried out within the project VIBRANT (No.2016/23/P/NZ8/04254) supported by the Polish National Science Center under POLONEZ programme which received funding from the European Union’s Horizon 2020 research and innovation programme under the Marie Skłodowska-Curie grant agreement No.665778.

## ANTS RESPOND TO TREE VIBRATIONS CAUSED BY BROWSER CHEWING

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Many plants engage in mutualism with ants, where plants provide food and shelter in exchange for protection against herbivores. The mutualism between African *Crematogaster* ants and acacias is particularly impressive, as ants can deter large mammalian herbivores such as elephants. Herbivores can have devastating effects on trees by browsing, breaking tree branches, stripping bark, and pushing over entire trees. However, mutualistic ants substantially reduce the amount of damage. The ants become alarmed when the plant takes some sort of damage. They immediately emerge from their plant shelter and aggressively defend the plant. For an efficient protection of the tree, ants need to react united and rapidly when the tree is under attack.

Here we show that the acacia ant *Crematogaster mimosae* defends its host tree by exploiting plant-borne vibrations caused by browsers feeding on the tree. Ants discriminate browser-induced vibrations from those induced by wind, become alarmed and patrol on the branches. Vibrations induced by browsers propagate through the whole acacia, reaching branch tips on the other side of the tree with amplitudes high enough to be sensed by ants. Furthermore, the ants make use of directional vibration sensing to orientate to the attacked part of the tree and fight back the attacker.

## TINY ANTS, WHISTLING ACACIAS AND DEVASTATING ELEPHANTS

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The common name of the whistling thorn acacia (*Acacia zanzibarica*) originates from the whistling noise that is audible if it is windy. The whistling sound is linked to the presence of ants (*Crematogaster* spp.) that defend the tree against mammalian browsers. It is emitted when the wind strikes the tree's bulbous thorns in which ants have made holes. The typical whistling sounds characterizing acacia trees could be an information source to mammalian browsers: a whistling tree is protected by ants and a strong defense must be expected. The whistling has a good potential of shortening the time needed to teach browsers about the defense. Mammalian browsers, such as elephants, can have a devastating effect on the survival of trees and consequently on the survival of ants. An auditory warning signal could add to the visual clarity of the thorns and to the aggressive attacks by *Crematogaster* ants.

We investigated various aspects of the acacia's whistling in a wind channel and in the field. Here we provide strong evidence that the whistling sound is a warning signal addressed to mammalian browsers.

## TRAPPING OF MALE AND FEMALE ASIAN CITRUS PSYLLIDS EXPOSED TO A 6-H SERIES OF SYNTHETIC VIBRATIONAL COMMUNICATION CALLS

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Asian citrus psyllids (ACP) communicate on citrus tree stems and leaves by buzzing their wings in vibrational duets. Typically, a duet begins with a male call, after which a female replies within 0.3-0.5 s, helping to direct the male's navigation along the branch to her location. It has been demonstrated that mimics of female replies that are broadcast immediately after each male call attract males to a trap and disrupt mating on small citrus trees in tests conducted over 1-h periods. Other researchers also have demonstrated that mating in other hemipteran species which communicate with vibrations can be disrupted by continuous playback of female calls over 8-h periods.

An experiment was conducted to assess whether the percentages of trapped males decline when female reply mimics are broadcast in continuous loops rather than being timed precisely in relation to ACP male calls. Males and females were released into a cage with a citrus tree on which a yellow sticky trap was hung to collect flying ACP and a small electrocutor device was attached to the tree to collect walking ACP. A piezoelectric buzzer was attached to the tree, next to the buzzer. The buzzer broadcast synthetic female reply vibrations in a continuous loop for 6-h periods in each test.

The numbers of males captured per test by the electrocutor device was significantly lower than numbers of females per test. In contrast, the numbers of males per test flying to the sticky trap was not significantly different from the numbers of females per test. This suggests that male searching along the branches might be inhibited by the continuous loop of vibration playbacks, or that the females are attracted to the vibration source.

Additional bioassays are in progress to examine male and female behaviors associated with the broadcast of a continuous loop of vibration playbacks.

## ACOUSTIC COMMUNICATION AND MATING BEHAVIOUR IN THE TOMATO POTATO PSYLLID, *BACTERICERA COCKERELLI* (SULC)

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The tomato potato psyllid (*Bactericera cockerelli*, TPP) is a pest on plants in the economically important family Solanaceae and transmits a pathogenic liberibacter (*Candidatus Liberibacter solanacearum*, CLso) which putatively causes Zebra chip disease in potatoes. The tomato potato psyllid was discovered in New Zealand in 2006 and was widespread throughout the country by 2009. Observations of the insect in the laboratory indicated that sound was potentially an important communication channel for this insect. To test this we used a laser vibrometer to record substrate vibrations made by male TPP. These provided a basis to develop an algorithm for an Arduino clone microprocessor which produced a vibrational pattern approximating the male TPP call.

The synthetic TPP call was transmitted through a capsicum plant using a small speaker to psyllids confined on a capsicum leaf mounted on a stage. Video and sound recordings were captured for an hour before during and after call transmission with a web camera and a laser vibrometer, recording insect movement and leaf surface vibrations.

Analysis of the video data demonstrated a decrease in male movement for the hour after call transmission ( $p=0.02$ ), and a significant increase in female movement in the hour after call transmission ( $p=0.02$ ). We propose a male call - fly behavioural strategy to explain our observations, with the female response call stimulating male searching and mating. Trials are needed with a synthetic female call with the long-term goal of developing a "lure and kill" system for males.



## VIBRATION SENSITIVITY IN A CERAMBYCID PEST AND ITS POTENTIAL FOR PEST MANAGEMENT

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Many insects exhibit vibration sensitivities as substrate-borne vibrations carry information about approaching predators and conspecifics. Since vibrations induce various insect behaviors, vibration sensitivities can potentially be used for pest management to reduce the damage caused by pest insects to plants. Previously, we have demonstrated that the leg chordotonal organs detect low-frequency vibrations in a cerambycid pest, *Monochamus alternatus*, the vector of the lethal pine wilt disease.

In this study, we investigated behavioral manipulations by artificial vibrations and their potential for pest management using *M. alternatus*.

The beetles exhibited startle responses, such as small bodily movements and freezing, in response to vibrations <1 kHz. Based on our findings of vibration sensitivities in the cerambycid beetles, we are currently developing a pest control method using vibrations that can be used as an environment-friendly alternative to pesticides. We demonstrated that a prototype of a vibration exciter generated vibrations with large amplitudes intermittently on a pine tree to suppress various behaviors of *M. alternatus*. Thus, vibrations can potentially be applied for the management of other pests exhibiting vibration sensitivities.

## VIBRATIONAL SIGNALS AS SEMIOPHYSICALS: A COMPARISON BETWEEN PHEROMONAL AND VIBRATIONAL MATING DISRUPTION

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Until a few years ago, the concept of mating disruption had been exclusively associated with the use of pheromones to reduce population density of insect pests. Since the early 2000s, a novel approach has been proposed to the scientific community: vibrational mating disruption (VMD). The novelty is the use of disturbance vibrations to disrupt the mating behavior of insect pests that communicate by means of substrate borne vibrations. This research falls within the new field of biotremology and it brought the VMD from a theoretical concept to practical open field experimentation: in 2017, VMD was applied in an organic vineyard in Northern Italy to control leafhopper pests' population density. In 2018 other two vibrational vineyards have been established.

These achievements gave us the opportunity to report the state of the field for the method, to discuss the ongoing research and to make a comparison between pheromone mating disruption (PMD) and VMD. We discuss VMD characteristics and we provide a benchmark, using as reference the traditional PMD to discuss similarities and differences. We analyze advantages and disadvantages of applying VMD to commercial crops and we introduce the novel term of "semiophysicals" for vibrational signals to underline their similarity, in terms of functions, with "semiochemicals".

The field VMD experiments conducted starting from 2017 and 2018 will require a long observation period, during which we will work on technological and methodological improvements and perform constant monitoring of insects and plants. In this regard, the half century of experience with PMD could be an essential source of information to boost VMD advancement.

## **INSECT PHONOLOGY: COMBINATORIAL SIGNAL PROCESSING IN *ENCHENOPA* TREEHOPPERS (HEMIPTERA: MEMBRACIDAE)**

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Human language is combinatorial: phonemes are grouped into syllables, syllables into words, words into phrases, and so on. Rules regarding acceptable signal element combinations (phonology) are present to different degrees in some mammals and birds, besides humans. We tested the phonology hypothesis in a vibrational insect against competing alternatives of non-combinatorial sequential processing (whereby early signal portions play a stronger role in acceptability) and non-sequential processing.

We worked with *Enchenopa* treehoppers, whose signals consist of a whine (W) followed by pulses (P). The phonology hypothesis predicts females will prefer natural-order (WP) over reverse-order signals (PW). The sequential hypothesis predicts females will prefer natural WP signals over signals with modified beginnings (PWP). The non-sequential hypothesis predicts no preference according to signal element order (WP vs PW).

*Enchenopa* females preferred natural-order signals regardless of the beginning element (WP or PWP) and discriminated against reverse-order signals (PW). The finding of phonology in insects broadens our understanding of the cognitive toolkit afforded by small brains and suggests an explanation for how small animals process complex signals.

## BIOTREMOLOGY IN HEELWALKERS: POPULATION DIFFERENTIATION, INFORMATION CONTENT AND FEMALE CHOICE

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Mantophasmatodea was first described in 2002. Species-specific vibrational communication, restriction to selected biomes, and limited dispersal abilities make these flightless insects an exciting taxon in which to study speciation and adaptive divergence. The morphologically cryptic *Karoophasma biedouwense* and *K. botterkloofense* were studied to infer the drivers of population differentiation and investigate the role of vibrational communication signals.

We took a multidisciplinary approach incorporating a fine-scale population genetic study (17 microsatellite loci), vibrational recordings, and biometric data of 339 individuals from 30 sites in South Africa. Behavioural experiments explored if males convey their quality to females via their signals, as well as female responsiveness to them.

Our results indicate that some population-level differences (e.g. morphology) are not driven by evolutionary forces but rather by e.g. food availability. Our genetic data show that both species are highly geographically structured, with limited inter-population genetic exchange, even over short distances. Male vibratory signals differed slightly between species, were stable irrespective of experimental feeding regime or age, but varied with male condition. Females, in contrast, responded to a variety of artificial male signals, far beyond the range of the natural patterns. However, they rejected calls having too short pauses or pulse-train durations as well as unnaturally long or short pulse rates.

Our data suggest that diversification was likely driven by vicariance caused by climatic changes in southern Africa, which also gave rise to the megadiverse vegetation types in the Cape Floristic Region. Due to restricted dispersal abilities, isolated lineages evolved in allopatry and achieved complete speciation in a short time frame. A combination of male vibrational signal parameters appears to contain the species recognition coding reinforcing species boundaries in Mantophasmatodea.

## **TROPICAL VIBES: VIBRATION USE AND DETECTION BY THE HERMIT CRAB *COENOBITA COMPRESSUS***

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Crustaceans are known to produce acoustic signals in the air and within water, however much less is understood about their capabilities to use and detect vibrations within substrates. In the terrestrial crabs, research has focussed upon those species easily accessible for research such as fiddler and ghost crabs (*Uca* and *Ocypode*), which produce vibroacoustic signals during key social interactions. The land hermit crabs of the genus *Coenobita*, which occupy a similar environment, stridulate within their shell causing a vibration which can be felt and heard. The role of these vibrations and other environmental vibroacoustic cues in their day to day activities are relatively unknown.

Here we provide an overview of experiments which we have recently conducted with *Coenobita compressus* at our field site on the Osa peninsula, Costa Rica. These range from fine-scale manipulations, medium-scale playbacks in the sand to large-scale measurements of the vibrational environment (the vibroscape). We assess the role of vibration within the context of essential activities at each scale, such as resource investigation, the formation of social groupings and as a key environmental cue within the landscape.

## THE USE OF BIMODAL ACOUSTIC SIGNALS BY RED-EYED TREEFROGS, *AGALYCHNIS CALLIDRYAS*

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Airborne calls and songs produced by animals have long been a focus of research, and the study of these airborne signals has shaped our understanding of the behavioral ecology and evolution of these species. Individuals signaling from, or nearby, vegetation or the ground inevitably also excite vibrations within those substrates. This raises the possibility that air- and substrate-borne components of calls can function together as bimodal acoustic signals. While this has been demonstrated in a handful of species, it has, in general, received very little research attention, particularly among vertebrates.

I conducted bimodal playback experiments to test whether the well-known sexual advertisement call of red-eyed treefrogs functions as a bimodal signal with receivers attending to both airborne sound and substrate vibration components.

My results indicate that both during female mate choice and during agonistic interactions between males the presence of the substrate vibration component substantially alters receiver responses to this signal. In two-choice preference trials, females choose calls with air- and substrate-borne components more than twice as often as the air-borne component played alone. When presented with both airborne sound and substrate vibration signal components, males respond more aggressively and with a greater variety of aggressive behaviors.

These results indicate that red-eyed treefrogs, previously assumed to be producing purely airborne calls, actually communicate with bimodal, sound and vibration, signals. As selection on either component of bimodal acoustic signals will shape the evolutionary trajectory of the signal as a whole, it may be wise to reevaluate even well studied communication systems in other taxa to determine whether receivers attend to previously unstudied components of acoustic calls.

## MATING BEHAVIOUR IN SPOTTED WING SPECIES SUGGESTS A MODULAR MODEL OF VIBRATORY COMMUNICATION IN *DROSOPHILA*

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Like in many other insects, *Drosophila* males produce acoustic signals to facilitate female's mating acceptance: some of these signals are clearly substrate-borne and are produced by abdominal vibrations. Compared to *D. melanogaster*, individuals from the *suzukii* subgroup are further characterised by the emission of a specific signal, called "toot", which is characterized by harmonic frequency structure and is associated with wing movements. We tested whether the "toot" signal is a specific characteristic of the *D. suzukii* group and if it co-evolved with the presence of spots on wings, by studying the courtship strategy and associated acoustic signals in spotted (*D. suzukii*, *D. subpulchrella*, *D. biarmipes*, *D. elegans*) and unspotted wings species (*D. takahashi*, *D. melanogaster*, *D. ananassae*).

We show that only spotted wings species, including *D. elegans* which does not belong to the *suzukii* subgroup, are capable of producing a toot or toot-like signal: with the exception of *D. biarmipes*, spotted wing males combine wing exposure with sound emission so that visual and acoustic cues work together to increase female acceptance. We also show that the "quivering" signal is a recent acquisition of the melanogaster group, while other type of signals such as "sine" and "pulsed" are scattered along the *Drosophila* phylogeny.

Our results advance our understanding of *Drosophila* signalling and make us hypothesize that some signals including the "toot" might be ancient characters that have been secondary lost in some lineages; alternatively these signals could be peculiar cases of convergent evolution. We propose a modular model of *Drosophila* vibratory signal based on the recruitment of a species-specific set of signals taken from a collection of at least 5 main signals.

## VIBRATION LANDSCAPES: THE ROLE OF MATERIALS IN INFORMATION TRANSFER

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Vibrations that propagate through materials and along surfaces are a ubiquitous and important form of biological information. I argue that Biotremology is an intrinsically interdisciplinary field, where physical sciences approaches can be used to answer important biological questions on the mechanisms and constraints acting on vibrational information transfer. Substrates form a platform over which information can be transmitted, what I call a 'vibration landscape'.

Often heterogeneous with complex geometries, substrates impose constraints on propagating information, including distortion, filtering and energy loss. Here I present two case studies that probe how substrate properties affect information transfer. Whereas spiders make and adjust their substrate to control vibration propagation, other organisms such as elephants have a degree of choice regarding which substrates are used for information transfer. The degree of choice or control an organism has over their vibration landscape are two strategies that organisms can use to mitigate physical constraints acting on vibrational information transfer.



## **BIOACOUSTICS AND ECOACOUSTICS FOR THE ANALYSIS OF THE SOUNDSCAPE**

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Bioacoustics and ecoacoustics are emerging disciplines in biodiversity science and biodiversity conservation: from the recognition and monitoring of individual species through to soundscape analysis and description, they provide new insights and approaches for science, conservation, and education.

Ecoacoustics is a recently defined interdisciplinary science, derived from bioacoustics and ecology that investigates natural and anthropogenic sounds and their relationship with the environment over a wide range of study scales, both spatial and temporal, at individual, community and population level. Ecoacoustics operates in all types of terrestrial and aquatic (freshwater and marine) ecosystems, extending the scopes of acoustics and bioacoustics and providing tools for the monitoring and the management of the environment. Ecoacoustics also concerns the study of possible acoustic interactions with animals in a wide range of frequencies, from infrasounds to ultrasounds. Current research trends will be presented with the support of sound samples.

**“BAD VIBES”: ANTHROPOGENIC VIBRATIONS AFFECT ANURAN CALLING ACTIVITY****Valentina ZAFFARONI CAORSI<sup>1</sup>, Raíssa FURTADO<sup>2</sup>, Vinicius GUERRA<sup>3</sup>, Diego LLUSIA<sup>4</sup>, Livia R MIRON<sup>5</sup>, Márcio BORGES-MARTINS<sup>1</sup>, Camila BOTH<sup>6</sup>, Peter M. NARINS<sup>7</sup>, Rafael MÁRQUEZ<sup>8</sup>**<sup>1</sup>Dep. Zoologia, Inst. de Biociências, Universidade Federal do Rio Grande do Sul (UFRGS), Porto Alegre, RS, Brazil<sup>2</sup>Programa de Pós-Graduação em Ecologia, UFRGS, Porto Alegre, RS, Brazil<sup>3</sup>Laboratório de Herpetologia e Comportamento Animal, Universidade Federal de Goiás, Goiânia, GO, Brazil<sup>4</sup>Terrestrial Ecology Group, Departamento de Ecología, Universidad Autónoma de Madrid, Madrid, Spain<sup>5</sup>Laboratório de Herpetologia, Universidade Federal de Santa Maria (UFSM), Santa Maria, RS, Brazil<sup>6</sup>Programa de Pós-Graduação em Biodiversidade Animal, UFSM, Santa Maria, RS, Brazil<sup>7</sup>Dept. Integrative Biology & Physiology, and Ecology & Evolutionary Biology, University of California LA, CA, USA<sup>8</sup>Dept. de Biodiversidad y Biología Evolutiva, Museo Nacional de Ciencias Naturales-CSIC, Madrid, Spain

Anthropogenic disturbance has been singled out as one of the major causes of the world biodiversity crisis. In particular, noise pollution is a potentially underestimated threat, expected to increase with urban expansion. Anthropogenic activity also produces substrate vibrations, but their possible impact on animal communication is poorly understood. Amphibians are the terrestrial vertebrates most sensitive to vibrations and are widely used for bioacoustics studies, which make them a suitable model for investigating this subject. Using playback tests, we assessed how vibrations produced by two sources of anthropogenic activity (road traffic and wind farms) affect anuran calling activity. Experiments were conducted in June 2017 in Somiedo Natural Park, Spain, with the midwife toad, *Alytes obstetricans*. Traffic and wind farm vibrations were recorded, and synthetic copies were generated digitally prior to the experiment. In their natural habitat, 2-minute recordings of each seismic source were randomly presented to focal toads: (a) traffic, (b) wind farms (c) synthetic traffic, (d) synthetic wind farm, and (e) silence as a control. Playback vibrations were generated with a tactile sound transducer buried 5-10 cm below ground. We analyzed three parameters of the toad advertisement calls: call rate, call duration and dominant frequency. We used general linear mixed-effects models to test the relationship between call parameters and the vibrational stimuli. Our results showed a negative effect of both anthropogenic vibrations on the call rate of *A. obstetricans*, while call duration and frequency remained stable. Furthermore, call rate was more affected by original traffic and wind farm recording than by the synthetic sounds. Since anurans use calls to defend territories and attract reproductive partners, our study suggests that anthropogenically derived substrate-borne vibrations could reduce individual reproductive success and thus merits further investigation.

## EFFECT OF WIND ON TREMULATORY SIGNALING OF A NEOTROPICAL KATYDID (*COPIPHORA BREVIROSTRIS*)

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Some Neotropical katydids are known to use complex, species-specific tremulations during courtship and/or male-male interactions. These tremulations, which complement their acoustic calls, are thought to have evolved as a response to strong predation pressure from eavesdropping foliage-gleaning bats. Tremulatory signals have a generally low frequency range, which places katydids out of a bat's reception radar. However, low frequency signals can easily be masked by noise in their natural environment. The Neotropics like most natural environments is a noisy place, especially during the rainy season, which brings along substantial wind and rain. Wind is considered to be the major source of environmental noise for invertebrates that communicate using plant-borne vibrations. This type of noise is not only acoustic, but also vibratory, potentially affecting the production, transmission and perception of katydid tremulatory signals.

In this study we look at the effects of wind on the production and transmission of tremulatory signals in *Copiphora brevirostris*. We exposed pairs to wind and recorded their tremulations overnight. We expect pairs that were exposed to wind to tremulate less than those in the absence of noise, a mechanism commonly employed by other animals to overcome noise interference. To determine how the transmission of their signals through the substrate (i.e., a plant where *C. brevirostris* is usually found) is affected by wind, we did a vibratory transmission experiment playing back tones encompassing the frequency range of their signals, and we exposed the plant to a wind treatment during the playback. We expect lower frequencies, characteristic of katydid tremulatory signals, to propagate further than higher frequencies. Masking by wind noise is expected to be more severe for vibrations recorded further from the vibratory source. From the perspective of a female searching for a male on a windy night, this would pose a problem.

## IS IT TIME FOR ECOTREMOLOGY?

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Adapting a definition of soundscape, vibroscape is composed from substrate-borne vibrations originating from biological, geophysical and anthropogenic sources present in the environment. Anthropogenic vibrations are produced by human activity, while geophysical vibrations result from natural abiotic sources. Biological vibrations result from activity of living organisms – either from intraspecific communication or as a by-product of other activity and include also vibrational component of air-borne sounds present in the environment.

While soundscape ecology and ecoacoustics are already recognized as interdisciplinary research fields, studies targeted specifically to characterize natural vibroscape are still lacking. In our ongoing study we used laser vibrometers to characterize vibroscape in a hay meadow. Regular recordings were carried out from May till October and included shorter 4 hour periods, as well as 7-day 24h recordings. In the analysis we focused on the biological component of the vibroscape and we assigned vibrational signals to vibrational taxonomic units (VTUs).

In total, we identified more than 60 different VTUs, from which 11 were assigned to known taxa. Results revealed seasonal changes in vibroscape composition and complexity, reaching its highest abundance (total number of VTUs per time) in beginning of July, and its peak richness (number of different VTUs per time) in the end of July. Changes throughout the day were even more pronounced - almost no activity was present during the night and in early morning, while the highest vibrational activity was observed in the afternoon. Vibrational signals were often overlapped in time and frequency domain. Our results show a complex and dynamic vibroscape and we can start not only asking questions about interactions shaping the evolution of vibrational communication, but also how can vibroscape help us to understand ecological processes and ecosystem dynamics, thus extending biotremology to ecotremology.

# POSTERS / FLASH TALKS





## NEURONAL REGRESSION OF CHORDOTONAL VIBRORECEPTOR ORGANS IN THE CAVE CRICKET *DOLICHOPODA ARANEIFORMIS* (ORTHOPTERA: RHAPHIDOPHORIDAE)

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The efficacy of vibrational signals and cues strongly depends on the transmission medium. In cave habitats, rock substrates limit the efficiency of substrate-borne vibrations. For this reason, vibrational signaling can be expected to be severely limited in troglobitic arthropods. For cave arthropods, the sensory environment is often drastically altered, and they show reduction in eye size and pigmentation and the elaboration of touch receptors and chemosensory organs. So far, internal mechanosensory organs including chordotonal sensilla detecting substrate vibrations have rarely been studied from species living in caves. An important vibration receptor system in Orthoptera is the subgenual organ complex in the proximal tibia of all legs. In Orthoptera, this sensory complex contains two to four different chordotonal organs.

Here, we document the neuroanatomy of the subgenual organ complex in the troglobitic cave cricket, *Dolichopoda araneiformis*. By using axonal tracing, we document the neuroanatomy to study (a) the presence of different sensory organs, (b) the numbers of sensory organ sensilla, and (c) the innervation pattern of the organs.

The findings are compared to the troglophilic cave cricket *Troglophilus neglectus* to identify sensory adaptations or reduction in the troglobitic species. In *D. araneiformis*, the subgenual organ complex consists of three chordotonal organs (subgenual organ, intermediate organ, and accessory organ) with a similar innervation pattern to other Orthoptera, including *Troglophilus*. Thus, the overall structure of the subgenual organ complex is conserved in cave crickets. However, the sensillum numbers in the subgenual organ and the accessory organ are about half of those in the organs in *Troglophilus*, indicating regression of sensory elements in two vibrosensitive organs. This reduction can be explained by a lower requirement to detect conspecific or predator vibrations in the cave habitat due to decreased transmission efficacy.

## INVESTIGATION OF THE VIBRATORY INTERNEURONS IN THE VENTRAL NERVE CORD OF *HALYOMORPHA HALYS*: MORPHOLOGY AND PHYSIOLOGY

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The invasive Brown Marmorated Stink Bug, *Halyomorpha halys* (Stål), is a notorious agricultural and household pest. It relies on substrate-borne vibrations during courtship for mate finding and recognition. Here, we tackled the neuronal mechanisms involved in vibratory communication of *H. halys* to increase our understanding of the mating process of this invasive pest, needed to develop reliable behavioral manipulation techniques.

Adult *H. halys* of both sexes from the North of Italy were used. The animals were fixed on a holder dorsal side-up, central ganglion was exposed and the leg tarsi were attached with wax on the mini-shaker as the source of vibratory stimuli. Sine-wave pulses of frequencies ranging from 30–2000 Hz and intensities from 0.001–7 m/s<sup>2</sup> were used for stimulation, and glass electrodes of resistance between 90–130 MΩ were used for registering neuronal activity. Following physiological characterization, the recorded neuron was filled iontophoretically with Lucifer Yellow dye. The ganglion was excised, fixed, dehydrated and cleared in methyl salicylate. The neurons were analyzed morphologically using fluorescent microscopy.

So far, we physiologically and morphologically characterized two neuron types, labeled CG-AC-1 and CG-AC-2, with the soma in the cortex between the 2nd and 3rd thoracic neuromere and a soma-contralateral ascending axon. In the supra-threshold range, these neurons are tuned to 50 and 200 Hz, respectively. Their thresholds, tested at 80 and 160 Hz, lie at 0.18 and 0.32 m/s<sup>2</sup>, and at 0.032 and 0.018 m/s<sup>2</sup>, respectively. Here, the responses of both neurons increase throughout the tested intensity range (40–60 dB). For CG-AC-1, the shortest response latency (12.5 ms) suggests a direct synaptic connection to receptors. At 50–100 Hz of high intensities, CG-AC-2 receives a delayed inhibition. Both neurons have clear homologues of nearly the same response properties identified in the green stink bug, *Nezara viridula*.



## VIBRATIONAL BEHAVIOR OF PSYLLOIDS (HEMIPTERA: PSYLLOIDEA): FUNCTIONAL MORPHOLOGY AND MECHANISMS

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Vibrational behavior of psylloids has been described for more decades: however, the exact mechanism remains unclear. Several speculations of signal-producing mechanisms of psylloids have been proposed, which have not been tested thus far. The aim of this study is to determine the specific signal-producing structure and mechanisms of the psylloids. We raised six hypotheses of signal-producing mechanisms according to the previous studies that include wing-beating, wing-wing rubbing, wing-thorax rubbing, wing-leg rubbing, leg-abdomen rubbing, and axillary sclerite rubbing.

Through cutting off possible signal producing structures and observing wing-beat frequency with a high-speed camera, the six hypotheses were tested. Extensive experiments were implemented on the species *Macrohomotoma gladiata* Kuwayama, while other species belonging to different families, i.e., *Trioza sozanica* (Boselli), *Mesohomotama camphorae* Kuwayama, *Cacopsylla oluanpiensis* Yang, and *Cacopsylla tobirae* Miyatake were involved to examine the prevalence of signal-producing mechanisms in Psylloidea. Further, scanning electron microscopy (SEM) was used to observe possible rubbing structures.

The results from the high speed camera showed that wing-beating frequency did not match the dominant frequency of vibrational signals, resulting in the rejection of the wing-beating hypothesis. As to cutting off experiments, the axillary sclerite-rubbing hypothesis is accepted and wing-thorax rubbing hypothesis is supported partially, while others are rejected. The SEM showed that the axillary cord on the mesothorax possesses a rough surface and the secondary axillary sclerite bears many protuberances that would be suitable for stridulation.

In conclusion, the signal-producing mechanism of psylloids involves two set of morphological structures. The first is stridulation between axillary cord and anal area of forewing. The second is stridulation between axillary sclerite of forewing and the mesothorax.

## **LEAFHOPPERS GOT EARS! THE ABDOMINAL CHORDOTONAL ORGAN OF LEAFHOPPERS - A NEW TYPE OF VIBRATION RECEIVER IN THE HEMIPTERA (HEMIPTERA: TYMBALIA: CICADOMORPHA: CICADELLIDAE).**

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The morphology and function of chordotonal organs in insects is extremely diverse. The organs can be found on different body parts and can serve as receivers of different acoustic and/or vibrational signals. Depending on chordotonal organ type, organs consist of 1 to 1300 mechanoreceptor units, the scolopidia. Leafhoppers have a complex communication system using substrate-borne vibrations. Yet, very little is known about the vibration receptor except a single publication by K. Vondráček (1949) on *Typhlocyba ulmi* [*Ribautiana ulmi* (L., 1858)], describing an abdominal chordotonal organ. Prompted by his findings, I started to investigate those “ears” in the rhododendron leafhopper, *Graphocephala fennahi* Young, 1977.

The analysis of a series of histological semi-thin sections confirmed the existence of chordotonal organs in the first and second abdominal segments. In combination with a morphological investigation of the exoskeleton, I created a graphical reconstruction of the chordotonal organs and the exoskeleton structures. I hypothesize the membrane connecting the first and second abdominal sclerites, to which the chordotonal organs attach, acts as the vibration receiver. The general configuration shows some similarities with the prosternal tympanal organ in tachinid flies and, thus, constitutes an interesting case of parallel evolution.

This study evinces the existence of a sophisticated chordotonal organ in the leafhoppers’ abdomen. The 3D-reconstruction provides a foundation to further investigate the function and the interaction of the chordotonal organs among each other and with the exoskeleton. A comparative analysis covering a wide range of taxa across the Auchenorrhyncha is expected to provide valuable insights into the evolution of the vibration receptor organs.

## **FROM WING VIBRATION TO LOUDSPEAKER DESIGN, A NETLOGO MODELLING APPROACH**

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The sound generated by insect wings dominates the acoustic landscape in many European meadows and tropical forests. Ensiferan insects generate acoustic signals via a file and scraper system that sets wings into vibration. The wings of field crickets show several anatomical structures that are formed by veins. Most relevant are the harp, mirror and chord regions that shape the resonance properties of the cricket wing. Depending on the engagement of the file, the carrier frequency of acoustic signals can be lower (calling songs) or higher (courtship songs), which mainly depends on the resonant properties of distinct wing regions. Recently, the study of wing vibrations using laser vibrometry and modern modelling approaches provided new insights into sound production, which can be inspiring for the design of miniature loudspeakers (e.g. U-sound Inc.).

In this study I developed a novel computer model of cricket wing vibration in the 3D version of Netlogo, a freely available agent-based modelling environment. It aims to simulate the resonance properties of wing regions as it can be found in real insects. Different from a finite element approach, where equations are solved by all elements of the anatomical model, the Netlogo approach simulates the spread of substrate-borne sound in a network of interconnected elements. The simulated stiffness and friction of veins (grey), the wing surface (red) and the mirror (blue) shapes the resonance properties of the wing. Parameter tuning of the model is possible by means of artificial evolution offered in the accompanying software "Behavioursearch".

## CALLING, COURTSHIP, AND POST-MATING TREMULATIONS IN A NEOTROPICAL KATYDID

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Male *Docidocercus gigliotosi* (Tettigoniidae: Pseudophyllinae) tremulates spontaneously to attract females, and both sexes tremulate during courtship. Here, we characterize both calling and courtship tremulations and report high amplitude post-copulatory tremulation by the male katydid. To our knowledge, similar post-copulatory vibrational signals have not previously been reported in *D. gigliotosi* or other katydids, though mate guarding is common in Orthoptera and these tremulations may be an elaboration of such behavior.

We used microaccelerometers to detect and characterize each type of vibrational signal produced by *D. gigliotosi* captured on Barro Colorado Island, Panama. To characterize calling signals, male katydids were recorded in isolation on nylon arenas. To characterize courtship signals, matings were staged and recorded both on nylon and on the terrestrial bromeliad *Aechmea magdalenae*, which is a preferred roost plant—and known signaling substrate—for *D. gigliotosi*. A non-staged mating was observed and filmed in a bromeliad patch, though not recorded with an accelerometer.

We found that female signals are lower in frequency and amplitude than male signals, and are produced only in reply to the male during the courtship duet. Calling tremulations produced by males in isolation do not differ markedly from male courtship tremulations in peak frequency or duration; although, bouts of tremulations are produced more often during courtship. However, the vibrations produced by the male post-copulation have a much higher amplitude and lower pulse rate relative to all pre-mating tremulations.

Further research into the function of these high amplitude vibrations is warranted, as post-copulatory signals have the potential to mediate both mate guarding interactions among males and spermatophore-based conflict between the sexes. Future experiments will provide insight about the role of vibrational signaling in nuptial gift mating systems.

## INTERFERENCE OF FEMALE RIVALRY SIGNAL OF STINK BUGS (HEMIPTERA: PENTATOMIDAE) IN THE COURTSHIP AND MATING BEHAVIOR OF THEIR CONSPECIFICS

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Male pheromone attracts stink bugs to meet on the same plant and triggers females to call for males by the emission of substrate-bone vibratory signals. During female call and male-female courtship emissions, some vibratory signals emitted by conspecifics can interfere in communication and mating behavior. These are the rivalry calls, or signals, emitted by individuals competing to have access to a sexual partner. Recently, rivalry signals emitted by females of *Euschistus heros* were described for the first time. The aim of this work was to evaluate how female rivalry signals can affect vibratory communication during the mating behavior of *E. heros*.

Experiments were performed using plants vibrated with playback of female rivalry signals, and as control plants with no experimental signal applied. Tests were performed by placing couples (male and female, N = 30) on different leaves of vibrated and non-vibrated plants. The behavior and vibratory signal emission of insects were monitored for 15 minutes.

There was no significant difference in the latency time (time to start to sing) of males and females of *E. heros* when they were placed on control or treatment plants. Signal parameters of females and males were affected by rivalry signals in opposite ways. In females, signal pulse duration was shorter in treatment plants and pulses were emitted in intervals where the rivalry songs were absent. In males, signal pulse duration and repetition time were longer in treatment plants. Couples in treatment plants sang for a longer time in comparison with control plants and the percentage of mates was higher in control compared to treatment plants, suggesting that rivalry signals interfere with the normal signal interchange required for satisfactory mating.

**THE ROLE OF VARIABILITY OF THE DIFFERENT ELEMENTS OF COURTSHIP SONGS IN MATING RECOGNITION AND SELECTION OF CONSPECIFICS PARTNER IN *CARPOCORIS FUSCISPINUS***

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Vibrational communication is one of the main mechanisms of recognition of sexual partners in the family Pentatomidae.

In *Carpocoris fuscispinus* we have discovered a new type of song containing both stable and very variable elements. The first part of the song is very stable in all parameters, and the second is highly variable in duration (the number of pulses in the series). We assumed that a stable part of the signal is necessary for successful mating recognition, and a variable part can encode information about individual characteristics. In play-back experiments, the change of stable parameters reduced the success of communication. And the change of the variable parameters had no effects on the effectiveness of the stimulus compared to the natural song.

**AGE-RELATED CHANGES IN FEMALE RESPONSIVENESS IN *APHRODES MAKAROVI*****Nastja FRAS, Anka KUHELJ, Meta VIRANT-DOBERLET**

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In the leafhopper *Aphrodes makarovi*, mate recognition and location is mediated exclusively via substrate-borne vibrational signals. Sexual communication is based on a coordinated exchange of species- and sex-specific vibrational signals initiated by a long and complex male advertisement call to which a sexually receptive virgin female responds, thus triggering the male search for the female on the plant. In this species, female replies are often longer than male calls and vary substantially. Studies showed that the duration of the female reply is negatively correlated with male calling effort invested in finding the female, and males with higher calling effort in early life die younger due to indirect costs associated with high energy expenditure.

In our study we investigated whether the variations in the duration of female reply are associated with female identity and/or with the age-associated changes in the structure of male call. We tested female responsiveness in playback experiments once a week throughout their lifetime. Females were tested with stimulation sequences that included male calls emitted by males from three different age groups (young, middle, old). We scored the number of female replies and for each female reply we measured the reply latency and duration.

## **TELLTALE VIBRATIONS – ARE MALE SIGNALS IN THE SPIDER *PISAURA MIRABILIS* DEPENDENT ON CONDITION?**

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Male mating success in spiders depends on many factors. In *P. mirabilis*, males offer a wrapped prey item as nuptial gift to the female prior to copulation. The production of such gifts was shown to be dependent on male condition. Still, females ignored this condition-dependent information for mate choice. We therefore hypothesized that male vibratory communication signals differ between males, dependent on their condition, and that females use this information to choose their mates. Thus, good-conditioned males would face a greater mating success and a higher number of offspring than bad-conditioned ones.

To test this, we used 58 males, separated into two feeding regimes: 29 individuals were starved since the final moult, while all other males were regularly fed. To record male vibratory signals with a laser-vibrometer (PDV-100), each male was placed in a test arena, where a female had left her draglines before. After 700 s of recording, the female was put to the male to observe further courtship behaviour and copulation events. To prevent effects of nuptial gifts, we used well-fed males as donors of equally sized gifts for all tests. Females were then allowed to build a cocoon and the number of spiderlings that hatched used as a measure for reproductive success. Weight and size of males and females were measured as well.

We observed that males of the poor feeding regime showed a reduced vibratory courtship with 18.8 pulses per minute during the test phase, whereas good-conditioned males exhibited an average of 46.5 pulses per min. Moreover, starved males were more often rejected by females, and if they could mate, copulation-duration was reduced. Additionally, their number of offspring was lower than that of good-conditioned males. Our results show that the feeding-condition of a male significantly affects vibratory courtship signals and subsequently mating success. Females are probably able to use this information encoded in the vibrational signals for mate choice.



## I'VE GOT THAT SILKY TOUCH! IMPACT OF FEMALE STATUS ON MALE VIBRATORY COURTSHIP IN *PISAURA MIRABILIS* SPIDERS

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Vibratory courtship signals are very common in spiders and serve for e.g. species recognition, suppressing the female's aggression towards the male, stimulating her motivation to mate, or signal individual male quality. Males of the nursery web spider, *Pisaura mirabilis*, vibrate their opisthosoma when in contact with female silk, presumably reacting to pheromones contained in her draglines. We hypothesized that male *P. mirabilis* are able to differentiate (developmental) life-stages of females by contacting their silk, and subsequently adjust their vibratory courtship behaviour.

To test this, we recorded reactions of 30 male spiders towards draglines of subadult, adult virgin, and mated females with a Laser-Doppler-Vibrometer. Measurements included pulse intervals and dominant frequencies, as well as latency times and pulse rates.

Our results show that males were less likely to perform vibratory courtship when contacting subadult female silk in comparison to adult female silk. Of those males that vibrated in response to subadult female silk, courtship was initiated later compared to the other two treatments, resulting in a decreased total number of pulses emitted. In contrast to that, pulse intervals did not differ between the three treatments. Our investigation shows that males are able to discriminate the life stages of females by contacting their draglines only and adjust their vibratory mating behavior accordingly. However, as soon as courtship is initiated, *P. mirabilis* males exhibit similar pulse repetition times, irrespective of female status. Since mating with subadult females, as it has been reported from *Latrodectus geometricus*, does apparently not occur in *P. mirabilis*, it remains to be investigated why males court to this life stage. The temporal pattern of male vibratory signals was surprisingly stable and may thus be used for species recognition.

## DO TAP-DANCING BIRDS USE VIBRATIONAL SIGNALS? MUTUAL MULTIMODAL COURTSHIP DISPLAY IN A SOCIALLY MONOGAMOUS SONGBIRD

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Vocalizations have been elucidated in previous songbird communication studies, whereas less attention has been paid to other behavioral components and modalities. Our study species, the blue-capped cordon-bleu (*Uraeginthus cyanocephalus*), is a socially monogamous songbird and both sexes perform courtship dance by holding a piece of nesting material and bobbing up and down in addition to song. Notably, the bobbing produces rhythmical sounds.

By recording these displays with a high-speed video camera, we have discovered that in addition to bobbing, their visual courtship display includes quite rapid tap-dance like movements. The behavior is invisible to the human naked eye and appears as a single pulse on the spectrogram. Interestingly, this behavior can produce vibrations via the perch, in addition to visual and non-vocal acoustic signals.

We have found that they intensify their dance performances (i.e., the number of steps in one bobbing) when their mate stayed on the same perch, which suggests that they use vibrational signals via perch in the context of sexual communication. To understand the role of vibrations in cordon-bleu courtship display, we are now trying to quantify the vibrations using accelerometers attached under the perch. We found that the vibration amplitude positively correlates with the number of steps in one bobbing. It implies that cordon-bleus use vibrational signals to convey their motivation and quality to potential mates. We will discuss the efficacy of the vibrational signals in cordon-bleu courtship display by describing our previous findings and ongoing projects.

## RUSTLING ANTS: PERCUSSIVE COMMUNICATION PERFORMED BY TWO *CAMPONOTUS* SPECIES IN BORNEO

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Many carpenter ants use multi-modal alarm signals consisting of vibrational and chemical components when disturbed or threatened. So far, such vibrational communication has predominantly been studied in *Camponotus*-species of the temperate zones, whereas tropical ones have rarely been considered. In Borneo, two undescribed *Camponotus* spp. (species A and B), which inhabit the ocreas of the rattan palm *Korthalsia robusta*, have been reported to create loud rustling sounds by beating their mandibles and abdomens on the substrate when disturbed.

To describe the characteristics of these signals, we recorded the drumming with a Laser-Doppler-vibrometer and a microphone in the field (Gunung Mulu N. P., Sarawak, Malaysia). We analysed the recordings regarding repetitive patterns, peaks in frequency, time-intervals between pulses, and pulse-durations. Species A exhibits a long-lasting pattern of short vibratory signals ( $0.10 \pm 0.02$  s), repeated at equal intervals. Dominant frequencies range between 131 and 1280 Hz, depending on the respective measuring point on the plant. In contrast, main pulses created by species B are longer ( $0.54 \pm 0.08$  s) with less broadbanded dominant frequencies (144 - 576 Hz), preceded and followed by much shorter and less intense pulses. In order to assess transmission characteristics of the rattan palm, we conducted experiments under controlled conditions in the lab. We played a defined auditory signal (white noise, 0-100 kHz) to different parts of the plant, recording it with the vibrometer.

We show that the ocrea is an adequate structure for converting airborne sound into substrate vibrations, thereby acting as a mediator between these two modalities. However, whether the interaction of airborne and substrate vibration plays a role for ant communication remains to be investigated. We also hypothesize that the vibratory and airborne signals might act as a vibro-acoustic aposematism against predators or herbivores to protect the host plant.

## WHEN TIME IS TIGHT – DRUMMING IN SWARMING ALATES

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The East-African termite *Macrotermes subhyalinus* reproduces by annual dispersal flights of alates, which found new colonies. Dispersal is typically a seasonal phenomenon, with one major exodus triggered by the first heavy rains. The tremendous number of swarming termites inevitably attracts a diversity of predators, resulting in a very high predation risk. There should be a strong selection pressure on mechanisms allowing finding mates in time, before hunted down by predators. Following the nuptial flight, alates drop their wings and search for mates. So far, long-range olfactory attraction by sex-pairing pheromones has been described to mediate pairing.

Here we show for the first time, that the mechanical channel is also involved. Alates produce vibrational drumming signals while searching for mates. They drum by turning over on the back and hit the soil with different body parts by vibrating the triangular scales the wings were attached to. The soil-borne vibrations are broadband (0.3-4 kHz) with a pulse repetition rate of 19 Hz. The signal is up to several tens of seconds long and repeated in irregular intervals. The signals have a striking similarity with soil-borne vibrations induced by the first rain drops falling on dry soil or termite mounds. Interestingly, there are several ethnic groups in Africa that developed methods to attract and gather alates by imitating rain through drumming on the ground.

## VIBRATIONAL SIGNALS ALERT THE FORAGING FORCE

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Communication with nestmates is a key feature of social insects. In the context of foraging, recruitment communication considerably increases the efficiency of food intake. In many stingless bee species, recruitment communication is multimodal, including vibrational signals. Stingless bees produce pulsed thoracic vibrational signals when returning from a successful foraging trip. The vibrations are considered important information for potential foragers about a previously visited food source, mainly because temporal patterns correlate with food source profitability. The vibrations are believed to be an alerting signal, which increases the colony's foraging activity, but this has not been experimentally tested so far.

Here we show for the African stingless bee *Plebeina hildebrandti* that the foraging process is adjusted according to the food profitability in a way that may increase foraging efficiency. Our experiments show that foragers produce thoracic vibrations upon arrival inside of the hive. Vibrational signal duration, pulse duration, and duty cycle correlate with food quality. This may indicate that the temporal patterns of the vibrational signals are modulated to encode information about the food profitability. The playback of successful forager vibrations leads to more bees flying out of the nest. This strongly supports the idea that thoracic vibrations are a signal alerting the foraging force. Furthermore, the number of bees taking over collected nectar from foragers at the nest entrance increases during vibrational playback. The vibrational signal attracts hive bees to the forager, which might decrease the time foragers spend inside the hive and increase foraging efficiency.

## INTERSPECIFIC DIFFERENCES OF VIBRO-ACOUSTIC SIGNALS IN FOUR SPECIES OF BARK BEETLES OF THE GENUS *POLYGRAPHUS* ER. (COLEOPTERA: CURCULIONIDAE, SCOLYTINAE) INHABITING THE ISLAND OF SAKHALIN

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Bark beetles are an important component of forest ecosystems, but outbreaks of the aggressive species can lead to the destruction of entire woodlands. In favor of the presence of vibration communication in the bark beetles, several arguments are evidenced: the presocial organization of colonies on the host tree; the absence of organs to sense air-borne signals and the presence of femoral chordotonal organs that can participate in the perception of the vibrational component of signals produced by beetles. An important issue is the species specificity of signals used by bark beetles and the possibility of using them as an additional or main component of multimodal communication in bark beetles.

In our study, the temporal characteristics of chirps producing by males of *Polygraphus poligraphus*, *P. subopacus*, *P. nigrielytris* and *P. proximus* were compared. Due to technical limitations, we analyzed only the acoustic component of their signals. To record signals, we used a microphone (Behringer ECM 8000) and digital recorder (Zoom R16). The analysis of the records was carried out in the Raven pro 1.5 software. The imago pairs were recorded in test tubes for 15 minutes. The Kruskal Wallis test was used for comparing temporal parameters of bark beetle's chirps (Statistica 10).

The results revealed significant ( $p < 0.001$ ) differences in the following signal characteristics: chirp rate (n / s), duration, interchirp interval, number of strikes per chirp, interstrike intervals. Thus, the differences in temporal parameters of interspecific signals were revealed not only between sympatric species that breed on the gymnosperm plants, but also with *P. nigrielytris* that feeds on angiosperms. This research supported by the Russian Science Foundation (grant № 17-74-10034).

## CONTINUOUS OR NOT CONTINUOUS: INSIGHTS INTO SOME TEMPORAL FEATURES OF THE FEMALE SIGNALS OF BROWN MARMORATED STINK BUG (*HALYOMORPHA HALYS*) TO IMPROVE ATTRACTION TOWARDS MALES

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The Brown Marmorated Stink Bug (BMSB), *Halyomorpha halys* (Stål, 1855), is an alien insect belonging to the family Pentatomidae (Hemiptera), and a native of south-east Asia that was recently introduced into Italy. The control of this pest requires the massive use of insecticides, which however are not effective or sustainable. In this way, the adoption of IPM solutions by using a multi-strategy approach (e.g. aggregation pheromone lures, parasitoids, etc) could be an important alternative. Recent experiments have shown that BMSB communicates using vibrational signals and that males are attracted by a female song. The aim of our experiment was to select between two different song playback types (continuous and discontinuous) to find the more effective method to attracting the males.

A laser vibrometer was used to monitor the playback signals emitted by a mini-shaker from the surface of a bean plant leaf. The playbacks consisted of a pre-recorded female signal that was transmitted either continuously or with regular silent pauses (4s of song + 6s of silence) in trials of 5 min. Males (n = 30) were given 5 min to reach the tip of the mini-shaker on a bean plant

Our results indicated that, when stimulated with continuous signal, 13 males reached the target, while 25 males showed the typical searching behaviour ("run-listen-run"). The discontinuous signal proved to be significantly less efficient in attracting males: only 6 males reached the target and only 12 showed the "run-listen-run" behavior. We concluded that the use of a continuous playback signal looks more promising for the implementation of BMSB traps.

## PRELIMINARY RESULTS ON THE PAIR FORMATION PROCESS AND PEST MANAGEMENT STRATEGY OF A NEWLY DISCOVERED TREEHOPPER VECTOR

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Red blotch is a relatively new viral disease of grapevine that was first recognized less than ten years ago. Since then, the grapevine red blotch-associated virus (GRBaV), the causal agent of red blotch, has been detected in the United States, in Canada, Switzerland and South Korea. Two treehopper species have been associated with the spread of red blotch virus in Oregon vineyards, *Tortistilus albidosparsus* and *T. wickhami* (Hemiptera: Membracidae: Smilinae). Little is known about their biology. It is however likely that mating and intersex attraction is facilitated by substrate-borne vibrations, similar to other species of the Smilinae subfamily. In order to limit the spread of GRBaV, a management strategy based on the use of intra-specific signals to manipulate insect's behavior can be designed, if the intra-specific communication of the species is known. The goal of this study was to acquire the background knowledge of *T. albidosparsus* intra-specific communication.

Laboratory experiments were performed to study the mating pair formation. In order to relate each signal to the specific behavior, singles and pairs of adult treehoppers were simultaneously recorded by means of a laser vibrometer and a video camera.

The preliminary results from the mating communication of *T. albidosparsus* are compared to the vibrational duets of known treehopper species. Moreover, acquired information on the intra-specific communication is discussed together with ecological data about the distribution of the pest, allowing for the follow-up design of possible pest management strategies.



## EVALUATION OF SOCIAL BEHAVIOR BASED ON VIBRATIONAL SIGNALS OF *PHILAENUS SPUMARIUS* IN SEMI-FIELD CONDITIONS

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Communication by substrate-borne vibrational signals underlies insect social and ecological interactions in a complex vibrational environment containing interference from other species and sources of noise. In many hemipteran species, mating behavior is mediated by vibrational signals that are emitted in different social contexts: individuals, pairs, and groups. The meadow spittlebug *Philaenus spumarius* (Hemiptera: Aphrophoridae) is the main vector of *Xylella fastidiosa* that is the causal agent of the Olive Quick Decline Syndrome in the Apulia region (Italy). The aim of our study was to investigate the communication behavior between individuals of the same sex of *P. spumarius* and to evaluate the temporal pattern of the emitted vibrational signals.

To exclude sexual interactions, the studied groups consisted of only males and only females, from 6 to 10 individuals. They were observed simultaneously but in two different net-cages, each containing two grapevine plants. Recordings were performed outdoors, in semi-field conditions, via two laser vibrometers. To evaluate the seasonal pattern of the insect vibrational signaling activity the trials were carried out from June to the end of September in three periods of the day: from 06:30 to 11, from 11:30 to 16 and from 16:30-21.

Our results indicate that males interact significantly more than females. Since no clear aggregation behavior was observed through the day or the season, the signals seemed to underlie competition or territoriality. The next step will be to test the male signals as playback to either disrupt or disturb individuals on their host plants.

## VIBROSCAPE VARIABILITY WITHIN HAY MEADOW HABITAT

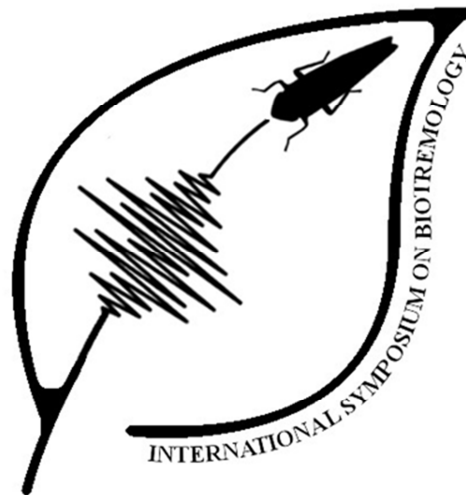
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In the environment, animals continuously encounter vast number of signals and cues from which they obtain information crucial for their survival and reproduction. In turn, the environment is shaping animal signals and communication strategies directly via its physical properties that affect signal transmission and reception, as well as indirectly by interactions with other species sharing the habitat that result in interference. Ongoing vibroscape study in a hay meadow revealed that the vibrational world is at least as complex and dynamic as the acoustic one. In analogy with soundscape, vibroscape is a combination of all vibrational components present in the environment (biological, geophysical, and anthropogenic). While the number of studies focusing on description of soundscape in different natural environments is rapidly increasing, the ecological context of substrate-borne vibrational communication has been almost completely neglected. The complexity of the natural vibroscape remains unknown and how species relying on substrate-borne vibrations interact at the level of community is virtually unexplored. We studied the variability of the vibroscape biological component encountered within the hay meadow in order to determine to what extent individual perception or experience of the vibrational community depends on the spatial position of the receiver at a given site.

Using laser vibrometers we recorded vibrations from several plants of different species on the same meadow and compared their diversity (identity of vibrational taxonomic unit (VTU)), abundance (total number of VTUs per time) and richness (number of different VTUs per time) between different plants within and between different plant species.

# SPECIAL SESSIONS



## **DRUMMER AWARDS 2018, PHILIP BROWNELL AND REGINALD COCROFT**

**Peggy SM HILL<sup>1</sup>, Rafael L Rodriguez<sup>2</sup>, Hannelore HOCH<sup>3</sup>**

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Although the importance of vibrational signals for inter- and intraspecific communication in animals, especially insects, such e.g., Hemiptera and Orthoptera, has been recognized at least since the middle of the 20<sup>th</sup> century, research on vibrational communication has received far less attention than the study of auditory communication by the scientific community. For a long time, vibrational communication was believed to be rare or subordinate to auditory systems. It is only within the last two decades that the ubiquity of vibrational signals among animals, invertebrates as well as vertebrates, and even plants, has become generally accepted.

In order to honour milestone achievements in the study of vibrational communication and to further draw attention to this field of research, the Insect Drummer Award was created in 2011. Here we give a brief overview of the contributions of previous laureates, and present laudations for the recipients of the Insect Drummer Awards 2018: Philip Brownell and Reginald B. Cocroft.

## **THE SECRET LIFE OF ELEPHANTS**

### **Caitlin O'CONNELL-RODWELL**

Department of Otolaryngology, Head & Neck Surgery, Stanford University School of Medicine, CA, USA

The Museum Alto Garda (MAG) will host one of the world's most renowned experts on elephants' behavior and communication: Caitlin O'Connell-Rodwell, who has spent long periods of her professional and personal life in Africa, closely observing the social dynamics of an elephant community. In addition to the curiosities about the life and behavior of the elephants, or the anecdotes related to her fascinating field studies, there will be an opportunity to learn about the results of one of Caitlin's most recent studies, which focused on how pachyderms communicate throughout long distances.

In a species that has so many similarities to humans, both physically and psychologically, Caitlin O'Connell-Rodwell will share striking parallels between elephant and human lives, including growing up in a tight-knit family with very similar family politics, and touch on lessons learned for conservation and human medicine.

This event is organized under the project E-STAR - Bando "I comunicatori star della scienza" - Provincia Autonoma di Trento Legge provinciale 2 agosto 2005, n. 14, articolo 22.

## **BUILDING A SOLID NETWORKING IN BIOTREMIOLOGY**

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To further promote the development of the community of biotremologists, we need a vibrant network that facilitates scientific communication, and helps us to claim and bundle resources for research, student training and public outreach. The workshop will be a platform for proposing and discussing ideas and for initiating (or to continue) efforts in international fund-raising. The goal is to coordinate all such efforts and define concrete steps to be taken in the near future, as well as appoint persons responsible.

Some points to discuss in our workshop are:

1. Résumé of the outcomes of the previous workshop in 2016 (outreach and training website for vibrational communication studies; application for a NSF(US) “Research Coordination Network” grant or matching European program): achievements and drawbacks?
2. Biotremology-database and mailing list: platform for news, job-opportunities, exchange
3. Thinking big: ERC Synergy Grants & COST, comparable other grants
4. European Innovative Training Networks or Staff Exchange (Marie Curie), DFG-Scientific Networks – possible funding sources?

Further ideas are very welcome, and can be communicated to the workshop organizers as well as presented during the workshop.

## RECORDING TECHNIQUES - CURRENT STATUS AND FUTURE DEVELOPMENTS

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The aim of this workshop is to give a review on current technologies for recording vibrational signals in animals based on practical expertise, discussing the problems and to elaborate on improvements and future developments. Laser-vibrometry has become a standard for daily research in biotremology, but in certain situations other (less-expensive) recording systems, such as piezoelectric microphones or accelerometers, are still useful.

Here, in this workshop, we invite people to discuss their daily experience with recording equipment and software for signal analysis. People are encouraged to bring their own field recording equipment and to demonstrate software or tools, especially those less known. Our workshop will have a rather informal format where people can exchange their knowledge and problems with various techniques.

Finally we want to approach POLYTEC and other companies with our bundled wishes and requests for future applications in laser-vibrometry.





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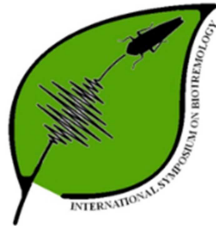
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